CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL-339 (r. 82/08)

Canada

PRESYNAPTIC RECEPTOR HYPOTHESIS AND TERMINATION OF RESPONSES TO CATECHOLAMINES

by

Chi-Chung Chan, B.Sc.

Thesis presented to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Department of Pharmacology,
University of Ottawa,
Ottawa, Ontario
Canada

May, 1981

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my research supervisor, Dr. Stanley Kalsner, for his guidance, constructive criticism and constant encouragement during the course of this study.

I thank Mr. Mohammad Suleiman and Mr. Robert Dobson for valuable technical assistance; and Miss Elizabeth Chen for her expert typing of this manuscript.

The financial support from the Ontario Heart Foundation is gratefully acknowledged.

Finally, I would like to express my special gratitude to my wife, Yan-Lap, for her help in typing the drafts of this thesis and in preparing the photographic artwork. I am indebted to her for her patience, understanding and encouragement, without which this work could not have been accomplished.

CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

I. GENERAL INTRODUCTION

In recent years much information has accumulated about the synthesis, storage and release of noradrenaline in sympathetic nerve endings. Tyrosine, the amino acid precursor, is taken up by the nerve endings from the extracellular fluid and converted to dihydroxyphenylalanine by tyrosine hydroxylase, the rate-limiting enzyme of the sequence. Dopamine is sequentially synthesized from dihydroxyphenylalanine by decarboxylation in the cytoplasm followed by its transformation into noradrenaline. This final synthetic step, mediated by the enzyme dopamine-beta-hydroxylase, takes place in dense-cored vesicles located in the varicosities of the adrenergic nerve endings. The transmitter, in association with adenosine triphosphate, is held in the vesicles protected from metabolic degradation by cytoplasmic monoamine oxidase until nerve stimulation.

A basal efflux of noradrenaline, perhaps due to random vesicular discharge, is converted by the arrival of the nerve impulses into massive transmitter discharge into the neuro-effector synapse. This liberation of noradrenaline is believed to occur by the process of exocytosis, a mechanism whereby the vesicles move toward and fuse with the neuronal membrane, followed by extrusion of the contents of the vesicles into the extracellular space. This process requires the extracellular presence of calcium ions. A high concentration of transmitter in the vicinity of the adrenergic nerve endings is produced by exocytosis since release is from a point source. It may be taken up into the nerve endings by an active transport process even before it reaches the postsynaptic receptors. The steep concentration gradient between the nerve

endings and the effector provides the driving force for diffusion of the transmitter to its sites of action. Once at the postsynaptic locus, the transmitter can either diffuse further into the extracellular fluid, or combine with the appropriate receptors and then exert a physiological response. The transmitter is vulnerable to a second uptake process, located postsynaptically. After transportation into neuronal or extra-neuronal tissues, the transmitter may either be definitely metabolized by monoamine oxidase or by catechol-O-methyl transferase separately or in sequence.

Although in a variety of tissues this complex of events serves a host of functions, two presently unresolved basic concerns are apparent. How is the neurosecretory process regulated and what reduces the quantity of the active transmitter once it is at the receptor sites and thereby terminates its action.

Adrenergic transmitter release in the cardiovascular system is regulated by vasomotor centres located in the brain. Vasomotor centres are tonically active and generate a slow rate of impulse discharge to efferent vasoconstrictor nerve fibers. Baroreceptors, which are sensory stretch receptors located in the walls of the large systemic arteries, detect changes in systemic pressure through a stretch sensitive system of afferent fibers. These receptors transmit signals to central vasomotor centres which adjust their activity and consequently the amount of transmitter released at the autonomic nerve terminals. The baroreceptor, the vasomotor centre and the efferent connections thus form a systemic negative feedback loop. The concept of regulation of transmitter release at the

nerve terminal level itself is recent and not well established, and forms the basis of this thesis. It is presumed that when the concentration of the transmitter at the synapse reaches a threshold level, neuronal receptors are activated thus inhibiting subsequent transmitter liberation. Feedback control is thought to be mediated by specific receptors located on the nerve terminals - the so-called presynaptic receptors. The present study will provide evidence to suggest strongly that the proposed presynaptic adrenergic receptors do not serve a routine physiological function under ordinary conditions of neurotransmission.

Termination of transmitter action refers to the reduction of the quantity of active molecules in the postsynaptic receptor region, and the end of the response. It was at first speculated that the process is analogous to the one described for the cholinergic system where the released acetylcholine is rapidly broken down by cholinesterase, and a metabolic equivalent to an esterase was sought. Later studies showed that the neuronal and extraneuronal uptake of intact adrenergic transmitter at the nerve-effector junction, processes that are not reported to be particularly eventful for the cholinergic system, participate directly in termination of noradrenaline action. Additionally, the physical diffusion of transmitter away from the receptor region is also involved in ending the response. Thus, the transmitter is exposed to several possible terminating mechanisms once it has reached the sites of action. Much effort has been made to determine the relative importance of neuronal and extraneuronal processes in terminating the action of noradrenaline. The present study will show that extraneuronal processes are of critical significance in terminating action in nerve activated vascular smooth muscle.

II. LITERATURE REVIEW

A. Historical background

In 1956, Brown and Gillespie set out to examine the relationship between the frequency of nerve stimulation and the amount of transmitter output (Brown and Gillespie, 1956). It was observed that the amount of noradrenaline in the splenic venous blood of cats, after nerve stimulation, increased with increasing frequency and reached a maximum at 30 Hz. It thereafter declined with frequency and at 300 Hz it was barely detectable. This output - frequency relationship was due neither to a variation in the duration of sample collection time nor to an increased noradrenaline degradation by monoamine oxidase, the only mode of transmitter inactivation recognized at that time. The most significant finding was that two haloalkylamines, namely phenoxybenzamine and Dibenamine, enhanced the noradrenaline output as much as ten-fold at 10 Hz, although they were ineffective at frequencies higher than 30 Hz. A similar enhancement was subsequently observed in the colon and the small intestine of the cat (Brown et al., 1958). Since these haloalkylamines were well known alpha-adrenergic receptor blocking agents (Nickerson, 1949; Furchgott, 1955), it was concluded that physical combination of the liberated noradrenaline with the postsynaptic alpha-receptors was a prerequisite for the destruction and removal of the transmitter, in a manner analogous to that once proposed for acetylcholine (Zupancic, 1953) and that this combination was prevented by covalent binding of the receptor with the antagonists. The decline of noradrenaline output, as well as the ineffectiveness of the adrenergic blocking agents at higher frequencies of stimulation, was attributed by Brown and Gillespie

primarily to the failure of impulse conduction by the small postganglionic nerve fibres.

The discovery, that phenoxybenzamine enhanced transmitter efflux, highlighted our ignorance of the specifics of adrenergic nerve function and initiated an era of research in autonomic pharmacology, still ongoing, directed towards the clarification and precise enunciation of neuronal mechanisms of transmitter release.

B. Preludes to the presynaptic alpha-adrenergic receptor hypothesis

Many possibilities were suggested by interested investigators in an attempt to explain the enhancement of stimulation-induced transmitter overflow by phenoxybenzamine. These efforts include: blockade of neuronal uptake; inhibition of monoamine oxidase and of catechol-O-methyl transferase; blockade of extraneuronal uptake; and even an antagonist-induced increase in flow rate of perfusate through the organ under study, washing out large amount of trapped transmitter.

1. Neuronal uptake

The first attractive alternative to the proposal of Brown and Gillespie that postsynaptic receptor binding is a prerequisite to transmitter inactivation appeared, almost casually, in 1960 at the CIBA Symposium on Adrenergic Mechanisms (Brown, 1960; Paton, 1960). W.D.M. Paton of Oxford claimed that since there is a similarity in the frequency-transmitter output relationship in the spleen and adrenal medulla, that postsynaptic effector events are irrelevant to transmitter secretion. For in the experiments with adrenal medulla, unlike that with the spleen,

catecholamines were collected before they reached the receptor sites at the target organs. Further, based on experiments showing that phenoxybenzamine enhanced transmitter output from the spleen to a greater extent at lower than at higher stimulation frequency, he reflected that a phenoxybenzamine-sensitive amine uptake process (presumably neuronal) is active at low rates of stimulation. Since these results could be explained without a linkage to postsynaptic receptor sites, Paton then suggested that the observations of Brown and Gillespie could represent a manifestation of the action of phenoxybenzamine to inhibit amine uptake back into the catecholamine-releasing tissues. This novel hypothesis readily "avoids the temptation to suppose that specific receptors themselves also destroy the drug they receive - a notion, to my mind, rendered exceedingly implausible ..." (Paton, 1960). The proposal of neuronal uptake was described by Brown as "a most attractive heresy" and no unanimous agreement was reached.

Support for the uptake of catecholamines into tissues continued to increase. ^{3}H-noradrenaline disappears rapidly from the blood of cat and mouse after intravenous injection and is found retained in the heart, spleen and the adrenal gland (Whitby et al., 1961), organs that are densely innervated by sympathetic nerves. This tissue uptake of noradrenaline was demonstrated to obey Michaelis-Menten Kinetics (Dengler, Spiegel and Titus, 1961; Dengler et al., 1962), suggesting that the process is saturable and that the amine is transported into a specific compartment. Following the removal of the superior cervical ganglia of the rat the accumulation of injected noradrenaline in the now denervated salivary gland

was much reduced (Stromblad and Nickerson, 1961), providing striking evidence for a neuronal locus of the uptake process. Similar findings were also reported by others that the uptake of ^{3}H-noradrenaline into cat salivary gland and skeletal muscle vasculature was reduced by 90% after chronic denervation (Hertting et al., 1961). These observations, added to compelling evidence from autoradiographic and electron microscopic studies (Wolfe et al., 1962), suggest strongly that sympathetic nerve endings are sites of catecholamine uptake and retention.

A variety of pharmacological agents have subsequently been shown to block the neuronal uptake of catecholamines (Axelrod et al., 1962), including the compound that received considerable early attention, namely phenoxybenzamine. The inhibitory effect of phenoxybenzamine on uptake has been shown in the heart, kidney, spleen and adrenal gland of cat (Hertting et al., 1961; Farrant et al., 1964); the heart, spleen, uterus and duodenum of rat (Axelrod et al., 1962; Farrant et al., 1964); and the skeletal muscle vasculature of dog (Rosell, Kopin and Axelrod, 1963). It is interesting to note one deviant observation: Dengler and co-workers failed to demonstrate an effect of Dibenamine, also a haloalkylamine, in concentrations from 10^{-6} to 10^{-4} M on the uptake of ^{3}H-noradrenaline in slices of cat spleen, although they demonstrated that cocaine at 10^{-6} M completely inhibited the uptake process (Dengler, Spiegel and Titus, 1961).

It was first believed by some investigators that uptake of catecholamine into neuronal tissue was mediated by special sites, e.g. particular cytological loci on nerve membranes, and that the binding of

the transmitter to these sites was a crucial part of the uptake process. Nevertheless, the identity of such sites was not clear and added confusion to the concept, since they were inappropriately termed "receptors" which related them to the alpha- and beta-adrenergic receptors that mediate physiological responses (Gillespie, 1968). It is unlikely, however, that adrenergic receptors in the physiological sense are by any means related to the neuronal uptake process. For example, phenoxybenzamine at a concentration that prevents the neuronal uptake of noradrenaline in the vasculature of cat kidney does not block the response of the tissue to the amine (Farrant et al., 1964), indicating that the effects of phenoxybenzamine on uptake and on postsynaptic responses are unrelated. Similarly, in the rabbit ear artery the beta-adrenergic blocker propranolol, at a concentration high enough to block beta-receptor mediated responses (5×10^{-5} g/ml), does not inhibit the neuronal uptake of noradrenaline (Avakian and Gillespie, 1968), mitigating against the likely involvement of beta-receptors, at least as we understand them, in the uptake process.

At first, the discovery that phenoxybenzamine inhibited neuronal uptake seemed to provide some guide to the interpretation of the overflow experiments. It was suggested, for example, that it accounts for the enhancement of transmitter overflow in the skeletal muscle vasculature of dog (Rosell, Kopin and Axelrod, 1963). This was based on the observation that phenoxybenzamine at a particular concentration (10^{-7} M) inhibited both neuronal uptake of amine and enhanced the stimulation-induced overflow of transmitters. However, it was soon realized that these two effects of phenoxybenzamine, if not totally unrelated, could at least be dissociated.

Other alpha-antagonists, such as phentolamine, do not affect the uptake process, in moderate concentrations, but increase stimulation-induced transmitter overflow in the same manner as does phenoxybenzamine. This was shown in rat iris by Farnebo and Hamberger (1971a) and in rabbit heart by Starke, Montel and Wagner (1971). Also, the potent neuronal uptake inhibitor cocaine was reported to have no effect on transmitter efflux in tissues such as the cat spleen (Blakeley, Brown and Ferry, 1963; Thoenen, Hürlimann and Haefely, 1964) and colon (Boullin, Costa and Brodie, 1967). The latter observation is particularly noteworthy since phenoxybenzamine does elevate transmitter efflux in cat colon (Brown, Davies and Gillespie, 1958). Another neuronal uptake inhibitor, desipramine, increased transmitter overflow in the rat iris, but to a considerably lesser extent than did phenoxybenzamine (Farnebo and Hamberger, 1970a). An interesting finding was that desipiramine, in a concentration (10^{-7} M) which was maximally effective in blocking neuronal uptake, produced an additional increase in transmitter overflow when given in the presence of an effective concentration of phenoxybenzamine. A similar result was reported by Starke and colleagues (Starke, Montel and Wagner, 1971) when desipiramine was given together with phentolamine in rabbit hearts. Several lines of evidence at that time did not support the view that alpha-adrenergic blocking agents enhance transmitter release by an inhibitory action on neuronal uptake of catecholamines. As summarized by one group "it appears very unlikely that interference with uptake and storage by nerve endings plays an important role in the augmentation by haloalkylamines of noradrenaline output from organs during stimulation of their adrenergic nerves" (Kalsner and Nickerson, 1969c).

2. Monoamine oxidase and catechol-O-methyl transferase

In 1928, Hare described an enzyme in liver extracts that oxidized tyramine. Following this work, Blaschko, Richter and Schlossmann (1937) identified an enzyme in liver which catabolized adrenaline. It is now known that both enzymes are the same entity, namely monoamine oxidase, which carries out oxidative deamination of monoamines. This enzyme was found routinely in sympathetically innervated effector organs (e.g. rabbit blood vessel, Thompson and Tickner, 1951; cat iris and nictitating membrane, Robinson, 1952; cat vasculature, Burn and Robinson, 1952) and based on the observation that its activity decreases following sympathetic nerve denervation, it was concluded that monoamine oxidase is located in sympathetic neurones (Burn and Robinson, 1952; Snyder, Fischer and Axelrod, 1965; Jarrott, 1971). Additional histochemical and pharmacological evidence indicate the presence of this enzyme in extraneuronal tissues, e.g. cardiac tissue (Horita, 1967; Lowe, Reichenbach and Horita, 1971) and vascular smooth muscle cells (Kalsner and Nickerson, 1969c; Su and Bevan, 1971; de la Lande and Johnson, 1972).

Three decades after the initial discovery of monoamine oxidase, Armstrong, McMillan and Shaw (1957) reported the presence of large amounts of an O-methylated metabolite 3-methoxy-4-hydroxymandelic acid, presumably converted from noradrenaline, in the urine of patients with pheochromocytoma. This initiated a search for an O-methylating enzyme and soon thereafter catechol-O-methyl transferase was indeed isolated from liver extracts (Axelrod and Tomchick, 1958). This enzyme is present in numerous tissues (Axelrod, Albers and Clemente, 1959) and species (Axelrod and Tomchick,

1958) and the denervation technique was used to reveal the anatomical location of the transferase. It appears to be located mostly in extra-neuronal tissues since the enzyme activity remained essentially unaffected after chronic nerve section (e.g. rabbit ocular tissues, Waltman and Sears, 1964; dog heart, Potter et al., 1965; dog kidney, Nagatsu, Rust and DeQuattro, 1969). However, it should be noted that a claim for an additional neuronal locus for the transferase was recently made (Jarrott, 1971).

Although both monoamine oxidase and catechol-O-methyl transferase are important in the degradation of circulating catecholamines, their function in the adrenergic neuro-effector system was for some time unclear. For instance, monoamine oxidase was once presumed to have a role to inactivate released transmitter equivalent to acetylcholinesterase in the cholinergic system. This concept was proposed by Burn (1958) who stated " .....that in the neighbourhood of the sympathetic nerve endings..... there is an enzyme - amine oxidase - which exerts the same function as cholinesterase at the endings of cholinergic nerves." It is now recognized, with the discovery of the extraneuronal uptake process for catecholamines (Kalsner, 1966; Iversen, 1967), that in the disposition and catabolism of adrenergic transmitter these enzymes do not function by themselves alone, but instead act together with the extraneuronal uptake process in a mutually dependent way.

In the early 1970's, as a result of various investigations, there emerged a picture of the metabolic inactivation of noradrenaline, applicable particularly to vascular smooth muscle (Kalsner and Nickerson, 1969a;

Levin and Furchgott, 1970; Kopin, 1972; Guldberg and Marsden, 1975). Noradrenaline, after release into the synaptic gap, and having reached the receptor region, is taken up into effector cells where it is predominantly O-methylated. If catechol-O-methyl transferase, a cytoplasmic enzyme, is blocked, monoamine oxidase, a particle bound enzyme, serves as an alternate extraneuronal mechanism and takes over the role of transmitter biotransformation (Kalsner and Nickerson, 1969a; Kopin, 1972). Uptake of noradrenaline into nerve terminals and the catabolism of the transmitter by neuronal monoamine oxidase are of less direct contribution to transmitter inactivation. Incidentally, storage of the re-captured transmitter into vesicles in nerve endings largely prevents the transmitter from destruction by monoamine oxidase (Kopin, 1972). Elsewhere it was proposed that neuronal catechol-O-methyl transferase (Jarrott and Langer, 1971) O-methylates the transmitter after its neuronal re-uptake (Langer, Stefano and Enero, 1972). However, considering the protection of the re-captured transmitter by vesicles, it is unlikely that catechol-O-methyl transferase, which is only questionably present in neuronal tissue, serves a role as important as does monoamine oxidase in the neuronal catabolism of adrenergic transmitter.

At present there is very little known about the effect of inhibition of individual enzymes on stimulation-induced transmitter overflow. Brown and Gillespie (1957) were unable to show any change in noradrenaline overflow, measured with a bioassay technique (blood pressure in the pithed rat), in the perfused cat spleen after administration of iproniazid, a monoamine oxidase inhibitor. Stinson (1961) failed to demonstrate an increase in nerve stimulation-induced efflux of pressor material (presumably

noradrenaline) in rabbit ear artery after treatment of the tissue with iproniazid or with the catechol-O-methyl transferase inhibitor pyrogallol. In contrast, Langer (1970) reported that inhibition of monoamine oxidase (by pargyline) or of catechol-O-methyl transferase (by pyrogallol) increased the efflux of ^{3}H-noradrenaline, isolated by chromatographic procedures, in cat nictitating membrane previously primed with ^{3}H-noradrenaline.

Stimulation-induced efflux of intact transmitter is enhanced after both metabolic enzymes for noradrenaline, catechol-O-methyl transferase and monoamine oxidase, are inhibited. Zimmerman, Liao and Gisslen (1971) observed, in a perfused dog kidney preparation, that after administration of iproniazid and the catechol-O-methyl transferase inhibitor tropolone, catecholamine efflux (detected biochemically) was significantly increased during renal sympathetic nerve stimulation by an effect not attributable to the blockade of the tissue uptake of noradrenaline. In other experiments Hughes (1972) reported that pretreatment of rabbit vas deferens or portal vein with pargyline and tropolone enhanced the efflux of noradrenaline (bioassayed) in response to field stimulation by 2.5- to 3-fold.

It should be noted that differences in the results between inhibition of the one or both enzymes may not be solely a consequence of different degrees of metabolic inhibition (i.e. in one case a single enzyme whereas in the other case both enzymes are inhibited). Other factors, such as type of tissue, experimental conditions and stimulation parameters, may also contribute to these contrasting obervations. For example, the length of the diffusional path between the site of transmitter release and

the superfusing medium is likely to be different in various tissues. Liberated transmitter is susceptible to different extents of enzymatic destruction, by passing through variable tissue spaces, before it is collected in the superfusate. The [redacted] of enzyme inhibition on stimulation-induced transmitter overflow thus represents not only the result of an interruption of transmitter inactivation at the receptor sites, but also a modification of its fate en route to the effluent. These effects and their implications, as of this moment, remain largely unexplored.

In any event, efflux of noradrenaline and its metabolites into the perfusate of organs or tissues cannot be a reliable index depicting the fate of the transmitter at the receptor region. It is the events occurring specifically at the receptor region that determine the magnitude and duration of tissue responses. For example, since there is an abundance of possible inactivation mechanisms, and some of them work in series, inhibition of one enzyme alone need not and does not as a rule alter transmitter-induced responses (Kalsner, 1977).

At the first CIBA Symposium on Adrenergic Mechanisms, Furchgott (1960b)made the suggestion that phenoxybenzamine may enhance stimulation-induced efflux by blocking metabolic inactivation of transmitter. The demonstration that phenoxybenzamine reduces tissue accumulation of metabolites of ^{3}H-noradrenaline in the perfused rat heart (Eisenfield, Axelrod and Krakoff, 1967) seemingly provided experimental support for this interpretation. However, it was soon recognized that the effect of phenoxybenzamine on the accumulation of ^{3}H-metabolites was critically dependent upon the integrity of the tissue under study. When the haloalkylamine at a

concentration of 10^{-5} M was administered to heart homogenates, no reduction in ^{3}H-noradrenaline metabolism by deamination or O-methylation was evident (Eisenfield et al., 1967). It thus appeared that phenoxybenzamine has no direct inhibitory effect on monoamine oxidase or catechol-O-methyl transferase but acts in some unique way to prevent catacholamine metabolism.

3. Extraneuronal uptake

Extraneuronal uptake as a terminating process in noradrenaline action was initially proposed by Kalsner (1966, 1969a) who, in an attempt to assess the importance of various processes in ending the action of sympathomimetic amines, showed that in rabbit aorta, the effector responses to noradrenaline, adrenaline and norefedrine were enhanced by haloalkylamines and by the steroids 17 beta-estradiol (referred to as estradiol hereafter), progesterone and desoxycorticosterone. The steroid potentiation was abolished by pretreatment of tissues with inhibitors of the extraneuronal enzyme catechol-O-methyl transferase but not easily attributable to direct enzyme inhibition (Kalsner, 1969a). The administration of the neuronal uptake inhibitor cocaine had no effect (Kalsner, 1969b). The results demonstrated that the augmented effector response is independent of blockade of the neuronal uptake process but instead is associated with extraneuronal processes of amine inactivation.

Subsequently, Kalsner and Nickerson (1969) used the oil immersion technique to study the effect of GD-131, a haloalkylamine similar to phenoxybenzamine but with less alpha-adrenergic blocking activity, on the inactivation of amines in the rabbit aorta. Under these conditions, by preventing the diffusion of amines away from the postsynaptic receptor

region, the rate of relaxation of the aorta strip is a primary index of the amine inactivation due to intrinsic factors. It was found that both cocaine and the compound GD-131 reduced the rate of relaxation of strips contracted by noradrenaline. When the strips were pretreated with the monoamine oxidase inhibitor iproniazid and the catechol-O-methyl transferase inhibitor tropolone, the effect of cocaine became more profound whereas that of GD-131 did not. This obervation, i.e. the effect of cocaine was additive to enzyme inhibition but that of GD-131 was not, suggested that these agents did not exert their effects by the same mechanism. Further, since the reduction of noradrenaline inactivation caused by exposure of the tissue to GD-131 and by inhibition of both enzymes were of the same extent, it was concluded that the major action of GD-131 is to impede access of amines to the sites of enzymatic degradation, a site which is "cocaine-resistant" in nature (uptake of amine into effector cells). Kalsner and Nickerson (1969c) then suggested that following sympathetic nerve stimulation "the largest part of the released mediator is distributed in the intracellular water of non-nervous elements of the tissue (presumably effector cells)".

Iversen studied the tissue uptake of catecholamines at various perfusion concentrations in the isolated rat heart (1965). He observed that there is a second phase of uptake of noradrenaline occurring at high concentrations (4-5 µg/ml), in addition to the one shown previously (Iversen, 1963) when low concentrations of the amine (0.4 µg/ml) were used. If rate of uptake is plotted against the noradrenaline concentration in the perfusate, the curve turns steeply upward following an initial plateau stage. Nevertheless, Iversen made no attempt to relate this second phase

of amine uptake with extraneuronal tissues but simply described it as a "novel" process. He suggested that a neuronal mechanism is involved in both phases of amine uptake, based on the observation that follwoing immunosympathectomy of the rat the uptake of noradrenaline in the heart is reduced with either low or high perfusion concentrations of the amine. He concluded "that both uptake processes are associated with sympathetic nerve fibres in the heart" (Iversen, 1965). It is now recognized that this second phase of amine uptake represents an extraneuronal process, earlier proposed by Kalsner and Nickerson, which is qualitatively and quantitatively different from its neuronal counterpart. Of particular importance is the fact that this process is susceptible to inhibition by pharmacological agents distinct from those which block neuronal uptake. For example, extraneuronal uptake is inhibited by normetanephrine but not by cocaine or metaraminol, two potent neuronal uptake inhibitors.

There is strong evidence to substantiate the claim that phenoxybenzamine inhibits the extraneuronal uptake of noradrenaline. Uptake of ^{3}H-noradrenaline in the rat heart, in the presence of cocaine, is further reduced by phenoxybenzamine (Eisenfeld, Axelrod and Krakoff, 1967). Since the concentration of cocaine (10^{-4} M) is high enough to maximally inhibit neuronal uptake, the action of phenoxybenzamine must be elsewhere. Additionally, since the tissue accumulation of metabolites of ^{3}H-noradrenaline, namely normetanephrine, deaminated catechols and O-methylated-deaminated products, was also decreased by the antagonist, it appears that phenoxybenzamine prevents the metabolism of ^{3}H-noradrenaline by impeding the entry of the ^{3}H-transmitter into extraneuronal tissues. Eisenfeld and colleagues

showed, by comparison of intact tissues and tissue homogenates, that direct enzyme inhibition was not involved. A similar finding was made with phentolamine in this study. Elsewhere, Lightman and Iversen (1969) showed that phenoxybenzamine reduces the accumulation and metabolism of ^{3}H-noradrenaline in the rat heart when the neuronal uptake process is blocked by metaraminol and suggested that phenoxybenzamine is among the most potent inhibitors of extraneuronal uptake again substantiating the earlier findings of Kalsner and Nickerson with GD-131. Langer (1970) also claimed an extraneuronal action of phenoxybenzamine in the cat nictitating membrane. These accumulated findings have led to speculation that the effect of phenoxybenzamine on transmitter overflow is at least partly due to its inhibitory action on extraneuronal uptake.

Separately, evidence not in favour of such an interpretation of phenoxybenzamine action, began to emerge. For example, some inhibitors of extraneuronal uptake devoid of alpha-adrenergic blocking action, have no effect on stimulation-induced transmitter overflow. Normetanephrine, at concentrations of up to 10^{-4} M, does not affect the overflow in either the rat iris (Farnebo and Hamberger, 1971a) or the guinea-pig atria (McCulloch, Rand and Story, 1972). Another extraneuronal uptake inhibitor, corticosterone (Kalsner, 1969a; Iversen and Salt, 1970), does increase overflow in vas deferens of rabbit (Hughes, 1972), however, the increase (36%) is much smaller than that induced by phenoxybenzamine (360-400%). When the vas deferens is pretreated with cocaine at a concentration (5 µg/ml) that eliminates possible complications caused by neuronal uptake of catecholamines, corticosterone induces a 4-fold increase, whereas phenoxybenzamine produces an 8-fold increase in transmitter overflow. This difference is not likely

to be due solely to the relative potencies of these agents as inhibitors of extraneuronal uptake. The concentration of phenoxybenzamine which inhibits extraneuronal uptake by 50% (IC_{50}) in the rat heart is estimated to be 2.5 μM (Lightman and Iversen, 1969). The IC_{50} for corticosterone in the same tissue is 2.6 μM (Iversen and Salt, 1970). These two agents therefore have a similar potency to block extraneuronal uptake, yet their effects on transmitter overflow are drastically different. This clearly suggests that phenoxybenzamine and corticosterone act via different mechanisms in increasing the overflow of noradrenaline.

Additional evidence supporting a lack of relationship between the inhibition of extraneuronal uptake of catecholamine and the enhancement of transmitter overflow is available. Kirpekar and Wakade (1970) reported that GD-131, described as a potent extraneuronal uptake inhibitor, had no effect on the overflow of transmitter from the intact cat spleen following sympathetic nerve stimulation. In fact, the overflow at high frequency of stimulation was decreased by GD-131. Control studies showed tht phenoxybenzamine increased the transmitter overflow by more than 10-fold under identical experimental conditions. A similar discrepancy between the effects of GD-131 and phenoxybenzamine on efflux was noted by Farnebo and Hamberger (1971a) on the isolated rat iris. These results cannot be meaningfully interpreted solely on the basis of extraneuronal uptake blockade.

4. Flow rate

The effect of blood flow through the organ under study on the amount of transmitter appearing in the venous effluent during nerve stimulation has been of concern to investigators. Carlsson and co-workers

(Carlsson, Folkow and Häggendal, 1964) have emphasized the importance of blood flow on the amount of noradrenaline recovered in the blood-perfused hind limbs of cat. Under control conditions the amount of noradrenaline in the venous effluent increases in response to sympathetic trunk stimulation. During exercise of the hind limb induced by stimulation of the sciatic nerve, sympathetic stimulation causes a greater increase in noradrenaline efflux. The authors concluded that normally the release of noradrenaline into the venous blood is restricted by the vasoconstriction accompanying sympathetic stimulation. This restraint can be overcome by counteracting the vasoconstriction (e.g. by muscle exercise-induced vasodilation), presumably due to local metabolites, thus producing a marked increase in noradrenaline efflux. Therefore, to confound possibilities, it was also thought that the enhancing effect of phenoxybenzamine on transmitter release might be due partly to its antagonism of an alpha-adrenergic receptor-mediated vasoconstriction. This possibility was dismissed subsequently by Häggendal (1970) who, in proposing the concept of trans-synaptic regulation of neurotransmission (to be discussed later), showed that in the cat hind limb phenoxybenzamine substantially increases transmitter overflow even when blood flow was maintained high by muscle exercise.

Further evidence against any speculation that the increase in blood flow is a cause of alpha-antagonist-induced enhancement of transmitter overflow is provided by the constant-flow experiments of Kirpekar and Puig (1971). They found that in the cat spleen, phentolamine and phenoxybenzamine both increased by nearly 4-fold stimulation-induced noradrenaline

overflow even when the tissue was perfused at a constant flow rate. A similar observation was reported for the vasculature of dog skeletal muscle (Resell, Kopin and Axelrod, 1963). Perhaps the most conclusive evidence against blood flow as a key factor in the effect of phenoxybenzamine was obtained when simple tissue models were employed (e.g. isolated vascular strips). Local circulatory influences are eliminated in such preparations but phenoxybenzamine still enhances transmitter overflow (Su and Bevan, 1970; present study).

The above described investigations illustrate vividly the complexity of the mechanisms of action of phenoxybenzamine on neurotransmission, yet none of these mechanisms taken alone, or perhaps even together, appear to explain satisfactorily the enhancing effects of the compound on transmitter overflow. This limitation has given incentive for another proposal - the presynaptic alpha-adrenergic receptor hypothesis.

C. The presynaptic alpha-adrenergic receptor hypothesis

1. The statements

The concept of peripheral presynaptic inhibition was initially introduced by Paton and Vizi who found that in guinea-pig ileum the output of acetylcholine elicited by nerve stimulation was reduced by exogenous noradrenaline and adrenaline. They concluded that the decrease in acetylcholine output was a result of "presynaptic inhibition mediated by alpha-receptors" (Paton and Vizi, 1969). This postulate was later applied to adrenergic transmission and was advanced in the same year from laboratories in Sweden by Farnebo and Hamberger (1971a), in the United States by Kirpekar and Puig (1971) and in Argentina by Langer and co-workers (Langer et al.,

1971). It was proposed, based on known observations that phenoxybenzamine increases the efflux of tritium with nerve stimulation, that sympathetic nerve endings are endowed with inhibitory alpha-adrenergic receptors. Combination of these sites with noradrenaline is presumably inhibited by phenoxybenzamine, releasing the nerve endings from presynaptic inhibition by neurally liberated noradrenaline. Thus, the presynaptic alpha-receptors are presumed to mediate a negative feedback system triggered when the amount of the transmitter at the synaptic gap reaches a threshold level, restricting the quantity of transmitter released per pulse. This hypothesis was also elaborated by Starke (1971) in Germany on the basis of the converse finding that two alpha-agonists, namely phenylephrine and naphazoline, decrease noradrenaline overflow from the rabbit heart on cardiac nerve stimulation.

Although the four groups referred to above used different tissue models, the foundations of the hypothesis were similar and it was based mainly on two sets of observations: 1) phenoxybenzamine enhances noradrenaline output elicited by nerve stimulation, 2) noradrenaline and some other alpha-agonists inhibit transmitter efflux. Since these agents are known to act on postsynaptic alpha-adrenergic receptors to block and elicit physiological responses respectively, and since the inhibitory actions of the agonists on transmitter release could be partially or completely blocked by known postsynaptic alpha-antagonists (Vizi et al., 1973; Langer, 1973), it was presumed that these two classes of drug interact with a presynaptic set of alpha-receptors to regulate neurotransmission. However, experimental evidence to substantiate the hypothesis is very limited.

That phenoxybenzamine enhances transmitter efflux is not in doubt and has been confirmed by investigators using a variety of tissues, as summarized in the following table:

	Tissue	Reference
Mouse	vas deferens	Jenkins, Marshall and Nasmyth, 1975
	atria	Farnebo and Hamberger, 1974
Rat	portal vein	Häggendal et al., 1972
	superior cervical ganglia	Vogel et al., 1972
Guinea-pig	aorta	Bell, 1974
	vas deferens	Stjärne, 1973
	atria	McCulloch, Rand and Story, 1972
	intestine	Henderson, Hughes and Thompson, 1972
Rabbit	pulmonary artery	Su and Bevan, 1970
	portal vein	Hughes, 1972
	superior cervical ganglia	Noon and Roth, 1975
	vas deferens	Hughes, 1972
Dog	spleen	de Potter et al., 1971
	kidney	Zimmerman, Liao and Gisslen, 1971
	adipose tissue	Fredholm and Hedqvist, 1973

Additionally these early data with phenoxybenzamine were supported by other observations with the alpha-blocker phentolamine, e.g. in cat aorta (Langer et al., 1975) and dog spleen (de Potter et al., 1971),

confirming stimulation-induced overflow enhancement of ^{3}H-transmitter by it as well. However, similar studies with still other alpha-antagonists and under more precise test conditions were not available. According to the presynaptic alpha-receptor hypothesis, phenoxybenzamine and phentolamine modify transmitter release during sympathetic stimulation by an interruption of the negative feedback loop which would normally be activated when the noradrenaline concentration in the synapse reaches a threshold level. Thus transmitter release is "disinhibited" by presynaptic alpha-receptor blockade and consequently an increased amount is collected from the overflow.

During the development of the hypothesis, two common concerns were apparent: 1) is the increased overflow of tritium after phenoxybenzamine a real measure of noradrenaline release? 2) is the effect of the antagonist due to an action on the postsynaptic effector cell or on the presynaptic nerve terminals?

That the increase of overflow reflects an actual increase of noradrenaline release has been implied by the inadequacy of other explanations related to neuronal uptake, extraneuronal uptake, enzymatic degradation and flow rate. Direct evidence comes from the finding of de Potter and co-workers that efflux of both noradrenaline and dopamine-beta-hydroxylase is increased during nerve stimulation in the dog spleen after administration of phenoxybenzamine (de Potter et al., 1971). Dopamine-beta-hydroxylase is present in the noradrenaline-containing vesicles (dense-cored vesicles) and is released concomitantly with noradrenaline during stimulation of sympathetic nerve endings, presumably by exocytotic expulsion (Gewirtz and Kopin, 1970; de Potter et al., 1969). Control

experiments with cocaine showed that the uptake inhibitor enhances, although to a lesser extent than does the adrenergic antagonists, the overflow of noradrenaline but has no effect on the output of dopamine-beta-hydroxylase, a macromolecule which is not a substrate for the uptake process. These observations suggest that phenoxybenzamine facilitates the process of exocytosis by some undefined mechanisms and that the increase in noradrenaline overflow after treatment of tissues with these agents represents an actual increase in transmitter release.

An early, now discarded hypothesis related the effect of adrenergic antagonists on efflux to trans-synaptic regulation. Häggendal (1970) observed that the amount of noradrenaline released during sympathetic nerve stimulation in the vasculature of cat skeletal muscle is negatively correlated with the peripheral vascular resistance; and that this relationship remains unchanged following administration of phenoxybenzamine. Also, phenoxybenzamine enhances transmitter output and such enhancement is much greater than that induced by another alpha-antagonist, ergotamine. Since ergotamine was reported to have a direct stimulatory action on some effector organs, namely nictitating membrane and spleen of cat (Salzmann et al., 1968), Häggendal reasoned that the smaller enhancement of efflux observed after the ergot alkaloid is due to a greater "reactive state of the effector cell" (as compared to that after phenoxybenzamine). On elaboration of this conclusion he then put forward the theory of trans-synaptic regulation which proposed that the effector cell modulates transmitter release by means of chemical mediator crossing the synaptic gap (Häggendal, 1970; Folkow, Häggendal and Lisander, 1968). According to this scheme the response magnitude of the effector cell is of prime importance: the amount

of transmitter released per nerve impulse will be reduced if the effector is at a supra-excited state. It was presumed that adrenergic antagonists interrupt the proposed trans-synaptic regulation by "depressing" the effector cell response and that more transmitter is released as a result of the disinhibition.

This theory was seemingly supported by an observation of Farnebo and Malmfors (1971) on mouse vas deferens. They reported that papaverine, a non-specific smooth muscle relaxant devoid of alpha-adrenergic receptor blocking activity, at high concentration (10^{-4} M) enhances stimulation-induced overflow of ^{3}H-transmitter and concommitantly decreases mechanical responses. Further, in contrast to its potentiating effect on the guinea-pig vas deferens (Ohlin and Stromblad, 1963), phenoxybenzamine inhibits the contractile responses of the mouse vas deferens to field stimulation. More importantly, such inhibition of mechanical responses is associated with an increase in ^{3}H-transmitter overflow. Conversely, the alpha-adrenergic agonist methoxamine increases mechanical responses but reduces efflux of tritium. Thus, it was concluded that transmitter release is modified by alpha-antagonists and alpha-agonists indirectly via their primary effect on altering postsynaptic effector responses. This conclusion also implied that alpha-adrenergic receptor is not necessarily an essential component of the regulatory mechanism for transmitter release, a contention that is in fact opposed to the presynaptic alpha-adrenergic receptor hypothesis which assigns a crucial role to the adrenergic site.

The chemical mediator that functionally connects the effector cell and the nerve terminals was thought to be prostaglandin E which could

be detected in the perfusate of various tissues after sympathetic stimulation or after infusion with exogenous noradrenaline (Junstad and Wennmalm, 1973; Davies, Horton and Withrington, 1967; Bennett, Freidmann and Vane, 1967). However, unlike phenoxybenzamine, exogenous prostaglandin of the E series produce all three possible effects on the stimulation-induced release of transmitter. Prostaglandin E may increase (Davies and Withrington, 1968), decrease (Hedqvist, Stjärne and Wennmalm, 1970) or have no effect (Fredholm and Hedqvist, 1973) on the overflow of noradrenaline. While the theory of trans-synaptic regulation may be applicable in some tissue models, it has not been widely accepted. In 1975, Häggendal, who originally proposed the theory, reported the failure to demonstrate changes in stimulation-induced transmitter release form the portal vein following mechanical manipulation of the vessel (Häggendal, 1975). He then declared a "partially changed view", and with regard to regulation of transmitter release he stated "the mechanism of the greatest importance, ..., appears to be localized presynaptically".

Most investigators believe that the site of action of phenoxybenzamine to alter transmitter efflux is presynaptic. This is supported in part by the repeated finding that phenoxybenzamine enhances stimulation-induced noradrenaline release in cardiac tissues (Starke, Montel and Schumann, 1971; McCulloch, Rand and Story, 1972; Farah and Langer, 1974), where the postsynaptic adrenergic receptors are mainly of the beta-type. In these preparations phenoxybenzamine does not inhibit the postsynaptic responses to nerve stimulation, thus demonstrating the irrelevance of the postsynaptic effector response to transmitter release. Further evidence

in support of a presynaptic phenoxybenzamine-sensitive site was provided by studies on cultured cells from the rat superior cervical ganglia. Several days after organ culture axonal sprouts, which resemble sympathetic nerve endings and take up ^{3}H-noradrenaline, start to grow from the ganglionic cells. Phenoxybenzamine at high concentration (3×10^{-5} M) doubled the field-stimulation-induced overflow of noradrenaline from such sprouts (Vogel et al., 1972). Since postsynaptic effector cells are absent in the cultured axonal sprouts, the site of action for phenoxybenzamine is concluded to be presynaptic.

Although phenoxybenzamine increases transmitter overflow consistently, the amount of the enhancement is far from uniform. The maximal enhancement induced by the haloalkylamine varies from 2-fold in the dog kidney to 38-fold in the rabbit vas deferens. According to Hughes (1972), this variation is presumably due to different densities of adrenergic nerve innervation in these tissues. More recently, Bevan (1978) has emphasized this issue and suggested that the concentration of released transmitter in the synaptic gap during nerve stimulation is a function of the neuro-effector distance. In the densely innervated tissues the concentration of released transmitter would be higher than that in the sparcely innervated preparations due to the high discharge concentration of transmitter and the restriction of physical diffusion of the transmitter away from the synaptic gap. In that case phenoxybenzamine, by removing the presynaptic alpha-receptor-induced restraint on transmitter release, produces a proportional greater magnitude of effect on stimulation-induced efflux of ^{3}H-transmitter in densely than in thinly innervated tissues.

However, differences in efflux enhancement occur without seeming relevance to densities of nerve innervation. For example, phenoxybenzamine increases the stimulation-induced overflow of ^{3}H-noradrenaline by 2.44-, 1.52-, 1.95- and 1.90-fold in the cat nictitating membrane (Langer, 1970), guinea-pig vas deferens (Stjärne, 1973d), rabbit pulmonary artery (Borowski, Ehrl and Starke, 1976) and rat portal vein (Dahlof, Ljung and Ablad, 1978), respectively, yet the nerve densities of the two former tissues are known to be much greater than that of the other two.

As emphasized earlier, another key observation repeatedly employed in support of the presynaptic receptor hypothesis is the finding that the stimulation-induced overflow of transmitter in several diverse tissues is decreased by noradrenaline and some other alpha-agonists. The observations are summarized as follows:

	Tissue	Agent	Reference
Mouse	vas deferens	noradrenaline	Farnebo and Malmfors, 1971
Rat	vas deferens	noradrenaline	Vizi et al., 1973
Guinea-pig	vas deferens	noradrenaline methoxamine	Stjärne, 1973a, 1973b
Rabbit	heart	noradrenaline oxymetazoline naphazoline phenylephrine	Starke, 1972a, 1972b
	ear artery	noradrenaline	McCulloch, Rand and Story, 1973
	pulmonary artery	noradrenaline	Taube et al., 1976
	superior cervical ganglion	methoxamine	Noon and Roth, 1975

	Tissue	Agent	Reference
Cat	aorta	isoproterenol	Langer et al., 1975
	spleen	noradrenaline phenylephrine methoxamine isoproterenol	Kirpekar et al., 1973
	nictitating membrane	noradrenaline	Langer, 1973

In many of these studies cocaine and/or normetanephrine were incorporated routinely into the physiological saline to eliminate possible interference of neuronal and/or extraneuronal uptake on the transmitter release (e.g. Stjärne, 1973a, 1973b; McCulloch, Rand and Story, 1973; Starke, 1972a, 1972b). Thus, presumably, the decrease in transmitter overflow in the presence of noradrenaline or other alpha-agonists is not due to an action on disposition and biotransformation of the transmitter. The possibility of an inhibitory action of these agents on nerve impulse conduction was excluded by Starke (1972b) based on the observation that oxymetazoline and naphazoline, both at a concentration (10^{-7} g/ml) which has no apparent local anaesthetic activity, reduce significantly the stimulation-induced transmitter overflow in the rabbit heart. In fact, most alpha-agonists employed in these overflow studies do not have local anaesthetic effects. A dilution by the exogenous unlabelled noradrenaline of the pre-loaded ^{3}H-noradrenaline tissue store is also unlikely. In the rabbit heart following incubation with ^{3}H-noradrenaline, the administration of cocaine at a high concentration (2.5×10^{-5} g/ml) to inhibit the neuronal uptake of subsequently administered unlabelled noradrenaline did not prevent the unlabelled amine from inhibiting the stimulation-evoked overflow of ^{3}H-transmitter (Starke, 1972a).

Since its proposal in 1971, the presynaptic negative feedback hypothesis has gained widespread and generally uncritical acceptance by the scientific community. Interest in the hypothesis is increasing and other facets of presynaptic regulation and the implications which derive from them are being explored.

2. The presynaptic alpha-receptors as a separate class of receptors

The presynaptic alpha-adrenergic receptor is postulated to be similar to but different from its "classical" postsynaptic counterpart. The idea stems from the finding that for many alpha-agonists and alpha-antagonists there are discrepancies between their potencies in altering transmitter release (i.e. presynaptic effect) and in modifying effector responses (i.e. postsynaptic effects). The results are difficult to interpret assuming a homogenous group of alpha-receptors, and it has been proposed that two classes of alpha-receptors exist: one mediates postsynaptic mechanisms and is designated alpha-1 receptor, the other mediates presynaptic mechanisms and is designated alpha-2 receptor (Langer, 1974).

For example, it was found that in the isolated rabbit pulmonary artery (Starke, Endo and Taube, 1975), the concentrations of alpha-agonists which produced 20% of the maximal contraction showed an order of potency of: adrenaline > noradrenaline > oxymetazoline > naphazoline > phenylephrine > tramazoline > alpha-methylnoradrenaline > methoxamine; and for the agonist concentrations which reduced the stimulation-induced overflow by 20%, it was: adrenaline > oxymetazoline > tramazoline > alpha-methylnoradrenaline > noradrenaline > naphazoline > phenylephrine > methoxamine. Differences in

pre- and postsynaptic "potencies" were also reported for alpha-antagonists: phenoxybenzamine is more potent in blocking postsynaptic responses than presynaptic sites in the rat portal vein (Häggendal et al., 1972) and in the cat spleen (Dubocovich and Langer, 1974). In both preparations, phenoxybenzamine at 10^{-8} to 3×10^{-8} M abolishes the mechanical response to nerve stimulation, but the increase in transmitter overflow is submaximal: a further increase in phenoxybenzamine concentration results in additional enhancement of the transmitter overflow. Monitoring the heart rate of the pithed rat, the presynaptic potency for the rate-increasing effect of alpha-antagonists was phentolamine > piperoxan > yohimbine > tolazoline > chlorpromazine > phenoxybenzamine > thymoxamine (Drew, 1976). This effect is presumed due to a presynaptic action since postsynaptic alpha sites are sparse in rat myocardium. However, in this study the comparison of potency was made using doses of antagonist which reversed 50% of the bradycardia induced by a standard dose of clonidine (100 μg/kg), a presynaptic receptor agonist, during continuous cardiac sympathetic stimulation. The experiment fails to account for the possible changes in sensitivity of the cardiac tissue with prolonged nerve stimulation. Also it does not deal with complications resulting from the interaction between the antagonists and a mixed "biophase" pool of exogenous and endogenous agonist, nor does it account for a possible lack of specificity in the effect of clonidine on cardiac nerve stimulation (Scriabine et al., 1970; Scriabine and Stavorski, 1973; Robson and Antonaccio, 1974). The postsynaptic potency of antagonists was also determined by blood pressure measurements in response to clonidine and it was reported that phentolamine

was the most potent and chlorpromazine the least effective antagonist. Nevertheless, this potency relationship may not necessarily be correlated with presynaptic potencies since it was determined by comparison of the shift of the clonidine vasopressor dose-response curves, an index which is procedurely different from that utilized to assess the presynaptic potencies.

Recently, other investigators examined the heterogeneity of the pre- and postsynaptic alpha-receptors. Steppler and colleagues (Steppler, Tanaka and Starke, 1978) examined the pre- and postsynaptic effects of phenylephrine and tramazoline in the auto-perfused hind leg of rabbit in which nerve stimulation was carried out by stimulating the distal portion of a sectioned lumbar sympathetic chain. They observed that phenylephrine, but not tramazoline, is more potent in the activation of postsynaptic receptors (which resulted in an enhancement of perfusion pressure) than in stimulating presynaptic receptors (which resulted in an inhibition of stimulation-evoked blood pressure rise). While the order of pre- and post-synaptic potencies for phenylephrine were in accord with those reported earlier in the isolated rabbit pulmonary artery (Starke, Endo and Taube, 1975), that for tramazoline were not. Over a moderate concentration range (10^{-6} to 3×10^{-8} M), tramazoline increased the basal perfusion pressure, in a dose-dependent manner, from 7.5- to 43-fold of the control value. With the same concentration range the agonist inhibited the stimulation-evoked pressure rise by an average of 34% to 62% (at 1 Hz). Tramazoline thus behaves as a potent postsynaptic and also a potent presynaptic alpha-agonist. In the rabbit pulmonary artery, however, this compound was among

the most potent presynaptic but the weakest postsynaptic alpha-agonists. The discrepancy in the postsynaptic potencies of tramazoline in different tissues has led the authors to raise the concern that "it would be premature to consider all presynaptic alpha-receptors as one pharmacologically homogenous group and all postsynaptic alpha-receptors as the second, distinct homogenous group".

Earlier it was also reported that in the auto-perfused hindquarters and hindlegs of cat an inconsistency was noted in the pre- and postsynaptic effects of clonidine when compared with in vitro experiments (Haeusler, 1976). It was demonstrated that a very high dose of clonidine (100 μg/kg, i.v.) is required to block the stimulation-evoked increase in perfusion pressure but a significantly lower dose of clonidine (30 μg/kg, i.v.) elevates blood pressure and perfusion pressure, and does so without any detectable effect on the response to sympathetic stimulation. The results indicated that clonidine has a preferential postsynaptic action. This is contradictory to the conclusion of in vitro experiments on rabbit pulmonary artery (Starke, Endo and Taube, 1975) and ear artery (Steinsland and Nelson, 1975). In their experiments clonidine behaved as a preferential presynaptic alpha-agonist. There is at present no satisfactory explanation for these conflicting results although species differences are frequently cited; as one group of investigators comments "perhaps cats in general are usually resistant to presynaptic inhibition by clonidine" (Steppler, Tanaka and Starke, 1978). It should be noted that in the in vivo experiments cited above the accessibility of circulating clonidine to the pre- and postsynaptic sites of action was not considered as a possible factor contri-

buting to the contrasting observations. In the superfused vascular strips the agonist is equally accessible to both pre- and postsynaptic sites of action, whereas in the auto-perfused hindleg the intravenously injected clonidine would have to overcome barriers (e.g. vascular smooth muscle) to reach the nerve terminals. This may explain why a higher dose of clonidine is required to have a detectable effect on neurotransmission than on effector responses.

The evidence supporting the existence of a distinct class of presynaptic alpha-receptors in contrast to the "classical" postsynaptic alpha-receptors is only preliminary and circumstantial. Further, the possibility that some compounds achieve their presynaptic effects via loci unrelated to the proposed presynaptic alpha-receptors has not been considered seriously.

3. Mechanism of efflux inhibition and enhancement

A critical role for calcium in adrenergic neurotransmission has been well established. In the cat spleen and the guinea-pig vas deferens the amount of transmitter release on sympathetic stimulation is a function of external calcium concentration (Kirpekar and Misu, 1967; Johnson et al., 1971). Elimination of the calcium from the perfusate almost completely inhibits the release of noradrenaline. In guinea-pig vas deferens, the adrenergic transmitter overflow with changing calcium concentration, in the presence of phentolamine or phenoxybenzamine, reflects the function of external calcium in the release of transmitter (Stjärne, 1973c).

Few substantial proposals are available about the mechanism of presynaptic feedback inhibition. One which is widely accepted is the

"calcium theory" which postulates that the negative feedback loop, activated by alpha-agonists, operates by inhibition of calcium influx into the nerve endings, restricting the amount of transmitter released with each pulse. Adrenergic antagonists, by blocking these otherwise activated presynaptic sites, enhance transmitter release. The theory is based mainly on the observations that presynaptic antagonists increase transmitter overflow induced by nerve stimulation or potassium, actions which require calcium; but not that by tyramine, which is known to release transmitter by a non-exocytotic and non-calcium dependent process (Brandão et al., 1980). For example, in the perfused rabbit heart oxymetazoline decreases and phentolamine increases the release of noradrenaline induced by potassium, but these compounds have no effect on transmitter release elicited by tyramine (Starke and Montel, 1974).

The effectiveness of alpha-agonists and alpha-antagonists in altering potassium-induced transmitter overflow also implies that these adrenergic compounds do not interfere with the conduction of nerve impulses, since high potassium depolarizes the nerve endings through a mechanism which does not involve the conduction of impulses, namely by reducing the electrogenic potassium gradient across the plasma membrane (Haeusler et al., 1968; Kirpekar and Wakade, 1968).

One notable facet of the calcium theory is that excessive intraneuronal accumulation of the divalent cation at high frequencies of stimulation, or after alpha-antagonists, is purported to "desensitize" a hypothetical calcium receptor thus accounting for the ineffectiveness of the presynaptic feedback loop at very high frequencies (Kirpekar, Prat and

Wakade, 1975). This is based on observations in the cat spleen, perfused with low calcium (0.5 mM) Krebs solution, that phenoxybenzamine induces about 5-times more ^{3}H-noradrenaline output at a high (30 Hz) than at a low (5 Hz) frequencies (Kirpekar, Prat and Wakade, 1975). In control spleens perfused with normal Krebs solution, however, phenoxybenzamine increases the release of noradrenaline most at 5 Hz, an effect about 3 times greater than at 30 Hz. The authors concluded that the alpha-antagonist facilitates release by allowing large amounts of calcium to enter the neurone and that the decreased output observed at high frequency is a result of flooding of the nerve terminals with the divalent cation. This would be avoided if the calcium level in the bathing medium is maintained artificially low. This proposal has intentionally been advanced to account for the frequency-output relationships observed with the alpha-antagonists and alpha-agonists.

Langer and co-workers (Langer, Dubocovich and Celuch, 1975) reported greater enhancement of efflux in low calcium by phenoxybenzamine with cat spleen, supporting further the theory that calcium is an intermediate link between presynaptic receptor activation and transmitter release. During perfusion with standard calcium Krebs solution the enhancing effect of phenoxybenzamine on transmitter release (expressed as the ratio of total radioactivity detected during the treated and control stimulation periods) at 5 Hz was about 2.5 times that at 30 Hz. Lowering the calcium concentration to 0.26 mM increased the effectiveness of phenoxybenzamine at 30 Hz as compared to that observed in spleens perfused with standard Krebs solution. He interpreted this as reflecting "a re-

covery in the regulatory role of prejunctional alpha-adrenoceptor mechanism", presumably by avoiding a calcium-induced desensitization (Langer, Dubocovich and Celuch, 1975).

Another piece of evidence supporting the calcium theory is the parallel observation that some alpha-agonists, e.g. clonidine in guinea-pig vas deferens (Westfall and Leighton, 1976) or noradrenaline in cat spleen (Langer, Dubocovich and Celuch, 1975), conspicuously decrease the transmitter overflow at moderate and high stimulation frequencies (5 and 30 Hz) when the tissue is exposed to low calcium perfusate; whereas in contrast these agents do not have any effect on overflow at these frequencies if the tissue is perfused with standard or high calcium medium. This presumably reflects a potentiation of the action of alpha-agonists to limit calcium influx into adrenergic nerve terminals by the low level of the divalent ion in the extracellular medium. The failure of these agents to alter transmitter overflow under standard or high calcium condition is attributed, as described above, to a desensitization of the calcium receptors. This proposed mechanism for the presynaptic alpha-receptor feedback system will be analyzed later in the discussion chapter.

4. Physiological and pharmacological significance of presynaptic alpha-adrenergic receptors

There are many studies on the physiological role of the presynaptic alpha-receptors. However, a clear picture has not yet emerged and the evidence accumulated so far is controversial. Since alpha-antagonists block postsynaptic effector responses in most innervated organs, there have been technical difficulties in monitoring post-synaptic effects

secondary to nerve stimulation. For this reason most work designed to study the physiological role of presynaptic receptors was performed on preparations containing primarily beta-receptors such as cardiac tissue. In the search for a physiological role for presynaptic receptors, investigators often made the assumption that if the presynaptic negative feedback loop is functionally important in regulating adrenergic neurotransmission, then the selective interruption of the loop should enhance the effector cell response as a result of augmented transmitter release (Lokhandwala and Buckley, 1976; Langer, 1977; Drew, 1979). However, there is some evidence opposing a physiological role of the presynaptic system. For example, studies with the perfused heart showed that phentolamine has variable effects on the heart rate during sympathetic stimulation. Depending on concentration, phentolamine may increase (Langer, Adler-Graschinsky and Giorgi, 1977) or have no effect on (Farah and Langer, 1974; Langer, Alder-Graschinsky and Giorgi, 1977) the positive chronotropic responses to nerve stimulation. However, this compound significantly enhances stimulation-induced transmitter overflow in both instances. Also, although a moderate dose of phenoxybenzamine (8.7×10^{-7} M) increases the transmitter release by 5.7-fold in the perfused cat heart, the postsynaptic effector response (heart rate) was not affected (Farah and Langer, 1974). Similar discrepancies were evident in experiments with isolated accelerans nerve - atrial preparation of guinea-pig. Phentolamine increased more than 3-fold the transmitter release during sympathetic stimulation but produced no significant effect on the mechanical response (Adler-Graschinsky and Langer, 1975). This

observation was assumed due to a negative chronotropic action of phentolamine which compensated precisely for the effects of an augmented transmitter release. Apparent support for this interpretation was subsequently obtained from the finding that phentolamine antagonizes the responses of the atrial pacemaker to noradrenaline. It was reported that phentolamine significantly decreases the basal heart rate in guinea-pig atria and that it shifts to the right the dose-response curve for exogenous noradrenaline on heart rate (Langer, Adler-Graschinsky and Giorgi, 1977). However, no such explanation is available for phenoxybenzamine.

Studies in vivo were also performed to elucidate the significance of the presynaptic system. Most often the cardiac chronotropic response to sympathetic stimulation in the vagotomized, spinal sectioned animals was taken as a measure of presynaptic activity. In dogs, phentolamine significantly potentiated the positive chronotropic responses to stimulation of the cardioaccelerator nerve (Lokhandwala and Buckley, 1976; Constantine, Weeks and Muschane, 1978). Additionally, agonists such as noradrenaline, adrenaline and phenylephrine decreased heart rate upon nerve stimulation (Lokhandwala, Coats and Buckley, 1977). Although these findings are suggestive for a physiological role of the presynaptic alpha-receptors, it would be premature to reach such a verdict before an examination of some other contradictory observations. For example, it has been reported that phenoxybenzamine has no effect on heart rate during sympathetic stimulation in the pithed rat (Chang and Lee, 1973) and vagotomized dog (Yamaguchi, de Champlain and Nadeau, 1977). The potent adrenergic agonist xylazine was shown not to reduce the cardiac rate response to nerve

stimulation in the vagotomized dog (Antonaccio and Robson, 1973). In the rabbit heart, another alpha-agonist Bay a6781 (2 - (2-methyl-6-ethyl-cyclohexylamino) - 2 - oxazoline) does reduce the heart rate, however, the inhibitory effect of the agonist on stimulation-induced noradrenaline output and on heart rate are not correlated (Werner, Starke and Schuman, 1972). Bay a6781 at 5×10^{-10} M inhibits the output of transmitter in a dose-dependent manner with a maximum inhibition of 60% at 5×10^{-9} M. However, the heart rate is not reduced by the compound until the concentration reaches 1.6×10^{-9} M. The lack of parallelism between the presynaptic and postsynaptic events would suggest that the presynaptic feedback serves no immediately definable physiological role.

It has been observed that the effectiveness of alpha-agonists to inhibit and that of alpha-antagonists to potentiate transmitter release are most at low rate of stimulation, reflecting interference of these compounds with the presynaptic feedback loop (Stjärne and Brundin, 1977; Dubocovich and Langer, 1974; Haefely, Hurlimann and Thoenen, 1965). However, at the moment there is no unanimous view on the frequency range at which the presynaptic feedback loop should operate most efficiently. For instance, Yamaguchi and co-workers (Yamaguchi, de Champlain and Nadeau, 1977) suggested that the feedback mechanism is mainly activated at high frequencies of stimulation (10 to 30 Hz), based on the observation that the catecholamine level in the coronary sinus blood of the dog decreases in that frequency range, following cardioaccelerator nerve stimulation.

One early report that scruntinized the presynaptic alpha-receptor

hypothesis was that of Antonaccio and co-workers (Antonaccio, Halley and Kerwin, 1974). They attempted to clarify the functional significance of the presynaptic control of noradrenaline release. The effects of several alpha-agonists and of phentolamine on the cardiac response to postganglionic sympathetic stimulation at 0.3 to 10 Hz were examined in the vagotomized dog. Their observations are in contrast to those of most other workers. Of these alpha-agonists tested (namely, noradrenaline, phenylephrine, clonidine and naphazoline), only clonidine at high doses decreased the heart rate on sympathetic stimulation; the other agonists had no significant effect. Phentolamine similarly did not alter the heart rate. The authors concluded that "these results do not support the hypothesis that sympathetic nerves are under a functionally significant feedback loop mediated by alpha-receptors."

On the other hand, Lokhandwala and co-workers (Lokhandwala, Coats and Buckley, 1977) claimed a clear inhibitory effect of noradrenaline and phenylephrine on canine heart rate during sympathetic stimulation. They used a similar animal model (vagotomized dog) and experimental procedures. In fact, the conditions were almost identical in the two studies. For example, the anasthetics (pentobarbital) and the dose (35 mg/kg) used; the site of sympathetic stimulation (cardiac nerve posterior to stellate ganglion); the route of drug administration (intravenously into the femoral vein), the dose (0.1 - 0.2 μg/kg/min for noradrenaline and 2.25 μg/kg/min for phenylephrine) and exposure time (15 minutes). Also similar were the stimulation frequencies (an overlapping range from 0.3 to 1.0 Hz) and voltage (supramaximal voltage). The only different parameter

was the pulse duration: in Antonaccio's experiment 5 msec was used, a value which is ten times greater than that employed by Lokhandwala's group. It is presently unclear what sort of influences such a variation has on the presynaptic effectiveness of alpha-agonists, and if it is a contributing factor to explain the marked difference in the observations made in the two studies.

It has been suggested that the proposed feedback loop provides an explanation for the therapeutic effects of some drugs. Examples are the alpha-agonist clonidine (Starke and Altmann, 1973) and the alpha-antagonist prazosin (Constantine, Weeks and Meshane, 1978), both used clinically as antihypertensives and presumed to act via presynaptic mechanisms. However, until the controversy concerning the physiological significance of the presynaptic feedback loop is resolved, any pharmacological implications are difficult to accept.

5. Current status of the Hypothesis

Von Euler (1979) in a recent conference on presynaptic receptors commented "from the observations made in recent years it appears that activation of presynaptic receptors represents a mechanism having a wide application in the control of autonomic neurotransmission". Current considerations of the hypothesis have seemingly circumvented theoretical concerns and diverged into two aspects: 1) application of the hypothesis to various clinical situations; 2) ramification of the concept to still other receptor and organ systems.

The most commonly quoted clinical relevance of presynaptic receptors is perhaps the explanation of the anti-hypertensive effects of

clonidine by feedback inhibition (Yamaguchi, de Champlain and Nadeau, 1977; Starke and Altmann, 1973; Roach et al., 1979; Van Zwieten, 1980). Even the central hypotensive action of clonidine is postulated to be mediated presynaptically (Starke and Altmann, 1973; Van Zwieten, 1980), based on the finding that clonidine stimulates central alpha-adrenergic receptors (Anden and Strombom, 1975). This latter action is blocked by the preferential presynaptic alpha-antagonists rauwolscine and yohimbine (Van Zwieten, 1980). Elsewhere the hypothesis is suggested to explain facets of mood regulation (Puech, Lecrubier and Simon, 1979), narcotic tolerance and dependance (Starke, 1977; Schwartz, 1979), antidepression (Langer, 1979) and treatment of schizophrenia with neuroleptics (Langer, 1979). The trend for the widespread clinical application of the hypothesis is nicely shown by quoting Langer from a recent article in which he stated ".....it is therefore possible that highly selective, presynaptic receptor agonists or antagonists may become a generation of new drugs with different and, hopefully, useful therapeutic properties" (Langer, 1980).

That the concept of presynaptic regulation is not limited to the peripheral tissues has already been pointed out. It has been extended to the central nervous system (Starke and Montel, 1973; Farnebo and Hamberger, 1971; Strombom, 1975; Taube, Starke and Borowski, 1977) on the basis that alpha-agonist decreases and alpha-antagonist increases ^{3}H-transmitter release during field stimulation of brain slices. The hypothesis also serves to contain observations with many other receptor systems. These include the presynaptic beta-adrenergic (Adler-Graschinsky

and Langer, 1975), dopamine (Hertting et al., 1979), adenosine (Hedqvist and Fredholm, 1976) and presynaptic prostaglandin receptors (Hedqvist, 1970; 1976). These systems follow the same theoretical principles exemplified by the alpha-adrenergic receptor hypothesis and are thought to play a local regulatory role, either inhibitory or facilitory, at different types of synapses. Although a detailed review on these receptor systems is not the intent here, it should be pointed out that there are numerous inadequacies in the experimental support for these receptor systems as functional entities.

6. Challenges to the Hypothesis

The first challenge to the presynaptic receptor concept appeared in 1979 when Kalsner discovered that in guinea-pig vas deferens phenoxybenzamine increased both the transmitter output and the mechanical response elicited by a single pulse stimulation, a condition where the possibility of a negative feedback function is eliminated (Kalsner, 1979). More evidence was provided from the same laboratory to support a call for a re-examination of the hypothesis. These studies will be described later in the Discussion section. In short, after the evolution and the subsequent uncritical acceptance of the presynaptic alpha-receptor concept during the past two decades, a re-examination of the hypothesis at present seems appropriate and indeed imperative.

D. Termination of agonist action and sensitization of adrenergic effector response

By definition, termination of agonist action is the reduction

in number of active agonist molecules at the postsynaptic receptor sites (Kalsner, 1977), a process which, if impaired, may cause sensitization of the effector response to the agonist and prolongation of its duration (Kalsner, 1976). Diffusion of agonist away from the synaptic cleft, along with neuronal and extraneuronal uptake processes and enzymatic metabolism, can all influence termination of agonist action and induce sensitization of the effector response. There are contrasting views as to which of these processes plays the determinant role in terminating agonist action (de la Lande, 1975; Kalsner, 1975; Folkow, 1976). The effect of the neuronal uptake inhibitor cocaine on sensitization of effector responses to nerve stimulation or exogenous noradrenaline has been recognized for some time (Rosenblueth and Cannon, 1932; Fleckenstein and Burn, 1953; Trendenlenburg, 1959; de la Lande, 1978). This phenomenon is generally attributed to an action of the agent to inhibit the rapid uptake of amines into adrenergic nerve terminals and is considered as supporting evidence for the crucial role of neuronal uptake in agonist inactivation (Trendelenburg, 1959; de la Lande, 1975). An in vivo study demonstrated that cocaine significantly enhances the increase in blood pressure induced by noradrenaline as well as prolonging the half-life of injected noradrenaline in the cat. Since the increase in blood pressure is positively correlated to the concentration of noradrenaline in the plasma, it was concluded that "cocaine causes supersensitivity to norepinephrine by delaying its inactivation" (Trendelenburg, 1959).

Kalsner and Nickerson, on the other hand, disputed the unitary

(i.e. neuronal) mechanism of cocaine action based on results obtained from oil-immersion experiments. The oil-immersion technique involves the replacement of normal Krebs solution by mineral oil in the tissue bath. Under these conditions the diffusion of amines away from the "biophase" is prevented and an accurate assessment of the influence of the neuronal uptake on termination of agonist action made possible (Kalsner and Nickerson, 1968). A series of tests were conducted using rabbit aorta to clarify the mechanism of cocaine potentiation (Kalsner and Nickerson, 1969b). Cocaine potentiates the responses to noradrenaline, but it does the same to methoxamine, a sympathomimetic agent which is known to be resistant to neuronal uptake. In the aortic strips depleted of endogenous noradrenaline by reserpine, cocaine potentiated significantly the response to histamine but not 5-hydroxytryptamine. Oil immersion experiments revealed that the inactivation of 5-hydroxytryptamine (determined by the half-recovery time in oil), but not that of histamine, was decreased by cocaine. These observations clearly demonstrated that potentiation of effector response is not necessarily a consequence of impairment of agonist inactivation by nerves but that they appear to be two events sometimes unrelated to each other. It was concluded that, at least in the rabbit aorta, cocaine potentiates effector response also by an as yet unknown mechanism on the effector cell, and not only by its well-known action to block the neuronal uptake of amine.

The dissociation of cocaine-induced supersensitivity and its action to block neuronal uptake was also observed by some other investigators (Davidson and Innes, 1970; Varma and McCullough, 1969; Bevan and

Verity, 1967; Greenberg and Long, 1971). For example, Davidson and Innes reported that contractile responses to noradrenaline and the beta-adrenergic agonist isoprenaline were potentiated by cocaine in isolated spleen strips of cat (Davidson and Innes, 1970). In separate experiments in spleen of reserpine-pretreated cats, measuring uptake of noradrenaline and isoprenaline, it was found that cocaine effectively inhibited the tissue uptake of noradrenaline but did not affect that of isoprenaline. Further, cocaine produced sensitizations of similar magnitude in fresh rabbit aorta, nictitating membrane and spleen of cat and in the same tissues after prolonged storage at low temperature (Varma and McCullough, 1969), a confirmation of earlier findings by Kalsner and Nickerson (Kalsner, 1966; Kalsner and Nickerson, 1969b). Cold storage is known to allow selective loss of nerve but not effector-cells. This was confirmed by the finding of Varma and McCullough that cocaine inhibited the uptake of ^{3}H-noradrenaline in fresh but not in cold-stored tissues. In nerve-free preparations of rabbit aorta the potentiation of responses to noradrenaline by cocaine still remains and is comparable in size to that obtained with control tissues (Bevan and Verity, 1967), again suggesting a dissociation between the two prominent effects of cocaine. Greenberg and Long (1971) concluded that cocaine enhanced the response of rat vas deferens to noradrenaline by blocking the neuronal mechanism, but did not exclude the possibility of a direct action of cocaine on the effector cell. In fact, they suggested that cocaine increases the smooth muscle permeability to calcium and that this may explain the cocaine-induced supersensitivity.

There are other findings, however, which support the concept that potentiation of adrenergic responses by cocaine is necessarily a consequence of blockade of neuronal uptake. Guimãraes and Brandão studied the inactivation of noradrenaline in the cat spleen using the oil-immersion techniques of Kalsner and Nickerson (Guimãraes and Brandão, 1973). They employed different concentrations of cocaine and examined the effect of the agent on relaxation of the spleen after oil immersion. Significant positive correlations between cocaine concentrations and increases in relaxation time and also between increases in relaxation time and response sensitivity were observed, in disagreement with the experiments of Kalsner and Nickerson on rabbit aorta. The authors concluded that cocaine-induced supersensitivity is at least to some extent the result of an action preventing inactivation of agonist by neuronal uptake.

Still more support for the unitary hypothesis of cocaine action comes from Trendelenburg (1971; 1974). He criticized the oil-immersion technique as unsuitable for the study of amine inactivation because oil traps exogenous amine in the effector cell (Kalsner in a recent review (1977) has commented that with this technique no evidence for reflux of agonist from either neuronal or extraneuronal sites was obtained - see discussion below). Instead of using oil-immersion, Trendelenberg used a general wash-out method derived from earlier work of Kalsner and Nickerson (Kalsner, 1966; Kalsner and Nickerson, 1969b), which involved contracting the rabbit aorta strip with sympathomimetic amines and then recording the relaxation of the preparation in physio-

logical Krebs solution. It was found that inhibition of either catechol-O-methyl transferase or monoamine oxidase alone prolonged relaxation time after noradrenaline-induced contractions. In contrast, if the strip was contracted by amines which are not substrates for the enzymes, (e.g. methoxamine and phenylephrine for catechol-O-methyl transferase, or methoxamine for monoamine oxidase) then inhibition of the corresponding enzymes produces no effect on the relaxation. Additionally, if either the neuronal or the extraneuronal uptake was blocked by cocaine or corticosterone respectively before the strip was contracted by noradrenaline, an acceleration of the relaxation was observed. On the basis of these findings the author suggested that at first exposure to the sympathomimetic amines, the neuronal and extraneuronal compartments are "loaded" with amine which then spontaneously leaves the tissue sites during the subsequent wash-out period. The amount of amine accumulation depends on amine concentration, the activity of degradative enzymes and the functional capacity of neuronal and extraneuronal uptake processes. Inhibition of enzymes and uptake processes results in the respective prolongation and shortening of the relaxation due to corresponding but complex changes in the amount of intact amine retained in the nerve terminals and effector cells. There is other support for the efflux of unchanged amine from temporary neuronal (Löffelholz, 1972; Lindmar and Löffelholz, 1974) and extraneuronal (Bömisch and Uhlig, 1973) stores.

Trendelenberg concludes that the oil-immersion technique, because of the elimination of the diffusional path, would tend to "trap

the amine" in the temporary stores and therefore lead to over-estimation of the relaxation. In response to Trendelenburg's study, Kalsner (1971) has pointed out that; a) the concentration of amines employed in pertinent experiments were moderate (from 1×10^{-8} to 3×10^{-8} g/ml); b) relaxation after contractions induced by noradrenaline and adrenaline was not prolonged after monoamine oxidase inhibition; c) another study has shown that only after exposure to high concentration of noradrenaline (1×10^{-5} g/ml) was the amine detectable in extraneuronal tissues (Avakian and Gillespie, 1968); d) exposure to the extraneuronal uptake inhibitor GD-131 did not affect the relaxation rate of strips already contracted by noradrenaline after both monoamine oxidase and catechol-O-methyl transferase were inhibited (an increase in relaxation rate would be expected if intact noradrenaline were stored temporarily and then released from the neuronal and extraneuronal sites). All this does not support the claim of a neuronal or extraneuronal site of amine accumulation during oil-immersion as suggested by Trendelenburg.

In the late 1970's, some degree of compromise between the two groups was reached (Kalsner, 1977). Kalsner (1975) reported a prolongation of relaxation in rabbit aortic strips contracted with noradrenaline in Krebs solution when monoamine oxidase, catechol-O-methyl transferase and neuronal uptake are all inhibited. Under these conditions only two inactivation mechanisms, i.e. extraneuronal uptake and diffusion into the bathing medium, are left functional. It was concluded that the slow relaxation is caused by the efflux of the previously entrapped

agonist from the extraneuronal sites during washout period. The same conclusion was reached by Trendelenberg and Henseling (1976). However, when the diffusional path is also eliminated, i.e. in oil-immersion experiments, extraneuronal uptake serves as the most important terminating mechanism.

The claim of "bi-directional fluxes" of noradrenaline to and from extraneuronal compartments rests heavily on the observation that in rabbit aorta depleted of endogenous noradrenaline stores and with neuronal uptake as well as both metabolic enzymes inhibited, intact ^{3}H-noradrenaline is detected in the bathing medium during a washout period after priming the tissue with the labelled transmitter (Henseling, Eckert, and Trendelenburg, 1976). This observation, together with subsequent studies of the kinetics and efflux pattern of various metabolites of ^{3}H-noradrenaline during the wash-out period (Henseling, Graefe and Trendelenburg, 1978), is regarded as "direct" evidence for the efflux of noradrenaline from the extraneuronal sites. Speculation was made that such efflux also occurs in tissues immersed in oil (Henseling, Rechtsteiner and Trendelenberg, 1978). However, results from the efflux experiments described above do not reflect precisely the spatial and temporal dynamics of the accumulation and dissipation of noradrenaline at the receptor region. This has been recently emphasized by Kalsner (1979d) who commented that the overflow of radioactivity from tissues previously exposed to ^{3}H-transmitter is a parameter representing results of complex events, both known and unknown, occuring at neuronal and extraneuronal sites. It is therefore not a reliable index of events during the

course of response. An acceptable measure for the study of terminating mechanisms is the response duration (either in Krebs solution or in oil). The concept of extraneuronal "bi-directional fluxes", based on evidence obtained from overflow experiments, is less direct and unavoidably open to criticism.

The relative importance of neuronal and extraneuronal uptake in termination of agonist action obviously depends on many factors, in particular the experimental conditions and the type of tissue studies. The relative contribution of individual processes in termination of the action of released transmitter in autonomic effector organs, under in vivo conditions, is not presently clear. Perhaps the finding of Hughes (1972) can be used to illustrate the complexity of the situation. The author reported that in rabbit vas deferens, corticosterone, the extraneuronal uptake inhibitor, increases noradrenaline release upon sympathetic stimulation by 30 - 40%, but in preparations pretreated with cocaine the steroid causes a 300% increase in noradrenaline output. The effect of cocaine is also much enhanced in tissues pretreated with corticosterone. It was concluded that "there is a dynamic balance in the distribution of noradrenaline between the neuronal and extraneuronal uptake mechanisms. When one of these mechanisms is blocked, more noradrenaline becomes available to the remaining process and its relative importance increases". The conclusion is compatible with the concept of multiple mechanisms of inactivation proposed by Kalsner (1977). He suggested that terminating mechanisms may work either in parallel (multiple mechanisms) or in series (alternate or "back-up" mechanisms). The inhibition of different subgroups

will have different effects on the amplitude of the effector response.

The sole function of terminating mechanisms is to end agonist action, blockade of which may cause sensitization. An important determinant of sensitization, however, is the control of the availability of agonist to the receptor region. This is attained by either increasing the amount of transmitter released, or by eliminating possible access barriers along the agonist route to the receptor (Kalsner, 1976). One of the most common access barriers is the nerve plexus found in vascular preparations. For example, in the rabbit ear artery it has been shown that the nerve plexus is located circularly in the adventitia adjacent to the media (Waterson and Smale, 1967). When the agonist is coming from the extraluminal side, as in the case of noradrenaline administered extraluminally to isolated perfused preparations or by nerve release, the agonist is subject to neuronal uptake before it reaches the effector cell. Thus, the rabbit ear artery has provided a useful tissue model for the study of sensitization of effector response (de la Lande, Frewin and Waterson, 1967; de la Lande, 1975). In later chapters, discussions on response sensitization will be presented based on data obtained from perfused branches of bovine radial arteries.

In short, termination of agonist action and sensitization of effector response are two sometimes separate processes. Much controversy has arisen in the past because of lack of discrimination between these two processes, e.g. as in the case of the unitary hypothesis of cocaine-induced supersensitivity. More work is necessary for a better understanding of these processes.

III. METHODS AND MATERIALS

Experiments were performed on isolated arterial preparations obtained from cattle, dog and guinea-pig. Krebs-Henseleit (Krebs) solution aerated with a 95% O_2 - 5% CO_2 mixture and maintained at 37°C was used for superfusion, perfusion, incubation and bathing of tissues. The Krebs solution was of the following composition (mM): NaCl, 115.3; KCl, 4.6; $CaCl_2$, 2.3; $MgSO_4$, 1.1; $NaHCO_3$, 22.1; KH_2PO_4, 1.1; glucose, 7.8; with EDTA added (0.03 mM) to prevent heavy metal catalyzed oxidation of catecholamines. In some experiments cocaine and/or normetanephrine were routinely added to the Krebs solution to block neuronal and extraneuronal uptake of noradrenaline respectively.

A. Drugs

The drugs used and their sources were:

1. adrenaline bitartrate, K & K Laboratory, Plainview, California, U.S.A.
2. chlorpromazine hydrochloride, Poulene, Montreal, Quebec, Canada.
3. cocaine hydrochloride, May & Baker, Dagenham, England.
4. Dibenamine hydrochloride, Smith, Kline & French, Montreal, Quebec, Canada.
5. dopamine hydrochloride, Mann Research Laboratory, New York, U.S.A.
6. guanethidine, CIBA, Dorval, Quebec, Canada.
7. 17 beta-estradiol, Calbiochem, La Jolla, California, U.S.A.
8. isoproterenol hydrochloride, Winthrop Laboratory, New York, U.S.A.
9. methoxamine hydrochloride, Burroughs Wellcome Co., Research Triangle Park, North Carolina, U.S.A.
10. metoclopromide hydrochloride, McNeil Laboratory, Don Mills, Ontario, Canada.

11. l-noradrenaline bitartrate, Calbiochem, La Jolla, California, U.S.A.
12. dl-noradrenaline bitartrate, Calbiochem, La Jolla, California, U.S.A.
13. normetanephrine hydrochloride, Calbiochem, La Jolla, California, U.S.A.
14. oxymetazoline hydrochloride, Schering Corp., Bloomfield, New Jersey, U.S.A.
15. phenoxybenzamine hydrochloride, Smith, Kline & French, Montreal, Quebec, Canada.
16. phentolamine hydrochloride, Ciba-Geigy, Summit, New Jersey, U.S.A.
17. phenylephrine hydrochloride, Mann Research Laboratory, New York, U.S.A.
18. pimozide, McNeil Laboratory, Don Mills, Ontario, Canada.
19. tetrodotoxin (lyophilized), Sankyo, Tokyo, Japan.
20. tolazoline hydrochloride, K & K Laboratory, Plainview, California, U.S.A.

All drugs, except those described below, were diluted to stock solutions of 10^{-3} g/ml in 0.01 N HCl and incorporated directly into Krebs solution with proper dilution. Stock solutions were stored frozen up to three days except dopamine, which was always prepared fresh because of the high susceptibility of this agent to oxidation. The haloalkylamines phenoxybenzamine and Dibenamine, when used in high concentrations (e.g. 10^{-5} g/ml), were dissolved directly into a large volume of Krebs solution with intense agitation. For lower concentrations the haloalkylamines were dissolved in acidified propylene glycol to a concentration of 10^{-2} g/ml and stored at 0°C. Dilutions were made on the day of use with 0.01 N HCl. Pimozide and 17 beta-estradiol were made into stock solutions of 10^{-2} g/ml.

with absolute ethanol. Dilutions were performed fresh in the same manner as with the haloalkylamines. The amount of propylene glycol and ethanol used were kept to a minimum: the highest concentration of the vehicles employed was 10^{-4} vol % (0.1 ml in 1000 ml of Krebs solution), a concentration which produced no detectable effect on tissue response.

B. Perfusion of branches of radial artery of cattle

Front legs of cattle of either sex were removed immediately after slaughter and transported to the laboratory from a local slaughter house. The animals were executed either by gun shot to the head or by cutting open the throat and then bleeding them. The total transportation time from slaughter house to laboratory was routinely about 20 minutes. The main branch off the distal portion of the radial artery, approximately 50 cm in length, was dissected out and cleaned of visible fat and connective tissue. The vessel, with all its small branches tied, was then cannulated at both ends with polyethylene tubing essentially as described by De La Lande and Rand (1965) for the rabbit ear artery. The preparations were suspended in individual 30 ml muscle baths containing Krebs solution and connected to gravity-feed apparatus which perfused the vessels with Krebs solution at a constant pressure of 60 mm Hg (Fig. 1). With this apparatus the intraluminal perfusate and the extraluminal bathing fluid are separated and any leakage of perfusate from the lumen into the extraluminal space would be detected by an increase in the bath fluid level. If such leakage happened, the preparation was either taken out of the bath and its branches re-tied or it was discarded. A record of the

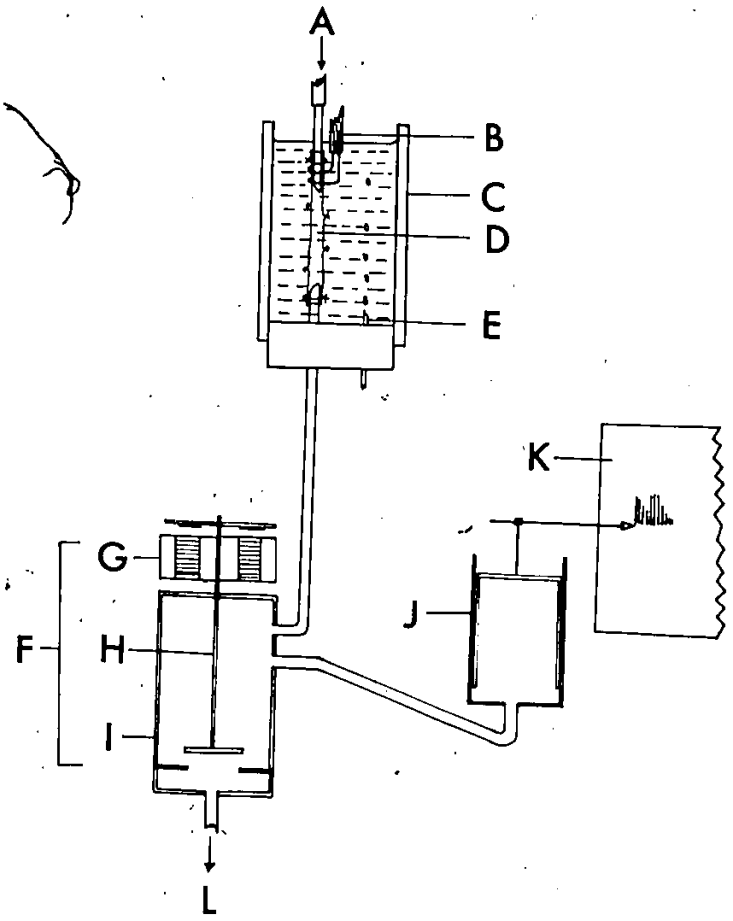

Figure 1. Diagram of the apparatus for perfusion of branches of bovine radial artery, showing flow recorder in off position. A, inlet for warm (37°C) and oxygenated Krebs perfusate (gravity feed); B, platinum wire electrodes; C, muscle chamber; D, artery; E, oxygen inlet; F, Andrews outflow recorder; G, electric magnet (on/off controlled by an electronic timer); H, stopper with spring recoil mechanism; I, chamber for fluid/air displacement; J, piston pen recorder; K, kymograph; L, perfusate outlet.

flow rate of perfusate through each artery was provided by a modified Gaddum outflow recorder (Andrews, 1952) writing on a slowly revolving smoked kymograph drum. The outflow recorder was a fluid/air displacement device with the on/off interval controlled by an adjustable electronic timer. The volume of air displaced over a fixed period (4 to 8 seconds) was transmitted to a piston recorder writing vertically on a kymograph drum. The height of each vertical stroke of the writing lever was proportional to the air volume and thus to the volume of perfusate flowing through the vessel lumen. The exact flow rate was determined by prior calibration of the recorder. Vasoconstrictor responses induced by nerve stimulation are represented by a reduction in flow rate.

Periarterial nerve stimulation was performed by means of a pair of platinum electrodes arranged around the proximal end of each artery over the area where the perfusion cannula lay within the artery. The nerves were stimulated at supramaximal voltage with biphasic pulses of 1 ms pulse width delivered by Grass model 5S stimulators. Supramaximal voltage was determined for each preparation by priming stimulations at 5 Hz and 30 volts with voltage increasing in 5 volt increments. A voltage which was 25% above that which produced maximal vasoconstriction (approximately 55 to 65 volts) was routinely used in all subsequent tests. Dose-response curves to noradrenaline administered extraluminally were evoked by adding the drug directly into the Krebs solution in the muscle chamber. Responses to intraluminally administered noradrenaline were obtained by injecting the agonist slowly, at a fixed vehicle volume of 0.2 ml., into the lumen using a needle inserted proximally into the Latex tubing connection.

After a 60 minute equilibrium period, vessels were stimulated for 2 minutes at each test frequency, 0.5, 1, 2, 5, 7.5, and 10 Hz. There was a 5 minute recovery time between stimulations and this period was sufficient for the vessel to re-establish the pre-stimulation flow rate. After completion of the frequency response series, dose response curves to extraluminally and intraluminally applied noradrenaline were obtained. Following the first series of tests either cocaine (10^{-5} g/ml, 3×10^{-5} M), or estradiol (10^{-5} g/ml, 3.6×10^{-5} M), or the two combined were introduced into the perfusion medium. The drugs were added extraluminally to the muscle chamber and intraluminally to the Krebs perfusate. A second series of tests, identical to the first one in sequence, was performed 30 minutes after exposure to, and in the presence of, the uptake inhibitors. Control experiments, in the absence of cocaine and estradiol, showed no significant difference between the first and the second series tests.

C. Isotonic mechanical responses of vascular strips in bath

Vascular strips were prepared according to the method of Furchgott (1960). Fresh vessels were obtained and placed in a Petri dish containing Krebs solution at room temperature. After removal of adherent fat and connective tissue, the vessels were cut into spiral strips using the following procedure. The preparation was held in one hand and was rotated towards a pair of fine scissors held in the other hand such that a continuous incision was allowed. For some vessels too small in size to be manipulated, e.g. the guinea-pig aorta used in superfusion experiments, a small polyethylene tubing was inserted into the lumen through the whole

length of the vessel and the vessels rotated while being cut with a fine scissors. The preparation was kept moistened with Krebs solution throughout the procedure. Strips obtained with this technique have an angle of about 15° to 20° between their circular smooth muscle layers and the long axis of the spiral coil.

For isotonic contraction experiments, strips of branches of bovine radial artery, 3 x 23 mm in length, were used. Both left- and right-hand spiral strips were used and responses from these two types of strips were not significantly different. The strips were attached at both ends with threads and immersed in 15 ml muscle chambers containing Krebs solution maintained at (37°C). One end of the strip was tied to a fixed glass rod which was also used to deliver oxygen into the bathing medium at a moderate rate (a few bubbles per second). The other end of the strip was connected, via the thread, to a free moving lever with a counterbalanced weight of 1 g. Isotonic mechanical responses of the strips were recorded by the lever which wrote on a slowly moving kymograph with a 6.8-fold magnification.

The strips were allowed two hours for equilibration in the bath. During this period the bathing medium was replaced with fresh Krebs solution every 30 minutes. The spontaneous muscle tone developed in the equilibration period was of insignificant amplitude compared to the noradrenaline- or potassium-induced contraction. Dose-responses of the strips were obtained by adding drugs directly into the bathing medium in single (potassium) or cumulative doses (noradrenaline). At the end of each experiment strips were treated with the non-specific smooth muscle relaxant sodium nitrite (10^{-3} g/ml, 1.5 x 10^{-2} M) to determine the total muscle tone.

D. Tissue accumulation of ^{3}H-noradrenaline

The accumulation of ^{3}H-noradrenaline in the branches of bovine radial artery, and the modification of it by drugs, was examined. Freshly obtained arteries were cut longitudinally into two halves each weighing approximately 100 to 150 mg. Each pair of vessel segments was placed in a glass vial containing 20 ml of warmed and oxygenated Krebs solution. The vials were incubated in a constant temperature water bath (37°C) and the vessels were equilibrated for 60 minutes with a change of the medium every 20 minutes. At the end of the equilibration period the preparations, selected randomly, were divided into four groups. One group was further incubated with cocaine (10^{-5} g/ml, 3 x 10^{-5} M), one group with estradiol (10^{-5} g/ml, 3.6 x 10^{-5} M), one group with both cocaine and estradiol at the above concentrations, and the final control group with drug-free Krebs solution. After incubation for 30 minutes, the preparations were exposed to ^{3}H-dl-noradrenaline bitartrate at 6 x 10^{-7} M for an additional 20 minutes. All drugs were replenished and kept in the respective incubation media during this period. The Krebs solution containing the labelled transmitter was prepared by diluting a stock solution of 3 x 10^{-6} M ^{3}H-dl-noradrenaline 20 times with non-radioactive dl-noradrenaline bitartrate. The tritiated amine was obtained from a batch with specific activity of 8.7 or 11.4 mCi/mol at a concentration of 4.5 x 10^{-5} M (New England Nuclear Corp.). The ^{3}H-noradrenaline stock solution was made with ascorbic acid (50 µg/ml) and stored frozen in small aliquots under nitrogen. Aliquots were thawed only once, immediately before use.

After the incubation, the tissues were rinsed, blotted, weighed and placed in scintillation vials containing 2 ml of tissue solubilizer (Protosol, New England Nuclear Corp.) and 0.2 ml of distilled water. The vials were left overnight in a water bath at 50°C and tissue radioactivity was subsequently determined by scintillation counting. Scintillation fluid was prepared by mixing 42 ml Liquifluor (New England Nuclear Corp.) with one litre of toluene, i.e. 4 g PPO (2, 5-diphenyloxazole) and 50 mg POPOP {1, 4-bis [2(5-phenyl-oxazolyl)] benzene} per litre of toluene. To each vial containing the tissue digest, 15 ml of the scintillation fluid was added and the samples were counted to a 1% error in a Beckman LS-150 counter with automatic external standardization to determine efficiency. Tissue radioactivity was expressed as disintegrations per minute (dpm) per gram of tissue (wet weight). The concentration of ^{3}H-dl-noradrenaline in the incubation medium was confirmed for each experiment by counting the radioactivity in 0.1 ml aliquots.

E. Superfusion of arterial strips previously labelled with ^{3}H-noradrenaline

1. Tissue preparation

A variety of arteries from different species was used. These included the radial, renal and facial arteries of cattle, the aorta of guinea-pig, the aortic, renal, femoral and carotid arteries of dog.

Bovine facial and renal arteries were dissected out promptly after execution of cattle at the slaughter house, immersed in cold (4°C) previously-oxygenated Krebs solution and transported immediately to the laboratory. The dissection of the radial arteries from the previously

removed front legs of cattle was done in the laboratory.

Albino guinea-pigs of either sex weighing between 400-600 g were used for some experiments. The animals were kept in the animal care centre and maintained on a commercial diet. Tap water was allowed ad libitum. Animals were killed by cervical dislocation and the segment of aorta from the heart to the diaphram dissected out. Aortic strips were always used in pairs, cut from the same vessel, of which one was tested with drug and the other used as control.

For the experiments with canine preparations, mongrel dogs weighing between 10-20 kg were used. The animals were initially anaesthetized with sodium pentobarbital (30 mg/kg) injected intravenously in the forelimb and then killed by introduction of air into the circulation. The aortic, renal, femoral and carotid arteries were dissected out and used promptly.

After dissection, all arteries were immediately immersed in warmed and previously oxygenated Krebs solution and cleaned of loose fat and connective tissues. According to the procedures described above, the preparations were cut spirally into strips of different sizes: 2 x 40 mm for bovine facial artery, 4 x 30 mm for bovine renal and radial arteries, 3 x 30 mm for the canine tissues and 2 x 30 mm for the guinea-pig aorta.

2. Incubation of tissues with ^{3}H-noradrenaline

Two types of radiochemicals were used. For the bovine tissues, l-[7-^{3}H]-noradrenaline hydrochloride with specific activity of 12 mCi/mol was employed. Another radiochemical, l-[7,8-^{3}H]-noradrenaline hydrochloride

with specific activity of 10-13 mCi/mol, was later found to give more satisfactory results and was therefore used in subsequent experiments with dog and guinea-pig tissues. Both radiochemicals were obtained from the Radiochemical Centre, Amersham, diluted to a stock solution of 100 μCi/ml in ascorbic acid (50 μg/ml) and stored at 4°C in 5 ml aliquots under nitrogen gas. The incubation medium was prepared by adding 0.4 ml of the stock radiochemical solution (100 μCi/ml) to 3.6 ml of Krebs solution.

Vascular strips were incubated for 60 minutes in 4 ml of warm and oxygenated Krebs solution containing the radiochemical with an activity of 10 μCi/ml and a concentration of $6.7-10.0 \times 10^{-7}$ M. They were then washed with fresh Krebs solution and mounted onto a superfusion apparatus.

3. Superfusion of vascular strips

Strips were superfused by the technique of Su and Bevan (1970). Each preparation was suspended vertically under tension between a force displacement transducer (or in some cases one end of a lever with counter balance weight) and a fixed glass rod. The initial tension was 1 g for the guinea-pig aorta, 4 g for the bovine renal artery and 2 g for all other tissues. A pair of platinum wire electrodes was placed on opposite sides of each strip which was superfused with warm and oxygenated Krebs solution at a constant flow rate under a gravity feed apparatus. The strip and the electrodes were carefully positioned such that the space between them was filled with superfusate to ensure proper electrical conductivity and nourishment of the tissue. Flow rate was 4 ml/min

except for facial artery where it was 5 ml/min. Strips and electrodes were enclosed in individual glass-jacketed chambers warmed with circulating hot water to minimize heat loss from tissues. Cocaine (3×10^{-5} M for bovine radial artery, 1×10^{-5} M for all others) was routinely added to the Krebs superfusate, and for experiments with guinea-pig and dog tissues, normetanephrine (1×10^{-5} M) was also incorporated into the superfusing medium.

Mechanical responses were recorded isometrically by Grass force displacement transducers (model FT03) which converted mechanical signals into electrical signals. The latter was amplified by Grass low-level D.C. pre-amplifiers (model 5P1) and recorded by a Grass polygraph (model 5D). In some experiments, only the efflux of ^{3}H-transmitter was of interest and thus the mechanical response was not recorded.

4. Stimulation parameters, efflux of ^{3}H-transmitter and general protocol

For equilibration, the strips were superfused for at least 90 minutes before the onset of the experimental protocol. This period was sufficient to obtain a steady basal efflux of ^{3}H-transmitter.

Transmural stimulation of the strips was performed using Grass model S6 stimulators. A fixed number of biphasic pulses of 1.0 ms duration at supramaximal voltage and at the desired frequencies were delivered to the strips. Depending on the size and thickness of the preparation, current passing through the tissues varied between 80 to 160mA as measured by an oscilloscope. After an initial 60 minutes of superfusion, strips were primed with a test stimulation at 5 Hz for 1 minute. This procedure enabled

an assessment of tissue viability and also eliminated employment of the possible erroneous results with the first stimulation as reported by others (Su and Bevan, 1970; Nedergaard and Schrold, 1973).

Basal and stimulation-induced efflux of radioactivity from the tissues was determined by assaying 1.0 ml aliquots of superfusate collected in vials by a fraction collector rotating every 2 minutes for the bovine radial artery or 4 minutes for all other preparations. The aliquots were then transferred to vials containing 10 ml of pre-mixed counting cocktail (Aqueous Counting Scintillant, Amersham) and counted to a 1% error in a Beckman LS-150 or LS-8100 scintillation counter with automatic external standardization to determine efficiency. Counts represent the total radioactivity from the superfusate. Basal efflux was expressed as disintegrations per minute (dpm) and referred to as the radioactivity detected in the sample collected immediately before stimulation. Stimulation-induced efflux was calculated as the difference between basal efflux and the total dpm detected in the samples collected during and immediately after stimulation. In most instances the radioactivity returned to basal levels in the second post-stimulation collection. However, in some cases (such as the bovine facial and radial arteries), where the overflow of ^{3}H-transmitter was relatively high, four post-stimulation samples were collected to enable the determination of an entire stimulation-induced efflux. Transmural stimulation was always begun at the onset of a 2 minute (or 4 minute) collection period.

In experiments involving the effects of a single drug on stimulation-induced efflux, each strip was stimulated first at one of the

desired frequencies, followed after at least 12 minutes by stimulation at a second frequency. In some preparations, a total of four different frequencies, given in random order, were tested. The strips were then treated with the drug under study for the desired period and a second series of stimulations identical in sequence to the first was performed. Depending on the nature of the drug, a wash-out period was introduced before the second stimulation series. Control strips received no drug treatment but had the identical sequence of transmural stimulations performed on them. Protocols for specific experiments will be described in the Results section.

5. Data evaluation

The effects of drugs on stimulation-induced efflux was, in some experiments, evaluated on the basis of the efflux ratios of the second and the first stimulation periods with the same frequency. This efflux ratio is widely used by others (Farnebo and Malmfors, 1971; McCulloch, Rand and Story, 1972; Rand et al., 1973) and the procedure eliminates the differences in absolute transmitter output due to preparation or species variation. However, in some tissues such as the bovine renal artery, the stimulation-induced efflux in the control strips was not consistent but instead declined or increased to some extent with time. The control efflux ratio was thus deviated considerably from unity, and to compensate for this variation a more sensitive procedure was employed. The ratios of absolute efflux of tritium (second versus first stimulation period) for the treated strips were compared to, and calculated as a percentage of, the corresponding ratios for the control strips. For

example, a ratio of 0.2 for a treated preparation and 0.8 for its matching control would give a value of 25% (rather than 20%) of control efflux of tritium. This procedure accounts for any spontaneous changes in stimulation-induced efflux due to time factor and identified more accurately the particular pharmacological effect.

6. Statistical evaluation

Mean values and their standard errors were calculated for all data which were compared by the "two-tailed" Student's t-test. The paired t-test was used for intra-preparation comparison whereas the unpaired t-test was used for comparisons of data between different groups of preparations. Probability values of 0.05 or less were considered significant. Correlation coefficients between two variables, when required, were determined according to the method of Goldstein (1964).

In experiments with perfused branches of bovine radial arteries the concentration of noradrenaline that produced half-maximal response (ED_{50}, or mean effective concentration) was used as an index for comparison of response sensitivity. However, the distribution of this parameter tends to deviate from normal on an arithmetic scale (Flemming et al., 1972), and therefore the geometric mean, i.e. the mean of the logarithm value, of the ED_{50} was employed instead.

IV. RESULTS

PART I. PRESYNAPTIC ALPHA-RECEPTOR HYPOTHESIS

A. Effect of phenoxybenzamine in bovine renal artery

The effect of phenoxybenzamine on transmural stimulation-induced mechanical responses and on efflux of tritium in isolated strips of bovine renal artery previously incubated with ^{3}H-noradrenaline was examined. Particular effort was directed to the relationship between stimulation frequency and the efflux of ^{3}H-transmitter in the presence and in the absence of the haloalkylamine.

Bovine renal arterial strips were superfused with Krebs solution containing routinely cocaine (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M) to eliminate possible complications resulting from neuronal and extraneuronal uptake of catecholamines respectively. Experiments were always done on four strips at a time, all taken from the same renal artery. After equilibration for 120 minutes, the preparations were stimulated with 300 pulses at 1, 2, 5, 10 and 15 Hz, frequencies spanning the physiological range. On completion of the first series of stimulation, two of each set of four tissues were exposed to phenoxybenzamine (3.3×10^{-5} M) for 30 minutes, followed, without antagonist washout, by a second stimulation series identical to the first one on all four arteries.

1. Stimulation-induced efflux of ^{3}H-transmitter

Figure 2 shows the overflow of ^{3}H-transmitter in response to transmural stimulation at various frequencies in the bovine renal artery. Transmural stimulation with 300 pulses induced a significant increase of

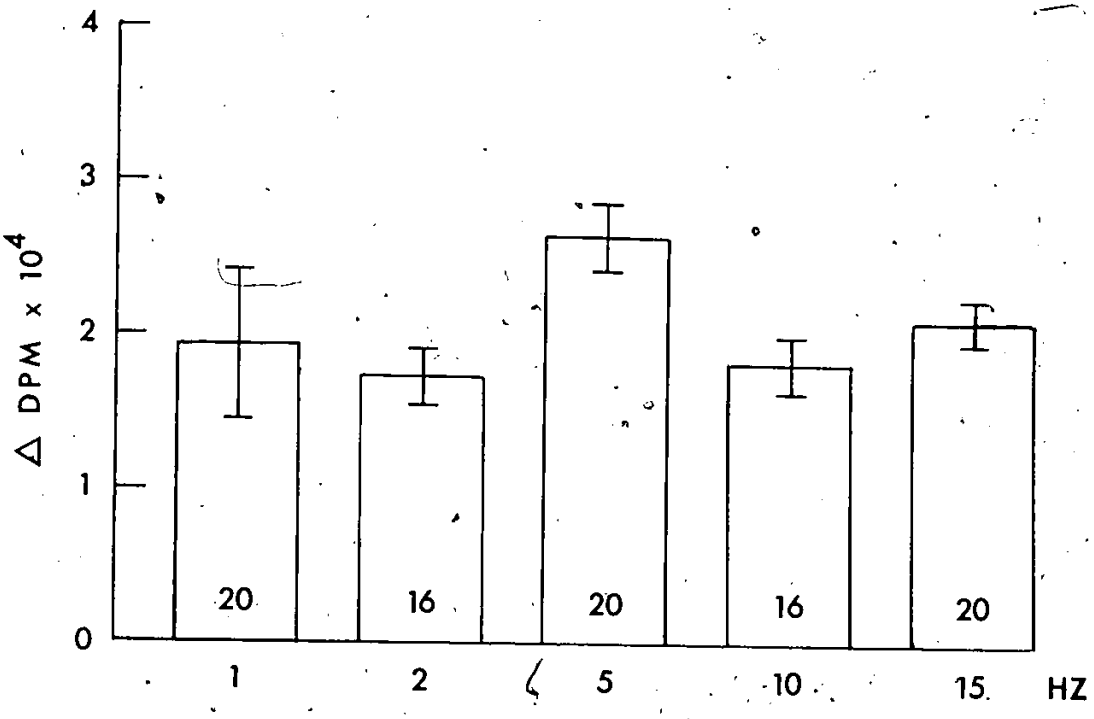

Figure 2. Relationship between stimulus frequency and efflux of tritium in bovine renal artery. Stimulation was with 300 pulses at each test frequency. Number of values in each group are shown within columns. Mean value at 5 Hz differed from that at 2 Hz ($p < 0.01$) and from that at 10 Hz ($p \leq 0.05$). All other values did not differ significantly from each other using the t-test for unpaired data.

tritium overflow above basal levels at all frequencies. However, a clear-cut relationship between the amount of tritium overflow and the stimulation frequency was not observed. The output of ^{3}H-transmitter did not differ significantly at 1, 2, 10 and 15 Hz, although at 5 Hz the efflux was materially higher than that at 2 and 10 Hz. The mean and 95% confidence limits for efflux at each of the frequencies (1 to 15 Hz) was 1.93±0.65, 1.67±0.34, 2.58±0.48, 1.83±0.40 and 2.06±0.29 x 10^{4} dpm, respectively. The minimum efflux values (i.e. the lower limit of the 95% confidence interval) at 10 and 15 Hz fell within the 95% confidence intervals for efflux at 1 and 2 Hz, indicating the uniform overflow of tritium regardless of stimulation frequency even when the lowest extreme values possible are taken into consideration.

Efflux during the second set of stimulations in the control strips did not differ significantly from those during the first (Table 1). In the preparations treated with phenoxybenzamine during the interval between stimulation periods, overflow of tritium was significantly enhanced by the haloalkylamine at all frequencies, as determined by the intrastrip comparison of the overflow ratio for the second versus the first period of stimulation. The enhancing effect of phenoxybenzamine was statistically greatest at the lowest frequency examined (1 Hz). At higher frequencies (2 to 15 Hz), the efflux ratios in the presence of phenoxybenzamine were not significantly different from each other except that at 10 Hz, where a slightly but significantly lower value was obtained. The correlation coefficient (r) between length

Table 1. EFFECT OF PHENOXYBENZAMINE (3×10^{-5} M, POB) ON THE STIMULATION-INDUCED EFFLUX OF TRITIUM IN BOVINE RENAL ARTERY.

Treatment group [1]	Stimulation frequency (Hz)	No. of values	Transmitter efflux dpm ($\times 10^4$) 1st run	Transmitter efflux dpm ($\times 10^4$) 2nd run	Efflux ratio 2nd run / 1st run
Control	1	10	1.91±0.57	1.44±0.20	(a) 1.05±0.16*
POB	1	10	1.94±0.27	5.25±0.82	(b) 2.88±0.25**
Control	2	8	1.58±0.31	1.45±0.26	(c) 0.95±0.05
POB	2	8	1.76±0.12	3.41±0.25	(d) 1.98±0.17*
Control	5	10	2.34±0.30	2.11±0.23	(e) 0.94±0.07
POB	5	10	2.81±0.30	5.33±0.48	(f) 2.01±0.16*
Control	10	8	1.76±0.32	1.66±0.36	(g) 0.93±0.06*
POB	10	8	1.90±0.23	2.69±0.40	(h) 1.43±0.10***
Control	15	10	1.99±0.20	1.70±0.13	(i) 0.89±0.06
POB	15	10	2.13±0.19	3.70±0.62	(j) 1.76±0.24*

[1]Phenoxybenzamine, when given, was administered in the interval between runs, as described in text. *Indicates ratios of treated groups significantly different from ratios of corresponding control groups with $p<0.01$. **Indicates group significantly different from groups (d), (f), (h), (j) with a $p<0.02$. ***Indicates group significantly different from groups (d) and (f) with a $p<0.02$. The ratios of all other treated groups do not differ significantly from each other. Untreated groups do not differ significantly from each other.

of stimulus interval and ratio of transmitter overflow in the presence of phenoxybenzamine was 0.929 with a $p < 0.01$.

2. Basal efflux

Basal efflux is the ^3H-transmitter liberated spontaneously during superfusion, and was measured immediately prior to each stimulation. Although the basal efflux reached a steady level after the initial equilibration period, it declined steadily but moderately during the course of the experiments. In the control strips the basal efflux obtained during the second stimulation series decreased to about two-thirds of that obtained initially, i.e. during the first stimulation series (Figure 3). In phenoxybenzamine-treated strips a similar decrease in basal efflux was also observed. However, this decline was significantly less than that in control strips as assessed by an intra-strip comparison of efflux ratios in the second versus first stimulation periods ($p < 0.001$). The efflux ratios for the control and treated groups were 0.64±0.01 and 0.76±0.02 respectively.

3. Mechanical response

The renal artery responded to transmural stimulation with increases in tension. Contractile responses to the first period of stimulation (i.e. in the absence of phenoxybenzamine) in all strips examined is shown in Figure 4 (solid line). As expected, the magnitude of response increased with increasing frequency. Peak tension developed gradually, with clear gradation, over the frequency range from 1 to 5 Hz, and maintained a steady level thereafter. This is in contrast to the

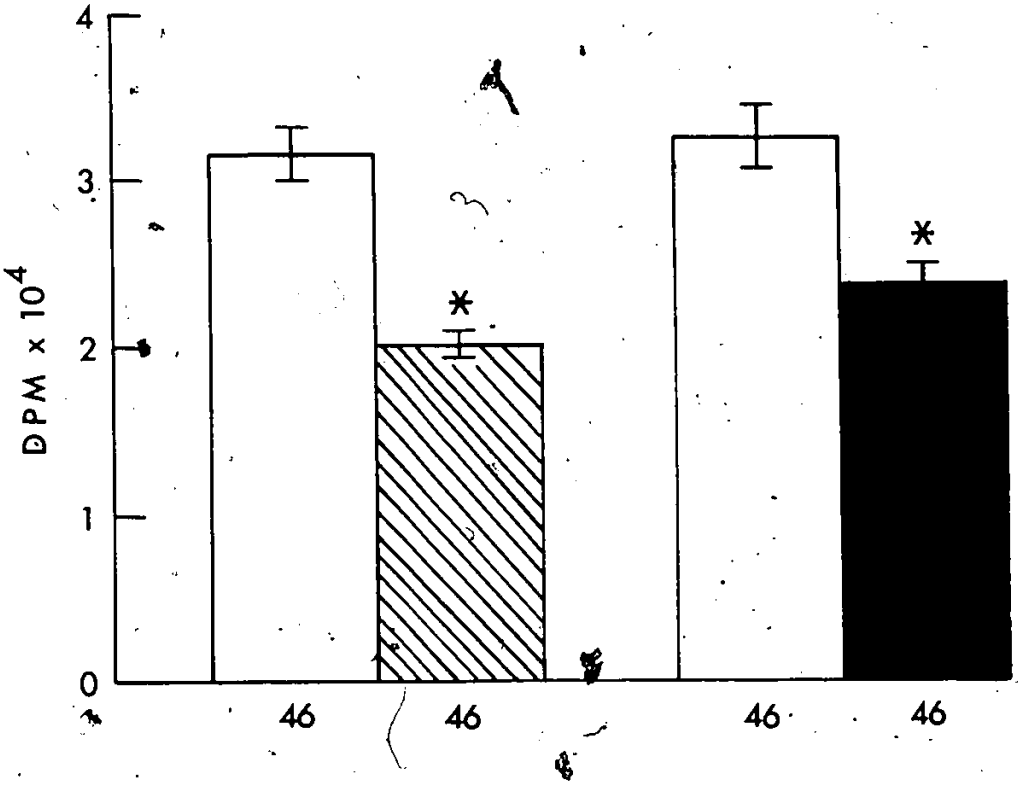

Figure 3. Basal efflux of tritium in bovine renal arteries pretreated with cocaine (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M). Basal efflux in two matched sets of arteries determined prior to each frequency test during first period of nerve-induced stimulations (open columns) and during second period in the absence (hatched column) and presence (filled column) of phenoxybenzamine (3.3×10^{-5} M). Number of values in each group are shown below column. Probability comparisons, by the paired t-test are between first and second values in each group, *$p < 0.001$.

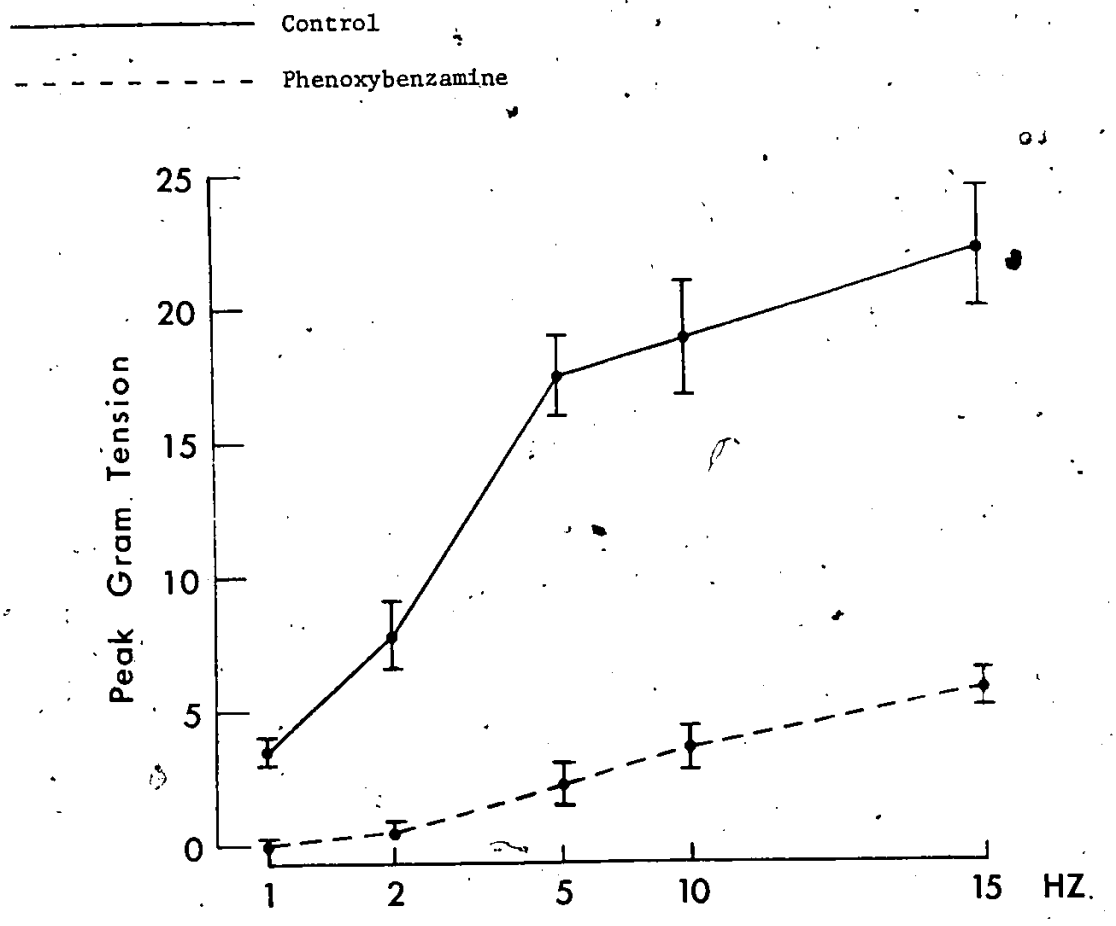

Figure 4. Frequency-response curves in bovine renal artery in the absence and presence of phenoxybenzamine (3.3×10^{-5} M). Stimulation was with 300 pulses at each test frequency. Mean values shown are those for initial control tests in all strips whose tritium efflux values are presented in Figure 2 and also those obtained during the second stimulation period in all strips pretreated with phenoxybenzamine in the interval between the two stimulation periods. Values of second stimulation period in untreated strips (not shown) did not differ significantly from those of the first period.

efflux data where a relationship between frequency of stimulation and efflux of tritium was not evident (compare Figures 2 and 4). It should be mentioned that although stimulations at 1 and 2 Hz allowed sufficient time (5 and 2.5 minutes respectively) for the responses to plateau, stimulations at the higher frequencies did not. The short duration of stimulation at high frequencies was unavoidable as a fixed number of pulses was intentionally delivered. Mean times taken for responses to peak were 115.9±15.7, 97.6±7.2, 62.4±1.7, 44.1±2.9 and 44.1±3.7 seconds for stimulations at 1, 2, 5, 10 and 15 Hz respectively.

The adrenergic neuronal blocking agent guanethidine was used to confirm that the contractile response was induced selectively by the activation of sympathetic nerve terminals. Exposure of strips to guanethidine (3×10^{-5} M) for 30 minutes eliminated completely the contractile responses to transmural stimulation at low frequencies. The response at the highest frequency (15 Hz) was reduced to only 22.4% of control values.

Treatment of strips with phenoxybenzamine significantly depressed the tissue response to transmural stimulation (Figure 4, broken line), as was expected with a typical alpha-adrenergic antagonist. Frequency-response curves after phenoxybenzamine were shifted to the right and their maxima reduced, showing the characteristics of a non-competitive non-equilibrium antagonism caused by formation of covalent bond between antagonist and some component of the alpha-receptor site (Nickerson and Collier, 1975).

B. Effect of noradrenaline in bovine renal artery

The effect of exogenous noradrenaline on stimulation-induced efflux of ^{3}H-transmitter and on mechanical response was studied in order to determine the extent of compliance of the noradrenaline inhibition with predictions of the presynaptic receptor hypothesis. These experiments were performed using the same conditions and same type of tissues as those described in section IV, A. with phenoxybenzamine; they thus provided parallel observations to those obtained with the antagonist.

Two moderate concentrations of noradrenaline (1×10^{-6} M, 3×10^{-6} M) were used. Strips were exposed to noradrenaline for 20 minutes after the first stimulation with 300 pulses, followed by a second stimulation series performed in the presence of the agonist. For each preparation only two frequencies, either 1 and 2, or 5 and 15 Hz (in random sequence); and one concentration of noradrenaline, were employed. Cocaine (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M) were routinely present in the Krebs superfusate.

1. Stimulation-induced efflux of ^{3}H-transmitter

Efflux of tritium elicited by the first stimulation series with 300 pulses at 1, 2, 5 and 15 Hz in strips treated with noradrenaline (1×10^{-6} M) and their controls was 2.97±0.30, 4.31±0.38, 5.84±0.50 and 5.59±0.38 $\times 10^4$ dpm respectively. The transmitter overflow increased with ascending frequency, with the values at 1 and 2 Hz significantly different from the others.

Corresponding efflux of tritium with stimulations at 1, 2, 5

and 15 Hz in strips treated with noradrenaline at the higher concentration (3×10^{-6} M) and their controls was 3.51±0.24, 4.83±0.50, 4.40±0.33 and 2.31±0.23 $\times 10^{4}$ dpm respectively. In this population the output of tritium at 2 and 5 Hz, although not significantly different between themselves, were significantly higher than the output at 1 and 15 Hz.

A 20 minute exposure of the strips to the low concentration of noradrenaline (1×10^{-6} M) decreased moderately but significantly efflux of tritium at 1 Hz, as determined by the intrastrip efflux ratio of the second versus the first stimulation (Table 2). However, noradrenaline at this concentration was not effective in inhibiting the efflux at 2, 5 and 15 Hz. The efflux ratios at these frequencies in the presence of noradrenaline were not significantly different from that of the control group. Noradrenaline at the higher concentration (3×10^{-6} M), on the other hand, inhibited significantly the overflow of tritium at all test frequencies (Table 3). Efflux at 1 Hz was decreased most by the agonist as the efflux ratio at this frequency was significantly lower than that observed at the other frequencies. The inhibitory effect of noradrenaline on efflux of tritium, however, was not significantly different with stimulation at 2, 5 and 15 Hz.

Figure 5 summarizes the effectiveness of noradrenaline at the two concentrations on the inhibition of ^{3}H-transmitter efflux in the bovine renal artery. Shown in this figure is the percentage inhibition of efflux calculated from the efflux ratios of the control and treated groups at each frequency, with the efflux of the control group taken as

Table 2. EFFECT OF NORADRENALINE (1×10^{-6}M) ON THE STIMULATION-INDUCED EFFLUX OF TRITIUM IN BOVINE RENAL ARTERY.

Treatment group [1]	Stimulation frequency (Hz)	No. of values	Transmitter efflux dpm ($\times 10^4$) 1st run	Transmitter efflux dpm ($\times 10^4$) 2nd run	Efflux ratio 2nd run / 1st run
Control	1	12	3.58±0.42	3.20±0.33	0.91±0.06
Noradrenaline	1	12	2.37±0.37	1.44±0.11	0.69±0.07*
Control	2	12	5.09±0.56	4.71±0.64	0.90±0.06
Noradrenaline	2	12	3.53±0.41	2.88±0.29	0.86±0.09
Control	5	5	6.26±0.92	5.87±0.15	1.05±0.20
Noradrenaline	5	5	6.22±0.57	5.42±0.62	0.88±0.08
Control	15	5	5.87±0.60	5.09±0.25	0.90±0.08
Noradrenaline	15	5	5.53±0.61	5.27±0.48	0.97±0.08

[1]Noradrenaline, when given, was administered in the interval between runs, as described in text.

*Indicates treated group significantly different from corresponding control group with a $p<0.05$.

Table 3. EFFECT OF NORADRENALINE (3×10^{-6}M) ON THE STIMULATION-INDUCED EFFLUX OF TRITIUM IN BOVINE RENAL ARTERY.

Treatment group [1]	Stimulation frequency (Hz)	No. of values	Transmitter efflux dpm ($\times 10^4$) 1st run	Transmitter efflux dpm ($\times 10^4$) 2nd run	Efflux ratio 2nd run / 1st run
Control	1	8	3.95±0.29	3.43±0.17	0.89±0.05
Noradrenaline	1	8	3.07±0.31	0.88±0.18	0.28±0.05*†
Control	2	8	5.46±0.82	4.27±0.30	0.85±0.08
Noradrenaline	2	8	4.21±0.53	2.00±0.37	0.47±0.05*
Control	5	6	4.66±0.62	4.22±0.54	0.91±0.08
Noradrenaline	5	6	4.14±0.25	2.28±0.19	0.55±0.03*
Control	15	6	2.30±0.38	2.52±0.30	1.21±0.17
Noradrenaline	15	6	2.33±0.28	1.46±0.28	0.63±0.11*

[1]Noradrenaline, when given, was administered in the interval between runs, as described in text.

*Indicates treated group significantly different from corresponding control group with a $p<0.02$.

†Indicates ratio significantly different from that obtained with other test frequencies in the presence of noradrenaline.

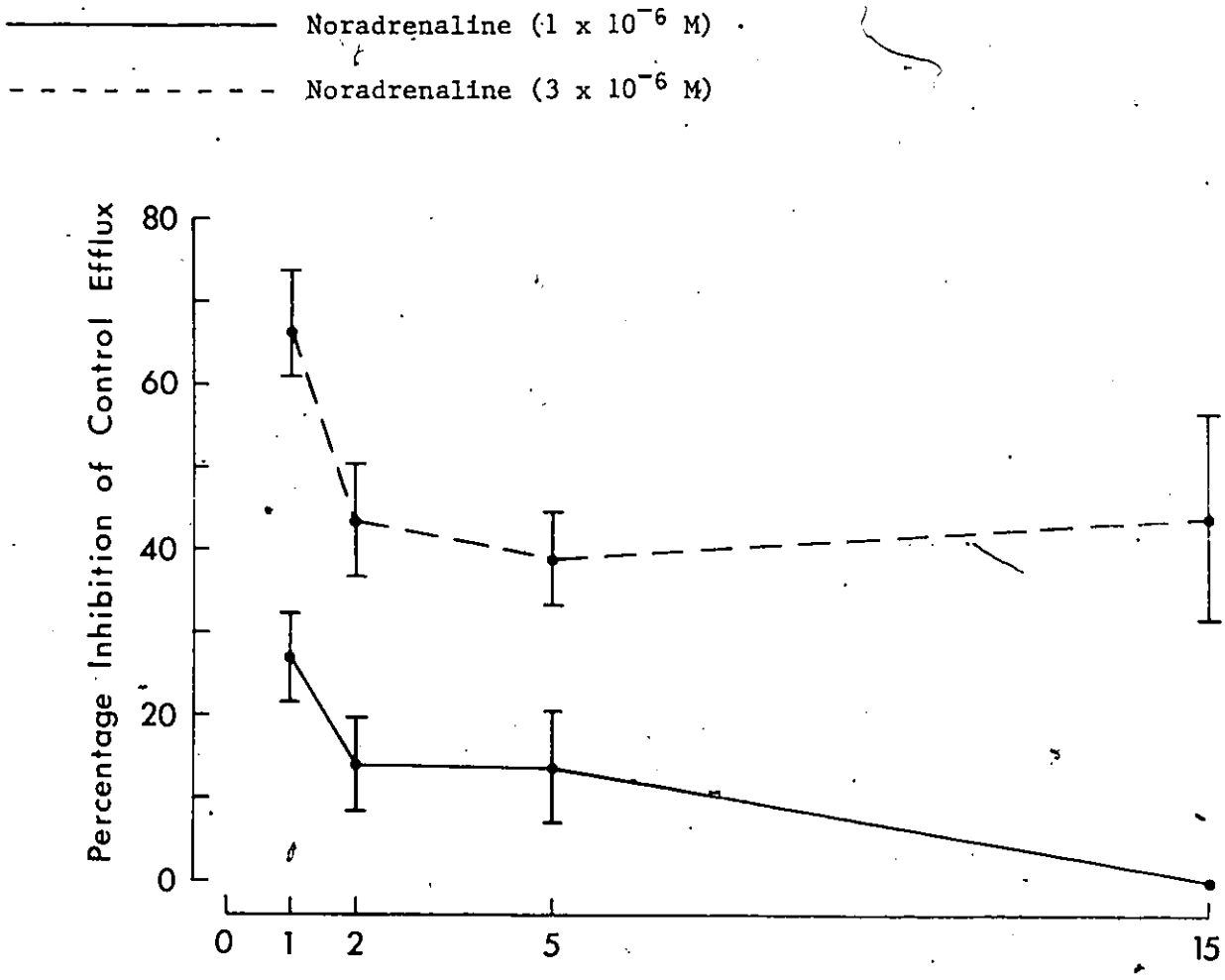

Figure 5. Effect of noradrenaline on the stimulation-induced efflux of tritium in bovine renal artery. Stimulation was with 300 pulses at each test frequency. Percentage inhibition of control efflux was obtained by comparison of individually determined efflux ratios for treated and matching control strips. In the group treated with noradrenaline at 1×10^{-6} M, percentage inhibition of flow at 1 Hz but not at 2 and 5 Hz was significantly different from that at 15 Hz ($p < 0.001$). In the group treated with noradrenaline at 3×10^{-6} M, values at 2, 5 and 15 Hz did not differ significantly from each other, but all three values were significantly different from that at 1 Hz ($p < 0.05$).

100%. Noradrenaline inhibited the overflow of ^{3}H-transmitter in a dose-dependent but not frequency-related manner. As assessed by the percentage inhibition of efflux, noradrenaline at 1 x 10^{-6} M inhibited efflux of ^{3}H-transmitter significantly only at 1 Hz. The percentage of inhibition of efflux at this lowest frequency was significantly different from that at 15 Hz ($p < 0.001$), but those at 2 and 5 Hz were not. The agonist at 3 x 10^{-6} M inhibited efflux significantly at all stimulation frequencies, with the greatest reduction observed at 1 Hz. However, the percentage of inhibition of efflux at 2, 5 and 15 Hz were not significantly different from each other.

2. Beta-adrenergic blockade and stimulation-induced efflux of ^{3}H-transmitter

The possibility that the ineffectiveness of noradrenaline at the lower concentration (1 x 10^{-6} M) on efflux at high frequencies of stimulation was due to compensation of transmitter overflow by some beta-receptor-mediated events was examined using the beta-adrenergic antagonist dl-propranolol. After initial stimulations with 300 pulses at 5 and 15 Hz, renal artery strips were exposed to dl-propranolol (1 x 10^{-7} M) for 20 minutes followed by additional exposure to noradrenaline (1 x 10^{-6} M) for another 20 minutes. At the end of this period a second stimulation series identical to the first was performed in the presence of both the agonist and the antagonist. Control preparations were treated only with propranolol at the same concentration and for the same duration.

A 40-minute exposure to propranolol did not alter stimulation-

induced efflux in the bovine renal artery: the efflux ratios at 5 and 15 Hz of the strips treated with propranolol were not significantly different from that of the control strips (i.e. control strips in Table 2). Efflux of tritium at these frequencies of stimulation was not significantly reduced by noradrenaline after treatment of strips with propranolol (Table 4). The strips treated with both propranolol and noradrenaline had an apparent lower value of efflux ratio compared to that of the control group with only propranolol treatment. However, the difference was not statistically significant ($p < 0.1$ for 5 Hz, $p < 0.2$ for 15 Hz) despite the moderate sample size examined.

3. Basal efflux

Mean basal efflux measured prior to the first and second stimulation series of the control strips in Table 2 was $4.76 \pm 0.26 \times 10^4$ and $3.15 \pm 0.18 \times 10^4$ dpm respectively. The ratio of basal efflux (second versus first period) was 0.67 ± 0.03. In strips treated with noradrenaline (1×10^{-6} M) the corresponding ratio was 0.63 ± 0.05, a value not significantly different from that of the control group.

The mean basal efflux of the control strips in Table 3 similarly declined with time. The corresponding value measured prior to the first and second stimulation series in the control strips was $6.13 \pm 0.31 \times 10^4$ and $3.72 \pm 0.16 \times 10^4$ dpm. This gave a ratio of 0.62 ± 0.01. Treatment of tissues with noradrenaline (3×10^{-6} M) increased the ratio significantly to 0.68 ± 0.02 ($p < 0.01$ compared to the corresponding control group, N=28). Thus, noradrenaline at 3×10^{-6} M was the highest

Table 4. EFFECT OF DL-PROPRANOLOL (1×10^{-7}M) ON STIMULATION-INDUCED EFFLUX OF TRITIUM IN THE ABSENCE AND PRESENCE OF NORADRENALINE (1×10^{-6}M) IN BOVINE RENAL ARTERY.

Treatment group	Stimulation frequency (Hz)	Transmitter efflux dpm ($\times 10^4$) 1st run	Transmitter efflux dpm ($\times 10^4$) 2nd run	Efflux ratio 2nd run / 1st run
Propranolol[1] (8)	5	3.36±0.52	3.40±0.23	1.22±0.21
Propranolol plus Noradrenaline[2] (8)	5	5.06±0.47	4.02±0.62	0.78±0.09 NS
Propranolol[1] (8)	15	2.78±0.33	2.95±0.26	1.11±0.09
Propranolol plus Noradrenaline[2] (8)	15	4.48±0.31	4.12±0.40	0.92±0.06 NS

[1]Strips were exposed to propranolol for a total 40 minutes in the interval between runs and the second run was performed in its presence. Number of values in each group are shown in parentheses.

[2]Strips were exposed to propranolol for 20 minutes and then, without washout, to noradrenaline for 20 minutes. Second stimulation was performed in the presence of both agents.

NS, no significant difference between value of group treated with both agents and the corresponding group treated with propranolol alone ($p<0.2>0.1$).

concentration that could be used. A higher concentration of the agonist would tend to elevate the basal efflux to an uncontrollable extent, even in the presence of cocaine.

4. Mechanical responses

Contractile responses to the first series of stimulations at 1, 2, 5 and 15 Hz in control renal artery strips used in these experiments were similar to those previously shown in Figure 4 for the experiment with phenoxybenzamine. The peak tensions were 4.53±0.70 (N=20), 9.18±0.94 (N=20), 11.36±5.36 (N=11) and 15.15±1.52 (N=11) g for stimulations at 1, 2, 5 and 15 Hz respectively.

In contrast to the antagonist, noradrenaline itself induced vasoconstriction in renal artery. Plateau gram tension of the strips treated with noradrenaline at 1×10^{-6} M and 3×10^{-6} M were 6.62±1.24 g (N=17) and 10.08±1.84 g (N=14) respectively, which were significantly higher than those of the matching controls (1.24±0.19 g for the former group and 1.19±0.22 g for the latter group). Because of the considerable magnitude of contraction induced by the agonist, which may mask the postsynaptic effects of the subsequent nerve stimulations, a satisfactory method to assess the effect of noradrenaline on stimulation-evoked contractile responses is currently unavailable.

C. Adrenergic antagonists and the presynaptic alpha-receptor hypothesis

The validity of the presynaptic alpha-receptor hypothesis was tested in experiments examining the effects of six alpha-antagonists at various concentrations on the stimulation-induced overflow of ^{3}H-transmitter

in strips of bovine radial artery.

The radial artery strips, similar in size to the renal artery preparation, were superfused with Krebs solution containing cocaine (3×10^{-5} M). After a 90 minute equilibration period the strips were stimulated with 600 pulses at 5 Hz, then were exposed to one antagonist for 30 minutes, followed by a second stimulation identical to the first one in the presence of the antagonist. In the experiments with the haloalkylamines phenoxybenzamine or Dibenamine, the antagonist was washed out for 20 minutes before the onset of the second stimulation. Only one antagonist at one concentration was tested on any single strip. Contractile responses of the radial artery to nerve stimulation were not measured in the superfusion experiments.

1. Stimulation-induced efflux of ^{3}H-transmitter

As expected, efflux of tritium elicited by transmural stimulation with 600 pulses at 5 Hz in bovine radial artery was elevated materially over the basal levels. In 20 control strips receiving no antagonist treatment but allowed a 30-minute lag period between the two stimulations, mean efflux for the first and second stimulation, which were not statistically different from each other, were $3.98 \pm 0.63 \times 10^{4}$ and $3.65 \pm 0.60 \times 10^{4}$ dpm respectively. This yielded an efflux ratio (second versus first stimulation) of 0.95 ± 0.08.

The effects of six antagonists at three selected concentrations on the stimulation-induced overflow of ^{3}H-transmitter was determined. Since there is an interstrip variation of efflux during the initial pre-drug stimulations, with efflux values varying between extremes of 20,000

dpm and 90,000 dpm, the absolute amount of tritium overflow is not suitable for use as an index to assess the effects of antagonist on efflux. Instead, effects are more appropriately assessed by an intra-strip efflux comparison procedure, as summarized in Table 5 and Figures 6, 7 and 8. It is of note that the pattern of the effectiveness of antagonists was not consistent. Treatment of tissues with different antagonists resulted in all three possible effects (i.e. increase, decrease or no change) on the stimulation-induced efflux.

The haloalkylamines phenoxybenzamine and Dibenamine both increased the stimulation-induced efflux maximally at 3×10^{-5} M, with the magnitude of enhancement comparable to that obtained in the bovine renal artery (section IV, A.). These agents were ineffective at lower concentrations, except for phenoxybenzamine at 3×10^{-7} M where a slight but significant effect on efflux was detected.

The imidazoline derivatives phentolamine and tolazoline both enhanced efflux in a dose-dependent manner, but interestingly had their greatest effects at the lowest concentration. These compounds at the lowest concentration increased significantly the overflow of tritium, but at the highest concentration employed they did not enhance efflux.

Chlorpromazine and yohimbine exhibited yet other patterns of effect - they decreased significantly the stimulation-induced overflow of ^{3}H-transmitter. Chlorpromazine was effective when administered at the highest but not at the lower concentrations. On the other hand, yohimbine decreased the output of tritium significantly at 3×10^{-6} M and 3×10^{-5} M concentrations.

Table 5. THE RATIOS OF TRANSMITTER EFFLUX IN THE FIRST AND SECOND PERIODS OF FIELD STIMULATION IN THE ABSENCE AND PRESENCE OF ADRENERGIC ANTAGONISTS IN BOVINE RADIAL ARTERY.

Antagonist[1]	Transmitter efflux ratio 2nd run / 1st run[2]		
	3×10^{-7} M Antagonist	3×10^{-6} M Antagonist	3×10^{-5} M Antagonist
Phenoxybenzamine	1.26±0.11 (8)*	1.21±0.11 (4)	1.79±0.37 (4)***
Dibenamine	1.07±0.13 (8)	1.13±0.08 (4)	1.73±0.09 (4)***
Chlorpromazine	1.02±0.11 (10)	1.40±0.33 (10)	0.59±0.04 (4)*
Yohimbine	0.64±0.15 (4)	0.41±0.12 (4)***	0.10±0.03 (6)***
Phentolamine	1.77±0.18 (12)***	1.13±0.12 (7)	0.71±0.16 (10)
Tolazoline[3]	2.06±0.70 (8)**	1.47±0.28 (8)*	1.01±0.08 (8)

[1]Antagonists were administered in the interval between first and second periods of stimulation as described in text. Number of values at each concentration are shown in parentheses.

[2]The ratio of second period versus first period stimulation values for 20 untreated control strips, to which all other groups were compared was 0.95 ± 0.08.

[3]The concentrations of tolazoline used was twice that indicated for the other antagonists.

*$p<0.05$; **$p<0.02$; ***$p<0.01$ compared to ratio for control strips.

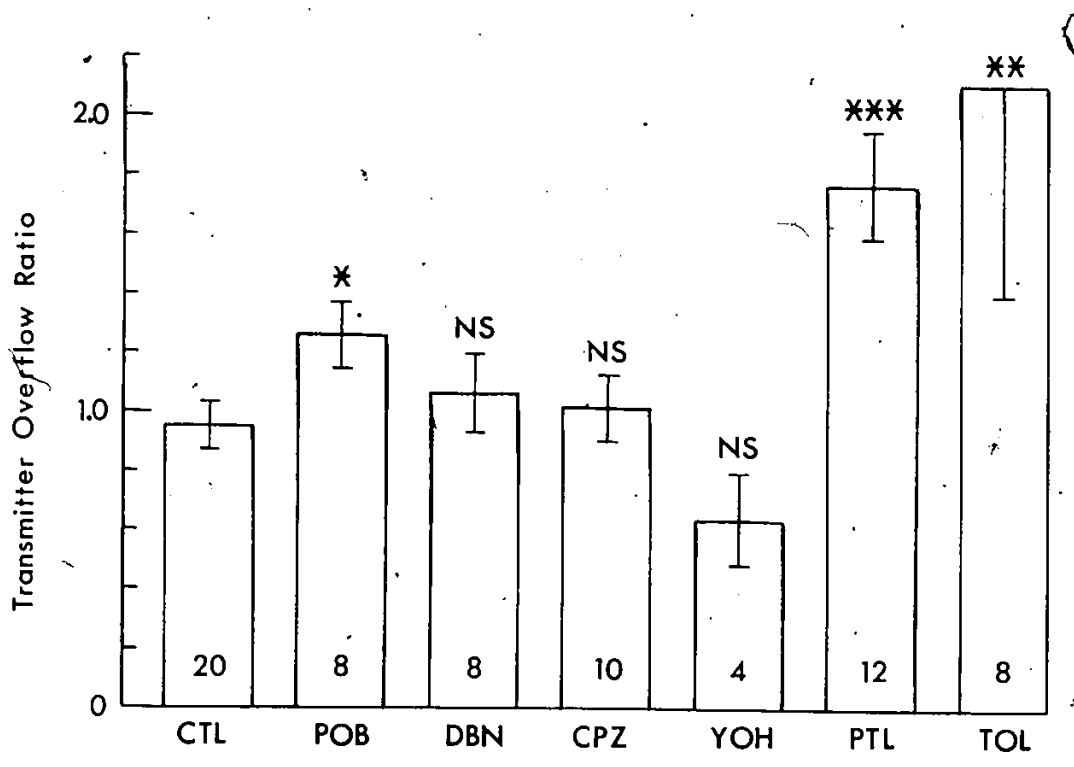

Figure 6. Profile of antagonist effects on stimulation-induced efflux in bovine radial artery. The ratios of transmitter efflux in the first and second stimulation with 600 pulses at 5 Hz in the absence and presence of six antagonists at 3×10^{-7} M (6×10^{-7} M for tolazoline) are shown, with number of values in each group indicated within column. Data are taken from Table 5. CTL, control; POB, phenoxybenzamine; DBN, Dibenamine; CPZ, Chlorpromazine; YOH, yohimbine; PTL, phentolamine; TOL, tolazoline. *$p < 0.05$; **$p < 0.02$; ***$p < 0.01$ compared to ratio for control strips, N.S., not significant.

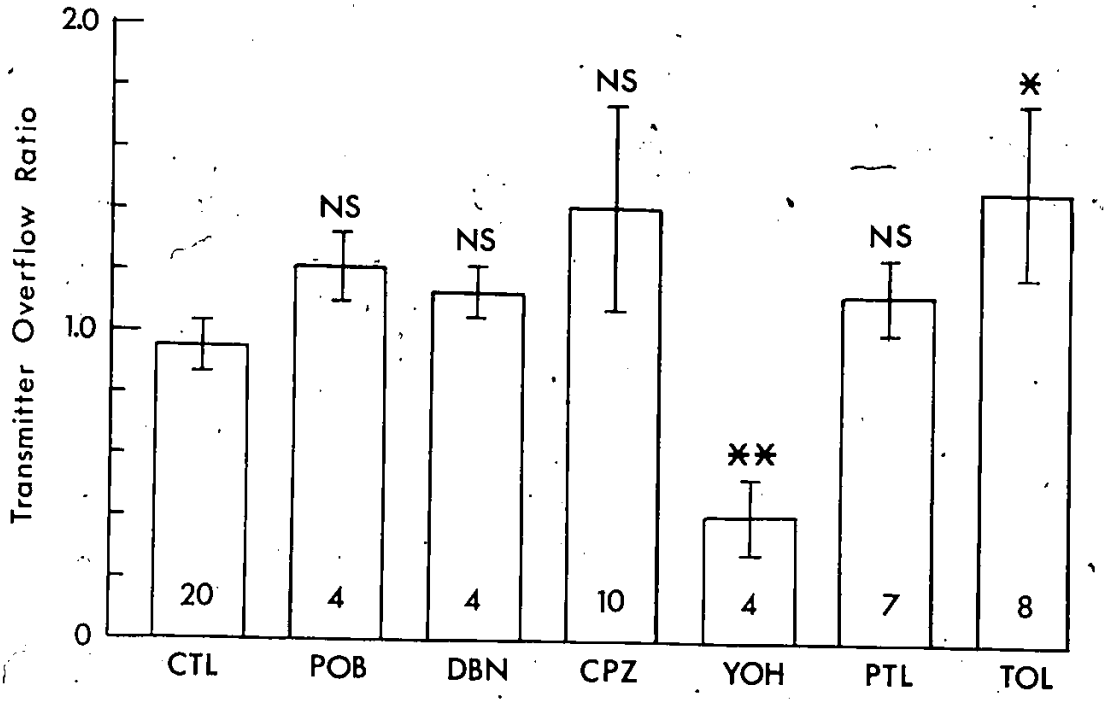

Figure 7. Profile of antagonist effects on stimulation-induced efflux in bovine radial artery. The ratios of transmitter efflux in the first and second stimulation with 600 pulses at 5 Hz in the absence and presence of six antagonists at 3×10^{-6} M (6×10^{-6} M for tolazoline) are shown, with number of values in each group indicated within column. Data are taken from Table 5. CTL, control; POB, phenoxybenzamine; DBN, Dibenamine; CPZ, chlorpromazine; YOH, yohimbine, PTL, phentolamine; TOL, tolazoline. *$p < 0.05$; **$p < 0.01$ compared to ratio for control strips; N.S., not significant.

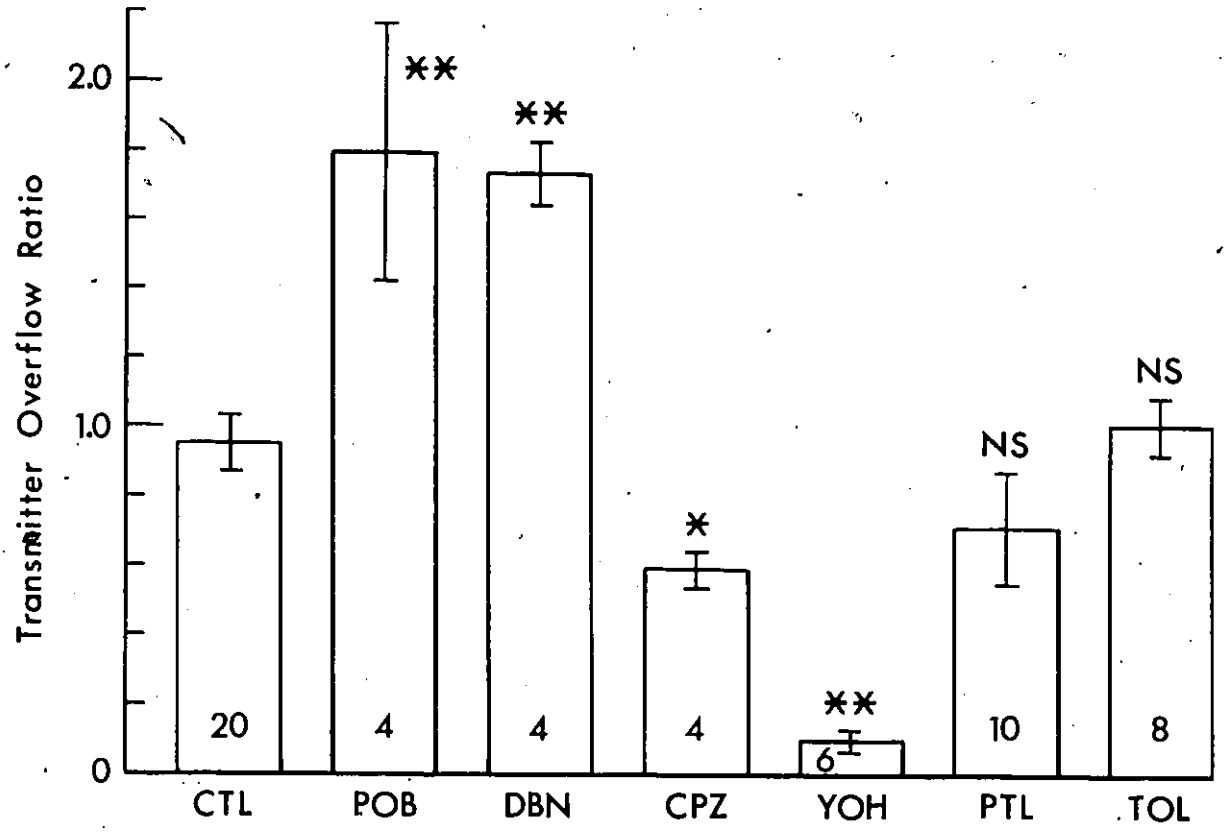

Figure 8. Profile of antagonist effects on stimulation-induced efflux in bovine radial artery. The ratios of transmitter efflux in the first and second stimulation with 600 pulses at 5 Hz in the absence and presence of six antagonists at 3×10^{-5} M (6×10^{-5} M for tolazoline) are shown, with number of values in each group indicated within column. Data are taken from Table 5. CTL, control; POB, phenoxybenzamine; DBN, Dibenamine; CPZ, chlorpromazine; YOH, yohimbine; PTL, phentolamine; TOL, tolazoline. *$p < 0.05$; **$p < 0.01$ compared to ratio for control strips, N.S., not significant.

Since in these experiments only the neuronal uptake inhibitor cocaine was routinely present in the Krebs superfusate, the possibility that extra-neuronal uptake contributed significantly to the observed effects was investigated. Eight radial artery strips were superfused with Krebs solution containing cocaine (3×10^{-5} M) and were stimulated initially with 600 pulses at 5 Hz. Four strips were subsequently exposed to the extraneuronal uptake inhibitor normetanephrine (1×10^{-5} M) for 30 minutes, and a second stimulation was performed in the presence of both uptake inhibitors. The other four strips served as control. Efflux of tritium in the first and second stimulations for the strips treated with cocaine alone were $6.51 \pm 0.55 \times 10^4$ and $7.20 \pm 0.42 \times 10^4$ dpm, respectively. This yielded an efflux ratio of 1.12 ± 0.05. The corresponding efflux values for the strips treated with both cocaine and normetanephrine were $5.13 \pm 2.09 \times 10^4$ and $5.72 \pm 1.49 \times 10^4$ dpm, respectively. This gave an efflux ratio of 1.27 ± 0.13. There was no statistical significant difference between the two efflux ratios ($p < 0.4 > 0.3$).

2. Basal efflux

Basal efflux determined immediately prior to the first and second stimulation periods in 20 control strips were $1.96 \pm 0.11 \times 10^4$ and $1.48 \pm 0.08 \times 10^4$ dpm, respectively, showing a significant decline in spontaneous overflow of tritium with time ($p < 0.001$). This gave a control efflux ratio of 0.76 ± 0.01. Table 6 summarizes the effects of different antagonists on basal efflux, as assessed by a comparison of the efflux ratios. Chlorpromazine, yohimbine and tolazoline consistently and and significantly enhanced basal efflux over the range of concentrations

Table 6. THE RATIOS OF BASAL EFFLUX OF TRITIUM PRIOR TO THE FIRST AND SECOND PERIODS OF STIMULATION IN THE ABSENCE AND PRESENCE OF ADRENERGIC ANTAGONISTS IN BOVINE RADIAL ARTERY.

Antagonist[1]	Basal efflux ratio 2nd run / 1st run[2,3]		
	3×10^{-7} M Antagonist	3×10^{-6} M Antagonist	3×10^{-5} M Antagonist
Phenoxybenzamine	0.75±0.02 (8)	0.82±0.03 (4)*	0.83±0.02 (4)**
Dibenamine	0.76±0.06 (8)	0.80±0.03 (4)	0.76±0.05 (4)
Chlorpromazine	0.85±0.04 (10)***	1.11±0.07 (10)***	2.25±0.50 (4)***
Yohimbine	0.83±0.02 (4)**	0.92±0.02 (4)***	1.01±0.06 (6)***
Phentolamine	0.79±0.02 (12)	0.77±0.04 (7)	1.08±0.03 (10)***
Tolazoline[4]	0.90±0.03 (8)***	0.93±0.06 (8)***	1.04±0.05 (8)***

[1]Antagonists were administered in the interval between first and second periods of stimulation as described in text. Number of values are shown in parentheses.

[2]The basal efflux values were determined in samples taken immediately prior to the onset of the first and second periods of stimulation of strips described in Table 5.

[3]The ratio of second period versus first period values for the 20 untreated control strips, to which all other groups were compared was 0.76 ± 0.01.

[4]The concentrations of tolazoline used was twice that indicated for the other antagonists.

*$p<0.05$; **$p<0.02$; ***$p<0.01$ compared to ratio for control strips.

employed. The effect of chloropromazine was the most profound among all the antagonists under study, whereas that of yohimbine and tolazoline were moderate. A dose-dependent effect of chlorpromazine on basal efflux was observed. Such a relationship was less clear for tolazoline or yohimbine. Phenoxybenzamine and phentolamine enhanced basal efflux only slightly at the moderate and the high concentration. On the other hand, Dibenamine at all test concentrations did not significantly alter the basal overflow of tritium.

3. Adrenergic blockade of mechanical responses of bovine radial artery to exogenous noradrenaline

The mechanical response of radial artery strips was not recorded concomitantly with the overflow of tritium in the superfusion experiments. Instead, it was measured isotonically in strips bathed in muscle chambers containing 15 ml of cocaine-Krebs solution (3×10^{-5} M cocaine) with a 6.8-fold magnification lever writing on a kymograph drum.

The purpose of these experiments was to confirm the post-synaptic alpha-adrenergic blocking effects of the antagonists used in the efflux experiments. After a 90-minute equilibration period with replacement of the Krebs solution every 30 minutes, the strips were contracted initially with a moderate concentration of noradrenaline (3×10^{-7} M). The response was allowed to peak, and then noradrenaline was washed out of the bath. Following the return of the contraction to pre-drug level, the preparations were exposed to one of the antagonists at the lowest test concentration used (i.e. 6×10^{-7} M for tolazoline

and 3 x 10^{-7} M for other antagonists) for 30 minutes and then the response to noradrenaline were re-tested in the presence of the antagonist, except the haloalkylamines which were washed out before the onset of the second noradrenaline response. The average contractile response to the initial administration of noradrenaline was 29.7±3.9 mm, which was reduced each by 98% after exposure to phenoxybenzamine and Dibenamine; and by 90%, 68%, 65% and 53% by phentolamine, chlorpromazine, tolazoline and yohimbine respectively even at the lowest concentration employed.

4. Yohimbine inhibition of efflux of ^{3}H-transmitter

The finding that yohimbine at moderate and high concentrations significantly inhibited the stimulation-induced efflux of tritium is at variance with the observations of Starke and co-workers, who reported that at concentrations from 3 x 10^{-8} M to 1 x 10^{-5} M the antagonist enhanced efflux in the rabbit pulmonary artery stimulated at 2 and 4 Hz (Starke, Borowski and Endo, 1975; Weitzell, Tanaka and Starke, 1979). The inhibitory effect of yohimbine on efflux observed here is similar to that reported for adrenergic agonists, an effect which is mediated via phenoxybenzamine-sensitive receptors. Experiments were designed to determine whether the effect of yohimbine is also mediated via the same receptors as noradrenaline and phenoxybenzamine utilize. Radial artery preparations, after initial stimulations at 5 and 15 Hz, were exposed to phenoxybenzamine (3 x 10^{-5} M) for 30 minutes and, after washout of the haloalkylamine, were subsequently exposed to yohimbine (3 x 10^{-5} M) for an additional 30 minutes. The second stimulation series was then repeated

Table 7. THE RATIOS OF STIMULATION-INDUCED TRANSMITTER EFFLUX IN THE PRESENCE OF ADRENERGIC ANTAGONISTS IN BOVINE RADIAL ARTERY.

Treatment group (N)	Stimulation frequency (Hz)	Transmitter efflux dpm (x 10^4) 1st run	Transmitter efflux dpm (x 10^4) 2nd run	Efflux ratio 2nd run / 1st run
Yohimbine[1] (4)	5	3.73±0.84	0.21±0.06	0.08±0.03
Phenoxybenzamine plus Yohimbine[2] (4)	5	4.34±0.65	0.52±0.08	0.13±0.04 NS
Yohimbine[1] (4)	15	3.35±0.60	0.27±0.08	0.07±0.03
Phenoxybenzamine plus Yohimbine[2] (4)	15	3.81±0.74	0.64±0.27	0.15±0.05 NS

[1]Strips were exposed to yohimbine (3×10^{-5} M) for 30 minutes in the interval between stimulation periods and the second stimulation was performed in its presence.

[2]Strips were exposed to phenoxybenzamine (3×10^{-5} M) for 30 minutes and 10 minutes after its washout, to yohimbine (3×10^{-5} M) followed 30 minutes later, without washout, by the second period of stimulation.

NS, no significant difference between efflux ratio of group treated with both antagonists and the corresponding group treated with yohimbine alone in the interval between stimulation periods with 300 pulses ($p<0.4>0.2$).

in the presence of yohimbine. As shown in Table 7 yohimbine inhibited stimulation-induced efflux at either frequency to the same extent regardless of whether the tissues were pretreated with phenoxybenzamine.

5. Effects of phenoxybenzamine and phentolamine on stimulation-induced efflux in bovine facial artery

Experiment with a bovine facial artery preparation provided additional information as to the general applicability to other vascular tissue models of the findings made in the radial artery. The facial artery is different from other tissues employed in the present study in that it responds to field stimulation and to exogenous noradrenaline with relaxation mediated via beta-receptors (Kalsner, 1979a). The effects of two antagonists, namely phenoxybenzamine and phentolamine, on stimulation-induced efflux were examined. As shown in Table 8 the absolute amount of tritium overflow elicited by stimulation with 600 pulses at 5 Hz in the facial artery was greater (as much as 10-fold) than that in the radial artery with the same number of pulses and frequency. Regardless of this difference, however, phenoxybenzamine (3×10^{-5} M) and phentolamine (3×10^{-5} M) exhibited the same pattern of effect as that observed with the radial artery preparation: phentolamine again failed to alter the overflow of ^{3}H-transmitter whereas phenoxybenzamine increased efflux to a comparable magnitude as it did in the radial artery. In fact, the effect of phenoxybenzamine on efflux in both preparations has the same level of statistical significance when the efflux ratio of the treated strips is compared to that of the control strips ($p < 0.01$). Further, experiments using the receptor protection

Table 8. THE RATIOS OF TRANSMITTER EFFLUX IN THE FIRST AND SECOND PERIODS OF FIELD STIMULATION IN THE ABSENCE AND PRESENCE OF ANTAGONISTS IN BOVINE FACIAL ARTERY.

Treatment group (N)	Transmitter efflux dpm (x 10^4) 1st run	2nd run	Efflux ratio 2nd run / 1st run[1]	p value
Control (6)	23.70±3.10	23.40±3.40	(a) 0.99±0.09	
Phentolamine[2] (8)	30.10±6.60	24.00±8.50	(b) 1.02±0.20	(a) vs (b) NS
Phenoxybenzamine[3] (12)	27.20±4.30	57.40±6.80	(c) 2.51±0.28	(a) vs (c) <0.01 (b) vs (c) <0.001
Phentolamine plus Phenoxybenzamine[4] (12)	23.10±4.20	39.00±5.50	(d) 1.98±0.20	(a) vs (d) <0.01 (b) vs (d) <0.01 (c) vs (d) NS

[1]The ratios between the efflux of tritium in the second period of stimulation at 5 Hz for 2 minutes and the first period of stimulation. Number of values in each group is shown in parentheses.

[2]Strips were exposed to phentolamine (3×10^{-5} M) for 20 minutes in the interval between stimulation periods and the second stimulation was performed in its presence.

[3]Strips were exposed to phenoxybenzamine (3×10^{-5} M) for 30 minutes and washed out for 20 minutes in the interval between stimulation periods.

[4]Strips were exposed to phentolamine (3×10^{-5} M) and 10 minutes later, in its presence, to phenoxybenzamine (3×10^{-5} M) for 30 minutes. The antagonists were then washed out for 20 minutes prior to the second period of stimulation.

technique were done to determine if the failure of phentolamine to increase efflux was related to some underfined action of the compound masking the expression of its proposed action to block presynaptic alpha-receptors. Facial artery strips were exposed to phenoxybenzamine, after the initial stimulations, in the absence and in the presence of phentolamine. A second stimulation was performed following the wash-out of both antagonists. As shown in Table 8 phentolamine did not protect against the phenoxybenzamine-induced enhancement of stimulation-induced efflux.

6. Inhibitory effects of phentolamine on stimulation-induced efflux and on mechanical responses in bovine renal artery

The effects of a high and a low concentration of phentolamine (i.e., 3×10^{-7} M and 3×10^{-5} M) on efflux of tritium elicited by stimulations at 1, 5 and 15 Hz were examined in the bovine renal artery. To compare the results with those obtained in experiments with phenoxybenzamine in this tissue as previously described in section IV, A., the strips were stimulated with 300 pulses (instead of 600 pulses) and superfused with Krebs solution containing cocaine (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M). The results are summarized in Table 9. In contrast to the observations on the radial artery, where phentolamine at 3×10^{-7} M enhanced efflux by 1.8-fold, the antagonist at this concentration did not alter the efflux with stimulation at any test frequency in the renal artery. At the higher concentration, phentolamine even significantly decreased the efflux of tritium elicited by stimulation at 5 or 15 Hz. Although efflux at 1 Hz was not significantly reduced by

Table 9. THE RATIOS OF TRANSMITTER EFFLUX IN THE FIRST AND SECOND PERIODS OF FIELD STIMULATION IN THE ABSENCE AND PRESENCE OF PHENTOLAMINE IN BOVINE RENAL ARTERY.

Treatment group (N)	Concentration (M)	Transmitter efflux ratio 2nd run / 1st run		
		1 Hz	5 Hz	15 Hz
Control (7)	-	1.17±0.08	1.09±0.26	1.00±0.10
Phentolamine[1] (7)	3×10^{-7}	1.30±0.22	1.22±0.15	1.01±0.07
Control (7)	-	1.09±0.14	0.96±0.08	1.10±0.12
Phentolamine[1] (6)	3×10^{-5}	0.74±0.14	0.51±0.08*	0.39±0.05*

[1]Strips were exposed to phentolamine beginning 30 minutes prior to the onset of the second period of stimulation with 300 pulses at the indicated frequencies.

*Indicates ratios of treated groups significantly different from ratios of corresponding control groups with $p<0.01$. All other treated groups do not differ significantly from their controls.

phentolamine at the high concentration, a tendency towards this direction was indicated ($p < 0.1 > 0.05$ when the efflux ratio of the treated group was compared to that of the control group).

Isometric contractile responses to nerve stimulation at 5 and 15 Hz in renal artery, measured simultaneously with tritium overflow, were clearly inhibited by phentolamine at both concentrations (Table 10), despite the fact that stimulation-induced efflux of transmitter was not affected by the antagonist at the lower concentration. The decrease of mechanical responses observed with phentolamine at the higher concentration, however, may be partly attributable to the effect of the compound to reduce transmitter overflow.

That the postsynaptic effects of phentolamine was due to a specific alpha-receptor blocking action of this agent was confirmed in experiments with a separate set of renal artery strips. Strips were bathed in individual muscle chambers and its contractile responses measured isotonically on kymograph drums. Cumulative concentration-response curves to exogenous noradrenaline and to potassium in the absence and in the presence of phentolamine (3×10^{-5} M) were obtained and the pertinent ED_{50}'s compared. The curve to noradrenaline was shifted materially to the right by the antagonist. The ED_{50} value of 1.23×10^{-7} M for two control strips was increased by 54-fold in two matching strips treated with phentolamine. On the other hand, responses to potassium were not affected materially by the antagonist. ED_{50} value of the potassium concentration-response curve in two control and two treated preparations was 12.2 and 10.1 mM respectively.

Table 10. THE RATIOS OF MECHANICAL RESPONSES IN THE SECOND AND FIRST PERIODS OF FIELD STIMULATION IN THE ABSENCE AND PRESENCE OF PHENTOLAMINE IN THE BOVINE RENAL ARTERY.

Treatment group (N)	Stimulation frequency (Hz)	Concentration (M)	Peak response 1st run[1] (g tension)	Peak response ratio 2nd run / 1st run
(a)				
Control (7)	1	-	3.14±0.87	0.93±0.21
Phentolamine (7)	1	3×10^{-7}	2.83±0.85	0.56±0.24
Control (7)	5	-	11.93±2.30	0.94±0.08
Phentolamine (7)	5	3×10^{-7}	9.76±2.97	0.51±0.09**
Control (7)	15	-	15.13±2.89	0.95±0.06
Phentolamine (7)	15	3×10^{-7}	11.64±2.79	0.52±0.08**
(b)				
Control (7)	1	-	2.39±0.90	0.97±0.36
Phentolamine (6)	1	3×10^{-5}	3.75±1.37	0.09±0.05*
Control (7)	5	-	10.80±1.71	0.73±0.08
Phentolamine (6)	5	3×10^{-5}	16.53±1.69	0.10±0.02***
Control (7)	15	-	19.04±3.22	0.74±0.07
Phentolamine (6)	15	3×10^{-5}	24.62±2.61	0.22±0.04***

[1]The responses shown are those of strips whose efflux of tritium is described in Table 9.

*$p<0.05$; **$p<0.01$; ***$p<0.001$ compared to ratios of corresponding control groups. Phentolamine was administered in the interval between the first and second periods of stimulation.

The inhibitory effect of phentolamine on stimulation-induced efflux observed in the renal artery was very similar to that of yohimbine in the radial artery (Table 5). Both compounds exhibited an effect which is frequently reported for adrenergic agonists. Experiments similar to those described above in section IV, C.4. for yohimbine in the radial artery were performed in renal artery strips utilizing phenoxybenzamine to determine if the inhibitory effect of the imidazoline derivative on efflux was related to blockade of presynaptic sites. Strips were initially stimulated with 300 pulses at two selected frequencies (5 and 15 Hz) and then exposed to phentolamine (3×10^{-5} M) in the absence and in the presence of phenoxybenzamine (3×10^{-5} M), and were subsequently re-stimualted. Table 11 shows that stimulation-induced efflux in renal artery was enhanced to approximately 2-fold that of control as previously reported in section IV, A.1. (Table 1). Phentolamine still exerted a clear-cut and significant inhibitory effect on efflux at either test frequency when administered after phenoxybenzamine.

7. Effect of tetrodotoxin

Since tetrodotoxin in micromolar concentration is known to selectively inhibit nerve impulse propagation without affecting the activity of muscle elements, through an action specifically blocking the sodium channels in nerve membrane (Kao, 1966; Hughes and Vane, 1967), the effect of this agent on stimulation-induced efflux in the bovine radial, renal and facial arteries was examined to confirm that this efflux of tritium was due to nerve activation. A 20-minute exposure to

Table 11. THE RATIOS OF STIMULATION-INDUCED TRANSMITTER EFFLUX IN THE PRESENCE OF ADRENERGIC ANTAGONISTS IN BOVINE RENAL ARTERY.

Treatment group (N)	Stimulation frequency (Hz)	Transmitter efflux dpm (x 10^4) 1st run	Transmitter efflux dpm (x 10^4) 2nd run	Efflux ratio 2nd run / 1st run
Phenoxybenzamine[1] (4)	5	11.21±3.38	22.20±5.05	2.13±0.18
Phenoxybenzamine plus Phentolamine[2] (4)	5	9.08±1.35	6.94±1.84	0.73±0.05*
Phenoxybenzamine[1] (4)	15	8.66±2.08	13.23±1.52	1.69±0.24
Phenoxybenzamine plus Phentolamine[2] (4)	15	7.57±1.01	4.58±0.30	0.63±0.07*

[1]Strips were exposed to phenoxybenzamine (3×10^{-5} M) for 30 minutes and it was washed out for 30 minutes in the interval between stimulation periods.

[2]Strips were exposed to phenoxybenzamine (3×10^{-5} M) for 30 minutes and after its washout, to phentolamine (3×10^{-5} M) followed 30 minutes later, without washout, by the second period of stimulation.

*Indicates efflux ratio of group treated with both antagonists differs significantly from corresponding group treated with phenoxybenzamine alone in the interval between stimulation periods with 300 pulses ($p<0.01$).

tetrodotoxin (1.57×10^{-6} M) reduced the efflux elicited by stimulation with 600 pulses at 5 Hz to 2.1% and 3.3% of the control values in radial and facial arteries respectively. In the renal artery, efflux with 300 pulses at 1, 5 and 15 Hz was similarly reduced to 1.5%, 1% and 0% of the matching control values respectively.

D. Adrenergic agonists and the presynaptic alpha-receptor hypothesis

As an adjunct to experiments with antagonists, the effects of adrenergic agonists on stimulation-induced efflux were investigated in a bovine radial artery preparation using the same experimental protocol as used with the antagonists. Six agonists were selected so as to include compounds having different selectivities in reacting with alpha- or beta-receptors. They included compounds that are efficacious alpha-agonist (phenylephrine), moderately efficacious alpha- and beta-agonists (adrenaline), and an efficacious beta-agonist (isoproterenol).

1. Stimulation-induced efflux of ^{3}H-transmitter

In eight control strips, the efflux of tritium elicited by the first and the second stimulations with 600 pulses at 5 Hz were $4.22 \pm 0.51 \times 10^4$ dpm and $4.12 \pm 0.68 \times 10^4$ dpm respectively. This gave an efflux ratio of 0.92 ± 0.09. Figures 9, 10, 11, 12, 13 and 14 show the absolute amount of stimulation-induced overflow of transmitter in radial artery strips before and after administration of different agonists at various concentrations. Three of the agonists, namely noradrenaline, adrenaline and oxymetazoline, significantly decreased the absolute amount of radioactivity at all test concentrations. However, if the effects are

assessed by the more sensitive efflux ratios, then only oxymetazoline consistently exhibited an inhibitory effect on efflux at all concentrations (Table 12). The effects of noradrenaline at 1×10^{-7} M and at 1×10^{-5} M, and adrenaline at 1×10^{-7} M, did not quite reach statistical significance, as determined by a comparison of the efflux ratios of the treated strips and the control strips. Other agonists, including methoxamine, the "pure" alpha-agonist phenylephrine and the "pure" beta-agonist isoproterenol, had no detectable effect on the absolute output of tritium at all concentrations employed. Efflux ratios of these treated groups were not significantly different from that of the control group.

2. Basal efflux

Basal efflux measured prior to the first and second stimulation in the control strips were $2.13 \pm 0.09 \times 10^4$ dpm and $1.60 \pm 0.07 \times 10^4$ dpm respectively. Table 13 shows the effects of the six agonists on basal efflux. Of all the agonists used only methoxamine, which is not taken up by adrenergic nerve terminals, had no detectable effects on basal efflux at all three concentrations. Other agonists, at the highest test concentration, enhanced the basal efflux to different extents, even in the presence of cocaine. Noradrenaline in particular showed a profound and consistent enhancing effect on efflux. This agonist at the highest test concentration almost doubled the basal efflux. Adrenaline, isoproterenol, oxymetazoline and phenylephrine did not have a consistent effect on the basal overflow. These agents increased the spontaneous overflow of tritium at some but not at other concentrations.

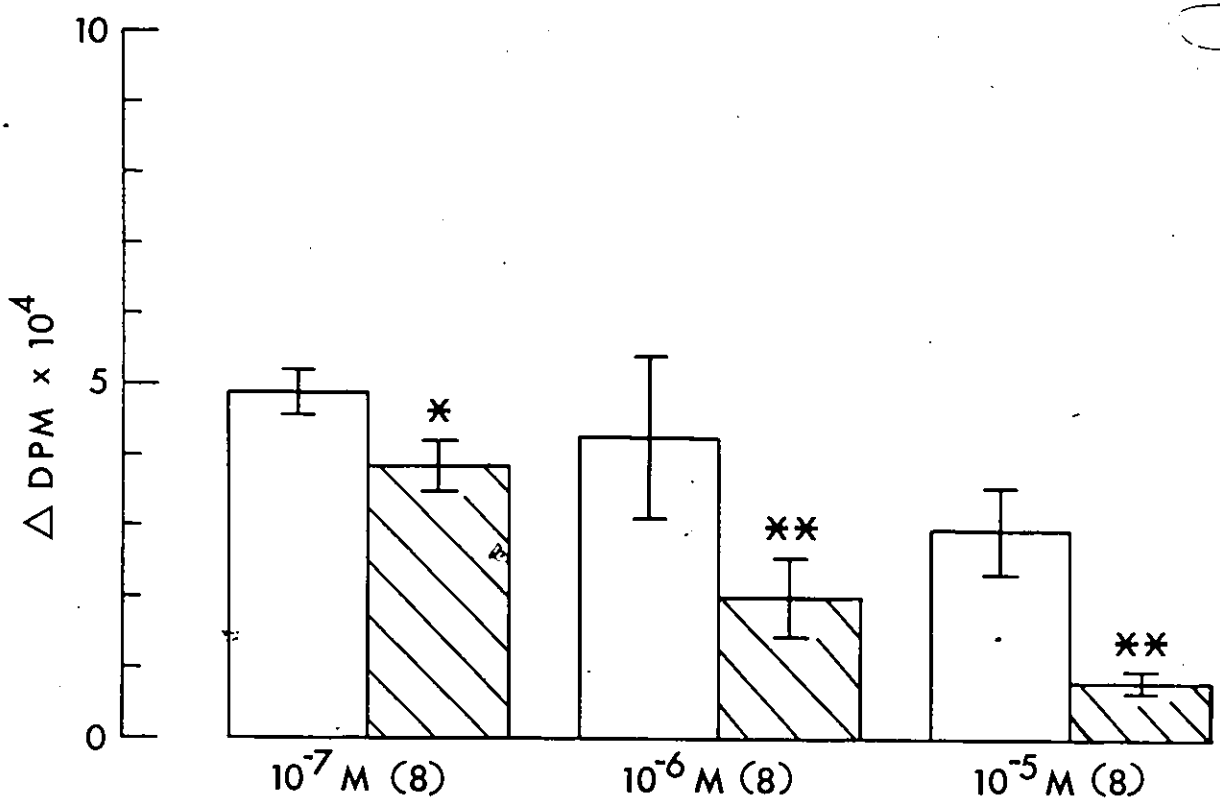

Figure 9. Effect of noradrenaline on stimulation-induced efflux of tritium in bovine radial artery. Stimulation was with 600 pulses at 5 Hz given in the absence (open columns) and presence (hatched columns) of noradrenaline. Concentration of noradrenaline is shown below columns and number of strips in each group in parenthesis. Probability comparisons, by the paired t-test, are between the control and treated values for each concentration, *$p < 0.05$; **$p < 0.02$.

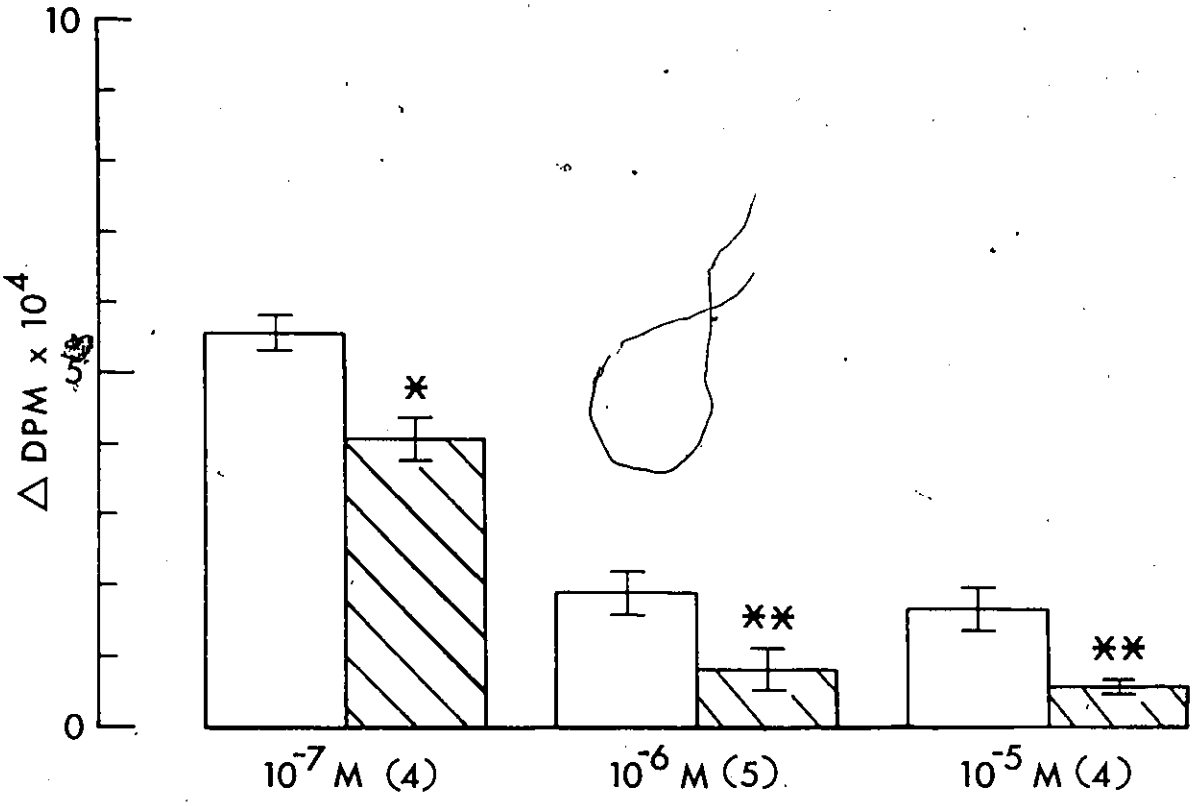

Figure 10. Effect of adrenaline on stimulation-induced efflux of tritium in bovine radial artery. Stimulation was with 600 pulses at 5 Hz given in the absence (open columns) and presence (hatched columns) of adrenaline. Concentration of adrenaline is shown below columns and number of strips in each group in parenthesis. Probability comparisons, by the paired t-test, are between the control and treated values for each concentration, *$p < 0.05$; **$p < 0.01$.

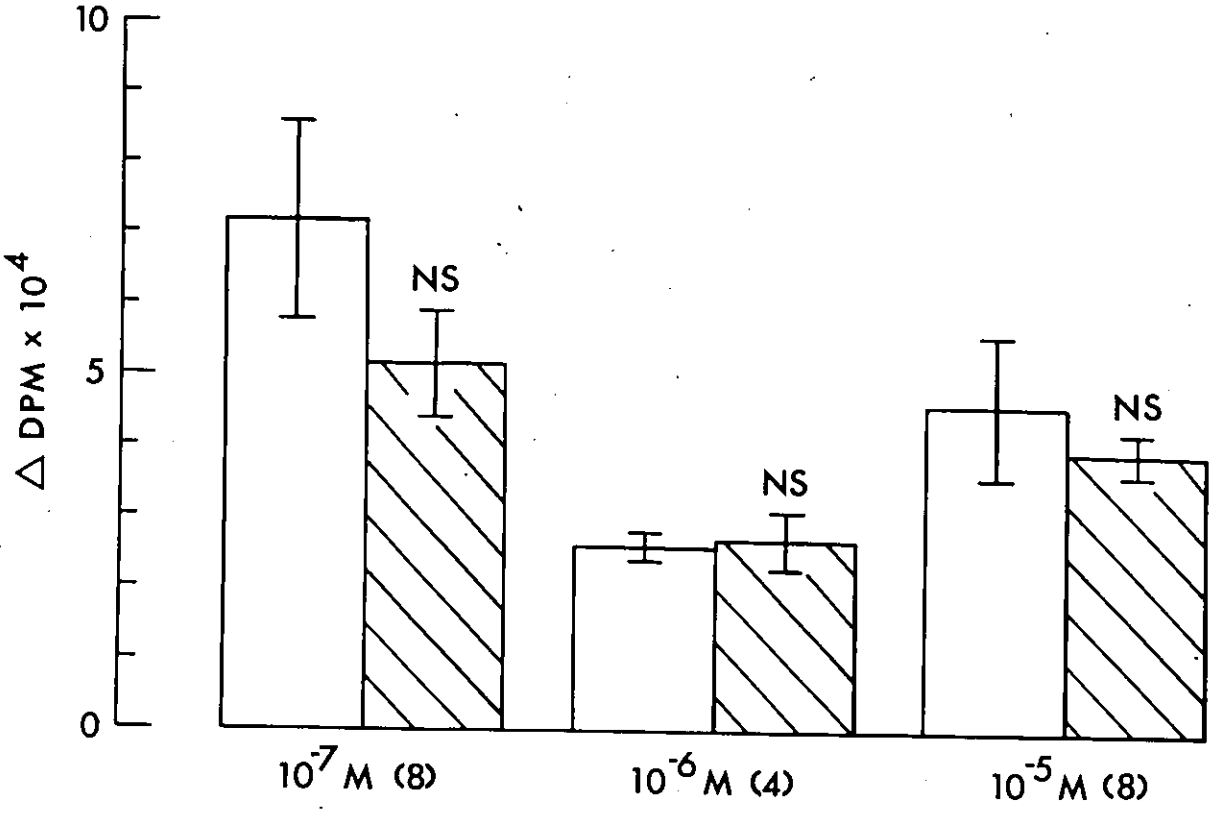

Figure 11. Effect of phenylephrine on stimulation-induced efflux of tritium in bovine radial artery. Stimulation was with 600 pulses at 5 Hz given in the absence (open columns) and presence (hatched columns) of phenylephrine. Concentration of phenylephrine is shown below columns and number of strips in each group in parenthesis. In all three groups efflux values obtained in the presence of phenylephrine did not differ significantly from that obtained in the absence of the drugs (NS), as compared by the paired t-test.

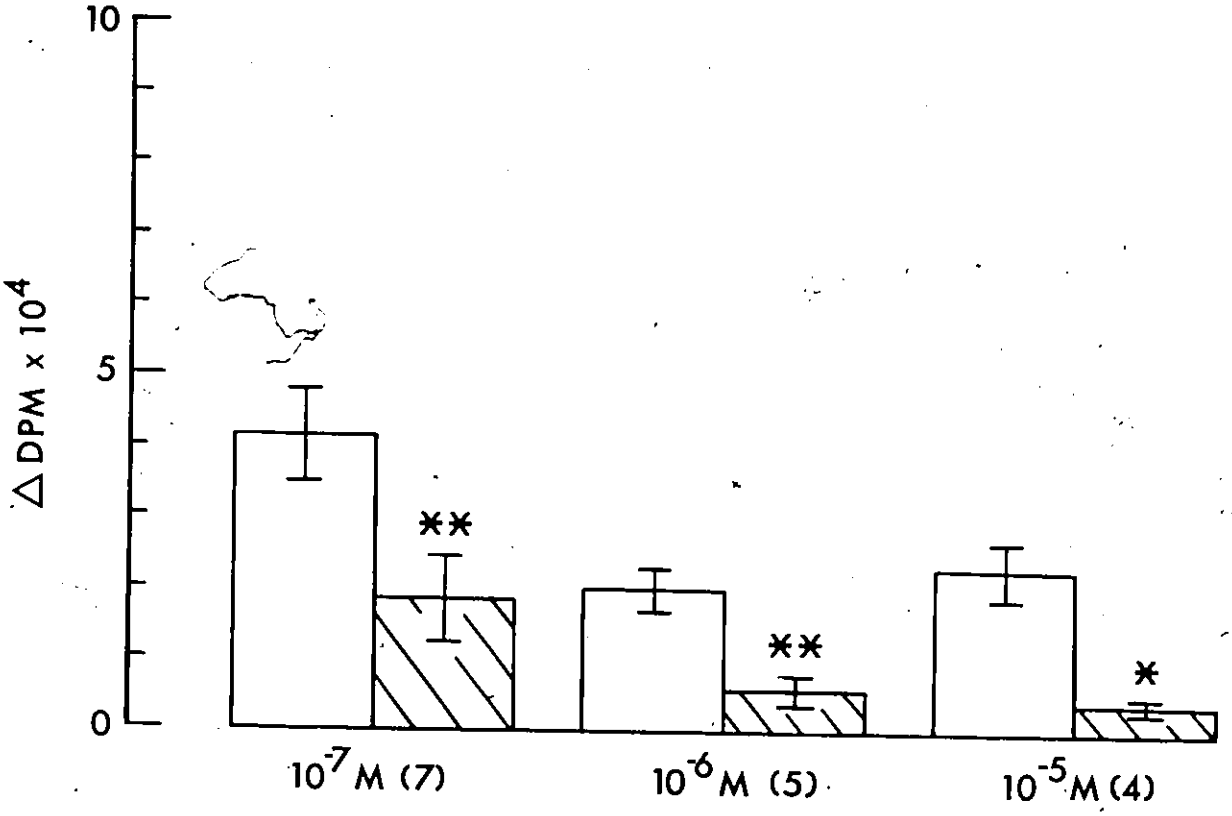

Figure 12. Effect of oxymetazoline on stimulation-induced efflux of tritium in bovine radial artery. Stimulation was with 600 pulses at 5 Hz given in the absence (open columns) and presence (hatched columns) of oxymetazoline. Concentration of oxymetazoline is shown below columns and number of strips in each group in parenthesis. Probability comparisons, by the paired t-test, are between the control and treated values for each concentration, *$p < 0.05$; **$p \leq 0.01$.

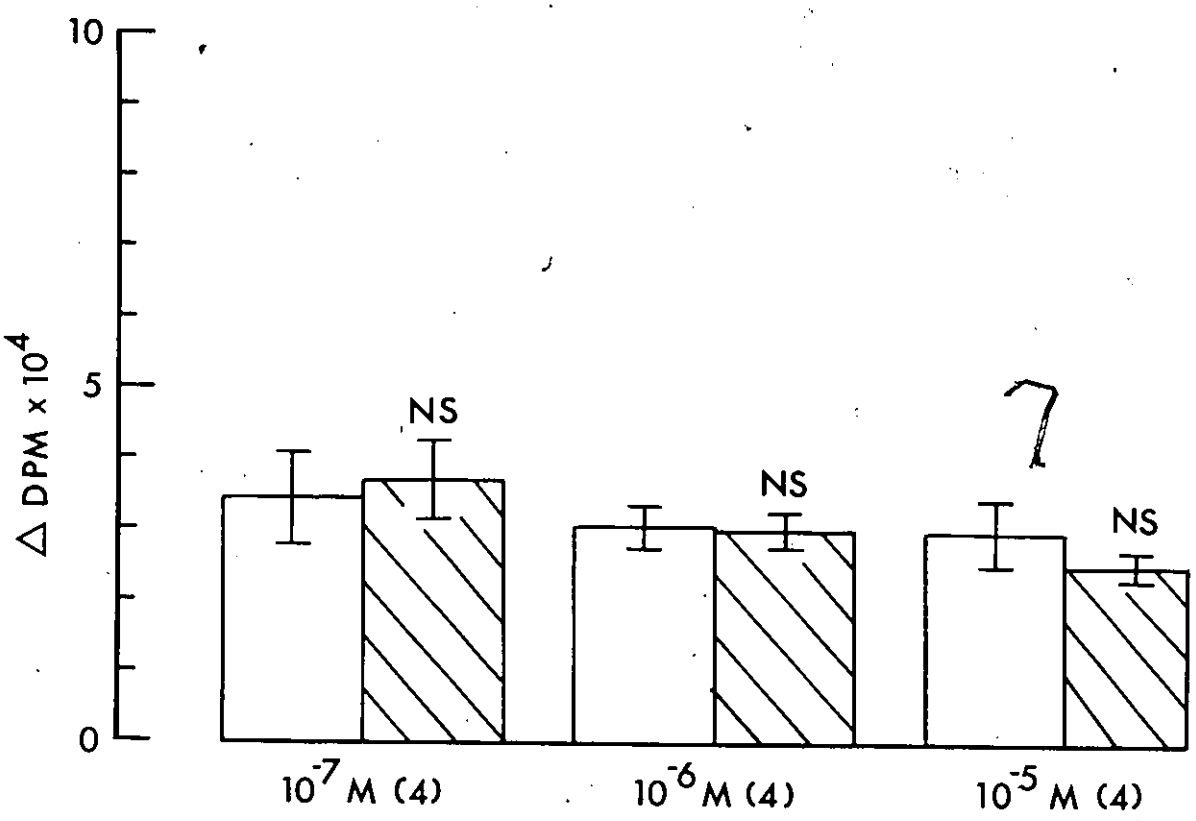

Figure 13. Effect of methoxamine on stimulation-induced efflux of tritium in bovine radial artery. Stimulation was with 600 pulses at 5 Hz given in the absence (open columns) and presence (hatched columns) of methoxamine. Concentration of methoxamine is shown below columns and number of strips in each group in parenthesis. Efflux values obtained in the presence of methoxamine did not differ significantly from that obtained in the absence of the drug in all three groups (NS), as compared by the paired t-test.

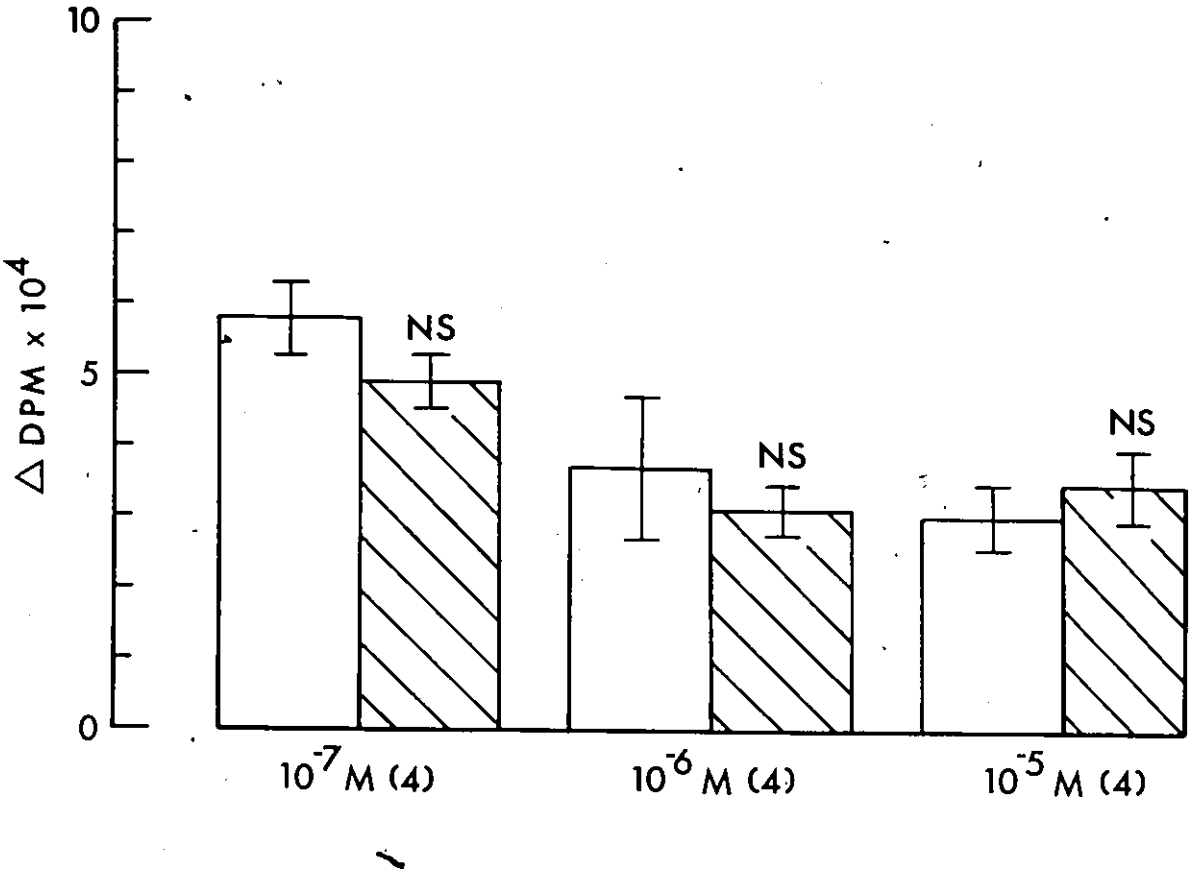

Figure 14. Effect of isoproterenol on stimulation-induced efflux of tritium in bovine radial artery. Stimulation was with 600 pulses at 5 Hz given in the absence (open columns) and presence (hatched columns) of isoproterenol. Concentration of isoproterenol is shown below columns and number of strips in each group in parenthesis. Efflux values obtained in the presence of isoproterenol did not differ significantly from that obtained in the absence of the drug in all three groups (NS), as compared by the paired t-test.

Table 12. THE RATIOS OF TRANSMITTER EFFLUX IN THE FIRST AND SECOND PERIODS OF NERVE STIMULATION IN THE ABSENCE AND PRESENCE OF ADRENERGIC AGONISTS IN BOVINE RADIAL ARTERY.

Agonist[1]	Transmitter efflux ratio 2nd run / 1st run[2]		
	1×10^{-7} M Agonist	1×10^{-6} M Agonist	1×10^{-5} M Agonist
Noradrenaline	0.75±0.09 (8)	0.52±0.06 (8)*	0.55±0.32 (8)
Adrenaline	0.74±0.05 (4)	0.35±0.10 (5)*	0.19±0.03 (4)**
Phenylephrine	0.83±0.12 (8)	1.04±0.14 (4)	1.05±0.13 (8)
Oxymetazoline	0.46±0.09 (7)*	0.28±0.08 (5)**	0.21±0.07 (4)**
Methoxamine	1.13±0.20 (4)	1.02±0.09 (4)	0.88±0.07 (4)
Isoproterenol	0.86±0.08 (4)	0.91±0.19 (4)	1.15±0.09 (4)

[1]Agonists were administered in the interval between first and second periods of stimulation as described in text.

[2]The ratio of second period versus first period stimulation values for 8 untreated control strips, to which all other groups were compared was 0.92 ± 0.09.

Number of values in each group are shown in parentheses.

*$p<0.01$; **$p<0.001$ compared to ratio for control strips.

Table 13. THE RATIOS OF BASAL EFFLUX OF TRITIUM PRIOR TO THE FIRST AND SECOND PERIODS OF STIMULATION IN THE ABSENCE AND PRESENCE OF ADRENERGIC AGONISTS IN BOVINE RADIAL ARTERY.

Agonist[1]	Basal efflux ratio 2nd run / 1st run[2,3]		
	1×10^{-7} M Agonist	1×10^{-6} M Agonist	1×10^{-5} M Agonist
Noradrenaline	0.85±0.06 (8)*	0.86±0.03 (8)***	1.40±0.06 (8)***
Adrenaline	0.88±0.02 (4)***	0.74±0.02 (5)	1.06±0.03 (4)***
Phenylephrine	0.80±0.03 (8)	0.75±0.01 (4)	1.03±0.05 (8)***
Oxymetazoline	0.78±0.02 (7)	0.76±0.02 (5)	0.92±0.01 (4)***
Methoxamine	0.78±0.06 (4)	0.78±0.02 (4)	0.81±0.03 (4)
Isoproterenol	0.87±0.05 (4)**	0.79±0.05 (4)	1.02±0.04 (4)***

[1]Agonists were administered in the interval between first and second periods of stimulation as described in text.

[2]The basal efflux values were determined in samples taken immediately prior to the onset of the first and second periods of stimulation of strips described in Table 12.

[3]The ratio of second period versus first period stimulation values for 8 untreated control strips, to which all other groups were compared was 0.73 ± 0.02.

Number of values in each group are shown in parentheses.

*$p<0.05$; **$p<0.01$; ***$p<0.001$ compared to ratio for control strips.

3. Lack of effect of oxymetazoline on stimulation-induced efflux in bovine renal artery

Of all six agonists examined, only oxymetazoline exhibited a clear-cut and consistent inhibitory effect on stimulation-induced efflux in the radial artery. To determine if this finding could be applied to other vascular tissue, experiments were performed to examine the effect of this agonist on efflux in the bovine renal artery. After initial stimulation with 300 pulses at 5 and 15 Hz, renal artery strips were exposed to oxymetazoline (1×10^{-6} M) for 20 minutes and then were re-stimulated in the presence of the agonist. In contrast to what was observed with the radial artery, where oxymetazoline at this concentration inhibited the stimulation-induced efflux by 72%, this agent produced no significant effect on efflux at either stimulation frequency in the renal artery (Table 14).

E. Effects of noradrenaline and phenoxybenzamine on stimulation-induced efflux of tritium in vascular preparations of dog

To extend the observations made with the bovine artery preparations, the effects of noradrenaline and phenoxybenzamine on stimulation-induced efflux were examined in a variety of vascular preparations in dog. Strips of canine abdominal aorta, carotid, femoral and renal arteries were superfused with Krebs solution containing cocaine (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M). To minimize the number of dogs required, a modified protocol which combined the study of effects of both the agonist and the antagonist on a single preparation was employed. After the initial stimulation series with 200 pulses at 1 and 5 Hz, preparations were exposed

Table 14. THE RATIOS OF STIMULATION-INDUCED TRANSMITTER EFFLUX IN THE PRESENCE AND ABSENCE OF OXYMETAZOLINE IN BOVINE RENAL ARTERY.

Treatment group (N)	Stimulation frequency (Hz)	Transmitter efflux dpm (x 10^4) 1st run	Transmitter efflux dpm (x 10^4) 2nd run	Efflux ratio 2nd run / 1st run
Control (4)	5	5.58±0.63	4.41±0.47	0.80±0.07
Oxymetazoline[1] (4)	5	4.68±0.56	4.45±0.87	0.93±0.11 NS
Control (4)	15	3.84±0.34	3.20±0.08	0.85±0.06
Oxymetazoline[1] (4)	15	3.68±0.26	3.37±0.53	0.90±0.10 NS

[1]Strips were exposed to oxymetazoline (1 x 10^{-6} M), after the first period of stimulation, followed 20 minutes later, without washout, by the second period of stimulation.

NS, no significant difference between efflux ratio of control group and the corresponding group treated with oxymetazoline in the interval between stimulation periods with 300 pulses ($p<0.5>0.4$).

to noradrenaline (3×10^{-6} M) followed 20 minutes later by another series of stimulations in the presence of the agonist. On completion of the stimulation, noradrenaline was washed out for 20 minutes and phenoxybenzamine (3×10^{-6} M) was introduced to the superfusate for an additional 30 minutes. The haloalkylamine was subsequently washed out and, 20 minutes later, a third stimulation series was performed. Matching control strips cut from the same artery received the same schedule of stimulation but were superfused with Krebs solution free of adrenergic agents. These control strips served to detect any time-related or spontaneous fluctuation in stimulation-induced efflux.

All four types of tissue responded to transmural stimulation at either 1 or 5 Hz with substantial elevations in transmitter efflux above basal levels (Table 15). Overflow of tritium at both frequencies in the femoral artery was materially greater than that in the other preparations. In the aortic, carotid and femoral arteries, efflux of tritium at the low frequency was not significantly different from that at the high frequency, a pattern similar to that found in the bovine renal artery stimulated with 300 pulses (section IV, A.1.). In the canine renal artery, however, efflux at 1 Hz is slightly but significantly higher than that at 5 Hz ($p < 0.05$).

Table 15 summarizes the effect of noradrenaline on stimulation-induced efflux in canine artery preparations. Noradrenaline significantly inhibited efflux at both 1 and 5 Hz in all preparations, as assessed by a comparison of the efflux ratios. However, the noradrenaline inhibition on overflow at the low frequency was not significantly different

Table 15. EFFECT OF NORADRENALINE (NA) ON STIMULATION-INDUCED EFFLUX OF TRITIUM IN VARIOUS VASCULAR STRIPS OF DOG.

Type of tissue	Treatment group [1]	No. of values	Stimulation frequency (Hz)	Transmitter efflux dpm (x 10^3)		Efflux ratio 2nd run/1st run	Present inhibition of efflux[2]
				1st run	2nd run		
Abdominal aorta	Control	4	1	8.40±2.85	7.13±2.01	0.82±0.02	
	NA	4	1	7.81±1.81	2.31±0.71	0.30±0.06**	63.5±6.7
	Control	4	5	9.34±2.29	7.11±2.30	0.73±0.06	
	NA	4	5	6.54±0.75	1.99±0.62	0.29±0.06*	59.5±9.4
Carotid artery	Control	4	1	8.45±0.89	9.94±0.67	1.19±0.05	
	NA	4	1	7.53±0.69	2.24±0.41	0.31±0.07**	73.6±6.0
	Control	4	5	10.43±1.11	12.86±1.86	1.23±0.09	
	NA	4	5	9.96±1.67	1.87±0.37	0.19±0.01**	84.8±1.2
Femoral artery	Control	4	1	20.52±1.74	20.11±0.96	0.99±0.05	
	NA	4	1	23.92±5.53	3.69±0.93	0.17±0.04**	83.2±3.6
	Control	4	5	20.45±1.46	16.99±1.28	0.83±0.03	
	NA	4	5	24.68±6.04	6.45±1.31	0.27±0.03**	67.6±2.2#
Renal artery	Control	4	1	10.11±2.77	8.51±2.58	0.91±0.16	
	NA	4	1	8.07±1.49	1.22±0.41	0.16±0.04*	78.7±8.9
	Control	4	5	6.10±1.60	4.89±1.71	0.78±0.10	
	NA	4	5	5.29±0.55	1.38±0.68	0.25±0.11*	69.1±11.6

Table 15. (Continued)

[1]Noradrenaline (3×10^{-6} M), when given, was administered in the interval between runs, as described in text.

[2]Obtained by comparison of individually determined ratios for control and treated strips.

*$p < 0.01$; **$p < 0.001$ compared to ratio for corresponding control strips.

#$p < 0.01$ compared to percent inhibition obtained at 1 Hz in femoral artery.

from that at the high frequency in the aortic, carotid and renal arteries, as determined by a comparison of the percentage of inhibition which accounted for spontaneous variation of efflux with time. In the femoral artery, noradrenaline had a significantly greater effect at the low than at the high frequency.

Ratios of basal efflux (second stimulation period versus first stimulation period) in control strips of abdominal aorta, carotid, femoral and renal arteries were 0.78±0.04, 0.85±0.02, 0.79±0.05 and 0.80±0.05 respectively. Corresponding values for strips treated with noradrenaline were 0.78±0.07, 1.42±0.04, 1.04±0.08 and 0.93±0.08 respectively. The basal efflux was significantly increased by noradrenaline in the carotid ($p < 0.001$) and femoral arteries ($p < 0.05$).

Table 16 shows the effect of phenoxybenzamine on stimulation-induced efflux in canine artery preparations. In accord with many other observations, phenoxybenzamine significantly enhanced efflux of tritium with 200 pulses in all preparations. In the abdominal aorta, efflux at 1 Hz did not quite attain statistical significance ($p < 0.1 > 0.05$ when efflux ratio of the treated group is compared to that of control), due to a large variation between individual values. When the effect of phenoxybenzamine was assessed by a comparison of the percentage of control efflux ratios, which is more appropriate for the present experiment as efflux in control strips showed a considerable extent of fluctuation (e.g. abdominal aorta and carotid artery), there was no significant difference in the phenoxybenzamine-induced enhancement of efflux at 1 and at 5 Hz in the aorta, femoral and renal arteries. In the carotid artery,

Table 16. EFFECT OF PHENOXYBENZAMINE (POB) ON STIMULATION-INDUCED EFFLUX OF TRITIUM IN VARIOUS VASCULAR STRIPS OF DOG.

Type of tissue	Treatment group[1]	No. of values	Stimulation frequency (Hz)	Transmitter efflux dpm (x 10^3)		Efflux ratio 3rd run/1st run	Percent increase of efflux[2]
				1st run	3rd run		
Abdominal aorta	Control	4	1	8.40±2.85	5.63±2.43	0.63±0.06	
	POB	4	1	7.81±1.81	12.52±3.27	1.88±0.65	321±120
	Control	4	5	9.34±2.29	5.38±1.59	0.59±0.09	
	POB	4	5	6.54±0.75	8.53±1.54	1.28±0.09**	245±60
Carotid artery	Control	4	1	8.45±0.89	13.62±1.57	1.70±0.33	
	POB	4	1	7.53±0.69	52.84±7.92	7.07±1.09**	427±38
	Control	4	5	10.43±1.11	14.34±1.83	1.40±0.16	
	POB	4	5	9.96±1.67	39.60±5.80	4.02±0.16***	295±21#
Femoral artery	Control	4	1	20.52±1.74	21.23±1.60	1.04±0.05	
	POB	4	1	23.92±5.53	43.76±6.02	1.96±0.23**	188±19
	Control	4	5	20.45±1.46	17.70±1.29	0.87±0.04	
	POB	4	5	24.68±6.04	38.33±2.82	1.75±0.29*	199±27
Renal artery	Control	4	1	10.11±2.77	9.09±3.30	0.88±0.13	
	POB	4	1	8.07±1.49	18.36±2.34	2.39±0.26**	277±25
	Control	4	5	6.10±1.60	6.60±2.28	1.01±0.09	
	POB	4	5	5.29±0.55	9.91±0.26	1.95±0.25**	203±44

Table 16. (Continued)

[1]Phenoxybenzamine (3×10^{-6} M), when given, was administered in the interval between runs, as described in text.

[2]Obtained by comparison of individually determined ratios for control and treated strips.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to ratio for corresponding control strips.

#$p < 0.02$ compared to percent increase obtained at 1 Hz in carotid artery.

the effect of phenoxybenzamine on efflux was significantly greater at the low than at the high frequency, confirming the finding made in the bovine renal artery (section IV, A.).

Ratios of basal efflux (third stimulation period versus first stimulation period) in control strips of abdominal aorta, carotid, femoral and renal arteries were 0.49±0.03, 0.58±0.02, 0.61±0.06 and 0.71±0.06 respectively. Corresponding values in strips treated with phenoxybenzamine were 0.59±0.06, 0.95±0.09, 0.82±0.07 and 0.82±0.06 respectively. The basal efflux was significantly elevated by phenoxybenzamine in the carotid ($p < 0.01$) and the femoral arteries ($p < 0.05$).

F. Combined effects of noradrenaline and phenoxybenzamine in guinea-pig aorta

In previous sections the effects of phenoxybenzamine and noradrenaline on stimulation-induced efflux were examined independently. In the present experiments with guinea-pig aorta, the effect of combined administration of noradrenaline and phenoxybenzamine on efflux was investigated to examine if the efflux pattern would adjust accordingly due to different extents of presynaptic receptor blockade. Guinea-pig aortic strips were superfused with Krebs solution containing cocaine (8.8×10^{-6} M) and normetanephrine (1×10^{-5} M) and were stimulated with 200 pulses at 1 and 5 Hz immediately after a 90-minute equilibration period. On completion of the initial stimulation, phenoxybenzamine at a selected concentration was administered for 30 minutes and then washed out for 20 minutes before the onset of a second stimulation set. One group of strips was further treated with noradrenaline (3×10^{-6} M) for

20 minutes and the strips were re-stimulated in the presence of the agonist. In a separate set of strips noradrenaline (3×10^{-6} M) was administered for 20 minutes between the first and second stimulations and the effect on efflux was examined and compared to that of a matching control group.

As shown in Table 17, efflux of tritium in four control strips exhibited no material decline between the two stimulations. Noradrenaline inhibited, whereas phenoxybenzamine enhanced dose-dependently, the stimulation-induced efflux at either 1 or 5 Hz. The inhibitory effect of noradrenaline on efflux at 1 Hz was reduced in strips treated with the antagonist at the moderate and the high concentration, as assessed by the ratio of efflux ratios (agonist-treated group versus untreated group). Nevertheless, this effect was not significantly attenuated by phenoxybenzamine at the low concentration (3×10^{-8} M), although efflux was significantly enhanced by the antagonist at this concentration ($p < 0.05$ when efflux ratio in strips treated with phenoxybenzamine was compared with that of the corresponding control strips). Efflux of tritium at 5 Hz was significantly reduced by noradrenaline in 4 control preparations, as determined by the efflux ratios of the treated and control groups. Such inhibitory effect of noradrenaline on efflux was not observed in strips pretreated with phenoxybenzamine at any of the test concentration.

In a separate set of experiments it was confirmed that phenoxybenzamine at 3×10^{-8} M effectively blocked the mechanical responses to nerve stimulation at 1 Hz and to exogenous noradrenaline in the guinea-pig

Table 17. EFFECTS OF NORADRENALINE (3×10^{-6} M, NA) AND/OR PHENOXYBENZAMINE (POB) ON THE STIMULATION-INDUCED EFFLUX OF TRITIUM IN GUINEA-PIG AORTA.

Treatment group	Concentration of POB (M)	No. of values	Stimulation frequency (Hz)	Transmitter efflux dpm ($\times 10^3$) 1st run	2nd run	Efflux ratio 2nd run/1st run	Ratio of ratios [5]
Control[1]	-	4	1	46.85±4.17	45.17±5.32	0.96±0.05	
NA[2]	-	4	1	28.09±1.86	8.02±1.21	0.29±0.04**	a) 0.30±0.03
Control[1]	-	4	5	73.08±6.10	66.27±5.29	0.92±0.08	
NA[2]	-	4	5	45.68±3.53	29.31±3.02	0.64±0.04*	b) 0.71±0.06
POB[3]	3×10^{-8}	6	1	20.30±1.52	30.60±3.30	1.49±0.10	
POB + NA[4]	3×10^{-8}	6	1	20.37±2.54	12.40±1.80	0.62±0.07**	c) 0.43±0.07
POB[3]	3×10^{-8}	6	5	32.30±3.92	47.66±6.34	1.49±0.14	
POB + NA[4]	3×10^{-8}	6	5	27.96±4.79	33.40±4.30	1.33±0.24	d) 0.91±0.16
POB[3]	3×10^{-7}	8	1	20.31±2.00	45.69±6.36	2.27±0.20	
POB + NA[4]	3×10^{-7}	8	1	18.48±1.61	24.72±3.65	1.45±0.27*	e) 0.62±0.09#
POB[3]	3×10^{-7}	8	5	31.75±2.72	69.48±7.70	2.18±0.15	
POB + NA[4]	3×10^{-7}	8	5	27.73±2.00	56.72±8.41	2.08±0.27	f) 0.95±0.11
POB[3]	3×10^{-6}	4	1	21.10±0.81	71.63±17.21	3.33±0.71	
POB + NA[4]	3×10^{-6}	4	1	22.96±3.80	111.72±38.60	4.49±1.12	g) 1.33±0.17#
POB[3]	3×10^{-6}	4	5	31.93±1.11	87.96±7.59	2.77±0.28	
POB + NA[4]	3×10^{-6}	4	5	35.24±4.71	122.91±12.51	3.55±0.16*	h) 1.31±0.12##

Table 17. (Continued)

[1]Untreated strips were stimulated with 200 pulses at either test frequency and, 50 minutes later, followed by a second period of identical stimulations.

[2]Strips were exposed to noradrenaline for 20 minutes in the interval between stimulation periods.

[3]Strips were exposed to phenoxybenzamine for 30 minutes in the interval between stimulation periods.

[4]Strips were exposed to phenoxybenzamine for 30 minutes after the first stimulation period and, after its washout, to noradrenaline for 20 minutes, followed by a second stimulation period.

[5]Obtained by comparison of individually determined ratios for groups with and without noradrenaline treatment, i.e., NA/control; POB + NA/POB.

*$p<0.05$; **$p<0.001$ compared to ratio for corresponding group without noradrenaline treatment.

#$p<0.05$ compared to the value of group a); ##$p<0.05$ compared to the value of group b).

aorta. Peak tension in response to stimulation at 1 Hz for 1 minute and to noradrenaline at 3×10^{-7} M in four strips averaged 150 mg and 250 mg respectively. After treatment of two strips with phenoxybenzamine at 3×10^{-8} M, the mechanical responses were completely eliminated, whereas in two matching control strips the contractile responses to nerve stimulation and to noradrenaline increased to 200 mg and 270 mg respectively.

G. Effects of dopamine in bovine renal artery

The effects of dopamine on stimulation-induced efflux and mechanical responses was investigated in a renal artery preparation of cattle. The agonist is known to inhibit efflux of ^{3}H-transmitter via an action on a discrete neuronal locus different from the presynaptic alpha-receptor site (Rand, McCulloch and Story, 1975; Langer, 1977). The frequency-related pattern of dopamine inhibition of transmitter efflux was compared to that induced by noradrenaline. The aim of these experiments was to determine if a specific pattern of inhibition of efflux by exogenous amine was a reliable index reflecting the consequences of adjustment of a functional autoinhibitory loop in response to the presence of a constant amount of the exogenous amine in the synaptic cleft. Experimental procedures and conditions are identical to those described previously in section IV, B. for noradrenaline.

1. Stimulation-induced efflux of ^{3}H-transmitter

Renal artery strips responded to transmural stimulation with 300 pulses at 1, 2, 5 and 15 Hz with efflux of ^{3}H-transmitter elevated

above basal levels (Tables 18 and 19). Further, as had been previously observed, the total efflux elicited by the first stimulation series at the lowest frequency did not differ significantly from that of the highest frequency in the twenty-eight strips examined ($p < 0.2 > 0.1$). Dopamine at 3×10^{-7} M and 3×10^{-6} M significantly inhibited the efflux at 1, 2 and 5 Hz but had no statistically detectable effect on efflux at 15 Hz, as assessed by a comparison of the efflux ratios. The effectiveness of dopamine (at either concentration) decreased with increasing frequency, as demonstrated by the correlation coefficient between the mean values for percentage of inhibition of transmitter efflux and the length of the stimulus interval. The correlation coefficients for groups treated with dopamine at 3×10^{-7} M and 3×10^{-6} M were 0.904 with a $p < 0.02$ and 0.957 with a $p < 0.01$ respectively.

2. Basal efflux

Basal efflux of tritium from eight control preparations was $6.12 \pm 0.27 \times 10^4$ dpm and $4.04 \pm 0.20 \times 10^4$ dpm for the first and second stimulation sets respectively. In another eight preparations treated with the low concentration of dopamine (3×10^{-7} M) in the interval between stimulations, the decline of basal efflux exhibited no significant difference from that of the control tissues as determined by the ratios of basal efflux: the ratios were 0.66 ± 0.01 and 0.62 ± 0.02 (N=32 for each group) for the control and the treated groups respectively. On the other hand, dopamine at 3×10^{-6} M materially elevated the basal efflux: the efflux ratio in six control preparations was 0.68 ± 0.02 whereas that in the matching treated preparations was 0.91 ± 0.04 ($p < 0.001$, N=24 for each group).

Table 18. EFFECT OF DOPAMINE (3×10^{-7} M) ON STIMULATION-INDUCED EFFLUX OF ^{3}H-NORADRENALINE IN BOVINE RENAL ARTERY.

Treatment group [1]	Stimulation frequency (Hz)	No. of values	Transmitter efflux dpm ($\times 10^4$) 1st run	Transmitter efflux dpm ($\times 10^4$) 2nd run	Efflux ratio 2nd run/1st run	Percent inhibition of efflux[2]
Control	1	8	4.96±0.81	4.05±0.88	0.79±0.06	
Dopamine	1	8	4.16±0.86	1.64±0.44	0.42±0.06**	42.60±10.00
Control	2	8	5.62±1.11	4.22±0.86	0.76±0.05	
Dopamine	2	8	5.06±0.81	2.46±0.52	0.45±0.04**	39.30± 7.00
Control	5	8	4.53±0.72	3.38±0.51	0.77±0.04	
Dopamine	5	8	4.42±0.79	2.56±0.47	0.58±0.03*	22.60± 5.70
Control	15	8	3.56±0.59	2.84±0.50	0.78±0.03	
Dopamine	15	8	3.53±0.47	2.44±0.29	0.71±0.03	12.60± 4.00

[1]Dopamine, when given, was administered for 20 minutes in the interval between the first and second stimulation periods with 300 pulses and the vessels were re-stimulated in its presence.

[2]Obtained by comparison of individually determined ratios for control and treated strips taken from the same renal artery.

*$p<0.01$; **$p<0.001$ compared to ratio for corresponding control group. The correlation coefficient (r) between the length of the stimulus interval and the mean values for percent inhibition of transmitter efflux was determined according to the method of Goldstein (1964). The correlation coefficient in the presence of dopamine (3×10^{-7} M) was 0.904 with a $p<0.02$.

Table 19. EFFECT OF DOPAMINE (3×10^{-6} M) ON STIMULATION-INDUCED EFFLUX OF ^{3}H-NORADRENALINE IN BOVINE RENAL ARTERY.

Treatment group [1]	Stimulation frequency (Hz)	No. of values	Transmitter efflux dpm ($\times 10^4$) 1st run	Transmitter efflux dpm ($\times 10^4$) 2nd run	Efflux ratio 2nd run/1st run	Percent inhibition of efflux[2]
Control	1	6	4.45±1.05	3.68±0.93	0.83±0.09	
Dopamine	1	6	3.78±0.85	0.95±0.46	0.19±0.07**	77.90±8.20
Control	2	6	5.21±1.20	4.30±0.83	0.87±0.08	
Dopamine	2	6	5.32±0.68	1.78±0.51	0.30±0.06**	61.30±9.30
Control	5	6	4.02±1.01	2.99±0.75	0.76±0.06	
Dopamine	5	6	4.05±0.67	2.03±0.40	0.49±0.02*	33.20±5.50
Control	15	6	2.92±0.73	1.85±0.55	0.60±0.05	
Dopamine	15	6	3.02±0.60	1.70±0.37	0.59±0.10	15.80±9.50

[1]Dopamine, when given, was administered for 20 minutes in the interval between the first and second stimulation periods with 300 pulses and the vessels were re-stimulated in its presence.

[2]Obtained by comparison of individually determined ratios for control and treated strips taken from the same renal artery.

*$p<0.01$; **$p<0.001$ compared to ratio for corresponding control group. The correlation coefficient (r) between the length of the stimulus interval and the mean values for percent inhibition of transmitter efflux was determined according to the method of Goldstein (1964). The correlation coefficient in the presence of dopamine (3×10^{-6} M) was 0.957 with a $p<0.01$.

3. Specific blockade by pimozide of dopamine inhibition of transmitter efflux.

The selective dopamine antagonist pimozide (Rand, McCulloch and Story, 1975) was employed to confirm that the dopamine inhibition of transmitter overflow was due to an action mediated by some dopamine-sensitive neuronal loci but not via the presynaptic alpha-adrenergic sites. Renal artery strips were stimulated 4 times with 300 pulses at 1 Hz (the stimulation periods were designated consecutively as S_1, S_2, S_3 and S_4). Pimozide at 1×10^{-8} M was administered to four strips for 20 minutes followed by additional exposure to dopamine at 3×10^{-6} M for another 20 minutes in the interval between S_1 and S_2. After completion of S_2 (in the presence of both agents), a 20-minute wash-out period with drug-free Krebs solution was allowed before the strips were exposed to a higher concentration (2×10^{-7} M) of pimozide for 20 minutes followed by S_3. During the interval between S_3 and S_4, strips were exposed to dopamine (3×10^{-6} M) in addition to the previously administered pimozide for 20 minutes. In other four strips the same schedule of transmural stimulation was followed but only the effect of dopamine was examined. Dopamine at the same concentration and with the same exposure time as that in the pimozide-dopamine treated strips was administered in the intervals between S_1 and S_2; and again between S_3 and S_4. Agonist and antagonist in both groups of strips were maintained throughout the relevant stimulation periods.

The effect of pimozide at the two selected concentrations on dopamine inhibition of efflux was assessed by the transmitter overflow

ratios of i) S_2 versus S_1, and ii) S_4 versus S_3. As shown in Figure 15, dopamine substantially reduced transmitter efflux of S_2 and S_4 and this effect was significantly blocked by prior administration of pimozide at either test concentration.

Another specific dopamine antagonist, namely metoclopromide, was also shown to have an inhibitory effect on the dopamine-induced inhibition of transmitter efflux. In six strips treated with dopamine (3×10^{-6} M) in the interval between two stimulations with 300 pulses at 1 Hz, efflux of the second stimulation was reduced by nearly 80%. In six matching strips treated with metoclopromide (1×10^{-8} M) prior to the administration of dopamine, the inhibitory effect of dopamine was significantly reduced, as efflux of the second stimulation was not reduced as much as that in the corresponding control strips (Table 21).

In separate experiments the effect of pimozide on noradrenaline-induced inhibition of efflux was examined. Strips were stimulated 3 times with 300 pulses at 1 Hz. After the first stimulation period one group of strips was treated with pimozide (1×10^{-8} M) for 20 minutes followed by the additional exposure to noradrenaline (3×10^{-6} M), and 20 minutes later a second stimulation set was given. In the interval between the second and the third stimulation periods the drugs administered previously were washed out for 20 minutes before a higher concentration of pimozide (2×10^{-7} M) and noradrenaline (3×10^{-6} M) were re-administered in the identical sequence and for the identical exposure time. Both the second and the third stimulation periods were performed in the presence of pimozide and noradrenaline. In a matching group of strips noradrenaline (3×10^{-6} M)

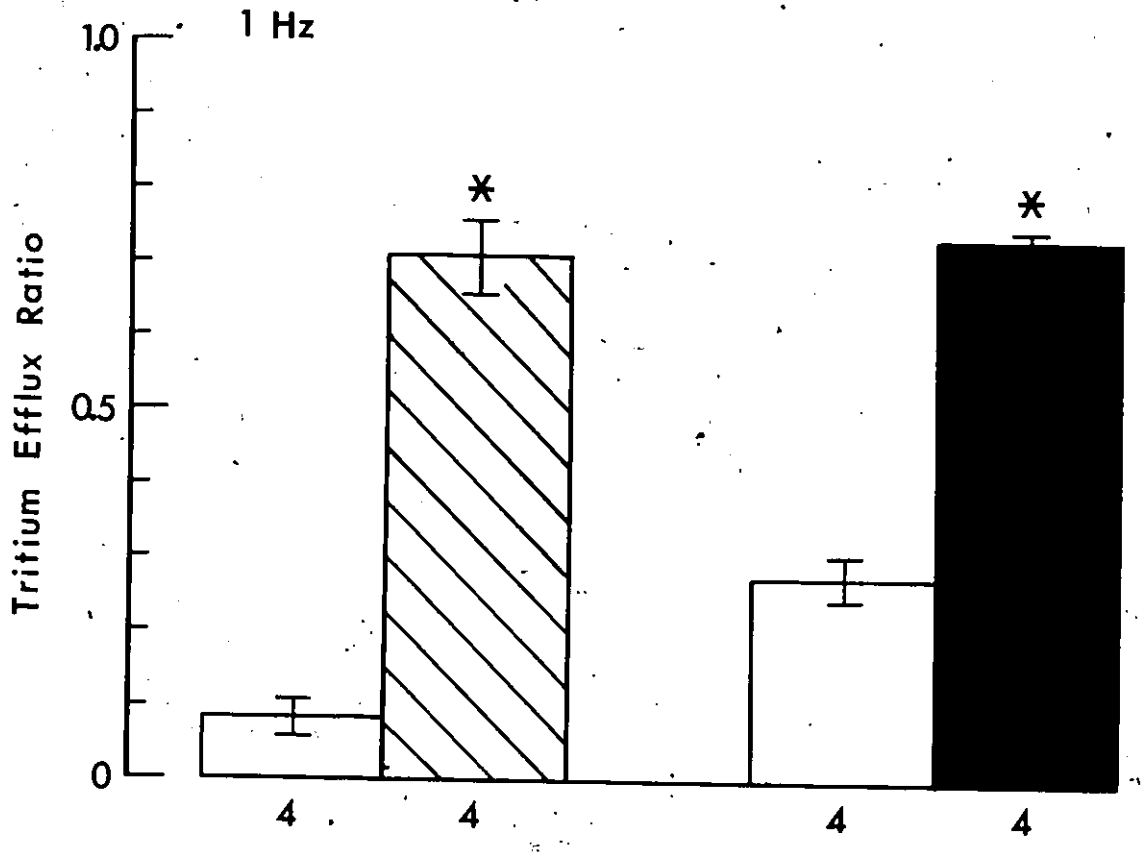

Figure 15. Effect of pimozide on the dopamine inhibition of stimulation-induced efflux of tritium in bovine renal artery. Tritium efflux ratio with 300 pulses at 1 Hz is shown in a group of strips treated with dopamine at 3×10^{-6} M (open columns, left, S_2/S_1; right, S_4/S_3) and in another group of matching strips treated initially with pimozide at 1×10^{-8} M and dopamine at 3×10^{-6} M (hatched column, S_2/S_1) and, after wash out of both drugs, again with pimozide at 2×10^{-7} M and dopamine at 3×10^{-6} M (filled column, S_4/S_3). The protocol in detail is described in the text (section IV, G.3). Number of values is shown below columns. Probability comparison, using unpaired t-test, was between control and treated groups, *$p < 0.001$.

was administered twice for 20 minutes in the interval between the three stimulation periods.

Results were assessed by comparison of transmitter efflux ratios of i) the second versus the first stimulation period; and ii) the third versus the first stimulation period. As shown in Figure 16, noradrenaline induced a clear inhibition of stimulation-induced efflux in both the second and the third stimulation periods. This effect was not reduced in strips treated with pimozide at either test concentration prior to the administration of noradrenaline.

4. Effects of dopamine antagonists on stimulation-induced efflux

Confirmation that under the present experimental conditions dopamine was not released in significant amount as to alter transmitter overflow during nerve stimulation was obtained in experiments with pimozide and metoclopromide. The effects of pimozide at three concentrations (1×10^{-8} M, 2×10^{-7} M and 1×10^{-6} M) and metoclopromide at 1×10^{-8} M on efflux of tritium elicited by stimulation with 300 pulses at 1 and 15 Hz were examined. The antagonists were administered for 20 minutes in the interval between two stimulation series and the second stimulation was performed in the presence of the relevant antagonist. As shown in Tables 20 and 21, efflux of tritium at 1 or at 15 Hz in renal artery was not enhanced by treatment with either pimozide or metoclopromide at any of the test concentrations, although as demonstrated previously these antagonists at these concentrations significantly blocked the effect of exogenous dopamine on efflux.

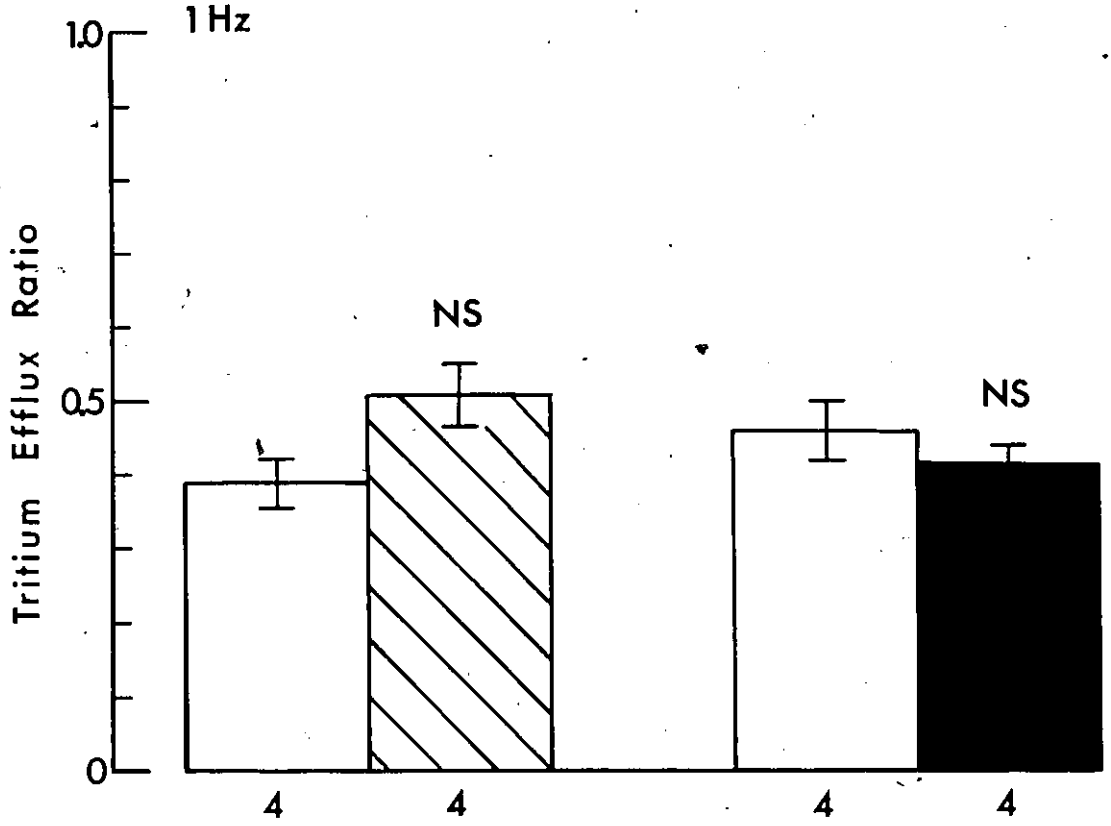

Figure 16. Effect of pimozide on the noradrenaline inhibition of stimulation-induced efflux of tritium in bovine renal artery. Tritium efflux ratio with 300 pulses at 1 Hz is shown in a group of strips treated with noradrenaline at 3×10^{-6} M (open columns, left, S_2/S_1; right, S_3/S_1) and in another group of matching strips treated initially with pimozide at 1×10^{-8} M and noradrenaline at 3×10^{-6} M (hatched column, S_2/S_1) and, after wash out of both drugs, again with pimozide at 2×10^{-7} M and noradrenaline at 3×10^{-6} M (filled column, S_3/S_1). The protocol in detail is described in the text (section IV, G.3). Number of values is shown below columns and probability value of the control groups did not differ significantly from that of the treated group (NS).

Table 20. THE EFFECT OF PIMOZIDE ON STIMULATION-INDUCED EFFLUX OF ^{3}H-NORADRENALINE IN BOVINE RENAL ARTERY.

Treatment group [1]	Concentration (M)	Stimulation frequency (Hz)	No. of values	Transmitter efflux dpm (x 10^4)		Efflux ratio 2nd run/1st run
				1st run	2nd run	
Control	-	1	4	6.10±1.73	5.27±1.38	0.97±0.15
Pimozide	1×10^{-8}	1	4	7.34±1.21	6.76±0.74	0.96±0.08
Control	-	15	4	5.19±1.00	3.56±0.47	0.74±0.09
Pimozide	1×10^{-8}	15	4	5.27±0.34	4.68±0.15	0.90±0.07
Control	-	1	4	3.52±0.62	3.40±0.88	0.96±0.14
Pimozide	2×10^{-7}	1	4	1.83±0.49	1.63±0.35	0.92±0.10
Control	-	15	4	4.27±0.97	2.94±0.57	0.70±0.02
Pimozide	2×10^{-7}	15	4	1.71±0.30	1.31±0.25	0.76±0.04
Control	-	1	6	3.62±0.62	2.75±0.19	0.83±0.10
Pimozide	1×10^{-6}	1	6	4.66±1.72	2.97±0.70	1.01±0.34
Control	-	15	6	2.76±0.16	2.50±0.32	0.93±0.14
Pimozide	1×10^{-6}	15	6	3.28±0.44	2.32±0.38	0.70±0.09

[1]Pimozide, when given, was administered for 20 minutes in the interval between the first and second stimulation periods with 300 pulses and the vessels were re-stimulated in its presence. There were no statistically significant differences between any of the treated groups and their corresponding controls.

Table 21. EFFECT OF METOCLOPROMIDE (1×10^{-8}M) ON STIMULATION-INDUCED EFFLUX OF ^{3}H-NORADRENALINE IN THE PRESENCE AND ABSENCE OF DOPAMINE IN BOVINE RENAL ARTERY.

Treatment group	Stimulation frequency (Hz)	No. of values	Transmitter efflux dpm ($\times 10^{4}$) 1st run	Transmitter efflux dpm ($\times 10^{4}$) 2nd run	Efflux ratio 2nd run / 1st run
Control	1	4	3.10±0.58	2.60±0.38	0.87±0.09
Metoclopromide[1]	1	4	2.87±0.26	2.54±0.54	0.86±0.10
Control	15	4	2.30±0.16	1.98±0.12	0.87±0.03
Metoclopromide[1]	15	4	2.24±0.22	1.91±0.21	0.85±0.05
Dopamine[2]	1	6	4.29±0.47	0.97±0.31	0.22±0.07
Metoclopromide plus Dopamine[3]	1	6	5.46±1.34	3.79±1.31	0.63±0.13*

[1]Strips were exposed to metoclopromide after the first period of stimulation with 300 pulses, followed 20 minutes later, without washout, by the second period of stimulation.

[2]Strips were exposed to dopamine (3×10^{-6} M) after the first period of stimulation with 300 pulses, followed 20 minutes later, without washout, by the second period of stimulation.

[3]Strips were exposed to metoclopromide (1×10^{-8} M) after the first period of stimulation with 300 pulses and 20 minutes later, in its presence, to dopamine (3×10^{-6} M) for an additional 20 minutes. The second period of stimulation was performed in the presence of both agonist and antagonist.

*$p<0.05$ compared to the ratio for the corresponding control group treated with dopamine alone.

5. Effects of dopamine and its antagonists on contractile responses to transmural stimulation

Contractile responses of renal artery to transmural stimulation, measured simultaneously with the efflux of tritium, was not significantly reduced by dopamine at the low concentration (3×10^{-7} M) over the frequency range examined (Table 22). Dopamine at the higher concentration (3×10^{-6} M), however, decreased gram tension rise of strips in response to stimulation at 1 or 2 Hz. This depressant effect of dopamine was not due to a direct action of the agent on smooth muscle since contractions of comparable magnitude to exogenous noradrenaline (3×10^{-6} M and 1×10^{-5} M) were not depressed by dopamine (3×10^{-6} M). The tension rise induced by noradrenaline at 3×10^{-6} M in the absence and in the presence of prior administration of dopamine was 3.2±0.3 g and 4.05±0.9 g ($p < 0.2$) in four strips respectively.

The antagonists pimozide and metoclopromide at any of the selected concentrations had no detectable effects on mechanical responses of the strips to nerve stimulation (Table 23). Neither dopamine nor its antagonists by themselves produced any spontaneous contractile responses in the renal artery.

Table 22. THE EFFECT OF DOPAMINE ON THE MECHANICAL RESPONSE TO NERVE STIMULATION IN BOVINE RENAL ARTERY.

Treatment group [1]	Stimulation frequency (Hz)	No. of values	Rise in tension (g)		Response ratio 2nd run / 1st run [2]
			1st run	2nd run	
a. DOPAMINE 3×10^{-7} M					
Control	1	8	2.40±1.31	2.40±1.49	1.29±0.28
Dopamine	1	8	1.61±0.68	1.08±0.32	0.96±0.15
Control	2	8	3.83±2.26	4.18±2.52	1.41±0.23
Dopamine	2	8	2.84±1.08	3.41±1.04	1.42±0.16
Control	5	8	6.49±3.13	7.03±2.99	1.40±0.15
Dopamine	5	8	5.74±2.00	7.16±2.21	1.35±0.08
Control	15	8	9.51±3.29	10.10±3.04	1.15±0.07
Dopamine	15	8	8.29±2.12	9.05±3.33	1.10±0.04
b. DOPAMINE 3×10^{-6} M					
Control	1	6	0.87±0.35	1.87±1.10	1.57±0.29
Dopamine	1	6	0.77±0.23	0.26±0.07	0.39±0.12**
Control	2	6	2.93±1.34	4.18±2.13	1.29±0.17
Dopamine	2	6	2.18±0.85	1.32±0.64	0.58±0.15*
Control	5	6	6.53±2.93	7.92±3.00	1.36±0.11
Dopamine	5	6	5.57±2.12	5.92±2.06	1.11±0.08
Control	15	6	10.32±3.51	11.27±3.26	1.18±0.06
Dopamine	15	6	8.27±3.07	8.55±2.94	1.06±0.08

Table 22. (Continued)

[1]Dopamine, when given, was administered for 20 minutes in the interval between the first and second stimulation periods with 300 pulses and the vessels were re-stimulated in its presence.

[2]Obtained by comparison of individually determined ratios for control and treated strips taken from the same artery.

*$p<0.02$; **$p<0.01$ compared to ratios for corresponding control groups.

Table 23. THE EFFECT OF PIMOZIDE AND METOCLOPROMIDE ON THE MECHANICAL RESPONSE TO NERVE STIMULATION IN BOVINE RENAL ARTERY.

Treatment group	Concentration (M)	Stimulation frequency (Hz)	No. of values	Response ratio 2nd run / 1st run
a. PIMOZIDE				
Control	-	1	4	2.75±0.48
Pimozide[1]	1×10^{-8}	1	4	1.50±0.29
Control	-	15	4	1.38±0.17
Pimozide[1]	1×10^{-8}	15	4	1.11±0.09
Control	-	1	4	1.67±0.31
Pimozide[1]	2×10^{-7}	1	4	1.04±0.14
Control	-	15	4	1.23±0.13
Pimozide[1]	2×10^{-7}	15	4	1.02±0.06
Control	-	1	6	1.52±0.23
Pimozide[1]	1×10^{-6}	1	6	1.24±0.14
Control	-	15	6	1.01±0.06
Pimozide[1]	1×10^{-6}	15	6	0.88±0.10
b. METOCLOPROMIDE				
Control	-	1	4	3.08±1.00
Metoclopromide[2]	1×10^{-8}	1	4	2.19±0.41
Control	-	15	4	1.05±0.02
Metoclopromide[2]	1×10^{-8}	15	4	1.13±0.06

Table 23. (Continued)

[1]Pimozide, when given, was administered for 20 minutes in the interval between the first and second stimulation periods with 300 pulses and the vessels were re-stimulated in its presence. There were no statistically significant differences between any of the treated groups and their corresponding controls.

[2]Metoclopromide, when given, was administered for 20 minutes in the interval between the first and second stimulation periods with 300 pulses and the vessels were re-stimulated in its presence. There were no statistically significant differences between any of the treated groups and their corresponding controls.

PART II. SENSITIZATION OF VASCULAR EFFECTOR RESPONSES AND TERMINATION OF ACTION OF SYMPATHETIC TRANSMITTER IN BRANCHES OF BOVINE RADIAL ARTERY

A. Perfusion of branches of bovine radial artery

Branches of bovine radial artery were perfused with Krebs solution at constant pressure. Mechanical responses to noradrenaline and to nerve stimulation, and the modification of it by cocaine and/or estradiol, were examined. With the present preparation and experimental conditions, agonist could be administered to the tissue either intraluminally or extraluminally and thus would encounter known access barriers differently en route to the receptor region. The aim of these experiments was to study the relationship between routes of noradrenaline to the receptors and sensitization of effector responses. Additionally, prolongation of responses to noradrenaline or to nerve stimulation resulting from blockade of catecholamine uptake into neuronal and extraneuronal sites was also investigated.

1. Effects of cocaine and/or estradiol on responses to exogenous noradrenaline

Typical responses of the branches of bovine radial artery to extraluminal and intraluminal noradrenaline are shown in Figure 17. Vessels respond to noradrenaline with dose-dependent decreases in flow rate. In most preparations noradrenaline at 3×10^{-9} M (extraluminal) or a 30 ng bolus (intraluminal) was sufficient to elicit detectable responses. A complete obstruction of flow through the lumen was observed

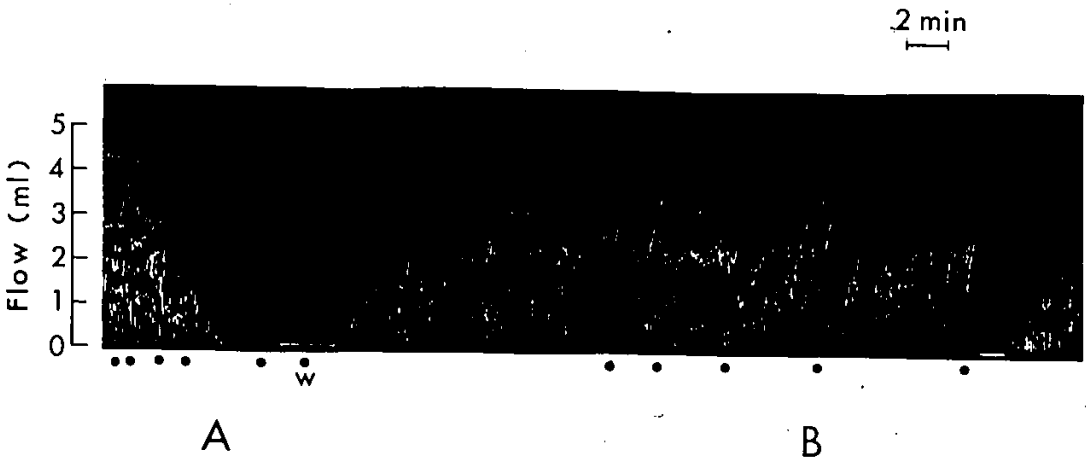

Figure 17. Typical responses of perfused bovine radial artery (branch) to noradrenaline administered extraluminally (A) and intraluminally (B). Contractile responses are recorded as reduction in outflow. Each vertical tracing represents the volume of perfusate flowing through vessel lumen over a 5 second period. Dots below tracings indicate doses of noradrenaline, which are 3×10^{-9}, 1×10^{-8}, 3×10^{-8}, 1×10^{-7} and 3×10^{-7} M (from left to right) for extraluminally added noradrenaline; and 10, 30, 100, 300 and 1000 ng (from left to right) for intraluminally added noradrenaline. Responses to extra-noradrenaline are cumulative. W, replacement of the extraluminal bathing medium with drug-free Krebs solution.

with extraluminally added noradrenaline at concentrations greater than 3×10^{-7} M. However, such obstruction was only occasionally observed with intraluminally added noradrenaline even at the highest dose employed.

Basal flow rate determined prior to the onset of the experimental protocol was 30.6±2.3 ml/min in 38 preparations examined. In six control preparations, the initial dose-response curves to extraluminally and to intraluminally added noradrenaline were not shifted significantly compared to the corresponding second set of dose-response curves obtained after the preparations were allowed a 60-minute period of perfusion with drug-free Krebs solution (Figure 18). On the other hand, a 30-minute exposure of the tissues to either cocaine (3×10^{-5} M) or to estradiol (3.6×10^{-5} M), which by themselves produced no substantial change in basal flow rate, shifted the noradrenaline dose-response curves (both extraluminal and intraluminal) significantly to the left (Figures 19 and 20).

The extent of drug-induced horizontal shift of the noradrenaline dose-response curves was determined by the ratio of geometric mean values for the mean effective concentration of noradrenaline which produced half-maximal response (ED_{50}) in the absence and in the presence of the drug under study (Table 24). Cocaine had a greater sensitizing effect than estradiol on responses to extraluminally added noradrenaline. The shift of ED_{50} for extraluminal noradrenaline induced by cocaine was 1.6-fold of that induced by the steroid. On the other hand, responses to intraluminal noradrenaline were sensitized by cocaine and by estradiol

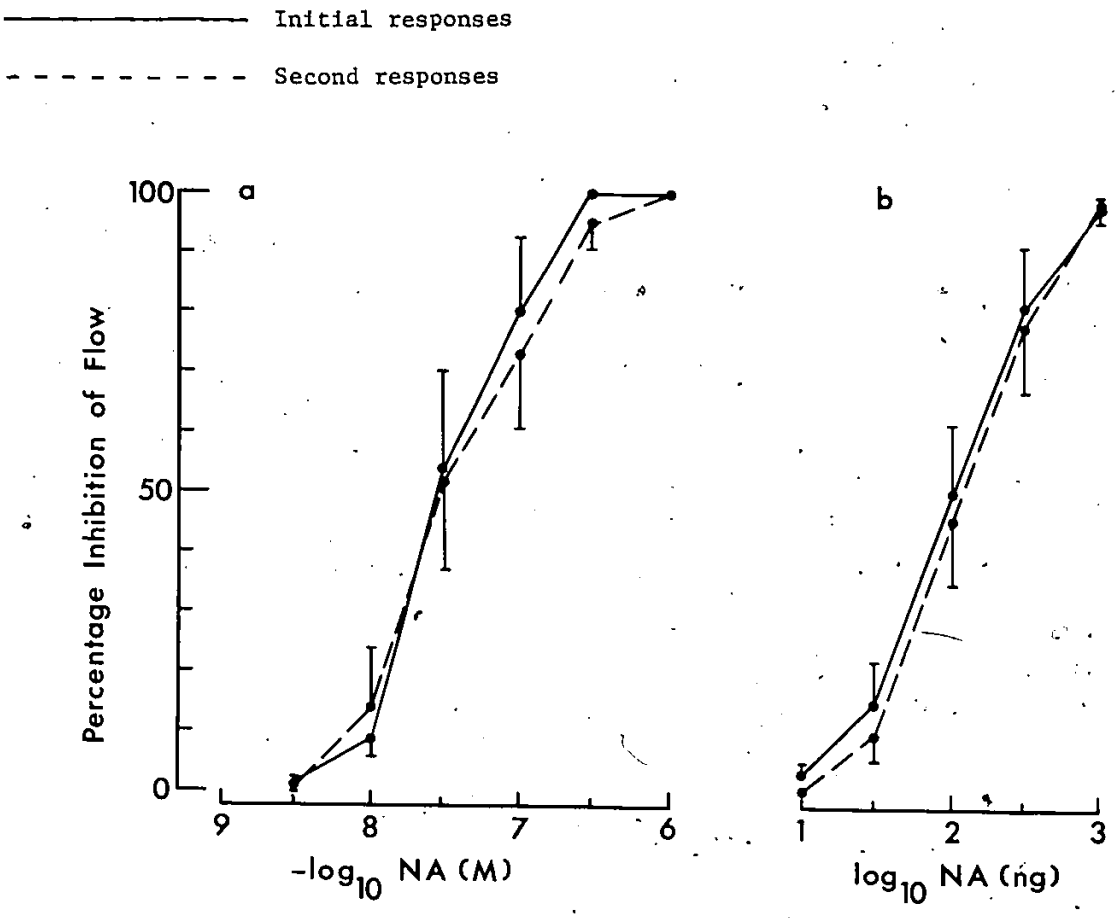

Figure 18. Dose-response curves to noradrenaline administered extraluminally (a) and intraluminally (b) in perfused branches of bovine radial artery. Mean values and their standard errors are shown for an initial and a second response to noradrenaline, obtained 60 minutes apart, at each dose. Responses to extraluminal noradrenaline are cumulative. N=6 in all groups. Initial responses did not differ significantly from the second responses.

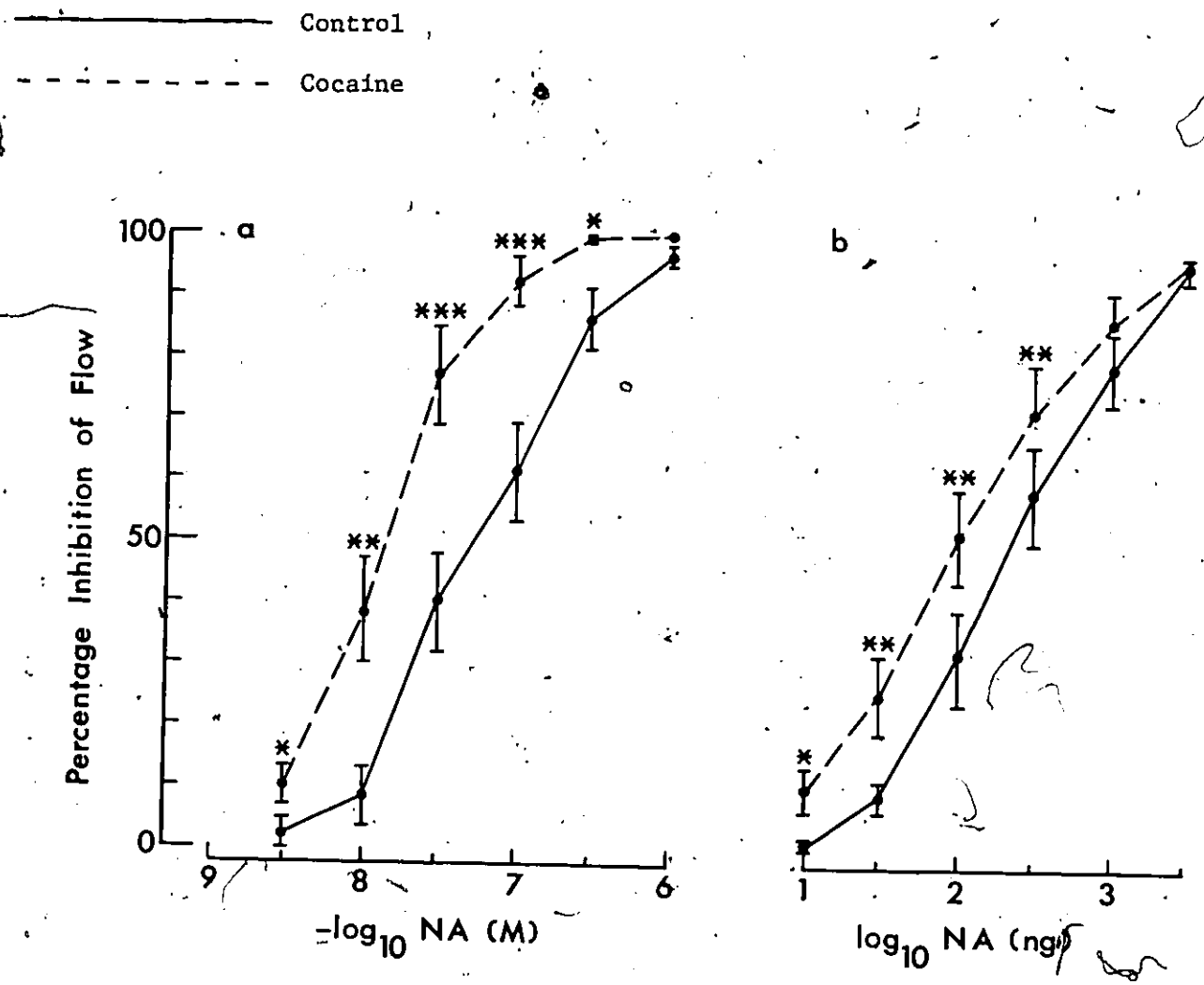

Figure 19. Effect of cocaine on dose-response curves to noradrenaline administered extraluminally (a) and intraluminally (b) in perfused branches of bovine radial artery. Dose-response curves obtained in the absence and presence of cocaine (3×10^{-5} M) are shown. Number of values in each group in (a) and (b) are 17 and 12 respectively. Probability comparisons, using paired t-test, were between values of control and treated groups, $*p < 0.5$, $**p < 0.01$, $***p < 0.001$.

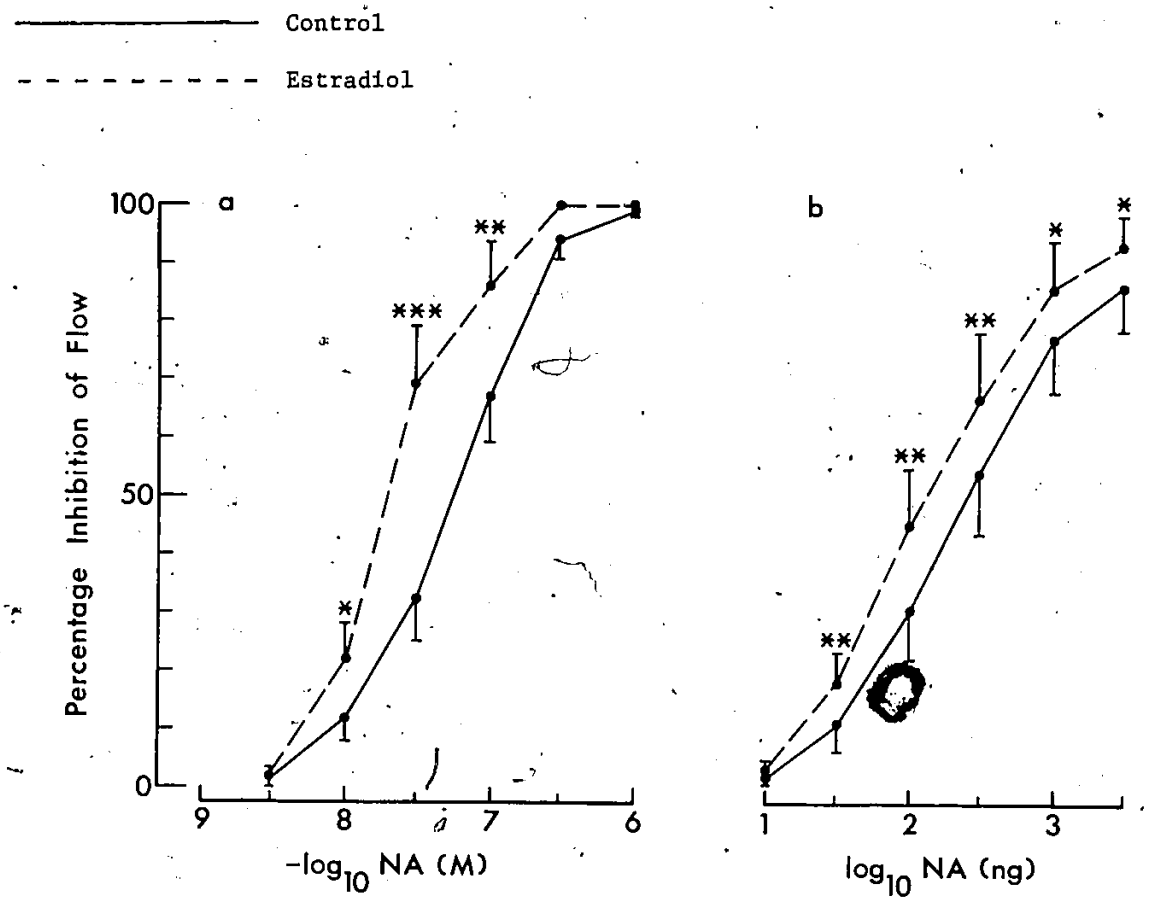

Figure 20. Effect of estradiol on dose-response curves to noradrenaline administered extraluminally (a) and intraluminally (b) in perfused branches of bovine radial artery. Dose-response curves obtained in the absence and presence of estradiol (3.6×10^{-5} M) are shown. Number of values in each group in (a) and (b) are 12 and 9 respectively. Probability comparisons, using paired t-test, were between values of control and treated groups, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 24. EFFECTS OF COCAINE (3×10^{-5} M) AND ESTRADIOL (3.6×10^{-5} M) ON THE DOSE-RESPONSE CURVES TO EXTRALUMINALLY AND INTRALUMINALLY ADDED NORADRENALINE IN PERFUSED BRANCHES OF BOVINE RADIAL ARTERY.

Route of noradrenaline	Treatment group	No. of values	Geometric mean ED_{50}[1]		Ratio of ED_{50}[2]	p value[3]
			Control	Treated		
			(x 3 nM)			
Extraluminal	Cocaine	17	18.32±1.35	4.53±1.26	4.04	< 0.001
	Estradiol	12	17.30±1.26	6.79±1.30	2.55	< 0.001
	Cocaine plus estradiol	9	16.60±1.70	3.31±1.41	5.01	< 0.01
			(x ng)			
Intraluminal	Cocaine	12	269.15±1.38	138.04±1.45	1.95	< 0.001
	Estradiol	9	299.91±1.80	162.18±1.62	1.85	< 0.05
	Cocaine plus estradiol	9	223.87±1.55	158.49±1.41	1.41	N.S.

[1] ED_{50} is the concentration of noradrenaline producing half maximal response; geometric mean ED_{50} is the antilog of the mean log values of individually determined ED_{50}s. Antilogs of the log mean and of the log standard error are shown.

[2] Ratio of geometric mean ED_{50} of the control versus the treated group.

[3] Comparison of the mean log values of ED_{50} for the control and the treated group using paired t-test. N.S., not significant.

to a similar extent. Ratios of ED_{50} for intraluminal noradrenaline in preparations treated with cocaine and that with the steroid were not substantially different from each other.

In preparations treated together with cocaine and estradiol, responses to extraluminally added noradrenaline, but not that to intraluminally added noradrenaline, were clearly potentiated (Figure 21). Assessment of ratios of ED_{50} for extraluminally added noradrenaline revealed that the sensitization induced by the combined administration of these agents was greater than that induced by estradiol alone (Table 24). The ED_{50} for intraluminally added noradrenaline, however, was not reduced significantly in tissues treated with both cocaine and the steroid ($p < 0.5 > 0.4$ when mean log values of ED_{50} for the control group was compared to that of the treated group).

It should be noted that in the initial series of test responses to intraluminally added noradrenaline (i.e. the dose-response curve obtained prior to exposure to cocaine and/or estradiol), some preparations either did not respond or responded poorly to even the highest dose of noradrenaline used (3,000 ng in bolus injection) and data from these tissues were rejected. Thus, the number of observations made with intraluminally added noradrenaline is not the same as that with extraluminally added noradrenaline, as shown in Table 24.

2. Effects of cocaine and/or estradiol on responses to periarterial nerve stimulation

Branches of bovine radial artery responded to stimulation at

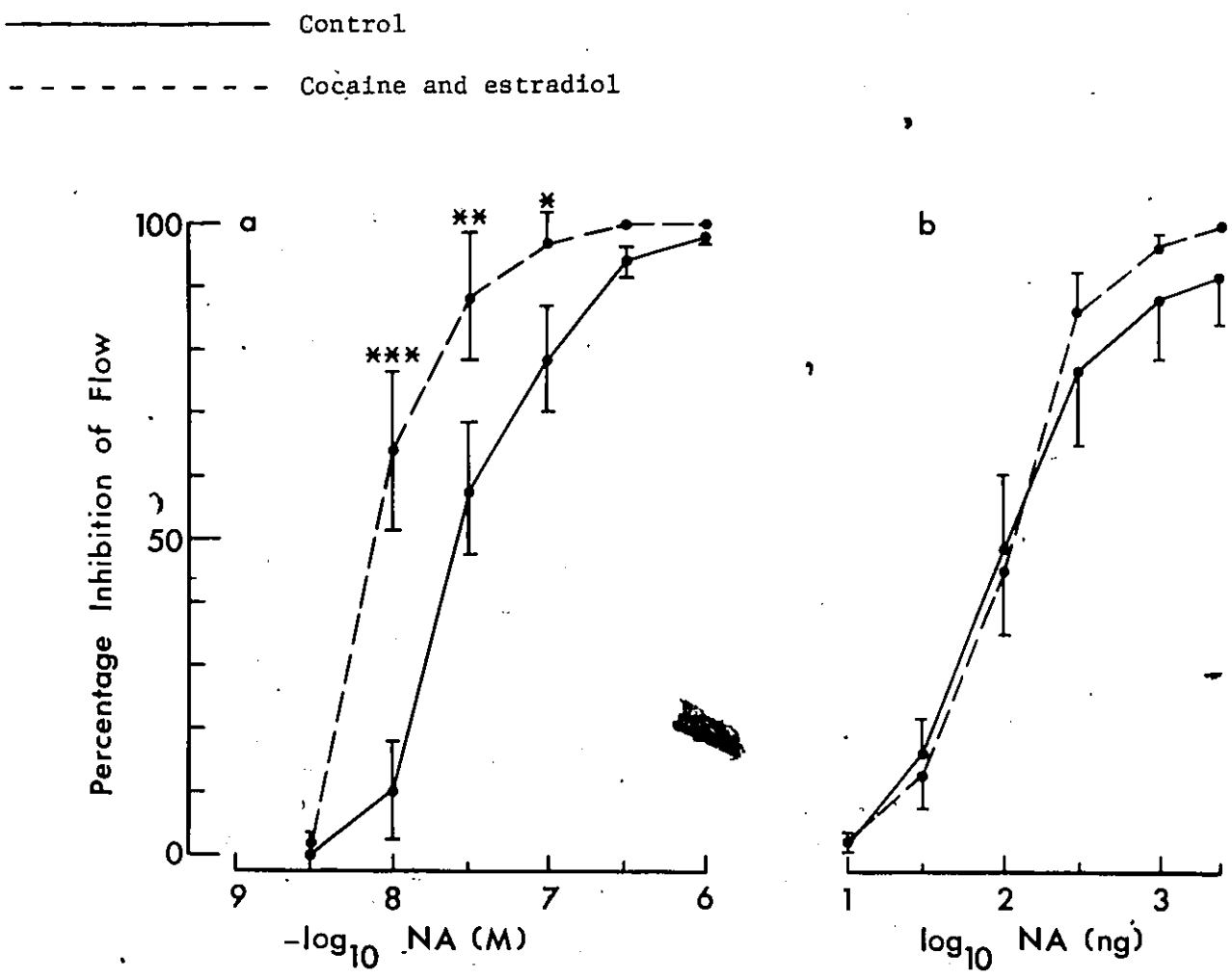

Figure 21. Effects of cocaine and estradiol on dose-response curves to noradrenaline administered extraluminally (a) and intraluminally (b) in perfused branches of bovine radial artery. Dose-response curves obtained in the absence and presence of cocaine (3×10^{-5} M) and estradiol (3.6×10^{-5} M) are shown. N=9 in both cases. Probability comparisons, using paired t-test, were between values of control and treated groups, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

the frequency range from 0.5 Hz to 5 Hz with a progressive reduction in flow through the lumen, reflecting a frequency dependent vasoconstriction. Figure 22 shows typical responses of the vessel. A plateau response, which in most cases was of the magnitude of 60% to 70% inhibition of basal flow, was obtained with stimulation at frequencies at or higher than 5 Hz (Figure 23). In five control preparations, no significant difference was evident in the two frequency-response curves obtained 60 minutes apart (Figure 23). In contrast, frequency-response curves in preparations treated either with cocaine (3×10^{-5} M) or with estradiol (3.6×10^{-5} M) for 30 minutes were shifted significantly to the left when compared to the curves obtained in the absence of the drug (Figures 24 and 25). Determination of the degree of horizontal shift were made at three mid-range response levels covering the linear portion of the curve (Table 25). The stimulation frequency required to produce a particular percentage inhibition of flow, namely 20%, 30% and 40%, was determined from each individual frequency response curves obtained in the absence and in the presence of cocaine or estradiol. Ratios of the arithmetic mean values of these frequencies were calculated and employed as an index for comparison. Table 25 shows that in all cases the stimulation frequency required to obtain a given magnitude of response was significantly lowered by the treatment of tissues with cocaine or with the steroid. Potentiation of responses by these two agents was not materially different from each other, as assessed by a comparison of the frequency ratios.

The combined administration of cocaine and estradiol to bovine

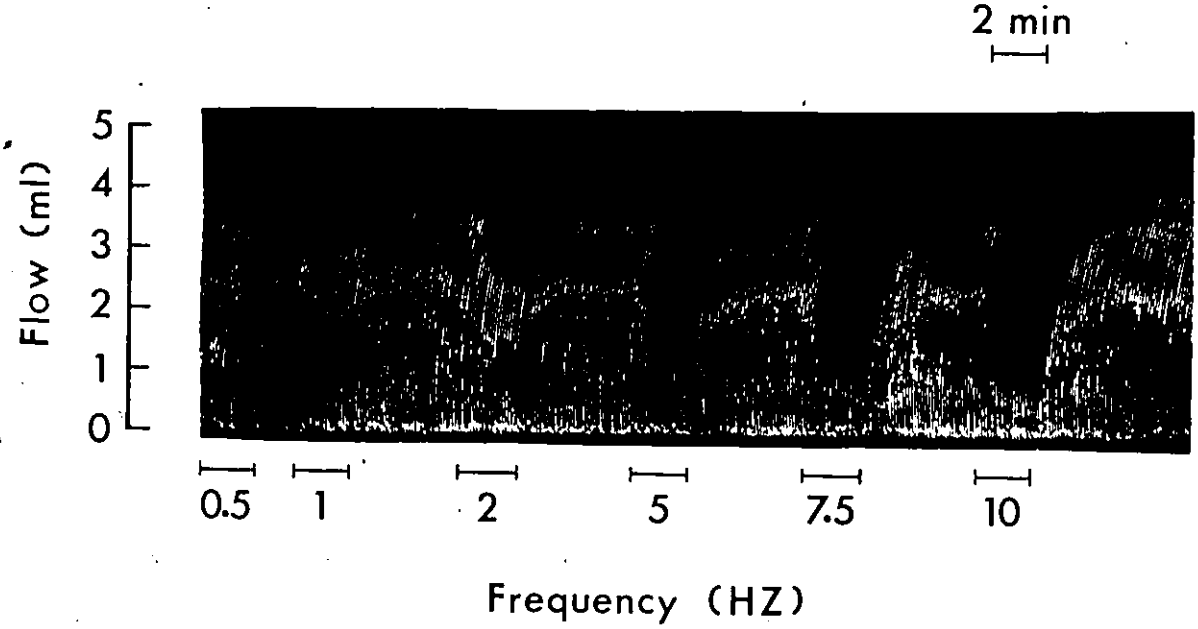

Figure 22. Typical responses of a perfused bovine radial artery (branch) to perfarterial nerve stimulation. Contractile responses are recorded as reduction in outflow. Each vertical tracing represents the volume of perfusate flowing through vessel lumen over a 5 second period. The duration of stimulation at each frequency is indicated by a horizontal bar below tracing.

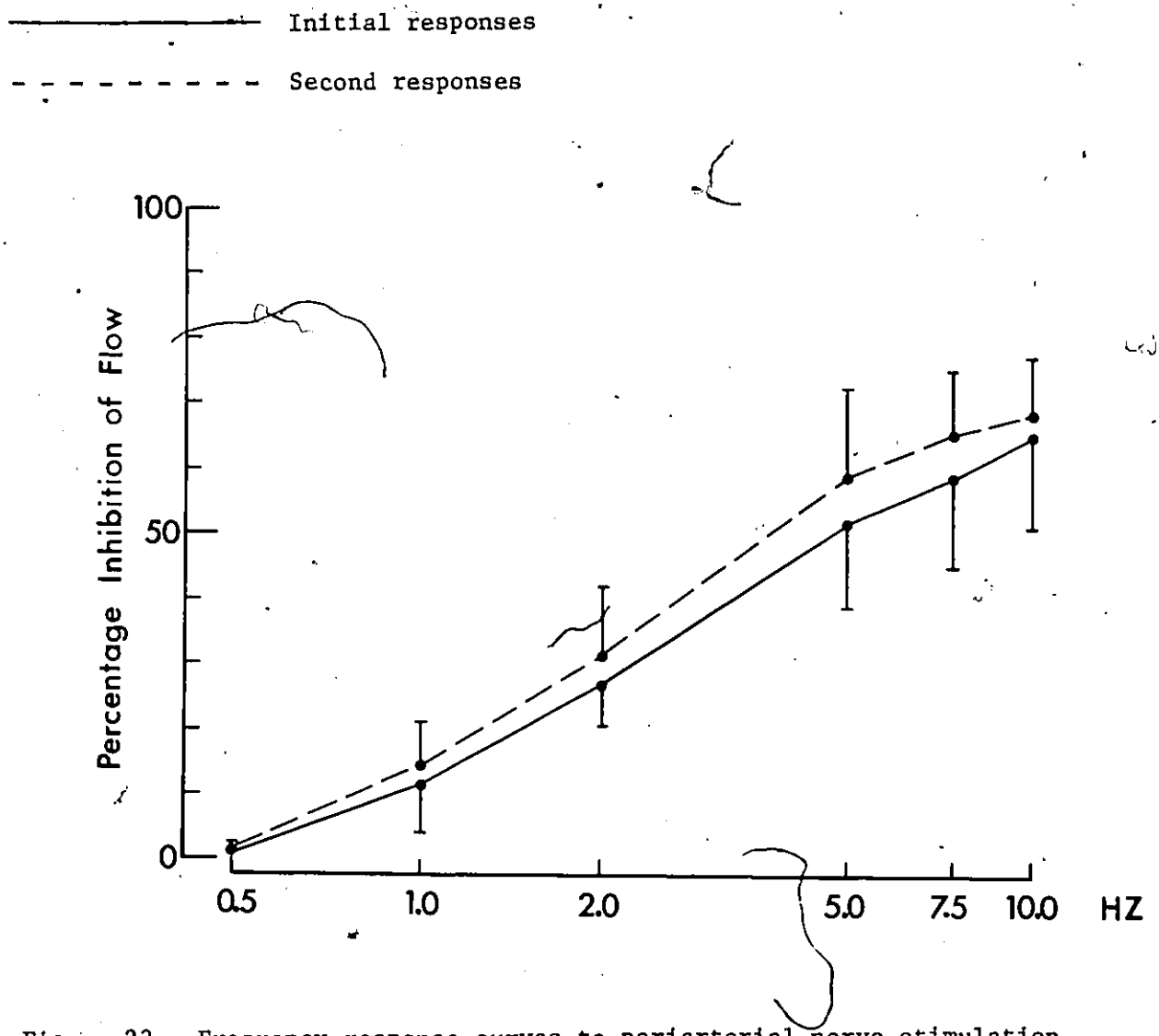

Figure 23. Frequency-response curves to periarterial nerve stimulation in perfused branches of bovine radial artery. Mean values and their standard errors are shown for an initial and a second response, obtained 60 minutes apart, to periarterial nerve stimulation at each frequency in 5 preparations. Stimulation was performed for 2 minutes at each test frequency. Initial responses did not differ significantly from the second responses.

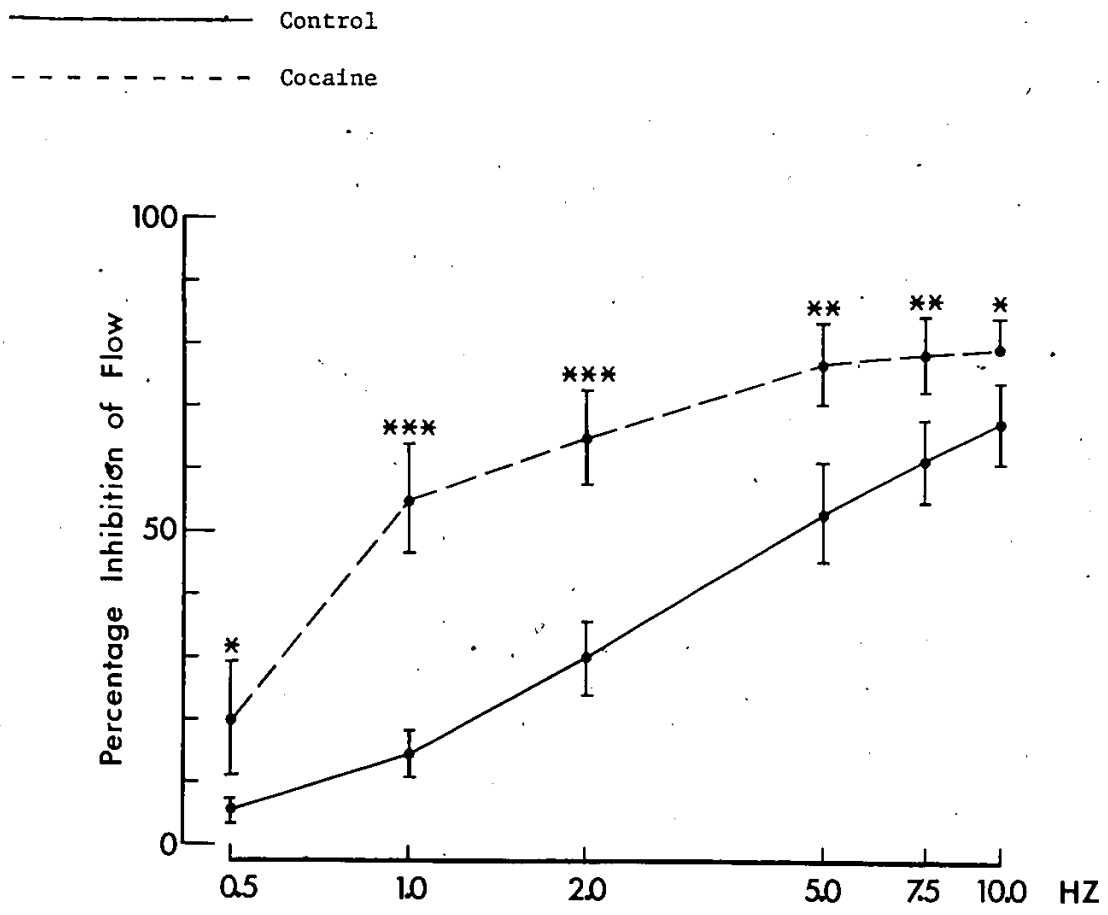

Figure 24. Effect of cocaine on response to periarterial nerve stimulation in perfused branches of bovine radial artery. Responses to stimulation for 2 minutes at each test frequency in the absence and then in the presence of cocaine (3×10^{-5} M) in 14 preparations are shown. Probability comparisons, using paired t-test, were between values of the control and treated groups, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

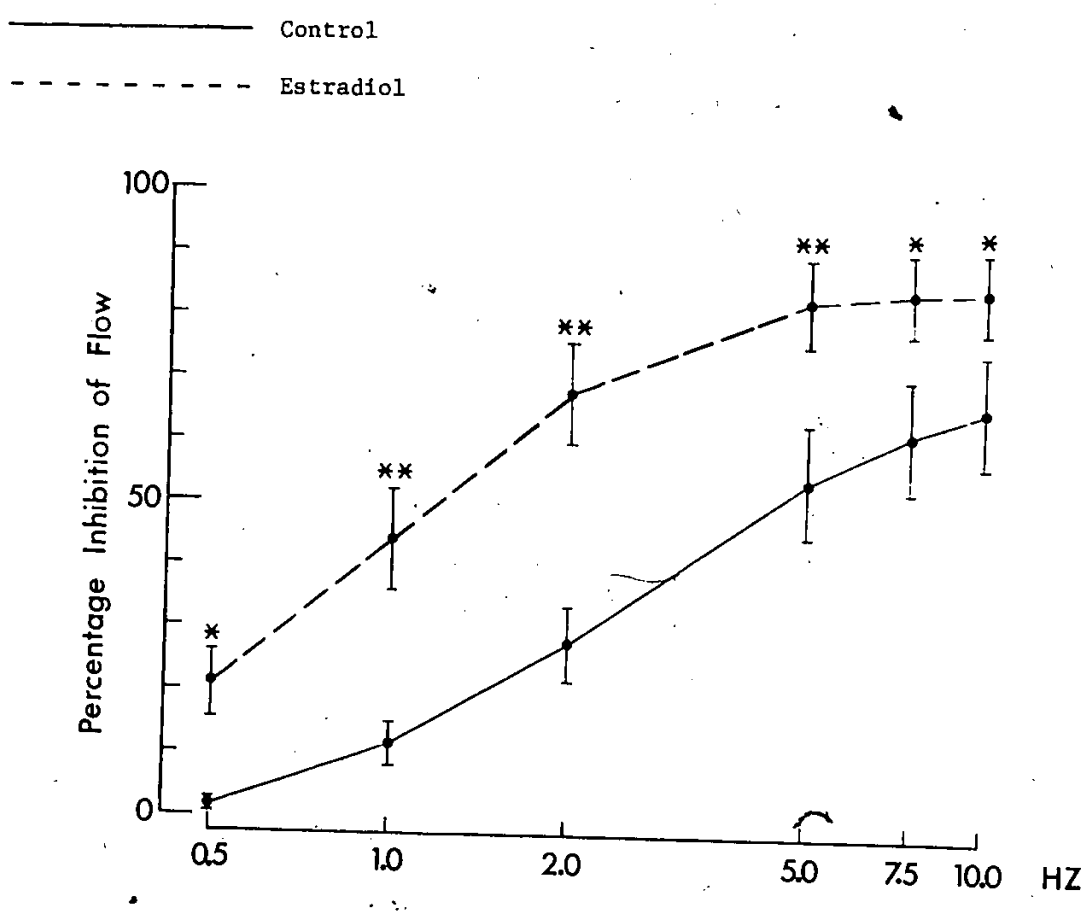

Figure 25. Effect of estradiol on response to periarterial nerve stimulation in perfused branches of bovine radial artery. Responses to stimulation for 2 minutes at each test frequency in the absence and then in the presence of estradiol (3.6×10^{-5} M) in 12 preparations are shown. Probability comparisons, using paired t-test, were between values of the control and treated groups, *$p < 0.01$, **$p < 0.001$.

Table 25. EFFECTS OF COCAINE (3×10^{-5} M) AND ESTRADIOL (3.6×10^{-5} M) ON THE FREQUENCY-RESPONSE CURVES TO PERIARTERIAL NERVE STIMULATION IN PERFUSED BRANCHES OF BOVINE RADIAL ARTERY.

Treatment group	Response level (% inhibition of flow)	No. of values	Stimulation frequency (Hz)[1]		Frequency ratio[2]	p value[3]
			Control	Treated		
Cocaine	20	14	2.0±0.4	0.4±0.1	5.0	< 0.001
	30	14	4.0±0.7	0.9±0.3	4.4	< 0.001
	40	14	6.6±1.0	1.4±0.4	4.7	< 0.001
Estradiol	20	12	2.2±0.6	0.5±0.1	4.4	< 0.01
	30	12	4.8±1.3	1.1±0.3	4.4	< 0.01
	40	12	7.4±2.2	1.7±0.5	4.4	< 0.01

[1]Arithmetic mean values of stimulation frequency required to produce the given response level, values are determined from each individual frequency-response curve.

[2]Ratio of frequencies of the control versus the treated group.

[3]Comparison made with the control and the treated values using paired t-test.

radial artery, nevertheless, did not produce a consistent potentiation of responses to nerve stimulation. In fact, all three possible results were observed: in nine preparations treated with both cocaine and the steroid, three exhibited potentiated responses after the drug treatment, four exhibited desensitized responses and the final two showed no substantial difference in responses. Thus, at this stage there is insufficient information to permit a rational analysis of these data.

3. Effects of cocaine and/or estradiol on duration of response

Duration of responses was assessed by the half recovery time (T_{50}) which was the time taken for responses to recover to 50% of the initial magnitude, i.e. 50% of the pre-stimulation flow rate in this case. In view of the concern brought up elsewhere (Trendelenburg and Henseling, 1976), as has been pointed out earlier by Kalsner and Nickerson (1969d), that drug-induced sensitization of response by itself may alter the duration of response, mathematical corrections were made to identify more accurately the direct drug effect. Percentage inhibition of flow (i.e. response magnitude) and T_{50} (i.e. response duration) of the initial responses to noradrenaline at each concentration, or to nerve stimulation at each frequency, were measured and their mean values calculated. Linear regression equations which correlated the magnitude and the duration of the initial responses were computed for which the x-variable was the percentage inhibition of flow and the y-variable was the T_{50}. After treatment of tissues with cocaine or estradiol, the magnitude and the duration of responses to noradrenaline and to nerve stimulation were measured. From the appropriate regression equation, a control T_{50}

corresponding to the same magnitude of response was calculated and compared to the T_{50} of the response obtained in the presence of the drug concerned. For example, flow rate in one preparation treated with cocaine was reduced to 86% of the pre-stimulation level on stimulation at 1 Hz. T_{50} of this response was measured to be 78 seconds. From the regression equation for this group (which is $y=10.45+0.41x$, see below), control T_{50} (i.e. value of y) was calculated to be 45.7 seconds when the response magnitude had a value of 86% inhibition of flow. Thus, in this individual preparation cocaine induced a 1.7-fold prolongation of the response to nerve stimulation at 1 Hz.

a. Responses to extraluminal and to intraluminal noradrenaline

It should be mentioned that the above method of mathematical correction could not be made with the responses to extraluminally added noradrenaline since, because of the cumulative administration of the agonist, only the maximal response was allowed to recover. Thus values of T_{50} of responses to noradrenaline at diverse concentrations were not available. Measured T_{50} values of the maximal response to noradrenaline i) before and after treatment with cocaine was 226.7±33.6 seconds and 288.8±14.8 seconds respectively ($N=17$, $p < 0.1 > 0.05$), ii) before and after treatment with estradiol was 190.3±17.6 seconds and 289.7±25.3 seconds respectively ($N=12$, $p < 0.001$), iii) before and after treatment with cocaine and estradiol was 187.3±32.3 seconds and 306.4±40.9 seconds respectively ($N=9$, $p < 0.001$). The corresponding T_{75} values, i.e. the time taken for responses to recover to 75% of the initial magnitude,

i) before and after treatment with cocaine was 272.4±34.0 seconds and 377.7±18.0 seconds respectively (N=17, $p < 0.01$), ii) before and after treatment with estradiol was 233.5±18.0 seconds and 371.9±25.5 seconds respectively (N=12, $p < 0.001$), iii) before and after treatment with cocaine and estradiol was 236.6±31.0 seconds and 426.2±36.9 seconds respectively (N=9, $p < 0.001$).

Table 26 shows the effects of cocaine and/or estradiol on the duration of response to intraluminally added noradrenaline. The linear regression equation, which correlated magnitude and duration of initial responses for the subpopulations of vessels subsequently treated with cocaine, estradiol or both compounds together was y=7.53+1.35x (r=0.963, $p < 0.01$, N=5), y=13.24+1.19x (r=0.985, $p < 0.01$, N=5), and y=17.93+1.36x (r=0.955, $p < 0.02$, N=5) respectively (where x=% inhibition of flow, $y=T_{50}$). In all preparations the value of T_{50} increased with increasing amount of injected noradrenaline. Cocaine and estradiol, when administered either alone, significantly prolonged the recovery of responses, as assessed by the ratio of T_{50}s. However, a combined administration of these agents did not materially alter the recovery of responses.

b. Responses to periarterial nerve stimulation

Table 27 shows the effects of cocaine and estradiol on the duration of response to periarterial nerve stimulation. The linear regression equation correlating the initial response magnitude and duration for the subpopulations of vessels subsequently treated with cocaine or estradiol was y=10.45+0.41x (r=0.979, $p < 0.01$, N=5) and y=11+0.36x (r=0.941, $p < 0.02$, N=5) respectively. Both neuronal and extraneuronal

Table 26. EFFECT OF COCAINE (3×10^{-5} M) AND ESTRADIOL (3.6×10^{-5} M) ON THE RECOVERY OF RESPONSES TO NORADRENALINE ADMINISTERED INTRALUMINALLY IN PERFUSED BRANCHES OF BOVINE RADIAL ARTERY.

Treatment group	Amount of noradrenaline injected (ng)	No. of values	Half recovery time (sec)[1]		Ratio of[4] T_{50}s	p value[5]
			Control[2]	Treated[3]		
Cocaine	30	12	46.49± 8.93	74.34±11.78	1.60	< 0.05
	100	12	61.77± 6.40	90.17±14.32	1.46	< 0.05
	300	12	127.20± 6.31	166.36±18.04	1.31	< 0.05
	1000	12	138.52± 2.22	209.82±23.40	1.51	< 0.01
Estradiol	30	9	38.55± 4.69	56.06± 7.29	1.45	< 0.05
	100	9	59.81± 9.86	93.07± 8.05	1.56	< 0.05
	300	9	96.30±10.90	136.66±17.99	1.42	< 0.05
	1000	9	128.19± 3.95	253.99±42.17	1.98	< 0.02
Cocaine plus estradiol	30	9	44.58± 8.05	48.60± 5.99	1.09	N.S.
	100	9	89.48±12.98	91.53±15.18	1.02	N.S.
	300	9	128.31± 9.36	151.31±14.53	1.18	N.S.
	1000	9	142.94± 4.94	169.93±18.41	1.19	N.S.

[1]Arithmetic mean value of the time required for responses to recover to half of the initial flow rate (T_{50}).

[2]Values were mathematically corrected, as described in text.

[3]T_{50} after treatment of tissues with the uptake inhibitor(s).

[4]Ratio of the treated versus the control group.

[5]Comparison made with the control and the treated groups using paired t-test. N.S., not significant.

Table 27. EFFECTS OF COCAINE (3×10^{-5} M) AND ESTRADIOL (3.6×10^{-5} M) ON THE RECOVERY OF THE RESPONSES TO PERIARTERIAL NERVE STIMULATION IN PERFUSED BRANCHES ON BOVINE RADIAL ARTERY.

Treatment group	Stimulation frequency (Hz)	No. of values	Half recovery time (sec)[1]		Ratio of[4] T_{50}s	p value[5]
			Control[2]	Treated[3]		
Cocaine	1.0	14	32.58±3.50	62.86± 9.99	1.93	< 0.01
	2.0	14	36.57±3.07	70.71±13.90	1.93	< 0.02
	5.0	14	40.12±2.57	72.32±11.40	1.80	< 0.01
	7.5	14	41.85±2.43	74.54±12.25	1.78	< 0.01
	10.0	14	42.27±2.34	81.35±13.60	1.93	< 0.01
Estradiol	1.0	12	26.01±2.90	71.50±11.87	2.75	< 0.01
	2.0	12	33.97±3.14	90.50±10.08	2.66	< 0.001
	5.0	12	39.07±2.71	133.67± 9.37	3.42	< 0.001
	7.5	12	38.01±2.42	157.50±12.14	4.14	< 0.001
	10.0	12	38.80±2.54	173.50±12.43	4.47	< 0.001

[1]Arithmetic mean value of the time required for responses to recover to half of the initial flow rate (T_{50}).

[2]Values were mathematically corrected, as described in text.

[3]T_{50} after treatment of tissue with the uptake inhibitor.

[4]Ratio of the treated versus the control group.

[5]Comparison made with the control and treated groups using paired t-test.

uptake inhibitors prolonged significantly the duration of response to nerve stimulation at all frequencies examined. T_{50} values were enhanced somewhat less than 2-fold after treatment of tissues with cocaine and between 2.7- and 4.5-fold after estradiol, as determined by the ratios of the mean values of T_{50} in the presence and in the absence of the inhibitors. The effect of cocaine did not show great variation over the range of frequencies examined. For example, the ratio of T_{50} at 1 Hz was not substantially smaller than that at 10 Hz. On the other hand, the effect of estradiol appeared to be frequency dependent. The ratio of the T_{50}s in preparations treated with the steroid increased progressively with frequency from 1 to 10 Hz.

B. Effects of cocaine and/or estradiol on isotonic contractile responses in strips of bovine radial artery branches

1. Responses to noradrenaline

Experiments were conducted using strips of bovine radial artery (branches) set up in individual muscle chambers. Strips were equilibrated for 90 minutes and isotonic contraction to a test concentration of noradrenaline (1×10^{-7} M) was evoked. Preparations of similar initial sensitivity were matched and, after noradrenaline was washed out, one group of strips was treated with cocaine (3×10^{-5} M), one group with estradiol (3.6×10^{-5} M), one group with both agents (at the above concentrations) and the last group served as control receiving only drug-free Krebs solution. Thirty minutes later, cumulative dose-response curves

for noradrenaline were obtained in all strips.

Strips of bovine radial artery (branches) did not demonstrate detectable spontaneous tone over the 90-minute equilibration period. Noradrenaline induced concentration-dependent contractions in all strips. In preparations treated with cocaine, dose-response curve for noradrenaline was shifted significantly to the left (Figure 26). Geometric mean values of ED_{50} obtained in the absence and in the presence of cocaine was 4.8×10^{-6} M and 1.4×10^{-6} M respectively, giving a ED_{50} ratio of 3.42. In contrast, treatment of tissues with estradiol did not shift significantly the dose-response curves (Figure 26). Additionally, the maximal response to noradrenaline was decreased by the steroid. Because of this latter phenomenon, assessment of drug effect (or the lack of it) cannot be made by using ED_{50} as an index. The combined administration of cocaine and estradiol produced almost a mixed effect on the dose-response curve (Figure 27). For example, the lower part of the curve was shifted considerably to the left, reflecting the potentiation of responses to noradrenaline by cocaine. On the other hand, the maximal responses to noradrenaline was reduced, showing the desensitizing effect of estradiol.

2. Responses to potassium

Responses of radial artery strips (branches) to potassium, and its modification by the uptake inhibitors, were examined using the same protocol as those with noradrenaline. Branches of radial artery responded to potassium with progressive increase in contraction. These responses were not significantly enhanced by cocaine (Figure 28), suggesting that

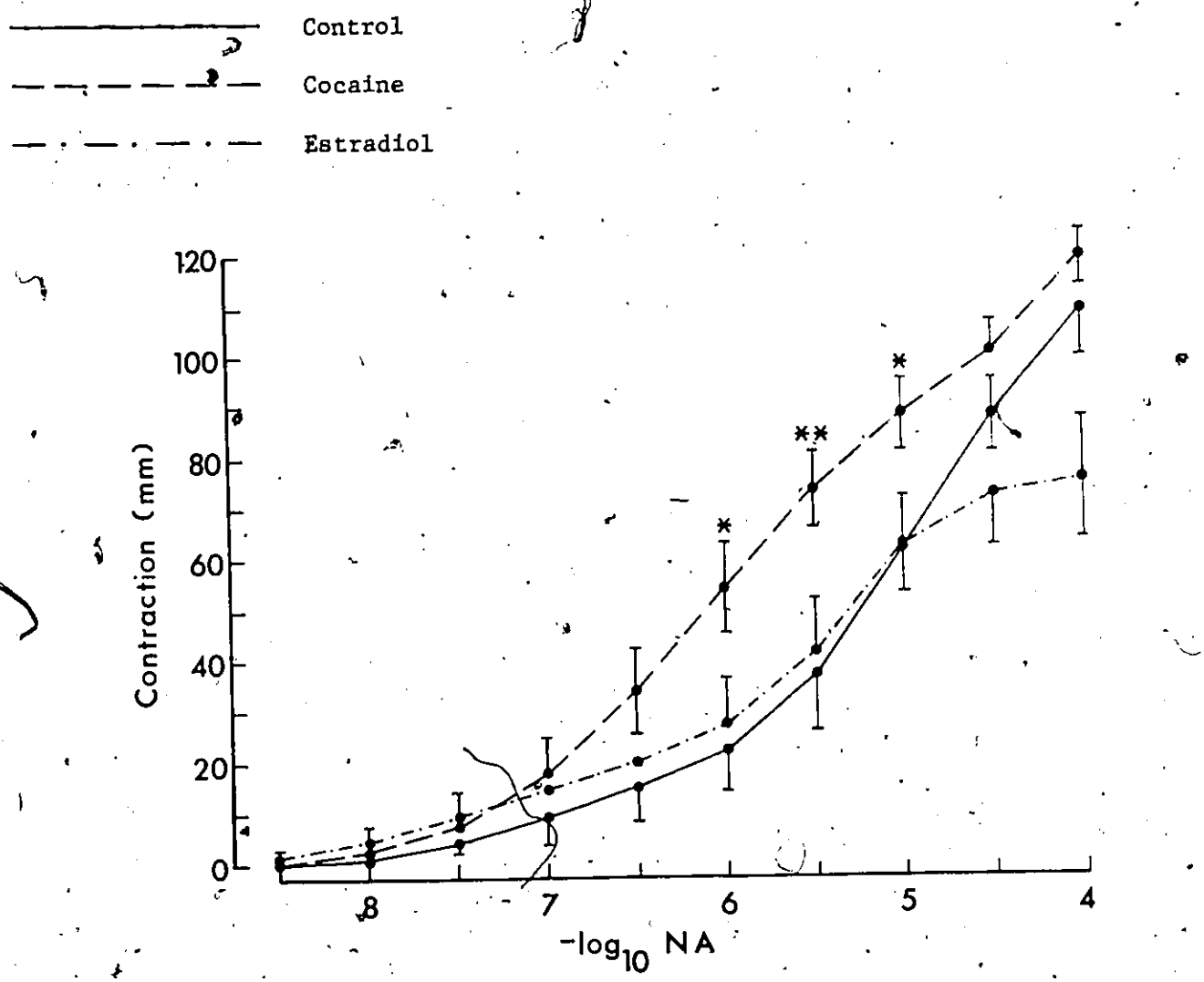

Figure 26. Cumulative dose-response curves in the absence and in the presence of cocaine (3×10^{-5} M) or estradiol (3.6×10^{-5} M) for noradrenaline-induced contractions of bovine radial artery strips (branches). Number of values in the control, cocaine-treated and estradiol-treated groups is 15, 13 and 14 respectively. Probability comparisons, using unpaired t-test, were between values of control and treated groups, $*p < 0.05$, $**p < 0.01$. Standard error bars are not shown wherever they reduce clarity.

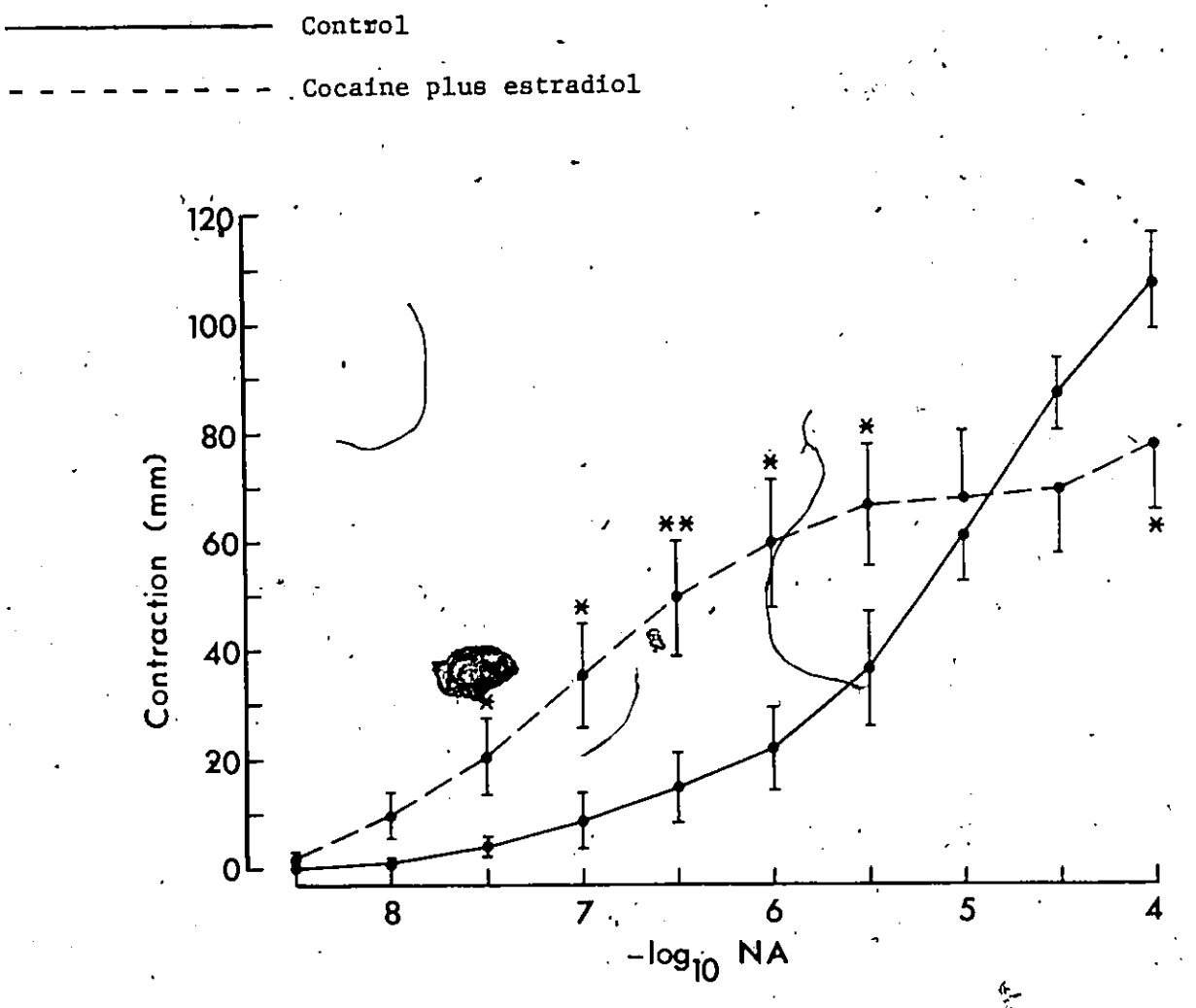

Figure 27. Cumulative dose-response curves in the absence and in the presence of cocaine (3×10^{-5} M) and estradiol (3.6×10^{-5} M) for noradrenaline-induced contractions of bovine radial artery strips (branches). Number of values in the control and treated groups is 15 and 14 respectively. Probability comparisons, using unpaired t-test, were between values of the control and the treated groups, *$p < 0.05$, **$p < 0.01$.

the potentiating effect of this agent on responses to noradrenaline was not due to an non-specific action. In strips treated with estradiol, however, responses to potassium were significantly reduced (Figure 28), reflecting a non-specific desensitizing effect of the steroid on smooth muscle cells. This effect appeared to contribute to the failure of this agent to potentiate the responses of the strips to noradrenaline. In strips treated with both cocaine and the steroid, the desensitization of responses by the latter compound was still predominant (Figure 29).

C. Accumulation of ^{3}H-noradrenaline in branches of bovine radial artery

To assess the effectiveness of cocaine and estradiol to block the accumulation of noradrenaline into tissues, branches of bovine radial artery were incubated with ^{3}H-dl-noradrenaline (6×10^{-7} M) in the absence and in the presence of the uptake inhibitors and the total radioactivity retained in the tissue was determined. Figure 30 shows the effects of cocaine and/or estradiol on the accumulation of ^{3}H-noradrenaline in the bovine tissue. As expected, the tissue radioactivity was significantly reduced (to half of the control value), by either cocaine (3×10^{-5} M) or by estradiol (3.6×10^{-5} M). In preparations treated with these agents together, there was a further decrease in tissue radioactivity, confirming two possibly overlapping sites of action for cocaine and for estradiol.

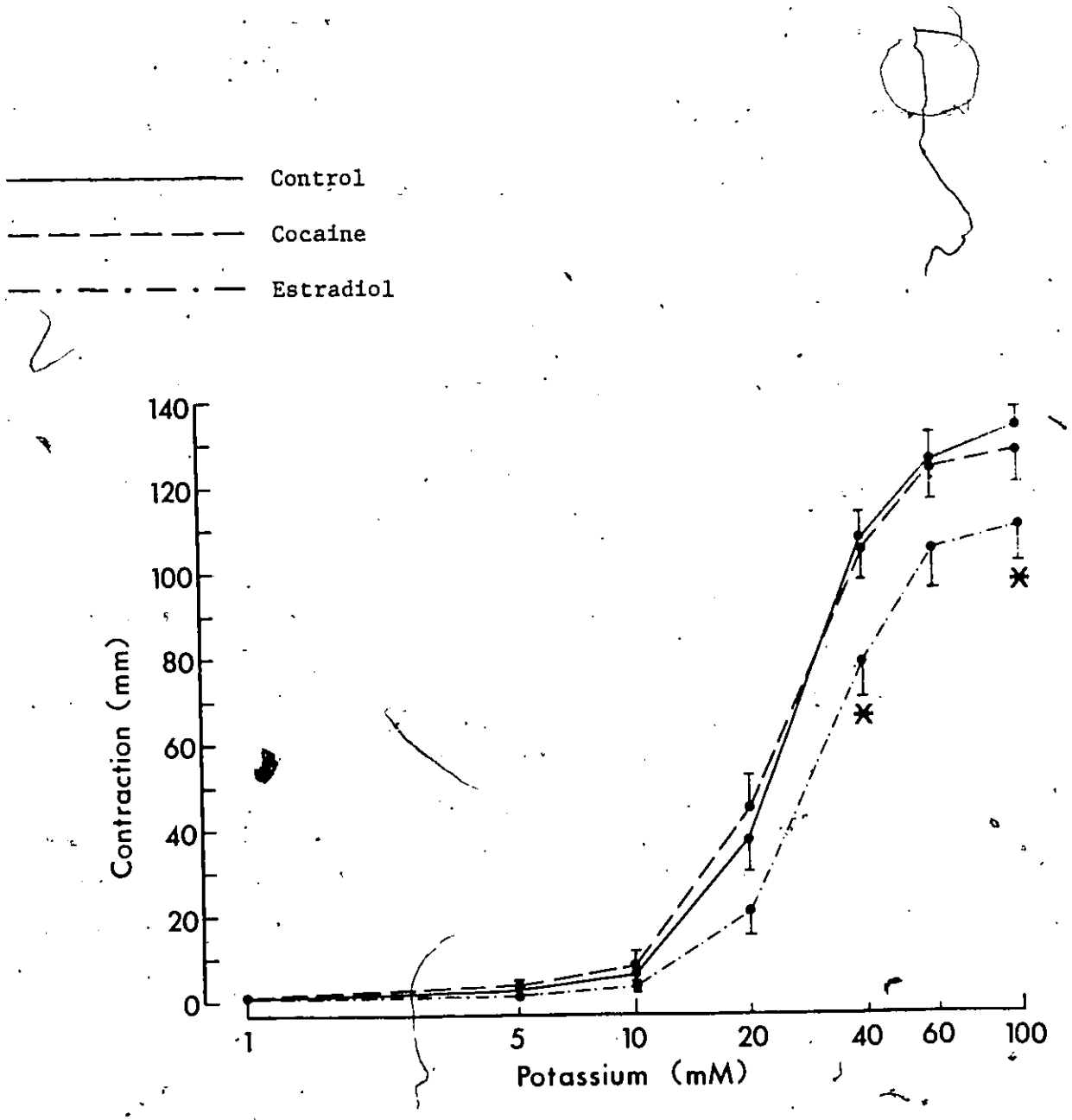

Figure 28. Effect of cocaine (3×10^{-5} M) or estradiol (3.6×10^{-5} M) on responses to potassium in bovine radial artery strips (branches). N=12 in all three groups. Asterisks indicate values of the treated group significantly different from that of the control group with a $p < 0.05$, as compared by the unpaired t-test. Standard error bars are not shown wherever they reduce clarity.

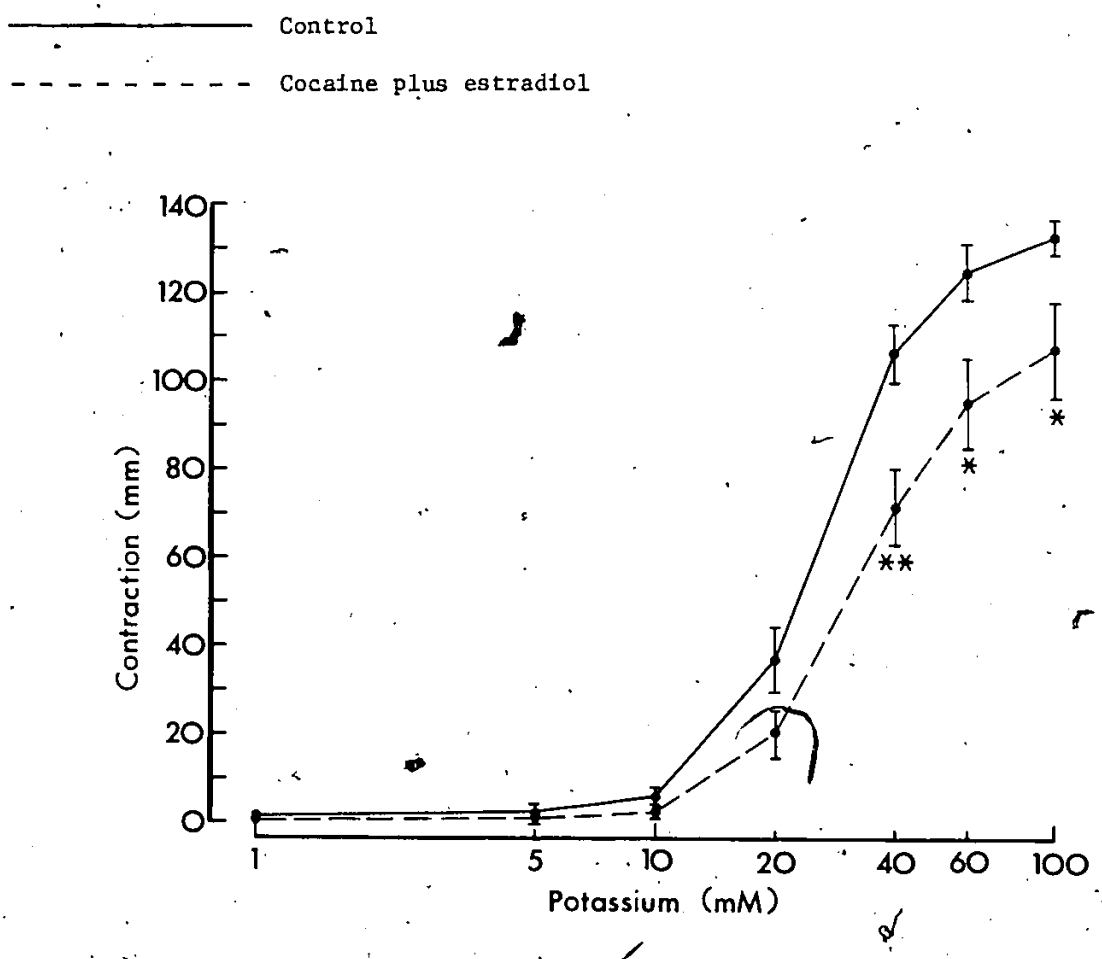

Figure 29. Effect of cocaine (3×10^{-5} M) and estradiol (3.6×10^{-5} M) on responses to potassium in bovine radial artery strips (branches). N=12 in both groups. Probability comparisons, using unpaired t-test, were between values of the control and the treated groups, *$p < 0.05$, **$p < 0.01$.

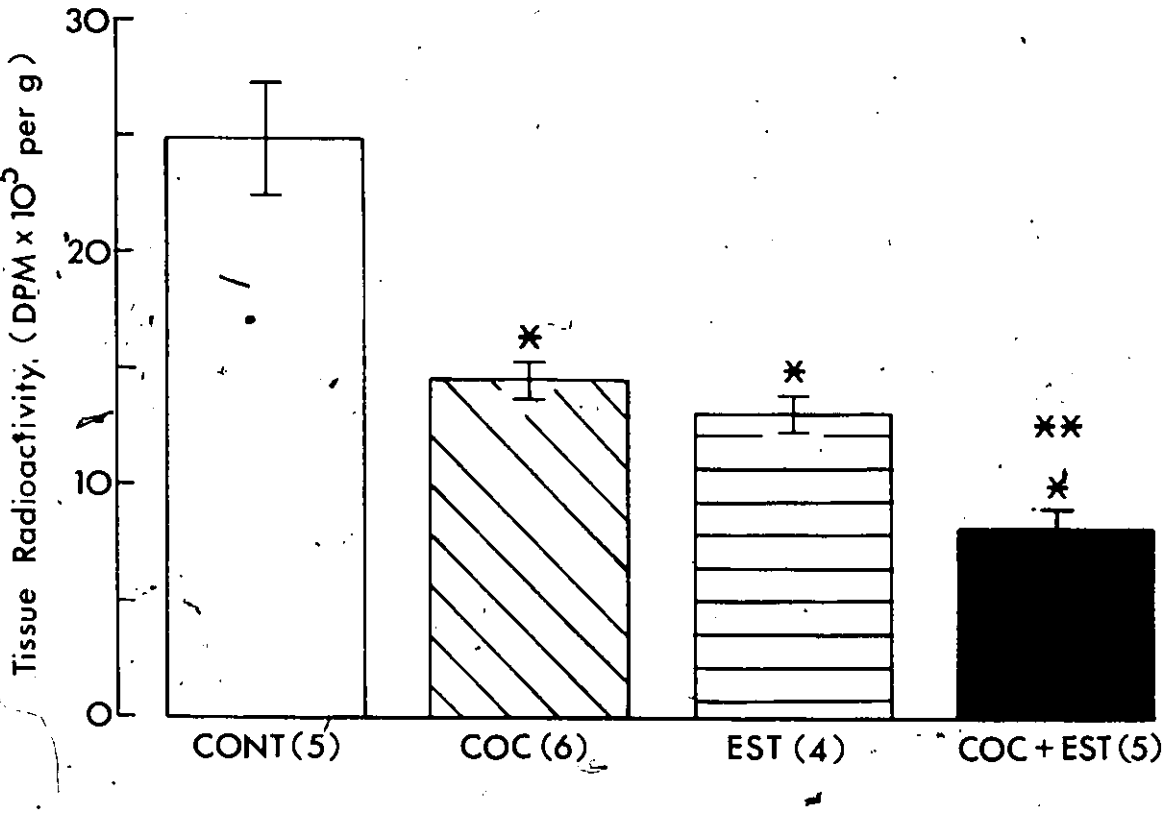

Figure 30. Effects of cocaine and/or estradiol on accumulation of ^{3}H-dl-noradrenaline in branches of bovine radial artery. Mean values of total tissue radioactivity are shown for four groups of preparations incubated with ^{3}H-dl-noradrenaline at 6 x 10^{-7} M in the presence of drug-free Krebs solution (CONT), cocaine (3 x 10^{-5} M, COC), estradiol (3.6 x 10^{-5} M, EST), or cocaine plus estradiol at the above concentrations (COC + EST). *Probability comparisons between values of the control and the treated groups with a $p < 0.01$. **Probability comparisons between values of the group treated with cocaine plus estradiol and groups treated with either agent alone with a $p < 0.05$. Number of values in each group is shown in parenthesis.

V. DISCUSSION

PART I. PRESYNAPTIC ALPHA-ADRENERGIC RECEPTOR HYPOTHESIS

According to the presynaptic alpha-adrenergic receptor hypothesis, endogenously released noradrenaline activates a negative - feedback system mediated by alpha-receptors located on sympathetic nerve terminals, thus providing a precise pulse-to-pulse physiological modulation of neurotransmitter release (Rand, McCulloch and Story, 1975; Langer, 1977; Starke, 1977; Westfall, 1977). Ever since its initial proposal, this attractive hypothesis has been accepted rapidly and, surprisingly, without often being critically tested. The sole evidence in support of this hypothesis comes fundamentally from the repeated observations in a variety of species and tissues that noradrenaline and some alpha-agonists decrease stimulation-induced efflux of neurotransmitter from sympathetic nerve endings; and that this effect is blocked by phenoxybenzamine which itself increases transmitter efflux during nerve stimulation. Nevertheless, the establishment of an autoinhibitory system subserving the crucial role of regulation of transmitter release requires that many other demands be fulfilled and that the hypothesis itself survives vigorous experimental tests. The first part of the present study was undertaken, using different approaches and tissues, to examine the viability of the presynaptic alpha-receptor hypothesis.

Certain terminologies need to be clarified at this point. In the current text the term "transmitter release" refers to the amount of transmitter released from the sympathetic nerve terminals into the synaptic cleft. The terms "transmitter efflux" and "transmitter overflow" are used interchangably, and refer to the amount of transmitter collected

in the superfusate after flowing through the tissue under study. The amount of transmitter in the superfusate represents the net difference between the amount released and the amount taken up by neuronal and extraneuronal tissues. Since at present there is no available method to determine transmitter release, transmitter overflow obtained after neuronal and extraneuronal uptake processes have been blocked is taken as an approximation of transmitter release. This method has been widely used by others during the formulation of the presynaptic alpha-receptor hypothesis (Rand and Story, 1972; Starke, 1973; Langer, 1973). "Efflux of tritium" refers to the total radioactivity detected in the superfusate in tissues previously primed with ^{3}H-noradrenaline. This measurement, obviously, includes both intact ^{3}H-transmitter and its metabolites. The suitability of total tritium, as a measure of noradrenaline release, has been pointed out by Starke (1977) who stated "...the stimulation-evoked overflow of total radioactivity approximates release better than the overflow of noradrenaline alone."

If an autoinhibitory negative feedback system regulating transmitter release is operational, then by definition it would be expected that stimulation-induced transmitter release, reflected by the efflux of tritium, decreases with increasing perineuronal concentration of the transmitter. In the present study, bovine renal artery strips were stimulated with 300 pulses at different frequencies (1 to 15 Hz) spanning the physiological range. As the total amount of transmitter released by a constant number of pulses (300) is liberated into the neuro-effector cleft in a time period compressed from 5 minutes to 20 seconds, the

perineuronal concentration of noradrenaline should increase accordingly. This is confirmed by the frequency/responses of the tissue: the contractile responses showed a clear gradation with increasing frequency from 1 to 5 Hz and reached a plateau at higher frequencies. Information as to the temporal and spatial dynamics of the released transmitter at the neuromuscular cleft is reflected in the rate and the magnitude of these responses.

Although the perineuronal concentration of transmitter varied as expected, the efflux of tritium did not. It did not decline with increasing frequency as would be predicted by the presynaptic alpha-receptor hypothesis but instead was essentially unchanged over the entire test frequency span except at 5 Hz where output was somewhat higher. This relationship between efflux and stimulation frequency did not occur as an isolated example in the bovine renal artery but was also observed in four other tissues examined in the present study, namely, the aorta, carotid artery, femoral artery of dog, and the aorta of guinea-pig. In these tissues stimulated with a moderate number of pulses (200) at two physiological frequencies (1 and 5 Hz), efflux of tritium at the moderate frequency was not significantly less than that at the low frequency. These observations thus provided no support for the operation of a negative feedback system which is purported to decrease the per pulse release of transmitter as the concentration of transmitter in the biophase increases.

The present finding that the efflux of transmitter does not vary with stimulation frequency is in accord with the observation of

Henderson and colleagues in cat nictitating membrane (Henderson, Hughes and Thompson, 1972) and that of Farnebo and Malmfors (1971) in mouse vas deferens. Henderson and colleagues reported that the overflow of noradrenaline from the cat nictitating membrane stimulated with 100 pulses was not significantly different at 0.2, 1, 5 and 15 Hz, i.e. the same ferquency range employed in the present study. Farnebo and Malmfors (1971), although using a variable number of pulses, observed that the per pulse overflow of tritium in mouse vas deferens previously incubated with ^{3}H-noradrenaline remained at a steady level with stimulations at 1, 2, 4, 8 or 16 Hz. Yet, this is not the only observed pattern of frequency/transmitter output. Hughes (1972) demonstrated that the stimulation-induced overflow of noradrenaline in both the rabbit portal vein and in the vas deferens increased as frequency rose from 2 to 15 Hz, illustrating the facilitation of transmitter output at higher frequencies. On the other hand, Stjärne and Brundin (1977) observed that neurally-induced efflux of ^{3}H-noradrenaline in the human omental blood vessels stimulated with 300 pulses ascended to a peak at 10 Hz and then declined at 30 Hz. Thus, a consistent pattern of frequency/transmitter output relationship is not obtained. In the present experiment with the renal artery preparation of dog, efflux of tritium at the moderate frequency was indeed significantly smaller than that at the low frequency. However, this is the only observation of this sort in six selected preparations from three different species, its generalization is thus unwarranted and consequently this observation cannot be singled out to support any claim for the presynaptic alpha-receptor hypothesis.

Since it could be disputed that proportional facilitation of transmitter release with increasing frequency compensates precisely, and thus masks, the presynaptic alpha-receptor-mediated inhibition of transmitter output, experiments were done using preparations treated with phenoxybenzamine and/or noradrenaline to assess the contribution of the presynaptic system at each of the test frequencies.

In accord with many other observations, phenoxybenzamine enhanced the stimulation-induced efflux of tritium in all types of tissues examined, namely, renal and radial arteries of cattle, aorta of guinea-pig, aorta, carotid, femoral and renal arteries of dog. However, these experiments provide no support for the operation of a negative feedback system mediated by presynaptic alpha-receptors. According to the presynaptic alpha-receptor hypothesis, the transmitter release process should be subjected to increasing restraint due to activation of the autoinhibitory loop as the biophase concentration of transmitter increases. When a fixed number of pulses is delivered in ascending frequencies to the tissue, the synaptic concentration of transmitter increases. Phenoxybenzamine, by combining with the presynaptic alpha-receptors, removes the restraint on transmitter release and according to theory, would consequently increase efflux of transmitter during nerve stimulation (in the presence of neuronal and extraneuronal uptake inhibitors). Most importantly, the enhancement of transmitter efflux should be in direct proportion to the degree of functional autoinhibition imposed on the nerve terminals at each stimulation frequency. That the phenoxybenzamine-induced enhancement of transmitter efflux increases in magnitude with increasing

frequency is crucial to the viability of the presynaptic alpha-receptor hypothesis. Such a pattern of frequency-related enhancement of efflux was, however, not evident in the tissues examined in the present study.

The effect of phenoxybenzamine to increase efflux was negatively correlated to the frequency of stimulation in bovine renal artery excited with 300 pulses at 1, 2, 5, 10 and 15 Hz, a pattern of efflux enhancement contradictory to the expectation of the presynaptic alpha-receptor hypothesis. This relationship was also observed in the carotid artery of dog in which the antagonist enhanced efflux more at a low (1 Hz) than at a moderate (5 Hz) frequency. In still other preparations, namely, aorta of guinea-pig, aorta, femoral and renal arteries of dog, the effect of the haloalkylamine on efflux was not significantly different at 1 Hz and at 5 Hz. This latter finding was not only observed with phenoxybenzamine at a high concentration (3×10^{-5} M), but was also with the antagonist at lower concentrations (e.g., 3×10^{-8} M to 3×10^{-6} M, in guinea-pig aorta). Thus, based on the supposition that phenoxybenzamine combines specifically with a distinct class of presynaptic alpha-receptor to interrupt the operation of a presynaptic autoinhibitory loop, it is unlikely that such a loop contributes significantly to the regulation of adrenergic transmitter release under the present experimental conditions.

The concentrations of phenoxybenzamine employed in this study are sufficiently high and non-reversible to rule out a possible break through of the adrenergic blockade by the released noradrenaline. A maximal blockade of the presynaptic alpha-receptor, as assessed by the magnitude of the stimulation-induced efflux of transmitter after treatment

of tissues with phenoxybenzamine, was reported by others to be in the concentration range of 10^{-5} M to 3 x 10^{-5} M (Rand et al., 1973; Henderson, Hughes and Kosterlitz, 1975). Hughes (1972) observed that the increase in stimulation-induced overflow of noradrenaline in rabbit portal vein and vas deferens by phenoxybenzamine (1.5 x 10^{-5} M) was maintained over a period of 6 to 8 hours with only a 10 to 15% decline in noradrenaline output. Starke (1972a) has demonstrated that exogenous noradrenaline (3 x 10^{-7} M) cannot break through the blockade of presynaptic alpha-receptors by phenoxybenzamine (3 x 10^{-6} M), as evidenced by the efflux of tritium in perfused rabbit heart, although the concentration of the antagonist is ten times lower than that used here in the bovine renal artery. In fact, as observed in the present experiments with the guinea-pig aorta, even 3 x 10^{-6} M noradrenaline cannot overcome a blockade of presynaptic alpha-receptors by the haloalkylamine at 3 x 10^{-6} M (Table 17).

The phenoxybenzamine-induced enhancement of transmitter overflow during nerve stimulation has been repeatedly reported in the literature. However, evidence substantiating a negative feedback function of the presynaptic alpha-receptor is very limited. In the majority of studies, the effect of phenoxybenzamine on efflux has been examined only at one moderate and one unphysiologically high frequency, providing little insight into mechanisms. For instance, it was observed that phenoxybenzamine enhanced efflux at the lower but not at the higher frequency in the guinea-pig parametrial artery (5 and 25 Hz, Bell and Vogt, 1971), the rat vas deferens (2 and 50 Hz, Vizi, Somogyi, Hadhazy and Knoll, 1973) and the cat spleen (5 and 30 Hz, Langer, Dubocovich and Celuch, 1975; 10 and 30 Hz, Brown and

Gillespie, 1957; Kirpekar and Gervoni, 1963). To interpret these results, Langer (1977) suggested that the presynaptic negative feedback loop does not function at high frequencies of nerve stimulation. If this is indeed the case, however, one question becomes immediately apparent: when does it play an important role? If the feedback loop were non-functional at high frequencies of nerve stimulation, autonomic nerve activity would be restricted within the low frequency range but not during intense high frequency stimulation. The latter situation may, in fact, result in the flooding of certain critical peripheral neuro-effector junctions with the transmitter.

In the few studies in which more modest parameters of stimulation were employed, the haloalkylamine did not show materially different effects on the enhancement of efflux with different frequencies. For example, Hughes (1972) reported that phenoxybenzamine, in the presence of cocaine, increased efflux of noradrenaline to a similar extent with stimulations at 2, 6 or 16 Hz (constant number of pulses) in the rabbit vas deferens. Likewise, the effect of the haloalkylamine on efflux was not significantly different at 4 and 8 Hz in the rabbit pulmonary artery (McCulloch, Bevan and Su, 1975). The present observations made with a variety of tissues are in accord with these findings and suggest that the proposed presynaptic negative feedback system, if it exists at all, is insensitive to the biophase concentration of transmitter under ordinary conditions of nerve activity.

An argument against the present result is that the presynaptic feedback system is already maximally activated even at the lowest test

frequency (i.e. 1 Hz) and thus efflux of transmitter in the presence of phenoxybenzamine does not increase with ascending frequency. If this were the case, then on this argument alone it can be concluded that such feedback system serves no important physiological role in the regulation of transmitter release, since most autonomic effector responses are elicited at frequencies higher than 1 Hz.

Examination of the effects of noradrenaline on efflux in bovine renal artery, guinea-pig aorta and a variety of vascular preparations of dog, again, provides no substantiation for an operational presynaptic autoinhibitory system. According to the presynaptic alpha-receptor hypothesis, the effect of a constant amount of exogenous noradrenaline to inhibit transmitter release, as assessed by the efflux of tritium in the absence and presence of the agonist, should decrease when the concentration of neurally-released transmitter in the synaptic cleft increases, reflecting a gradually diminishing contribution of the exogenous amine to an expanding pool of liberated transmitter. In the present study, it is evident, as inferred from the graded peak tension development during nerve stimulation, that the biophase level of active amine does increase with increasing frequency in the bovine renal artery stimulated with 300 pulses at four physiological frequencies. Under such circumstances, however, exogenous noradrenaline did not alter the stimulation-induced efflux in accordance with the presynaptic receptor hypothesis.

The effect of noradrenaline at two moderate concentrations on efflux in bovine renal artery was essentially insensitive to stimulation frequency. The agonist at 10^{-6} M inhibited efflux significantly at 1 Hz,

but not at 2, 5 and 15 Hz, as assessed by a comparison of efflux ratios. Raising the concentration of noradrenaline to 3×10^{-6} M caused significant reductions in efflux at all frequencies, however, the reduction was not significantly different at 2, 5 or 15 Hz. These results, evident in the bovine renal artery and in the aorta, carotid and renal arteries of dog, are incompatible with the operation of a presynaptic autoinhibitory loop.

In the femoral artery of dog and the aorta of guinea-pig stimulated with a low and a moderate frequency (1 and 5 Hz), however, noradrenaline did show a greater inhibitory effect on efflux at the lower than at the higher frequency. This finding is seemingly in support of the presynaptic feedback hypothesis. However, results from parallel experiments with phenoxybenzamine, obtained under the same conditions as that with noradrenaline, provided no support for the operation of a presynaptic feedback system. In neither the femoral artery of dog nor the guinea-pig aorta did phenoxybenzamine have a greater enhancing effect of efflux at 5 Hz than at 1 Hz, reflecting the lack of a regulatory role of presynaptic alpha-receptors. Instead, the haloalkylamine increased efflux to the same extent at both frequencies, and in the guinea-pig aorta it (at 3×10^{-6} M) even had a greater enhancing effect on efflux at 1 Hz than did at 5 Hz. These observations, in contrast to that with noradrenaline, suggest that the feedback inhibitory mechanism occurs without discrimination at both 1 and 5 Hz.

According to the presynaptic hypothesis, the effect of agonist should be most when that of antagonist is the least (or vice versa),

indicating the addition to and the blockade of the pool of neurally-released transmitter. Such a relationship was not evident in the present study. Both phenoxybenzamine and noradrenaline had their greatest effects on efflux at 1 Hz in the bovine renal artery stimulated with four different frequencies. Also, phenoxybenzamine enhanced efflux to a greater extent at 1 Hz than did at 5 Hz in the carotid artery of dog, but the inhibitory effect of noradrenaline on efflux was not significantly less at 1 Hz than at 5 Hz.

The inhibitory effect of noradrenaline on stimulation-induced efflux has been examined in many preparations, but surprisingly, only one selected frequency was employed in most studies. (e.g. guinea-pig vas deferens, Stjärne, 1975; guinea-pig atria, McCulloch, Rand and Story, 1972; rabbit pulmonary artery, Endo et al., 1977; rabbit ear artery, Hope et al., 1976; rabbit heart, Starke, 1972a and cat nictitating membrane, Enero and Langer, 1975). These studies demonstrated that transmitter efflux is inhibited by exogenous noradrenaline but they provided no information on the underlying mechanisms of inhibition. It is not clear from these studies whether the inhibition is mediated by a presynaptic autoinhibitory system that is sensitive to the perineural concentration of transmitter, since no comparative data on efflux at frequencies other than the selected one is available. One study in which a range of physiological frequencies was employed is that of Langer, Dubocovich and Celuch (1975). These investigators, in proposing the "Calcium theory" as a mechanism of presynaptic inhibition of efflux (to be discussed later), reported that noradrenaline inhibited efflux of tritium at 1, 2 and 5 Hz with decreasing effectiveness in the cat spleen.

In addition to the observations that noradrenaline inhibits and phenoxybenzamine enhances stimulation-induced efflux, another observation frequently cited as supporting evidence for the presynaptic alpha-receptor hypothesis is that noradrenaline-induced inhibition of efflux is blocked by alpha-antagonists, reflecting competition between these agents for a common presynaptic receptor site.

To examine the interaction of phenoxybenzamine and noradrenaline with presynaptic alpha-receptors, experiments were conducted on guinea-pig aorta in which the presynaptic autoinhibitory system was routinely blocked by phenoxybenzamine at several concentrations and the effect of a fixed quantity of exogenous noradrenaline on stimulation-induced efflux was examined. Efflux of tritium at 1 Hz was enhanced to 149%, 227% and 333% of control values by the haloalkylamine at three concentrations, (3×10^{-8} M, 3×10^{-7} M and 3×10^{-6} M, respectively) suggesting that the phenoxybenzamine - sensitive locus of the presynaptic autoinhibitory loop was blocked and that the degree of blockade was dependent on the concentration of the antagonist. If this locus also mediates the inhibitory action of noradrenaline, tissues pretreated with phenoxybenzamine should exhibit diminished stimulation-induced efflux in the presence of noradrenaline in increase proportion to the magnitude of phenoxybenzamine enhancement. That is, as the concentration of phenoxybenzamine decreases, the effect of noradrenaline on inhibition of efflux should increase. More importantly, as the concentration of phenoxybenzamine decreases, a point should be reached where the effect of the haloalkylamine is negligible and the agonist exerts its full potency. This is, however, not clearly evident

in the guinea-pig aorta stimulated with a moderate and probably physiological frequency (1 Hz) at which the presynaptic autoinhibitory system is expected to be functional.

Noradrenaline (3×10^{-6} M) inhibited efflux of transmitter at 1 Hz by 70%, and this was blocked by phenoxybenzamine (3×10^{-8} M, 3×10^{-7} M, 3×10^{-6} M). As the concentration of phenoxybenzamine decreased, the effectiveness of noradrenaline gradually recovered. However, a full recovery of the agonist effect was obtained, as assessed by the ratio of efflux ratios, under conditions where the antagonist (at 3×10^{-8} M) still partially blocked the presynaptic receptor site, as evident by a significant enhancement of efflux. Observations of this sort has never been reported for the postsynaptic alpha-receptors, and in fact, the now accepted concept of postsynaptic adrenergic receptors would be seriously challenged if such an observation were made. Thus, the present data from guinea-pig aorta, although preliminary, raises concern as to whether the presynaptic alpha-receptor should be appropriately designated as a common binding site for both noradrenaline and phenoxybenzamine.

Investigations on the ability of phenoxybenzamine to block noradrenaline-induced inhibition of efflux are scarce. This is surprising as the phenoxybenzamine-induced enhancement of transmitter overflow is one of the crucial element in presynaptic alpha-receptor theory. It is imperative to show that this antagonist in particular blocks the presynaptic alpha-receptors for which the natural transmitter competes. Two studies in this direction were those of Starke (1972a) and of McCulloch and colleagues (McCulloch, Bevan and Su, 1975). Starke reported that the

haloalkylamine at 10^{-6} g/ml blocks the inhibitory effect of exogenous noradrenaline (at concentrations up to 10^{-7} g/ml) on stimulation-induced efflux in the perfused rabbit heart. McCulloch and colleagues showed that phenoxybenzamine at 10^{-5} M prevents the inhibition of efflux by noradrenaline at a moderate (2.5×10^{-7} M) but not at a 10-fold higher noradrenaline concentration in the rabbit pulmonary artery. They concluded that the agonist at the high concentration broke through the haloalkylamine blockade of presynaptic alpha-receptors. It is of note that in both studies only one selected concentration of phenoxybenzamine, but several concentrations of noradrenaline, were used. Since phenoxybenzamine binds "irreversibly" to the presynaptic alpha-receptor by formation of covalent linkage, increasing the quantity of biophase noradrenaline while maintaining a fixed concentration of the haloalkylamine would unlikely change the degree of presynaptic alpha-receptor blockade. These limited studies were not performed under experimental conditions where the presynaptic alpha-receptors were differentially blocked. Such a protocol is essential for the demonstration that two compounds, one designated agonist and the other antagonist, both bind to a common receptor site. Thus, although these studies have shown that the inhibitory effect of noradrenaline on efflux was reduced by pretreatment of tissues with phenoxybenzamine, they provide no compelling evidence to support the view that these agents are competing for a common receptor site.

Other experiments were conducted on the bovine radial artery in which the effects of six alpha-adrenergic antagonists and of six agonists on transmitter efflux were examined. The effects of antagonists are

especially important since these agents are supposed to nullify the autoinhibitory system and therefore increase stimulation-induced overflow of transmitter.

All six antagonists employed in the present study, namely, phenoxybenzamine, Dibenamine, chlorpromazine, yohimbine, phentolamine and tolazoline, are effective postsynaptic alpha-adrenergic blocking agents. In the present study, they consistently antagonized the contractile responses of the bovine radial artery to noradrenaline. In contrast, a consistent blockade of presynaptic alpha-receptors by these antagonists, as assessed by enhancement of transmitter efflux, was not evident.

At the highest test concentration, only phenoxybenzamine and Dibenamine significantly increased the efflux of ^{3}H-transmitter in the radial artery stimulated with 600 pulses at a physiological frequency (5 Hz). Yohimbine and chlorpromazine significantly inhibited transmitter efflux whereas phentolamine and tolazoline had no detectable effect. This is interesting as all three possible effects on efflux were obtained while, according to the concept of presynaptic feedback inhibition, only the enhancing effect should have been observed.

Phenoxybenzamine and Dibenamine always act to enhance transmitter efflux. The magnitude of enhancement by phenoxybenzamine (almost two-fold) was comparable to that observed in the bovine renal artery and the renal and femoral arteries of dog as described previously. A similar magnitude of enhancement was observed in tissues treated with Dibenamine. Thus, Dibenamine is not less potent than phenoxybenzamine (since a same magnitude

of enhancement of efflux were obtained with these agents at the same concentration), although the latter is about 10-fold more potent than Dibenamine in vascular tissues (Furchgott, 1954; 1972).

Yohimbine and chlorpromazine at the highest test concentration both decreased stimulation-induced efflux of transmitter. The observation with yohimbine is particularly noteworthy as this agent was reported by Starke and colleagues (Starke, Borowski and Endo, 1975) to be an alpha-antagonist having a preferential action on presynaptic alpha-receptor sites in rabbit pulmonary artery. These investigators demonstrated that yohimbine at 3×10^{-6} M, a concentration at which the compound inhibits efflux by 60% in the bovine radial artery, caused more than a 400% increase in transmitter overflow (at 2 Hz) in the rabbit pulmonary artery. Under the same experimental conditions, however, phenoxybenzamine caused maximally only a 200% increase in efflux. It is of interest to question why in the rabbit pulmonary artery the maximal effect of a reversible antagonist is so much higher than that of a irreversible antagonist which forms a covalent linkage with the alpha-adrenergic receptors. In the bovine radial artery, yohimbine at both moderate and high concentrations drastically reduced the stimulation-induced efflux of transmitter. This agent thus exhibits a profile of effect, characteristic of an agonist rather than an antagonist. At present it is still unclear why the effect of yohimbine on efflux is so markedly different in two different vascular preparations. It is clear, nevertheless, that the effect of yohimbine in the bovine radial artery is not mediated through the phenoxybenzamine-sensitive sites, as administration of phenoxybenzamine (3×10^{-5} M) prior to exposure of tissues to yohimbine (3×10^{-5} M) did not attenuate the

inhibitory effect of yohimbine.

A very recent publication also indicates that yohimbine does not act as a typical presynaptic alpha-antagonist. Measuring renal blood flow as an index for vascular responses, Robie (1980b) showed that yohimbine at a wide dose range (1 to 10^4 ng, injected into the renal artery during nerve stimulation) had no detectable effect on contractile responses of renal vasculature of dog to renal nerve stimulation at 1, 2, 4 or 8 Hz. According to the claim of a preferential presynaptic blocking action of yohimbine, it is expected that responses would be potentiated as a result of an increased transmitter release. The possibility that the potentiation was precisely balanced by a postsynaptic blocking action of the antagonist could be excluded since no such action was evident under these experimental conditions. Yohimbine at the same dose range did not block the vascular responses to noradrenaline, which amounted maximally to a 80% inhibition of blood flow. The author declared, based on this and additional observations with yet other alpha-agonists and antagonists, that no evidence for a physiologically significant alpha-receptor-mediated negative feedback mechanism was obtained in the dog renal vasculature in support of the present findings.

Chlorpromazine at the two lower concentrations had no detectable effect on efflux but at the highest test concentration it significantly reduced transmitter overflow. The mechanism by which chlorpromazine inhibits efflux of transmitter is not known. However, the possibility that this compound blocks presynaptic dopamine receptors can be eliminated because if this were the case then an enhancement of efflux would be

manifested since dopamine inhibits efflux in vascular tissues (Rand, McCulloch and Story, 1975). The present observation in the radial artery that chlorpromazine inhibits efflux is at variance with those of Hedqvist (1973) and of Enero and Langer (1975). Hedqvist reported that chlorpromazine increased efflux of tritium by 1.5-fold in the guinea-pig vas deferens, and also, Enero and Langer (1975) showed that this antagonist caused a 2-fold increase of transmitter overflow in the cat nictitating membrane. Thus, chlorpromazine is similar to yohimbine in that it enhances efflux in some but not in other preparations. Clearly, then, neither of them could be classified as a typical presynaptic alpha-antagonist.

The two imidazoline derivatives, namely phentolamine and tolazoline, exhibited a profile of concentration-dependent effect on efflux that has not been previously reported. Both compounds at the low concentration (3×10^{-7} M for phentolamine and 6×10^{-7} M for tolazoline) significantly increased transmitter overflow, whereas at a 100-fold higher concentration they did not have any effect on efflux. This finding, i.e. the presynaptic alpha-receptor is blocked by a low but not by a high concentration of antagonist, deserves further investigation. Phentolamine was selected as representative antagonist and its effect on efflux was examined in two other vascular preparations of cattle. In the bovine facial artery, phentolamine at 3×10^{-5} M had no detectable effect on efflux, although under the same condition phenoxybenzamine increased the transmitter overflow by 2.5-fold. Further experiments using receptor protection technique revealed that phentolamine did not compete with phenoxybenzamine for the phenoxybenzamine-sensitive sites.

In the bovine renal artery stimulated with 300 pulses at three different frequencies (1, 5 and 15 Hz), phentolamine at the low concentration (3×10^{-7} M) did not enhance efflux at any of the test frequencies, whereas interestingly, at the high concentration (3×10^{-5} M) this compound even significantly inhibited efflux of transmitter (5 and 15 Hz). On the other hand, postsynaptic responses of the renal artery strips to transmural stimulation were consistently reduced by phentolamine. Although the reduction of mechanical responses may be partly explained by the finding that phentolamine at the high concentration inhibits transmitter efflux, this explanation is not always valid as in some instances this compound has no effect on efflux but still significantly reduces mechanical responses. For example, in renal artery treated with phentolamine at 3×10^{-7} M, efflux at 5 and 15 Hz were not significantly different from the control values, yet the mechanical responses were substantially depressed. Employing the receptor protection technique, it was found that phenoxybenzamine enhanced efflux as expected, but it did not attenuate the ability of phentolamine (3×10^{-5} M) to reduce efflux. The inhibitory effect of phentolamine on efflux is therefore not achieved via an action on the presynaptic phenoxybenzamine-sensitive sites. Thus, these studies with phentolamine and tolazoline raise further doubts as to whether the presynaptic receptors proposed for the regulation of adrenergic neurotransmitter release should be clearly designated as alpha-adrenergic. The clarification of the identity of these receptor sites is important, as during the evolution of the presynaptic alpha-receptor hypothesis an assumption about the action of antagonists has been made. It is presumed

that they have a unitary presynaptic site of action, and that is at the presynaptic alpha-receptors (Starke, 1977; Langer, 1977; Westfall, 1977). The present investigations on the interactions of phenoxybenzamine and phentolamine, and that of phenoxybenzamine and yohimbine as previously described, challenge the validity of such an assumption.

Recently, Robie (1980b) demonstrated that phentolamine at a low dose (1 ng, intra-arterial injection into the renal artery) enhanced significantly the contractile responses of the dog renal vasculature to renal nerve stimulation at 1, 2 and 4 Hz. However, at higher concentrations (up to 10^4 ng) phentolamine failed to enhance the stimulation-induced vasoconstriction and at a still higher concentration (10^5 ng) it even significantly reduced the responses to nerve stimulation while exerting no blocking effect on responses to exogenous noradrenaline. This study complements and confirms the present observations on the effect of phentolamine on stimulation-induced efflux with the bovine renal artery.

Elsewhere, it has also been shown that phentolamine enhances stimulation-induced efflux. Starke and coworkers (1974) observed that the antagonist at 10^{-6} to 10^{-5} M induced a 1.5- to 1.8-fold increase in overflow of noradrenaline in rabbit pulmonary artery. Langer et al (1975) reported a 2.0- and 1.8-fold increase in rat mesenteric artery and cat aorta after treatment with phentolamine (3×10^{-6} M). Also, in the rabbit heart (Starke, 1973) and the guinea-pig atria (Rand, McCulloch and Story, 1975) treated with phentolamine at 10^{-7} M, efflux was increased respectively to 1.8- and 2.8-fold of control values (corrected for a possible effect on neuronal uptake in the guinea-pig atria). The magnitude of enhancement is

thus not great in these cardiovascular tissues and, surprisingly, only one or two concentrations of the antagonist was employed. Until recently only one study was available in which a broad range concentrations of phentolamine were employed. Using isolated strips of dog saphenous vein, Sullivan and Drew (1980) reported that phentolamine at concentrations from 10^{-7} to 10^{-5} M increased dose-dependently efflux of tritium by 1.5- to 4-fold that of controls. This finding, the implication of which is still unknown, is in marked contrast to the one reported here with the bovine artery preparations. It is interesting to note that in another recent investigation, Lorenz and colleagues (1979) employed the same preparation and stimulation frequency as those in the above-cited study, and observed that the irreversible antagonist phenoxybenzamine at 3×10^{-6} M enhanced transmitter overflow to only about 2.0-fold that of control. The magnitude of enhancement by phenoxybenzamine is substantially smaller than that by phentolamine at the same concentration, which amounted to a 4-fold increase as determined from the phentolamine dose-response curve in Sullivan and Drew's study. If these two compounds enhance efflux via a common mechanism by blocking the presynaptic alpha-receptors, then it remains to be clarified why the effect of a compound which is irreversibly blocking the presynaptic receptors is only half of that of a reversible antagonist.

There are other reports which suggest that the action of phenoxybenzamine and phentolamine are not identical. In the rat iris, phenoxybenzamine increased stimulation-induced efflux of tritium over a wide dose range from 10^{-8} to 10^{-5} M in a dose-dependent manner, whereas phentolamine

at the same dose range had an effect which was seemingly independent of dose (Farnebo and Hamberger, 1971a). In fact, the effects at 10^{-8}, 10^{-7} and 10^{-5} M did not appear to be significantly different from one another, although no probability comparison was available. Also, Garcia and colleagues (1978) reported that phenoxybenzamine enhanced potassium-induced efflux by 50% in cat spleen but phentolamine at a wide dose range (10^{-7} to 10^{-5} M) did not increase efflux and instead at higher concentrations it materially decreased overflow of noradrenaline. Although potassium releases catecholamines by a mechanism different from that with nerve stimulation, the process is nevertheless calcium-dependent and is presumably sensitive to the operation of adrenergic presynaptic mechanisms (Starke, 1977; Langer, 1977).

Basal efflux of ^{3}H-transmitter in bovine radial artery employed here was increased to varying extents by five of the selected antagonists at different concentrations. Dibenamine was the only compound that had no effect on basal efflux. There is, however, no evidence for a causal relationship between effects on stimulation-induced efflux and basal efflux. For example, yohimbine, phentolamine and phenoxybenzamine each increased basal efflux at 3 x 10^{-5} M but the first antagonist decreased, the second was without effect and the third increased stimulation-induced efflux. Also, tolazoline enhanced basal efflux to a similar extent at all three concentrations, but only at the lowest concentration did it increase stimulation-induced efflux.

In addition to the study with adrenergic antagonists, the effects of six agonists, namely noradrenaline, adrenaline, phenylephrine,

oxymetazoline, methoxamine and isoproterenol (isoprenaline), on efflux were also examined in the bovine radial artery. Stimulation-induced efflux was inhibited by oxymetazoline consistently at the three selected concentrations, by adrenaline and noradrenaline at some but not all concentrations; and was not affected at all by phenylephrine, methoxamine and isoproterenol.

The lack of effect of isoproterenol, a beta-adrenergic agonist, suggests that beta-adrenergic receptors do not play a significant role in the regulation of transmitter release. The presynaptic beta-receptor hypothesis, which proposed that transmitter release is subjected to a positive feedback regulation mediated by presynaptic beta-adrenergic receptors, was originally proposed by Langer and coworkers (1974) and was subsequently elaborated by other investigators (Stjärne and Brundin, 1975; Hedqvist and Moawad, 1975; Dahlof, Ljung and Åblad, 1978). This concept, similar to that of presynaptic alpha-receptor feedback mechanism, does not have sound experimental basis and has also been re-examined recently. Kalsner (1980b) observed that both the l- and the d-isomers of propranolol blocked the enhancing effect of isoproterenol on stimulation-induced efflux in guinea-pig atria. Since the d-isomer is devoid of any beta-blocking activity, as has been shown by the lack of a blocking effect on the mechanical responses of the atria to isoproterenol, it was concluded that the presynaptic site of action for isoproterenol is non-specific and is different from the stereospecific beta-adrenergic receptors.

Neither methoxamine nor phenylephrine at any of the selected concentrations (10^{-7} to 10^{-5} M) inhibited stimulation-induced efflux in the bovine radial artery. Recently, it was also reported that these two agonists,

at the same concentration range as used here, failed to inhibit efflux in the isolated saphenous vein of dog stimulated with 360 pulses at 2 Hz (Sullivan and Drew, 1980). One interesting comparison to be made is that between the effectiveness of phenylephrine and methoxamine in the bovine radial artery or in the dog saphenous vein and that reported for cat spleen. Kirpekar and colleagues (1973) observed that phenylephrine and methoxamine at 2×10^{-7} to 2×10^{-6} g/ml inhibited efflux of tritium maximally by 79% and 57%, respectively, in the cat spleen stimulated with 200 pulses at 30 Hz, a frequency at which the presynaptic feedback mechanism is proposed not to play an important role in this preparation (Langer, 1977). In the present study with the bovine radial artery, and also that by Sullivan and Drew (1980) with dog saphenous vein, more moderate conditions of nerve stimulation were used (5 and 2 Hz) but yet no detectable effect of phenylephrine or methoxamine was observed. The question then arises as to why these two agonists fail to inhibit efflux of transmitter at low stimulation frequencies where the autoinhibitory mechanism is proposed to be functional, but at the same concentration range, they significantly depress transmitter overflow under conditions where the presynaptic negative feedback loop has presumably been rendered inoperational.

As have been shown for many other preparations, noradrenaline, adrenaline and oxymetazoline all inhibited stimulation-induced efflux in bovine radial artery. In terms of the concentration required to achieve a maximal inhibition, oxymetazoline is of approximately the same potency as adrenaline. This is in contrast to the finding in rabbit pulmonary

artery where adrenaline was reported to be more potent than oxymetazoline (Starke, Endo and Taube, 1975). Although these agonists all inhibited efflux in the radial artery, the general applicability of these observations is still in doubt. This is further indicated in the study of the effect of oxymetazoline in another bovine artery preparation. Oxymetazoline had no effect on efflux in the bovine renal artery stimulated with 300 pulses at 5 and 15 Hz. These observations sustain the concern raised in earlier sections that profiles of agonist and antagonist effects on efflux should not be prematurely acknowledged as a basis for a receptor theory.

The unitary presynaptic receptor hypothesis was also challenged by the experiments with dopamine on the bovine renal artery. The supposition of presynaptic regulation of transmitter release was substantiated by the observation in some preparations that the effectiveness of exogenous noradrenaline to inhibit transmitter overflow was less at high than at low frequency of stimulation (Langer, Dubocovich and Celuch, 1975; Starke, 1977). This profile of noradrenaline effect, as previously discussed, is presumably a reflection of the diminishing contribution of exogenous noradrenaline to the total inhibitory effect as a result of an increasing occupancy of the presynaptic alpha-receptors by neurally-liberated transmitter. However, the validity of this presumption is in doubt since it has been shown in this investigation, as described earlier, that noradrenaline does not inhibit transmitter overflow at different physiological frequencies with an expected pattern of variation in accordance with the hypothesis. The present experiments with dopamine, were

conducted to determine whether the above-mentioned profile of inhibitory effect of exogenous noradrenaline on efflux could be used confidently as evidence supporting the existence of an ongoing autoinhibitory feedback system. Dopamine was employed since it also inhibits stimulation-induced overflow of transmitter in a variety of preparations, but its presynaptic site of action was shown to be different from that of noradrenaline (Rand, McCulloch and Story, 1975; Fuder and Muscholl, 1978; Dubocovich and Langer, 1980).

As expected, dopamine inhibited efflux of tritium in the bovine renal artery stimulated with 300 pulses, and interestingly, it did so in a frequency-dependent manner. At both selected amine concentrations the inhibition was greatest at 1 Hz, less at 2 and 5 Hz, and undetectable at 15 Hz. This profile of effectiveness of dopamine, according to the established presumption, would imply that the exogenous amine competes with an ongoing dopaminergic feedback mechanism to inhibit transmitter release. However, experiments with two potent and specific dopamine antagonists, namely pimozide and metoclopromide (Rand, McCulloch and Story, 1975; Goldberg, Volkman and Kohli, 1978), did not reveal the existence of a functional dopaminergic autoregulatory system. The specificity of the action of pimozide, a representative dopamine antagonist, was demonstrated by the finding that this compound blocks the inhibitory effect of dopamine, but not that of noradrenaline, on stimulation-induced efflux. If a presynaptic dopaminergic autoregulatory mechanism for transmitter release was operational under the present conditions, then either pimozide or metoclopromide by itself should enhance stimulation-induced efflux of transmitter,

indicating the interruption of the autoregulatory system. However, this was not observed. Efflux of transmitter at a high and at a low stimulation frequency was not significantly altered by treatment of the strips with either pimozide or metoclopromide. Thus, it is highly unlikely that a presynaptic dopaminergic feedback mechanism is present in the bovine renal artery.

In the present study exogenous dopamine inhibited transmitter overflow clearly in a frequency-dependent manner even in the absence of a dopaminergic autoregulatory system. This finding strongly suggests that it is not necessary to attribute a particular profile of agonist effect with varying frequency as a consequence of the relative contributions of exogenously-added and neurally-liberated amine to a common pool of agonist at presynaptic sites in regulating transmitter release. Although it is unknown to what extent dopamine is released from sympathetic nerve terminals into the neuroeffector cleft during nerve stimulation in the renal artery, no evidence was detected that the released dopamine, if any, is of an amount which acts together with noradrenaline to inhibit transmitter efflux. Firstly, dopamine represents only a small fraction (about 2%) of the total catecholamine content of sympathetic nerves (Smith and Winkler, 1972; Costa et al., 1972). In a variety of innervated organs of cat, namely heart, spleen, iris and nictitating membrane, the content of dopamine in the tissue homogenate is only 2-6% that of noradrenaline, and in the heart and spleen of rat, dopamine is only bearly detectable (Thoenen et al., 1967). In peripheral tissues, dopamine functions more likely as precursor for noradrenaline biosynthesis rather than as releasable

transmitter under moderate conditions of nerve stimulation. Hope and colleagues (1979) have shown that 60 minutes after incubation of rabbit ear artery with ^{3}H-dopamine, the unchanged amine and ^{3}H-noradrenaline represent 4% and 94%, respectively, of the total radioactivity in the tissue. Only after impairment of the conversion of dopamine to noradrenaline, e.g. by inhibiting dopamine-beta-hydroxylase, does the amount of dopamine in sympathetic nerves and the stimulation-induced overflow of the amine becomes significant (Thoenen et al., 1967; Hope et al., 1979). Further, neither pimozide nor metoclopromide, in the present study, enhanced contractile responses of the renal artery during nerve stimulation. An increase in the size of postsynaptic response would be expected if a dopaminergic inhibitory mechanism was interrupted. Finally, dopaminergic antagonists, e.g. sulpiride, pimozide, chlorpromazine, flupentixol, haloperidol and metoclopromide, all failed to enhance stimulation-induced efflux of transmitter in sympathetically innervated preparations (Enero and Langer, 1975; Hope et al., 1977; Fuder and Muscholl, 1978; Dubocovich and Langer, 1980; present study). One exception is the study by Hope et al. (1978) who reported that pimozide, metoclopromide and haloperidol, by themselves, slightly but significantly enhanced stimulation-induced overflow of transmitter in the perfused ear artery of rabbit. However, this finding is controversial as it has been reported earlier by Bell and Matalanis (1977) that neuronal dopaminergic sites in this preparation are not activated during normal transmission at physiological frequencies. Elsewhere, Dubocovich and Langer (1980), observing no enhancement of efflux by the dopamine antagonist sulpiride in the cat spleen, pointed out that

their results "do not support the view that the presynaptic dopamine receptors might have a physiological role in the regulation of noradrenergic neurotransmission."

The finding here with exogenous dopamine and noradrenaline in the bovine renal artery contradicts directly an important supposition of the presynaptic alpha-receptor hypothesis. Dopamine inhibited transmitter efflux in a frequency-dependent manner when a dopaminergic feedback regulatory system was apparently lacking. On the other hand, noradrenaline failed to suppress, frequency-dependently, transmitter overflow when a presynaptic alpha-adrenergic feedback system was presumably functional under the present experimental conditions. Thus, no evidence is obtained to sustain the supposition that a pattern of agonist effect which deminishes with increases in frequency of stimulation is a consequence of the interference of the agonist with the operation of an ongoing autoinhibitory feedback system for transmitter release.

In addition to the present study, other recent investigations, all from the same laboratory, also questioned the validity and physiological relevance of the presynaptic alpha-adrenergic receptor hypothesis. These observations are summarized below. (1) Both the overflow of transmitter and the mechanical responses to a single pulse stimulation are enhanced by phenoxybenzamine in the guinea-pig vas deferens (Kalsner, 1979b). Under these conditions the operation of a presynaptic feedback system is not possible since transmitter released by a single pulse cannot retroactively modify its own release. This finding readily dissociates the linkage between phenoxybenzamine-induced enhancement of neurally-liberated

transmitter and the blockade of a functional presynaptic adrenergic feedback system. (2) On examination of the profile of phenoxybenzamine effect with changes in stimulation frequency in the guinea-pig vas deferens, it was found that transmitter overflow elicited by a small number of pulses (4 pulses) at 1, 5 and 15 Hz was enhanced to the same extent by the antagonist (Kalsner, 1979c). Since the experimental conditions were optimal for the operation of the negative feedback system, the failure of phenoxybenzamine to discriminate between frequencies suggested that the feedback mechanism, presumably functional in the absence of the antagonist, is unable to detect changes in biophase amine level and adjusts accordingly the degree of restraint on transmitter release during stimulation with physiological frequencies. (3) As an extention of the last study, the effects of noradrenaline and phenoxybenzamine on transmitter efflux elicited by a greater number of pulses (10 and 50) at four different frequencies (0.5, 1, 3 and 10 Hz) were examined in the guinea-pig vas deferens (Kalsner, 1980a). The magnitude of the noradrenaline-induced inhibition of transmitter efflux, with either 10 or 50 pulses, showed no significant variations between 1 and 10 Hz. Efflux at 1, 3 and 10 Hz with 10 pulses, however, was inhibited to a significant greater extent than that with 50 pulses. In contrast, phenoxybenzamine-induced enhancement of transmitter overflow exhibited a profile of diminishing effectiveness with increases in frequency. There was no statistically significant difference between enhancement of efflux with 10 pulses and that with 50 pulses at each frequency. Thus, the effect of noradrenaline was sensitive to pulse train length but not to stimulation frequency, whereas the opposite pattern

was observed with phenoxybenzamine. This finding suggests that these two compounds may not have a common neuronal site of action to modify transmitter overflow. Further, since both noradrenaline and phenoxybenzamine had their most intense effect at the lowest frequency (0.5 Hz), a pattern also observed here with the bovine renal artery, it is unlikely that these compounds act as agonist and antagonist to interfere with an underlying adrenergic mechanism regulating transmitter release. (4) In the guinea-pig atria, the effects of noradrenaline and phenoxybenzamine on transmitter efflux at different frequencies, again, were not in accord with the requirements of an autoinhibitory system (Kalsner et al., 1980). Percentage of noradrenaline inhibition of efflux with 100 pulses at the low frequency range of 0.5, 1 and 2 Hz did not differ significantly from each other nor did that between 5 and 10 Hz. A similar lack of discrimination among frequencies was observed with phenoxybenzamine which enhanced efflux at the three lower frequencies consistently by 230-240%. This observation further confirms the findings in guinea-pig vas deferens and bovine renal artery that both the agonist and the antagonist have the greatest effect at low frequency. Thus, the profile of effects of noradrenaline and of phenoxybenzamine do not conform to the demand of the hypothesis. Importantly, in none of the frequency-efflux studies, either the present one or those mentioned above, was a reciprocal pattern between the effects of noradrenaline and phenoxybenzamine observed. The demonstration of this pattern of effects is essential for the survival of the presynaptic alpha-receptor hypothesis as this is a prerequisite for the designation of noradrenaline and phenoxybenzamine as agonist and antagonist

which interact with presynaptic receptor sites.

Elsewhere, Robie (1980 a,b) observed no significant effects of three agonists (oxymetazoline, clonidine and noradrenaline) and two antagonists (yohimbine and phentolamine) on the vasoconstrictor responses to renal nerve stimulation in the in situ perfused kidney of dog. He stated "these studies failed to reveal a physiological significant alpha$_2$-receptor-mediated negative feedback mechanism for stimulation-induced vasoconstriction in the canine renal vascular bed.", in support of the present study.

Evidence which appears to sustantially support the presynaptic receptor hypothesis is that of Enero and Langer (1973) and Cubbedu and Weiner (1975) which shows that phenoxybenzamine enhancement of efflux was reduced after depletion of transmitter with either reserpine or alpha-methyl-p-tyrosine. This is presumably a result of a decreased amount of transmitter liberated into the synaptic cleft with each frequency. However, there are reservations on the interpretation of the data since before the mechanism of phenoxybenzamine action can be established, the actual mode of interaction between the antagonist and noradrenaline depleting agents must be explored.

It is worth mentioning, at this point, the proposed mechanism for the presynaptic feedback regulation of transmitter release, i.e. the "Calcium theory" which assigns to calcium the role of linkage between presynaptic receptor activation and transmitter release (Stjärne, 1978c; Kirpekar, Prat and Wakade, 1975; Langer, Dubocovich and Celuch, 1975; Starke, 1977; Westfall, 1977). According to this theory, the negative feedback loop operates by inhibition of calcium influx into the nerve

endings and, because of the absolute requirement of calcium ion in the electrosecretory coupling process, transmitter release is accordingly reduced. The basis of this hypothesis is the observation that presynaptic antagonists enhance transmitter efflux elicited by processes that are calcium-dependent, e.g. nerve stimulation or potassium-induced depolarization, but not by processes that do not require the presence of calcium, e.g. tyramine-induced release of transmitter. Such evidence is obviously highly indirect as it is not clear whether the difference in these observations is due to a lack of calcium, or of some other intermediate that may also be involved in the complex of events of transmitter release.

One attractive aspect of the calcium theory is that excessive accumulation of calcium ion in the nerve terminals at high frequencies of stimulation, or after alpha-antagonists, is presumed to "desensitize" the hypothetical calcium receptor and explain the ineffectiveness of the presynaptic feedback loop at very high frequencies (Kirpekar, Prat and Wakade, 1975; Langer, Dubocovich and Celuch, 1975). In the cat spleen perfused with standard calcium-Krebs solution, the effectiveness of phenoxybenzamine to enhance transmitter efflux at a low frequency (5 Hz) was about 2.5 times that at a high frequency (30 Hz), as assessed by the efflux ratios (Langer, Dubocovich and Celuch, 1975). On a ten-fold reduction of the calcium concentration, enhancement of efflux at 30 Hz was substantially increased as compared to that observed in spleen stimulated at 30 Hz and perfused with standard Krebs solution. This latter observation was interpreted to signify restoration of the regulatory role of the autoinhibitory mechanism which would otherwise be desensitized at high

frequencies of stimulation due to a marked influx of calcium. However, a genuine concern arises if the change in the effectiveness of phenoxybenzamine at 5 and 30 Hz during perfusion with low calcium Krebs solution are analyzed. In this case the effect of phenoxybenzamine (efflux ratio) at 30 Hz was one third lower than that at 5 Hz. If during perfusion with standard calcium the nerve terminal is desensitized at high frequency, then lowering the amount of calcium in the medium would reduce greatly the amount of desensitization. According to the calcium theory the only limiting factor under such circumstances then becomes the synaptic concentration of transmitter which should result in an inhibition proportional to the frequency of stimulation. Therefore, phenoxybenzamine should have a progressive increasing effect on transmitter overflow with rises in frequency. That the opposite pattern of effect was obtained is contradictory to the calcium theory and raises doubts concerning the validity of this theory as a proposed mechanism for presynaptic feedback regulation of transmitter release.

In conclusion, little evidence was obtained in the present study to support the presynaptic alpha-adrenergic receptor hypothesis. According to the present data, the presynaptic negative feedback system, if it exists at all, is unable to detect differences in biophase concentration of transmitter and thus serves no important physiological functions. The concept of a unitary presynaptic site of action for adrenergic agonists and antagonists, and also the possible linkage of a particular pattern of agonist effect to the operation of an ongoing feedback loop, cannot be confirmed. The mechanisms of action of noradrenaline and phenoxybenzamine

to modify transmitter overflow are more complicated than have been understood previously. These observations cannot be adequately accounted for by the presynaptic receptor hypothesis. A re-examination and a reconstruction of this hypothesis is necessary.

PART II. SENSITIZATION OF VASCULAR EFFECTOR RESPONSES AND TERMINATION OF ACTION OF ADRENERGIC TRANSMITTER

Sensitization of effector responses to noradrenaline by uptake inhibitors and termination of action of the adrenergic transmitter were studied in the bovine radial artery using the perfusion technique of De La Lande and colleagues as modified (De La Lande and Rand, 1965; De La Lande et al., 1967; Kalsner, 1972a). With this technique noradrenaline can be selectively administered to either the medial or the adventitial side of the vessel. Understandably, intraluminally added agonists would encounter less access barrier en route to effector cells than would the extraluminally added agonists, as in the latter case a portion of the agonist molecules is taken up by the nerve plexus located between the media and the adventitia before reaching the target cells. Thus, depending on the route of agonist, two different functions of neuronal uptake could be identified, i.e., to participate with extraneuronal uptake in terminating the action of noradrenaline, or to act as an access barrier impeding noradrenaline molecules en route to effector cells.

In the perfused bovine radial artery used here, responses to extraluminally added noradrenaline were sensitized by blockade of either neuronal or extraneuronal uptake. Cocaine and estradiol induced 4- and 2.5-fold shift respectively of the mean effective concentration of noradrenaline (ED_{50}). The much greater effect of cocaine implies that neuronal uptake functions mainly as an access barrier, since inhibition

of this barrier, as compared to that of a terminating mechanism, is more effective in increasing concentration of agonist at the receptor site. This is so because an open up access barrier would allow free passage of agonist molecules in perhaps unlimited amount to the receptor region, whereas a suppressed terminating mechanism could at most increase the biophase concentration of agonist by the amount which would otherwise be inactivated by that mechanism. The effect of cocaine, if attributed entirely to its action on terminating mechanism, would mean that 75% of the active amine molecules at the receptor site is removed by neuronal uptake. In other words, if this interpretation were valid then extraneuronal uptake and diffusive dilution of agonist, the two major terminating mechanisms in vascular tissues in general, together contribute to only a relatively small percentage (i.e. the remaining 25%) of the total tissue's capacity to inactivate noradrenaline. Considering the close proximity of effector cells to the receptor region, and the generally wide synaptic clefts in vascular preparations, it is unlikely that extraneuronal uptake and diffusive dilution of agonist play only an insignificant role to terminate the action of agonist.

Further, if the effect of cocaine is purely due to an action on terminating mechanism, then a rational interpretation could not be reached for the observation that potentiation of response was not further enhanced when cocaine and estradiol were concomitantly administered. Since both neuronal and extraneuronal uptake, as two discrete terminating mechanisms, are competing for the removal of a common pool of agonist, the additional blockade of one when the other is already

inhibited should result in a drastic increase in response potentiation. As has been emphasized by Kalsner (1976), the effect of combined inhibition of two terminating mechanisms on potentiation of response would be greater than the sum of the effects of the inhibition of each mechanism alone, since it is not the individual effects, but rather the reduction in the tissue's capacity to inactivate agonist that is additive. Thus, the present finding that a combined administration of cocaine and estradiol produced an effect which was not even as great as the sum of the individual effects of these two inhibitors suggests that a mechanism unrelated to termination of agonist action should be sought.

On examination of the data on recovery of responses, a direct assessment of termination of agonist action, it was revealed that the half recovery time (T_{50}) was prolonged by estradiol but not by cocaine. However, the corresponding T_{75} i.e. the time taken for responses to recover to 75% of the initial magnitude, was significantly but slightly increased by each of the uptake inhibitors. Thus, functioning as a terminating mechanism, neuronal uptake is only moderately influential and only when the biophase concentration of active amine has declined to a low level. In contrast, extraneuronal uptake is active as an inactivation process during the entire course of agonist dissipation. Recovery of responses was not prolonged much more by a combined administration of cocaine and estradiol, suggesting that one of the compounds, namely cocaine, participates to a relatively small extent in termination of agonist action by an action independent of estradiol.

Potentiation of responses to intraluminally added noradrenaline differs considerably from that of extraluminally applied noradrenaline. In both the cocaine- and estradiol-treated preparations, the ED_{50} was decreased nearly 2-fold. The cocaine-induced potentiation, as assessed by the ratio of ED_{50}s, was only half that observed with extraluminally added noradrenaline. This is expected as neuronal uptake is not the factor limiting the availability of agonist to the receptor region. Relatively, the estradiol-induced potentiation of responses to intraluminally added noradrenaline was not much different from that observed with extraluminally added noradrenaline, supporting the view that extraneuronal uptake serves mainly as a terminating mechanism since sensitization caused by inhibition of these mechanisms is essentially independent of the route of agonist (Kalsner, 1977).

The combined administration of cocaine and estradiol did not at all potentiate the responses to intraluminally added noradrenaline. A possible interpretation for this observation is that potentiation of responses was masked by a direct depressant effect of the sensitizing agents when they were given together. This depressant effect, the mechanism of which is still unknown, was also observed under other circumstances. In the present study, in four out of nine radial artery preparations treated with cocaine and estradiol, the responses to neurally-released noradrenaline were substantially desensitized as compared to the control. Also, Kalsner (1979a) found that the potentiation of responses in a bovine facial artery preparation to noradrenaline, in the presence of both cocaine and estradiol, were not significantly different from that

observed in the presence of either uptake inhibitor alone. It appears that in this preparation a further potentiation of response, which would otherwise be observed, was hindered by some other action of the uptake inhibitors when they were administered together.

Recovery of responses to intraluminally added noradrenaline in the radial artery were delayed by drug treatment in direct proportion to the extent of drug-induced enhancement of responses. T_{50} increases with increasing concentration of injected noradrenaline, and is significantly prolonged in the presence of either cocaine or estradiol. It should be noted that the T_{50} was increased in the cocaine-treated preparations to the same extent as in the estradiol-treated vessels (both to about 1.5-fold of control), a similar finding as in the case of the corresponding ED_{50}s. Further, a relationship of "no sensitization and no prolongation of responses" was clearly observed in preparations treated with both cocaine and estradiol. Thus, it is justified to correlate the increase in T_{50}s after uptake inhibitors with the corresponding extent of response potentiation.

Responses to nerve stimulation were significantly enhanced by either cocaine or estradiol, as assessed by a comparison of the shift of stimulation frequency required to elicit a given response in the absence and the presence of the uptake inhibitor. Unlike what was observed with extraluminally-added noradrenaline, the potentiating effect of cocaine was similar to that caused by estradiol. This effect of cocaine cannot be solely attributed to an action on access barrier. Rather, some other action, such as inhibition of terminating mechanism, which has a less

profound impact on the magnitude of potentiation is probably involved. It appears that cocaine has a mixed action involving both access barriers and terminating mechanisms. It is likely that a fraction of neurally-released transmitter is taken up into the nerve terminals immediately after its release (i.e. neuronal uptake as an access barrier). Another fraction of transmitter subsequently diffuses into the receptor region and, after producing a physiological response and then is subjected to neuronal uptake which then acts as a terminating mechanism.

That the cocaine-induced potentiation of responses to nerve stimulation is partly due to an action on terminating mechanism is supported by the observation that recovery of responses at all frequencies was significantly prolonged by cocaine, as determined by the ratio of T_{50}s. Further, prolongation of T_{50} by estradiol is substantially greater than that by cocaine, providing evidence for a crucial role of extraneuronal uptake as an inactivation process.

In the present experiments with radial artery strips set up in muscle chambers, cocaine induced a shift of 3.4 in ED_{50} ratio for noradrenaline, whereas estradiol did not potentiate the responses to the agonist but desensitized the maximal response to noradrenaline by a non-specific action and such an effect spread throughout the concentration-response curve could obviously obscure sensitization. The rather considerable magnitude of potentiation after cocaine may represent an effect on an access barrier. Although agonist was not selectively applied to one side of a spiral strip preparation, it is nevertheless subjected

to access limitations. Analysis of recovery of responses, showed previously, in the perfused radial artery, has revealed that extraneuronal uptake does play an important role in terminating the action of endogenous and exogenous noradrenaline. That combined administration of cocaine and estradiol did not produce a potentiation of responses greater than that of cocaine alone provides further support for an action of one of the compounds on an access barrier and the other on a terminating mechanism. Another possibility is that diffusive dilution of agonist may take over the inactivation role of extraneuronal uptake after the latter process has been inhibited; explaining the lack of potentiation.

Cocaine and estradiol each reduced approximately half the accumulation of ^{3}H-noradrenaline in the radial artery. However, percentage of tissue accumulation of ^{3}H-noradrenaline provides only a qualitative measure of the two uptake processes and cannot be correlated to the amount of sensitization of responses. For example, it is erroneous to speculate, based on uptake experiments, that the biophase concentration of agonist during the plateau response is doubled in the presence of either cocaine or estradiol and thus a 2-fold sensitization of response should be observed. This is because neuronal and extraneuronal uptake together do not represent the tissue's capacity to remove noradrenaline from sites of action. The contribution of diffusive dilution, and perhaps still other unknown processes, cannot be ignored. A further consideration compounding analysis is that the cocaine-sensitive and the estradiol-sensitive sites of action are sometimes overlapped, as

is the case in the radial artery strips. It is thus difficult to assess precisely what proportion of the total tissue accumulation of radioactivity should be attributed to neuronal or extraneuronal transport.

De La Lande and colleagues (1967) reported that, in the perfused ear artery of rabbit, sensitization of responses to extraluminally added noradrenaline by cocaine is materially greater than to the intraluminally added agonist. This observation was later confirmed by other workers (Yong and Chen, 1975). Since neuronal uptake is involved in one but not in the other agonist route, the finding has been taken as an important evidence to support the unitary hypothesis of cocaine sensitization. That is, cocaine sensitizes effector responses primarily by an inhibitory action on neuronal uptake which presumably represents most if not all the tissue's capacity to inactivate noradrenaline at the receptor region. These authors did not consider an action of cocaine on access barrier as an alternate or at least as a concomitant process. In the study of De La Lande and colleagues, the response duration after cocaine was not recorded and thus comparison between the recovery of response and the magnitude of cocaine sensitization cannot be made. This latter analysis is essential as it is the only means by which actions on access barrier and on terminating mechanisms could be distinguished (Kalsner, 1976). In the present study with the perfused radial artery, such analysis was made and it provides an alternate explanation for the cocaine-induced potentiation under the circumstance.

Studies on the relationship between response magnitude and the

duration of response in perfused preparations are scarce. Two such studies are those of Gillespie (1969) and of Kalsner (1972b). Gillespie reported that responses to nerve stimulation in the perfused rabbit ear artery were substantially prolonged after diffusive dilution of agonist was eliminated by replacing the Krebs solution (both extraluminal and intraluminal) with paraffin oil. However, magnitude of response was not altered under such conditions. Since diffusive dilution of agonist does play a significant role in the termination of agonist action, Gillespie's finding thus supports the view that inhibition of a single termination mechanism does not as a rule sensitize effector response. Kalsner observed that the decline of the response to continuous nerve stimulation in the rabbit ear artery was shortened after inhibition of tyrosine hydroxylase with alpha-methyl-p-tyrosine, but not after blockade of neuronal uptake by cocaine. He concluded that synthesis of noradrenaline, but not neuronal re-uptake of amine, is the major mechanism maintaining the response to continuous nerve stimulation.

In addition to the present investigation, there are other studies in which doubts were raised on De La Lande's view that neuronal uptake per se is a primary factor for the route-dependent sensitivity to agonist in perfused vascular preparations. Kalsner (1975b) pointed out that the enormous cocaine-induced sensitization of responses to extraluminally added noradrenaline in the rabbit ear artery, which varies from 3- to 17-fold increase in responses (De La Lande et al., 1967), could not be adequately accounted for by a presynaptic action of cocaine. Based on results obtained from rabbit aortic strips (Kalsner, 1966;

Kalsner and Nickerson, 1969b), he provided evidence that a postsynaptic action of cocaine unrelated to agonist disposition was responsible for the sensitization of responses. Although such action of cocaine was not sought in the present study with the bovine radial artery, abundant experimental support for a postsynaptic action of cocaine is now available (Bevan and Verity, 1967; Varma and McCullouch, 1969; Davidson and Innes, 1970; Greenberg and Long, 1971). These studies were discussed earlier in Literature Review. Recently, Simmers and Tillman (1979) reported that responses of cat spleen strips to noradrenaline and to potassium were both enhanced by cocaine. Since the potentiation of responses to noradrenaline could be eliminated by SKF-525A, a calcium influx inhibitor (Kalsner, Nickerson and Boyd, 1970), these authors thus concluded that cocaine sensitizes effector response mainly by an action facilitating the influx of calcium ion across the effector cell membrane.

The concept of access barrier as an alternate interpretation to terminating mechanism for cocaine-induced sensitization, although recent (Kalsner, 1977), is not without experimental support. O'Connor and Slater (1981) studied the isometric contractile responses to noradrenaline in the perfused mesenteric vein of rat, a preparation which has definable inner circular and outer longitudinal muscle fibre layers. In contrast to the present observation on the bovine radial artery and that of De La Lande on the rabbit ear artery, it was found that responses to intraluminally added noradrenaline was potentiated more by cocaine than those to extraluminally added noradrenaline. The dose ratio for

intraluminally and extraluminally added noradrenaline in the absence and presence of cocaine was 14.5 and 3.38 respectively. According to the authors the inner circular muscle layers in this preparation are more densely innervated than the outer longitudinal muscle layers. Thus, the route-dependent potentiation of responses by cocaine is most likely due to an action on access barrier. This becomes more obvious when the magnitudes of sensitization are considered. The drastic sensitization of responses to intraluminally added agonist is what would be expected if an access barrier is inhibited. This finding provides a complementary observation to the present one and further supports the present view of an action of cocaine on access barriers.

In short, the present experiments with cocaine and estradiol, using a novel preparation, show that these agents sensitize responses to both endogenously released or exogenously added noradrenaline. The magnitude of sensitization is dependent on many factors. Of these factors the most influential one is whether or not a barrier limits the accessibility of agonist to the receptor region. Based on an analysis of time for recovery from responses, it is concluded that extraneuronal uptake plays a more significant role than neuronal uptake in the inactivation of noradrenaline. The recognition of both functional access barriers and terminating mechanisms is essential and would avoid confusion in interpretating sensitization phenomena on the basis of a unitary and simplified hypothesis of cocaine action.

VI. SUMMARY

1. The hypothesis was examined that presynaptic alpha-adrenergic receptors mediate an inhibitory feedback mechanism regulating the release of adrenergic neurotransmitter during sympathetic nerve stimulation.

2. A variety of vascular preparations from guinea-pig, dog and cattle, preincubated with ^{3}H-l-noradrenaline, were stimulated with a constant number of pulses at different frequencies. Efflux of tritium was measured in the absence and the presence of adrenergic agents.

3. In the bovine renal artery strips stimulated with 300 pulses at 1, 2, 5, 10 and 15 Hz, frequencies spanning the physiological range, contractile responses to nerve stimulation exhibited a clear gradation with increasing frequency, reflecting the variation in biophase concentration of free and active noradrenaline. However, efflux of tritium was not at all correlated to the stimulation frequency. It was essentially unchanged over the entire test frequency span except at 5 Hz where the output was somewhat higher. As well, in the aorta, carotid and femoral arteries of dog, and the aorta of guinea-pig stimulated with 200 pulses at 1 and 5 Hz, efflux of tritium at the moderate frequency was not significantly less than that at the low frequency.

4. Phenoxybenzamine (3×10^{-5} M) increased the efflux of tritium in the bovine renal artery mostly at 1 Hz and to a similar extent at the other test frequencies, except at 10 Hz where its effect was slightly reduced. Similarly, the haloalkylamine at 3×10^{-6} M enhanced efflux in the carotid artery of dog more at 1 Hz than at 5 Hz. In still other preparations, namely aorta of guinea-pig, aorta, femoral and renal

arteries of dog, the antagonist (3×10^{-6} M) enhanced efflux at 1 Hz and 5 Hz to a similar magnitude.

5. The inhibitory effect of noradrenaline (3×10^{-6} M) on efflux in the bovine renal artery was essentially constant at 2, 5 and 15 Hz, except at 1 Hz where the effect is slightly greater. In the aorta, carotid and renal arteries of dog, the agonist inhibited efflux at 1 and 5 Hz to a similar extent. These observations with the agonist and antagonist suggest that the proposed presynaptic alpha-receptor negative feedback function is insensitive to changes in synaptic levels of transmitter.

6. In the bovine renal artery, both phenoxybenzamine (3×10^{-5} M) and noradrenaline (3×10^{-6} M) had their most pronounced effect on efflux at the lowest test frequency (1 Hz). In the guinea-pig aorta in which the presynaptic alpha-receptor are differentially blocked by pretreatment with phenoxybenzamine at a wide concentration range (3×10^{-8} M to 3×10^{-6} M), the inhibitory effect of noradrenaline (3×10^{-6} M) on efflux at 1 Hz was not accordingly diminished. In fact, phenoxybenzamine at 3×10^{-8} M, which by itself significantly enhanced efflux at 1 Hz, did not at all attenuate the inhibitory effect of noradrenaline. Thus, it appears that these two compounds possibly did not have a common site of action for their effects on stimulation-induced transmitter efflux.

7. The effects of six adrenergic antagonists at a wide concentration range were examined in the bovine radial artery to assess how they conform to the expectations of presynaptic alpha-receptor hypothesis. Efflux of tritium at 5 Hz (600 pulses) was enhanced by Dibenamine and

phenoxybenzamine, and decreased by yohimbine and chloropromazine. On the other hand, phentolamine and tolazoline increased efflux at a low but were without effect at a high concentration. Pretreatment of tissues with phenoxybenzamine did not diminish the inhibitory effect of yohimbine on efflux ruling out action as a partial agonist. In the bovine facial artery phenoxybenzamine (3×10^{-5} M) significantly enhanced the efflux of tritium but phentolamine (3×10^{-5} M) did not. Experiments in which a receptor protection technique was used indicated that phenoxybenzamine and phentolamine did not compete for a common presynaptic site. In the bovine renal artery phentolamine at 3×10^{-7} M did not enhance stimulation-induced efflux and at a high concentration (3×10^{-5} M) the antagonist even significantly inhibited transmitter output. This inhibitory effect was not reduced by pretreatment of the tissues with phenoxybenzamine, suggesting a phenoxybenzamine-insensitive site of action for phentolamine. These observations that only some but not all alpha-adrenergic antagonists act to enhance transmitter efflux raise doubts as to whether the proposed presynaptic site of action for these compounds should be appropriately designated adrenergic.

8. Similar to that with the antagonists, the effects of six alpha-adrenergic agonist at three concentrations (10^{-7} - 10^{-5} M) were examined in the bovine radial artery. Stimulation-induced efflux was decreased by oxymetazoline consistently at all concentrations by noradrenaline and adrenaline at high but not at low concentrations, and was not affected at all by phenylephrine, methoxamine and isoproterenol. The inhibitory effect of oxymetazoline was not detectable in the bovine

renal artery stimulated with 300 pulses at 5 and 15 Hz. These experiments with the agonists provide parallel observations to that of the antagonists and they together reinforce the concern that inhibition of efflux by agonists and its enhancement by antagonists should not be prematurely acknowledged as sufficient evidence forming a receptor theory.

9. The effect of dopamine on efflux was examined in bovine renal artery stimulated with 300 pulses at a wide frequency range (1 - 15 Hz). Dopamine (3×10^{-7} and 3×10^{-6} M) inhibited efflux of tritium in a frequency dependent manner. In fact, the percentage inhibition of efflux by dopamine at both concentrations was positively correlated to the length of the stimulus intervals. The inhibitory effect of dopamine but not that of noradrenaline was antagonized by pimozide and metoclopromide, two known antagonists of dopamine, confirming the specificity of dopamine action. Pimozide and metoclopromide by themselves neither enhanced stimulation-induced efflux nor increased the magnitude of contractile responses to nerve stimulation, revealing no evidence for the operation of an ongoing negative feedback loop mediated by dopamine. These results suggest that a profile of inhibitory effect of exogenous amine on transmitter efflux which exhibits a negative correlation between the degree of inhibition and increases in stimulation frequency is not due to an intervention of the foreign amine in the operation of an autoinhibitory feedback system.

10. The bovine radial artery (branches) was perfused through its lumen such that exogenous agonist could be administered selectively

via the intraluminal or the extraluminal route. Vasoconstrictor responses to noradrenaline and to periarterial nerve stimulation, and their modification by cocaine (3×10^{-5} M) and estradiol (3.6×10^{-5} M), were studied.

11. Cocaine and estradiol induced a 4- and a 2.5-fold shift, respectively, of the mean effective concentration of extraluminally added noradrenaline (ED_{50}). Contractile responses were only slightly further potentiated by a combined administration of both uptake inhibitors. The potentiating effect of cocaine cannot be adequately explained by an unitary action on terminating mechanisms.

12. Although the time required for responses to extraluminally added noradrenaline to recover to 15% of basal level (T_{15}) was prolonged by both cocaine and estradiol, the corresponding T_{50} was prolonged only by the steroid but not by cocaine, suggesting an important role of extraneuronal uptake as an inactivation mechanism.

13. Responses to intraluminally added noradrenaline were potentiated to approximately the same extent by cocaine and by estradiol. However, potentiation of responses was masked by an unknown depressant effect of cocaine and estradiol when they were administered together. Recovery of responses to noradrenaline added intraluminally was prolonged by either cocaine or estradiol in proportion to their individual potentiating effects on response magnitude.

14. Responses to periarterial nerve stimulation were potentiated by cocaine and by estradiol, as assessed by the shift of stimulation frequency at three response amplitudes. An approximately 4-fold

shift of stimulation frequency was observed in all cases. Estradiol delayed the recovery of responses to a greater extent than did cocaine.

15. In the bovine radial artery strips (branches) set up in muscle chambers, ED_{50} for noradrenaline was shifted 3.4-fold by cocaine. On the other hand, vasoconstrictor responses to noradrenaline were not potentiated by estradiol. The utilization of response potentiation as a reliable criterion for impairment of terminating mechanism is discussed.

16. Accumulation of radioactivity in bovine radial arteries (branches), preincubated with ^{3}H-dl-noradrenaline (6×10^{-7} M), was significantly reduced by cocaine and by estradiol. A further reduction in tissue radioactivity was observed when both uptake inhibitors were administered together. These observations confirm two possibly overlapping sites of action of cocaine and estradiol.

17. It is concluded that extraneuronal uptake plays a more important role than neuronal uptake in termination of action of adrenergic transmitter in the bovine radial artery, and that the drastic potentiating effect of cocaine is due to two independent actions on terminating mechanism and on access barriers.

VII. BIBLIOGRAPHY

ADLER-GRASCHINSKY, E. and LANGER, S.Z.: Possible role of a beta-adrenoceptor in the regulation of noradrenaline release by nerve stimulation through a positive feedback mechanism. Br. J. Pharmacol. 53: 43-50 (1975).

ANDÉN, N.-E. and STRÖMBOM, U.: Stimulation of control adrenergic alpha-receptors by L-dopa, alpha-methyldopa and clonidine. In "Central action of drugs in blood pressure regulation," (Eds. Davis, D.S. and Reid, L.J.), University Park Press, London, pp. 225-240 (1975).

ANDREWS, W.H.H.: A blood outflow recorder. J. Physiol. (Lond.) 117: 45p-46p (1952).

ANTONACCIO, M.J., HALLEY, J. and KERWIN, L.: Functional significance of alpha-stimulation and alpha-blockade on responses to cardiac nerve stimulation in anesthetized dogs. Life Sci. 15: 765-777 (1974).

ANTONACCIO, M.J. and ROBSON, R.D.: The effect of clonidine on adrenergic nerve function in vagotomized and nonvagotomized animals. J. Pharmacol. Expt. Ther. 184: 631-640 (1973).

ARMSTRONG, M.D., McMILLAN, A. and SHAW, K.N.F.: 3-Methoxy-4-hydroxyl-mandelic acid, a urinary metabolite of norepinephrine. Biochem. Biophys. Acta 25: 422-425 (1957).

AVAKIAN, O.V. and GILLESPIE, J.S.: Uptake of noradrenaline by adrenergic nerves, smooth muscle and connective tissue in isolated perfused arteries and its correlation with the vasoconstrictor response. Br. J. Pharmacol. 32: 168-184 (1968).

AXELROD, J.: Methylation reactions in the formation and metabolism of catecholamines and other biogenic amines. Pharmacol. Rev. 18: 95-113 (1966).

AXELROD, J., HERTTING, G. and POTTER, L.T.: Effect of drugs on the uptake and release of ^{3}H-norepinephrine in the rat heart. Nature (Lond.) 194: 297 (1962).

AXELROD, J. and TOMCHICK, R.: Enzymatic O-methylation of epinephrine and other catechols. J. Biol. Chem. 233: 702-705 (1958).

AXELROD, J., WEIL-MALHERBE, H. and TOMCHICK, R.: The physiological disposition of ^{3}H-epinephrine and its metabolite metanephrine. J. Pharmacol. Expt. Ther. 127: 251-256 (1959).

BELL, C.: Release of endogenous noradrenaline from an isolated elastic artery. J. Physiol. (Lond.) 236: 473-482 (1974).

BELL, C. and MATALANIS, G.: Dopamine-induced depression of adrenergic nerve-mediated contraction of smooth muscle. Br. J. Pharmacol. 61: 291-295 (1977).

BELL, C. and VOGT, M.: Release of endogenous noradrenaline from an isolated muscular artery. J. Physiol. (Lond.) 215: 509-520 (1971).

BENNETT, A., FREIDMANN, C.A. and VANE, J.R.: Release of prostaglandin E_1 from the rat stomach. Nature 216: 873-876 (1967).

BEVAN, J.A.: Norepinephrine and the presynaptic control of adrenergic transmitter release. Fed. Proc. 37: 187-190 (1978).

BEVAN, J.A. and SU, C.: Sympathetic mechanisms in blood vessels: nerve and muscle relationships. Ann. Rev. Pharmacol. 13: 269-285 (1973).

BEVAN, J.A. and VERITY, M.A.: Sympathetic nerve free vascular muscle. J. Pharmacol. Expt. Ther. 157: 117-124 (1967).

BLAKELEY, A.G.H., BROWN, G.L. and FERRY, C.B.: Pharmacological experiments on the release of the sympathetic transmitter. J. Physiol. (Lond.) 167: 505-514 (1963).

BLAKELEY, A.G.H. and SUMMERS, R.J.: The effects of piperoxan on uptake of noradrenaline and overflow of transmitter in the isolated blood perfused spleen of the cat. Br. J. Pharmacol. 63: 683-687 (1978).

BLASCHKO, H., RICHTER, D. and SCHLOSSMANN, H.: The inactivation of adrenaline. J. Physiol. (Lond.) 90: 1-17 (1937).

BÖNISCH, U. and UHLIG, W.: Uptake and metabolism of isoprenaline in the isolated perfused heart of the rat and guinea-pig. Arch. Pharmacol. 277: R6 (1973).

BOROWSKI, E., EHRL, H. and STARKE, K.: Relative pre- and postsynaptic potencies of alpha-adenolytic drugs. Arch. Pharmacol. 293: R2 (1976).

BOULLIN, D.J., COSTA, E. and BRODIE, B.B.: Evidence that blockade of adrenergic receptors causes overflow of norepinephrine in cat's colon after nerve stimulation. J. Pharmacol. Expt. Ther. 157: 125-134 (1967).

BRANDÃO, F., RODRIGUES-PEREIRA, E., MONTEIRO, J.G. and OSSWALD, W.: Characteristics of tyramine induced release of noradrenaline: mode of action of tyramine and metabolic fate of the transmitter. Arch. Pharmacol. 311: 9-15 (1980).

BROWN, G.L.: Release of sympathetic transmitter by nerve stimulation. In "Adrenergic mechanisms," (Ed. Vane, J.R.), J. & A. Churchill Ltd., London, pp. 116-130 (1960).

BROWN, G.L. and GILLESPIE, J.S.: Output of sympathin from the spleen. Nature 178: 980 (1956).

BROWN, G.L. and GILLESPIE, J.S.: The output of sympathetic transmitter from the spleen of the cat. J. Physiol. (Lond.) 138: 81-102 (1957).

BROWN, G.L., DAVIES, B.N. and FERRY, C.B.: The effect of neuronal rest on the output of sympathetic transmitter from the spleen. J. Physiol. (Lond.) 159: 365-380 (1961).

BROWN, G.L., DAVIES, B.N. and GILLESPIE, J.S.: The release of chemical transmitter from the sympathetic nerves of the intestine of the cat. J. Physiol. (Lond.) 143: 41-54 (1958).

BURN, J.H.: The enzyme at sympathetic nerve endings. Br. Med. J. 1: 784-787 (1952).

BURN, J.H. and ROBINSON, J.: Effect of denervation on amine oxidase in structure innervated by the sympathetic. Br. J. Pharmacol. 7: 304-318 (1952).

BURNSTOCK, G., McCULLOCH, M.W., STORY, D.F. and WRIGHT, M.E.: Factors affecting the extraneuronal inactivation of noradrenaline in cardiac and smooth muscle. Br. J. Pharmacol. 46: 243-253 (1972).

CARLSSON, A., FOLKOW, B. and HÄGGENDAL, J.: Some factors influencing the release of noradrenaline into the blood following sympathetic stimulation. Life Sci. 3: 1335-1341 (1964).

CHAN, C.C. and KALSNER, S.: An examination of the negative feedback function of presynaptic adrenoceptors in a vascular tissue. Br. J. Pharmacol. 67: 401-407 (1979).

CHAN, C.C. and KALSNER, S.: Noradrenaline inhibition of transmitter efflux in a renal artery preparation and the presynaptic receptor theory. Can. J. Physiol. Pharmacol. 57: 1192-1195 (1979).

CHANG, C.C. and LEE, M.C.: Studies of the effect of phenoxybenzamine on the uptake of noradrenaline and on the response to sympathetic stimulation of rat hearts. Arch. Int. Pharmacodyn. Ther. 201: 400-414 (1973).

CONSTANTINE, J.W., WEEKS, R.A. and McSHANE, W.K.: Prazosin and presynaptic alpha-receptors in the cardioaccelerator nerve of the dog. Eur. J. Pharmacol. 50: 51-60 (1978).

COSTA, E., GREEN, A.R., KOSLOW, S.H., LEFEVRE, H.F., REVUELTA, A.V. and WANG, C.: Dopamine and norepinephrine in noradrenergic axons: a study in vivo of their precursor product relationship by mass fragmentography and radiochemistry. Pharmacol. Rev. 24: 167-170 (1972).

CUBEDDU, L.X., BARNES, E.M., LANGER, S.Z. and WEINER, N.: Release of norepinephrine and dopamine-beta-hydroxylase by nerve stimulation. I. Role of neuronal and extraneuronal uptake and of alpha-presynaptic receptors. J. Pharmacol. Expt. Ther. 190: 431-450 (1974).

CUBEDDU, L.X. and WEINER, N.: Nerve-stimulation mediated overflow of norepinephrine and dopamine-beta-hydroxylase. III. Effects of norepinephrine depletion on the alpha presynaptic regulation of release. J. Pharmacol. Expt. Ther. 192: 1-14 (1975).

DÄHLOF, C., LJUNG, B. and ÅBLAD, B.: Evidence for the existence of a presynaptic beta-adrenoceptor mediated positive feedback mechanism. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.), Pergamon Press, Oxford, pp. 317-320 (1979).

DÄHLOF, C., LJUNG, B. and ÅBLAD, B.: Increased noradrenaline release in rat portal vein during sympathetic nerve stimulation due to activation of presynaptic beta-adrenoceptors by noradrenaline and adrenaline. Eur. J. Pharmacol. 50: 75-78 (1978).

DAVIDSON, W.J. and INNES, I.R.: Dissociation of potentiation of isoprenaline by cocaine from inhibition of uptake in cat spleen. Br. J. Pharmacol. 39: 175-181 (1970).

DAVIES, B.N., HORTON, E.W. and WITHRINGTON, P.G.: The occurrence of prostaglandins E_2 in splenic venous blood of dog following splenic nerve stimulation. J. Physiol. (Lond.) 188: 38p-39p (1967).

DAVIES, B.N. and WITHRINGTON, P.G.: The effects of prostaglandins E_1 and E_2 on the smooth muscle of the dog spleen and on its responses to catecholamines, angiotensin and nerve stimulation. Br. J. Pharmacol. 32: 136-144 (1968).

DE LA LANDE, I.S.: Adrenergic mechanisms in the rabbit ear artery. Blood Vessels 12: 137-160 (1975).

DE LA LANDE, I.S., FREWIN, D. and WATERSON, J.G.: The influence of sympathetic innervation on vascular sensitivity to noradrenaline. Br. J. Pharmacol. Chemother. 31: 82-93 (1967).

DE LA LANDE, I.S. and JOHNSON, S.M.: Evidence for inactivation of noradrenaline by extraneuronal monoamine oxidase in the rabbit ear artery. Aust. J. Expt. Biol. Med. Sci. 50: 119-121 (1972).

DE LA LANDE, I.S. and RAND, M.J.: A simple isolated nerve-blood vessel preparation. Aust. J. Expt. Biol. Med. Sci. 43: 639-656 (1965).

DE LA LANDE, I.S. and WATERSON, J.G.: Site of action of cocaine on the perfused artery. Nature 214: 313-314 (1967).

DENGLER, H.J., MICHAELSON, I.A., SPIEGEL, H.E. and TITUS, E.O.: The uptake of labelled norepinephrine by brain and other tissues of the cat. Int. J. Neuropharmacol. 1: 23-38 (1962).

DENGLER, H.J., SPIEGEL, H.E. and TITUS, E.O.: Effects of drugs on uptake of isotopic norepinephrine. Nature 191: 816-817 (1961).

DE POTTER, W.P., CHUBB, I.W., PUT, A. and DE SCHAEPDRYER, A.F.: Facilitation of the release of noradrenaline and dopamine-beta-hydroxylase at low stimulation frequencies by alpha-blocking agents. Arch. Int. Pharmacodyn. Ther. 193: 191-197 (1971).

DE POTTER, W.P., DE SCHAEPDRYER, A.F., MOERMAN, E.J. and SMITH, A.D.: Evidence for the release of vesicle-proteins together with noradrenaline upon stimulation of the splenic nerve. J. Physiol. (Lond.) 204: 102p-103p (1969).

DOCHERTY, J.R. and McGRATH, J.C.: A comparison of pre- and post-junctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygens muscle of the rat. Arch. Pharmacol. 312: 107-116 (1980).

DREW, G.M.: Effects of alpha-adrenoceptor agonists and antagonists on pre- and postsynaptically located alpha-adrenoceptors. Eur. J. Pharmacol. 36: 313-320 (1976).

DREW, G.M.: Presynaptic alpha-adrenoceptors: their pharmacological characterization and functional significance. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.), Pergamon Press, Oxford, pp. 59-65 (1979).

DUBOCOVICH, M.L. and LANGER, S.Z.: Negative feedback regulation of noradrenaline release by nerve stimulation in the perfused cat's spleen: differences in potency of phenoxybenzamine in blocking the pre- and postsynaptic adrenergic receptors. J. Physiol. (Lond.) 237: 505-519 (1974).

DUBOCOVICH, M.L. and LANGER, S.Z.: Dopamine and alpha adrenoceptor agonists inhibit neurotransmission in the cat spleen through different presynaptic receptors. J. Pharmacol. Expt. Ther. 212: 144-152 (1980).

EISENFELD, A.J., AXELROD, J. and KRAKOFF, L.: Inhibition of the extraneuronal accumulation and metabolism of norepinephrine by adrenergic blocking agent. J. Pharmacol. Expt. Ther. 156: 107-113 (1967).

EISENFELD, A.J., KRAKOFF, L., IVERSEN, L.L. and AXELROD, J.: Inhibition of the extraneuronal metabolism of noradrenaline in the isolated heart by adrenergic blocking agents. Nature 213: 297-298 (1967)

EISENFELD, A.J., LANDSBERG, L. and AXELROD, J.: Effect of drugs on the accumulation and metabolism of extraneuronal norepinephrine in the rat heart. J. Pharmacol. Expt. Ther. 158: 378-385 (1967).

ENERO, M.A. and LANGER, S.Z.: Influence of reserpine-induced depletion of noradrenaline on the negative feedback mechanism for transmitter release during nerve stimulation. Br. J. Pharmacol. 49: 214-225 (1973).

ENERO, M.A. and LANGER, S.Z.: Inhibition by dopamine of ^{3}H-noradrenaline release elicited by nerve stimulation in the isolated cat's nictitating membrane. Arch. Pharmacol. 289: 179-203 (1975).

ENDO, T., STARKE, K. BANGERTER, A. and TAUBE, H.D.: Presynaptic receptor systems on the noradrenergic neurones of the rabbit pulmonary artery. Arch. Pharmacol. 296: 229-247 (1977).

ENERO, M.A., LANGER, S.Z., ROTHLIN, R.P. and STEFANO, F.J.E.: Role of the alpha-adrenoceptor in regulating noradrenaline overflow by nerve stimulation. Br. J. Pharmacol. 44: 672-688 (1972).

FARNEBO, L.O. and HAMBERGER, B.: Effects of desipramine, phentolamine and phenoxybenzamine on the release of noradrenaline from isolated tissues. J. Pharm. Pharmacol. 22: 855-857 (1970).

FARNEBO, L.O. and HAMBERGER, B.: Drug-induced changes in the release of [^{3}H]-noradrenaline from field stimulated rat iris. Br. J. Pharmacol. 43: 97-106 (1971a)

FARNEBO, L.O. and HAMBERGER, B.: Drug-induced changes in the release of ^{3}H-monoamines from field stimulated rat brain slices. Acta Physiol. Scand. Suppl. 371: 35-44 (1971b)

FARNEBO, L.O. and HAMBERGER, B.: Influence of alpha- and beta-adrenoceptors on the release of noradrenaline from field stimulated atria and cerebral cortex slices. J. Pharm. Pharmacol. 26: 644-646 (1974).

FARNEBO, L.O. and MALMFORS, T.: ^{3}H-noradrenaline release and mechanical response in the field stimulated mouse vas deferens. Acta Physiol. Scand. Suppl. 371: 1-18 (1971).

FARAH, M.B. and LANGER, S.Z.: Protection by phentolamine against the effects of phenoxybenzamine on transmitter release elicited by nerve stimulation in the perfused cat heart. Br. J. Pharmacol. 52: 549-557 (1974).

FARRANT, J., HARVEY, J.A. and PENNEFATHER, J.N.: The influence of phenoxybenzamine on the storage of noradrenaline in rat and cat tissues. Br. J. Pharmacol. Chemother. 22: 104-112 (1964).

FLECKENSTEIN, A. and BURN, J.H.: The effect of denervation on the action of sympathomimetic amines on the nictitating membrane. Br. J. Pharmacol. 8: 69-78 (1953).

FLEMING, W.W., WESTFALL, D.P., DE LA LANDE, I.S. and JELLETT, L.B.: Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. J. Pharmacol. Expt. Ther. 181: 339-345 (1972).

FOLKOW, B.: Importance of adrenergic neuronal uptake in termination of action: some aspects. Blood Vessels 13: 253-255 (1976).

FOLKOW, B., HÄGGENDAL, J. and LISANDER, B.: Extent of release and elimination of noradrenaline at peripheral adrenergic nerve terminals. Acta Physiol. Scand. Suppl. 307: 1-38 (1968).

FOZARD, J.R.: Blockade of receptors for 5-hydroxytryptamine on the sympathetic nerves of the rabbit heart. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.), Pergamon Press, Oxford, pp. 327-332 (1979).

FREDHOLM, B.B. and HEDQVIST, P.: Role of pre- and postjunctional inhibition by prostaglandin E_2 of lipolysis induced by sympathetic nerve stimulation in dog subcutaneous adipose tissue in situ. Br. J. Pharmacol. 47: 711-718 (1973).

FUDER, H. and MUSCHOLL, E.: The effect of dopamine on the overflow of endogenous noradrenaline from the perfused rabbit heart evoked by sympathetic nerve stimulation. Arch. Pharmacol. 305: 109-115 (1978).

FURCHGOTT, R.F.: Dibenamine blockade in strips of rabbit aorta and its use in differentiating receptors. J. Pharmacol. Expt. Ther. 111: 265-284 (1954).

FURCHGOTT, R.F.: Spiral-cut strip of rabbit aorta for in vitro studies of responses of arterial smooth muscle. In "Methods in medical research," (Ed. Bruner, H.D.), Year Book Publishers, Inc., Chicago, Vol. 8, pp. 177-186 (1960a).

FURCHGOTT, R.F.: Discussion of the article presented by Brown, G.L. "Release of sympathetic transmitter by nerve stimulation". In "Adrenergic mechanisms," (Eds. Vane, J.R., Wolsterholme, G.E.W. and O'Connor, M.), J. & A. Churchill Ltd., London, pp. 116-130 (1960b).

FURCHGOTT, R.F.: Pharmacological characteristics of adrenergic receptors. Fed. Proc. 29: 1352-1361 (1970).

FURCHGOTT, R.F.: The classification of adrenoceptors (adrenergic receptros). An evaluation from the standpoint of receptor theory. In "Catecholamines," (Eds. Blaschko, H. and Muscholl, E.), Springer Verlag, New York, pp. 283-330, (1972).

GARCIA, A.G., KIRPEKAR, S.M. and PASCUAL, R.: Release of noradrenaline from cat spleen slices by potassium. Br. J. Pharmacol. 62: 207-211 (1978).

GEWIRTZ, G.P. and KOPIN, I.J.: Release of dopamine-beta-hydroxylase with norepinephrine during cat splenic nerve stimulation. Nature 227: 406-407 (1970).

GILLESPIE, J.S.: The role of receptors in adrenergic uptake. In "Adrenergic neurotransmission," (Eds. Wolstenholme, G.E.W. and O'Connor, M.), J. & A. Churchill Ltd., London, pp. 61-75 (1968)

GILLESPIE, J.S.: Intracellular uptake of noradrenaline in vascular smooth muscle. In "Proceedings of the fourth international congress on pharmacology vol. 2," (Ed. Eigenmann, R.), Schwake and Co., Basel, pp. 28-35 (1969).

GILLESPIE, J.S.: Extraneuronal uptake of catecholamines in smooth muscle and connective tissue. In "The mechanism of neuronal and extraneuronal transport of catecholamines," (Ed. Paton, D.M.), Raven Press, New York, pp. 325-354 (1976).

GOLDBERG, L.I., VOLKMAN, P.H. and KOHLI, J.D.: A comparison of the vascular dopamine receptor with other dopamine receptors. Ann. Rev. Pharmacol. Toxicol. 18: 57-79 (1978).

GOLDSTEIN, A.: Correlation. In "Biostatistics: An introductory text," (Ed. Goldstein, A.), MacMillan Co., New York, pp. 144-146 (1964).

GREENBERG, S. and LONG, J.P.: The effects of cocaine, norepinephrine and ionic stimulation on the isolated, superfused rat vas deferens: antagonism by "adrenergic neurone blockers" and reserpine. J. Pharmacol. Expt. Ther. 177: 136-145 (1971).

GUIMARÃES, S. and BRANDÃO, F.: Comparison between the effects produced by chronic denervation and by cocaine on the sensitivity to, and on the disposition of, noradrenaline in isolated spleen strips. Arch. Pharmacol. 277: 163-174 (1973).

GUIMARÃES, S. and PAIVA, M.Q.: The role played by the extraneuronal system in the disposition of noradrenaline and adrenaline in vessels. Arch. Pharmacol. 296: 279-287 (1977).

GULDBERG, H.C. and MARSDEN, C.A.: Catechol-O-methyl transferase: pharmacological aspects and physiological role. Pharmacol. Rev. 27: 135-206 (1975).

HAEFELY, W., HURLIMANN, A. and THOENEN, H.: Relation between the rate of stimulation and the quantity of noradrenaline libertated from sympathetic nerve endings in isolated perfused spleen of the cat. J. Physiol. (Lond.) 181: 48-58 (1965).

HAEUSLER, G.H.: Studies on the possible contribution of a peripheral presynaptic action of clonidine and dopamine to their vascular effects under in vivo conditions. Arch. Pharmacol. 295: 191-202 (1976).

HAEUSLER, G.H., THOENEN, H., HAEFELY, W. and HURLIMANN, A.: Electrical events in cardiac adrenergic nerves and noradrenaline release from the heart induced by acetylcholine and KCl. Arch. Pharmacol. 261: 389-411 (1968).

HÄGGENDAL, J.: Some further aspects on the release of the adrenergic transmitter. In "New aspects of storage and release mechanisms of catecholamines," (Eds. Schümann, H.J. and Kroneberg, G.), Springer-Verlag, Berlin, pp. 100-109 (1970).

HÄGGENDAL, J.: Some aspects on the activity of monoaminergic neurones and the response of the effector cell. In "Chemical tools in catecholamine research vol. II," (Eds. Almgren, O., Carlsson, A. and Engel, J.), North-Holland Publishing Co., Amsterdam, pp. 159-165 (1975).

HÄGGENDAL, J., JOHANSSON, B., JONASON, J. and LJUNG, B.: Effects of phenoxybenzamine on transmitter release and effector response in the isolated portal vein. J. Pharm. Pharmacol. 24: 161-164 (1972).

HARE, M.L.C.: Tyramine oxidase I. A new enzyme system in liver. Biochem. J. 22: 968-979 (1928).

HEDQVIST, P.: Studies on the effect of prostaglandins E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissues. Acta Physiol. Scand. Suppl. 345: 1-40 (1970a).

HEDQVIST, P.: Antagonism by calcium of the inhibitory action of prostaglandin E_2 on sympathetic meurotransmission in the cat spleen. Acta Physiol. Scand. 80: 269-275 (1970b).

HEDQVIST, P.: Prostaglandin mediated control of sympathetic neuroeffector transmission. In "Advances in the biosciences, vol. 9," (Ed. Bergström, S.), Pergamon Press, Oxford, pp. 461-473 (1972).

HEDQVIST, P.: Prostaglandin as a tool for local control of transmitter release from sympathetic nerves. Brain Res. 62: 483-488 (1973).

HEDQVIST, P.: Further evidence that prostaglandins inhibit the release of noradrenaline from adrenergic nerve terminals by restriction of availability of calcium. Br. J. Pharmacol. 58: 599-603 (1976).

HEDQVIST, P. and FREDHOLM, B.B.: Effects of adenosine on adrenergic neurotransmission: prejunctional and postjunctional enhancement. Arch. Pharmacol. 293: 217-223 (1976).

HEDQVIST, P. and MOAWAD, A.: Presynaptic alpha- and beta-adrenoceptor mediate control of noradrenaline release in human oviduct. Acta Physiol. Scand. 95: 494-496 (1975).

HEDQVIST, P., STJÄRNE, L. and WENNMALM, A.: Inhibition by prostaglandin E_2 of sympathetic neurotransmission in the rabbit heart. Acta Physiol. Scand. 79: 139-141 (1970).

HENDERSON, G., HUGHES, J. and KOSTERLITZ, H.W.: The effects of morphine on the release of noradrenaline from the cat isolated nictitating membrane and the guinea-pig ileum myenteric plexus-longitudinal muscle preparation. Br. J. Pharmacol. 53: 505-512 (1975).

HENDERSON, G., HUGHES, J. and THOMPSON, J.W.: The variation of noradrenaline output with frequency of nerve stimulation and the effect of morphine on the cat nictitating membrane and on the guinea-pig myenteric plexus. Br. J. Pharmacol. 46: 524-525 (1972).

HENSELING, M., ECKERT, E. and TRENDELENBURG, U.: The distribution of ^{3}H-(±) noradrenaline in rabbit aortic strips after inhibition of the noradrenaline-metabolizing enzymes. Arch. Pharmacol. 292: 205-217 (1976).

HENSELING, M., GRAEFE, K.-H. and TRENDELENBURG, U.: The rate constants for the efflux of the metabolites of noradrenaline from rabbit aortic strips. Arch. Pharmacol. 302: 207-215 (1978).

HENSELING, M., RECHTSTEINER, D. and TRENDELENBURG, U.: The influence of monoamine oxidase and catechol-O-methyl transferase on the inhibition of ^{3}H- (±) - noradrenaline in rabbit aortic strips. Arch. Pharmacol. 302: 181-194 (1978).

HERTTING, G., AXELROD, J. and WHITBY, L.G.: Effect of drugs on the uptake and metabolism of ^{3}H-norepinephrine. J. Pharmacol. Expt. Ther. 134: 146-153 (1961).

HERTTING, G., AXELROD, J., KOPIN, I.J. and WHITBY, L.G.: Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. Nature 189: 66 (1961).

HERTTING, G., REIMANN, W., ZUMSTEIN, A., JACKISCH, R. and STARKE, K.: Dopaminergic feedback regulation of dopamine release in slices of the caudate nucleus of the rabbit. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.), Pergamon Press, London, pp. 145-150 (1979).

HOPE, W., LAW, M., McCULLOCH, M.W., RAND, M.J. and STORY, D.F.: Effects of some catecholamines on noradrenergic transmission in the rabbit ear artery. Clin. Expt. Pharmacol. Physiol. 3: 15-28 (1976).

HOPE, W., MAJEWSKI, H., McCULLOCH, M., RAND, M.J. and STORY, D.F.: Dopamine as a false noradrenergic transmitter. Clin. Expt. Pharmacol. Physiol. 6: 206 (1979).

HOPE, W., McCULLOCH, M.W., RAND, M.J. and STORY, D.F.: Modulation of noradrenergic transmission in the rabbit ear artery by dopamine. Br. J. Pharmacol. 64: 527-537 (1978).

HOPE, W., McCULLOCH, M.W., STORY, D.F. and RAND, M.J.: Effects of pimozide on noradrenergic transmission in rabbit isolated ear arteries. Eur. J. Pharmacol. 46: 101-111 (1977).

HORITA, A.: Cardiac monoamine oxidase in rat. Nature 215: 411-412 (1967).

HUGHES, J.: Evaluation of mechanisms controlling the release and inactivation of the adrenergic transmitter in the rabbit portal vein and vas deferens. Br. J. Pharmacol. 44: 472-491 (1972).

HUGHES, J. and VANE, J.R.: An analysis of the responses of the isolated portal vein of the rabbit to electrical stimulation and to drugs. Br. J. Pharmacol. 30: 46-66 (1967).

IVERSEN, L.L.: The uptake of noradrenaline by the isolated perfused rat heart. Br. J. Pharmacol. 21: 523-537 (1963).

IVERSEN, L.L.: The uptake of catecholamines at high perfusion concentrations in the rat isolated heart: A novel catecholamine uptake process. Br. J. Pharmacol. 25: 18-33 (1965).

IVERSEN, L.L.: The uptake and storage of noradrenaline in sympathetic nerves. Cambridge University Press, Cambridge, pp. 253p (1967).

IVERSEN, L.L., GLOWINSKI, J. and AXELROD, J.: The physiologic disposition and metabolism of norepinephrine in immunosympathectomized animals. J. Expt. Pharmacol. Ther. 151: 273-284 (1966).

IVERSEN, L.L. and LANGER, S.Z.: Effects of phenoxybenzamine on the uptake and metabolism of noradrenaline in the rat heart and vas deferens. Br. J. Pharmacol. 37: 627-637 (1969).

IVERSEN, L.L. and SALT, P.J.: Inhibition of catecholamine uptake$_2$ by steroids in the isolated rat heart. Br. J. Pharmacol. 40: 528-530 (1970).

JARROTT, B.: Occurrence and properties of monoamine oxidase in adrenergic neurones. J. Neurochem. 18: 7-16 (1971a).

JARROTT, B.: Occurrence and properties of catechol-O-methyl transferase in adrenergic neurones. J. Neurochem. 18: 17-27 (1971b).

JARROTT, B. and LANGER, S.Z.: Changes in monoamine oxidase and catechol-O-methyl transferase activities after denervation of the nictitating membrane of cat. J. Physiol. (Lond.) 212: 549-559 (1971).

JENKINS, D.A., MARSHALL, I. and NASMYTH, P.A.: Is morphine inhibition of the twitch response of the mouse vas deferens mediated vai noradrenaline? Br. J. Pharmacol. 55: 267-268p (1975).

JOHNSON, D.G., THOA, N.B., WEINSHILBOUM, R., AXELROD, J. and KOPIN, I.J.: Enhanced release of dopamine-beta-hydroxylase from sympathetic nerves by calcaium and phenoxybenzamine and its reversal by prostaglandins. Proc. Nat. Acad. Sci. U.S.A. 68: 2227-2230 (1971).

JUNSTAD, M. and WENNMALM, A.: On the release of prostaglandin e from the rabbit heart following infusion of noradrenaline. Acta Physiol. Scand. 87: 573-574 (1973).

KALSNER, S.: Studies on the mechanisms terminating the action of the sympathetic nerve mediator (Noradrenaline). Ph.D. thesis, University of Manitoba (1966).

KALSNER, S.: Steroid potentiation of responses to sympathomimetic amines in aortic strips. Br. J. Pharmacol. 36: 582-593 (1969a).

KALSNER, S.: Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. Cir. Res. 24: 383-395 (1969b).

KALSNER, S.: The importance of the uptake mechanism of adrenergic nerves in blood vessels. In "Physiology and pharmacology of vascular neuro-effector systems," (Eds. Bevan, J.A., Furchgott, R.F., Maxwell, R.A. and Somlyo, A.P., S. Karger, Basel, pp. 126-129 (1971).

KASLNER, S.: Differential activation of the inner and outer muscle cell layers of rabbit ear artery. Eur. J. Pharmacol. 20: 122-124 (1972a).

KALSNER, S.: Effects of the inhibition of noradrenaline uptake and synthesis on the maintenance of the response to continuous nerve stimulation in the central artery of the rabbit ear. Br. J. Pharmacol. 45: 1-12 (1972b).

KALSNER, S.: Role of extraneuronal mechanisms in the termination of contractile responses to amines in vascular tissue. Br. J. Pharmacol. 53: 267-277 (1975a).

KALSNER, S.: The importance of adrenergic neuronal uptake in termination of action; another view. Blood Vessel 12: 316-322 (1975b).

KALSNER, S.: Sensitization of effector responses by modification of agonist disposition mechanisms. Can. J. Physiol. Pharmacol. 54: 177-187 (1976).

KALSNER, S.: Termination of effector responses to agonists: an analysis of agonist disposition mechanisms. Can. J. Physiol. Pharmacol. 55: 315-331 (1977).

KALSNER, S. Mechanisms of inactivation of noradrenaline in the iris sphincter, tracheal muscle and facial artery of cattle: implications for beta-adrenoceptor-mediated responses. Br. J. Pharmacol. 64: 545-552 (1979a).

KALSNER, S.: Single pulse stimulation of guinea-pig vas deferens and the presynaptic receptor hypothesis. Br. J. Pharmacol. 66: 343-349 (1979b).

KALSNER, S.: Adrenergic presynaptic receptors: Examination of a hypothesis in guinea-pig vas deferens. Can. J. Physiol. Pharmacol. 57: 717-724 (1979c).

KALSNER, S.: Termination of agonist action on autonomic receptors. In "Trends in autonomic pharmacology Vol. 1," (Ed. Kalsner, S.), Urban & Schwarzenberg, Baltimore, pp. 265-288 (1979d).

KALSNER, S.: Limitations of presynaptic adrenoceptor theory: The characteristics of the effects of noradrenaline and phenoxybenzamine on stimulation-induced efflux of ^{3}H-noradrenaline in vas deferens. J. Pharmacol. Expt. Ther. 212: 232-239 (1980a).

KALSNER, S.: The effects of (+)- and (-)-propranolol on ^{3}H-transmitter efflux in guinea-pig atria and the presynaptic beta-adrenoceptor hypothesis. Br. J. Pharmacol. 70: 491-498 (1980b).

KALSNER, S. and CHAN, C.C.: Adrenergic antagonists and the presynaptic receptor hypothesis in vascular tissue. J. Pharmacol. Expt. Ther. 211: 257-264 (1979).

KALSNER, S. and CHAN, C.C.: Inhibition by dopamine of the stimulation-induced efflux of ^{3}H-noradrenaline in renal arteries: limitations of the unitary hypothesis of presynaptic regulation of transmitter release. Can. J. Physiol. Pharmacol. 58: 504-512 (1980).

KALSNER, S. and NICKERSON, M.: A method for the study of mechanisms of drug disposition in smooth muscle. Can. J. Physiol. Pharmacol. 46: 719-730 (1968).

KALSNER, S. and NICKERSON, M.: Disposition of norepinephrine and epinephrine in vascular tissue, determined by the technique of oil immersion. J. Pharmacol. Expt. Ther. 165: 152-165 (1969a).

KALSNER, S. and NICKERSON, M.: Mechanism of cocaine potentiation of responses to amines. Br. J. Pharmacol. 35: 428-439 (1969b).

KALSNER, S. and NICKERSON, M.: Effects of a haloalkylamine on responses to and disposition of sympathomimetic amines. Br. J. Pharmacol. 35: 440-455 (1969c).

KALSNER, S. and NICKERSON, M.: Effects of reserpine on the disposition of sympathomimetic amines in vascular tissue. Br. J. Pharmacol. 35: 394-405 (1969d).

KALSNER, S., NICKERSON, M. and BOYD, G.: Selective blockade of potassium-induced contractions in aortic strips by SKF-525A. J. Pharmacol. Expt. Ther. 174: 500-508 (1970).

KALSNER, S., SULEIMAN, M. and DOBSON, R.E.: Adrenergic presynaptic receptors: an overextended hypothesis? J. Pharm. Pharmacol. 32: 290-292 (1980).

KAO, C.Y.: Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. Pharmacol. Rev. 18: 997-1049 (1966).

KIRPEKAR, S.M.: Factors influencing transmission at adrenergic synapses. Prog. Neurobiol. 4: 163-196 (1975).

KIRPEKAR, S.M. and CERVONI, P.: Effect of cocaine, phenoxybenzamine and phentolamine on the catecholamine output from spleen and adrenal medulla. J. Pharmacol. Expt. Ther. 142: 59-70 (1963).

KIRPEKAR, S.M. and MISU, Y.: Release of noradrenaline by splenic nerve stimulation and its dependence on calcium. J. Physiol. (Lond.) 188: 219-234 (1967).

KIRPEKAR, S.M. and PUIG, M.: Effect of flow-stop on noradrenaline release from normal spleen and spleen treated with cocaine, phentolamine or phenoxybenzamine. Br. J. Pharmacol. 43: 359-369 (1971).

KIRPEKAR, S.M. and WAKADE, A.R.: Release of noradrenaline from the cat spleen by potassium. J. Physiol. (Lond.) 94: 595-608 (1968).

KIRPEKAR, S.M. and WAKADE, A.R.: Effect of beta-haloalkylamines and ephedrine on noradrenaline release from the intact spleen of the cat. Br. J. Pharmacol. 39: 533-541 (1970).

KIRPEKAR, S.M., FURCHGOTT, R.F., WAKADE, A.R. and PRAT, J.C.: Inhibition by sympathomimetic amines of the release of norepinephrine evoked by nerve stimulation in the cat spleen. J. Pharmacol. Expt. Ther. 187: 529-538 (1973).

KIRPEKAR, S.M., PRAT, J.C. and WAKADE, A.R.: Effect of calcium on the relationship between frequency of stimulation and release of noradrenaline from the perfused spleen of the cat. Arch. Pharmacol. 287: 205-212 (1975).

KOBINGER, W.: Central cardiovascular actions of clonidine. In "Central action of drugs in blood pressure regulation," (Eds. Davies, D.S. and Reid, J.L.), University Park Press, London, pp. 181-193 (1975).

KOPIN, I.J.: Metabolic degradation of catecholamines. The relative importance of different pathways under physiological conditions and after administration of drugs. In "Catecholamines," (Eds. Blaschko, H. and Muscholl, E.), Springer-Verlag, Berlin, Heidelberg, pp. 270-282 (1972).

KOPIN, I.J. and AXELROD, J.: The role of monoamine oxidase in the release and metabolism of norepinephrine. Ann. N.Y. Acad. Sci. 107: 848-855 (1963).

LANGER, S.Z.: The effects of phenoxybenzamine on metabolism of ^{3}H-noradrenaline released from the isolated mictitating membrane. Br. J. Pharmacol. 34: 222p-223p (1968).

LANGER, S.Z.: The metabolism of [^{3}H] noradrenaline released by electrical stimulation from the isolated nictitating membrane of the cat and from the vas deferens of the rat. J. Physiol. (Lond.) 208: 515-546 (1970).

LANGER, S.Z.: The regulation of transmitter release elicited by nerve stimulation through a presynaptic feedback mechanism. In "Frontiers in catecholamine research," (Eds. Usdin, E. and Snyder, S.H.),Pergamon Press, New York, pp. 543-549 (1973).

LANGER, S.Z.: Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23: 1793-1800 (1974).

LANGER, S.Z.: Presynaptic receptors and their role in the regulation of transmitter release. Br. J. Pharmacol. 66: 481-497 (1977).

LANGER, S.Z.: Physiological and pharmacological role of presynaptic receptor systems in neurotransmission. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.),Pergamon Press, Oxford, pp. 13-22 (1979).

LANGER, S.Z.: Presynaptic receptors and modulation of neurotransmission: pharmacological implications and therapeutic relevance. Trends. Neurosci. 3: 110-112 (1980).

LANGER, S.Z., ADLER-GRASCHINSKY, E. and ENERO, M.A.: Positive feedback mechanism for the regulation of noradrenaline released by nerve stimulation. Abstract of Jerusalem Satellite Symposia XXVI International Congress of Physiological Sciences, p. 81 (1974).

LANGER, S.Z., ADLER-GRASCHINSKY, E., ENERO, M.A. and STEFANO, F.J.E.: The role of the alpha-receptors in regulating noradrenaline overflow by nerve stimulation. Proc. Intern. Congr. Physiol. Sci. 25th, p. 335 (1971)

LANGER, S.Z., ADLER-GRASCHINSKY, E. and GIORGI, O.: Physiological significance of alpha-adrenoceptor-mediated negative feedback mechanism regulating noradrenaline release during nerve stimulation. Nature 265: 648-650 (1977).

LANGER, S.Z., DUBOCOVICH, M.L. and CELUCH, S.M.: Prejunctional regulatory mechanisms for noradrenaline release elicited by nerve stimulation. In "Chemical tools in catecholamine research Vol. II," (Eds. Almgren, O., Carlsson, A. and Engel, J.), North-Holland Publishing Co., Amsterdam, pp. 183-191 (1975).

LANGER, S.Z. and ENERO, M.A.: The potentiation of responses to adrenergic nerve stimulation in the presence of cocaine: its relationship to the metabolic fate of released norepinephrine. J. Pharmacol. Expt. Ther. 191: 431-443 (1974).

LANGER, S.Z., ENERO, M.A., ADLER-GRASCHINSKY, E., DUBOCOVICH, M.L. and CELUCH, S.M.: Presynaptic regulatory mechanisms for noradrenaline release by nerve stimulation. In "Central action of drugs in blood pressure regulation," (Eds. Davies, D.S. and Reid, J.L.), University Park Press, London, pp. 133-153 (1975).

LANGER, S.Z., STEFANO, F.J.E. and ENERO, M.A.: Pre- and postsynaptic origin of the norepinephrine metabolites formed during transmitter release elicited by nerve stimulation. J. Pharmacol. Expt. Ther. 183: 90-102 (1972).

LEVIN, J.A. and FURCHGOTT, R.F.: Interactions between potentiating agents of adrenergic amines in rabbit aortic strips. J. Pharmacol. Expt. Ther. 1[redacted] 320-331 (1970).

LEVITT, M., SPECTOR, S., SJOERDSMA, A. and UDENFRIEND, S.: Elucidation of the rat-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. J. Pharmacol. Expt. Ther. 148: 1-8 (1965).

LIGHTMAN, S.L. and IVERSEN, L.L.: The role of uptake$_2$ in the extraneuronal metabolism of catecholamines in the isolated rat heart. Br. J. Pharmacol. 37: 638-649 (1969).

LINDMAN, R. and LÖFFELHOLZ, K.: Neuronal and extraneuronal uptake and efflux of catecholamines in the isolated rabbit heart. Arch. Pharmacol. 284: 63-92 (1974).

LÖFFELHOLZ, K.: Nicotinic drugs and post-ganglionic sympathetic transmission. Arch. Pharmacol. 267: 64-73 (1970).

LÖFFELHOLZ, K.: Neuronal efflux of exogenous catecholamines from the isolated rabbit heart. Arch. Pharmacol. 274: R74 (1972).

LOKHANDWALA, M.F. and BUCKLEY, J.P.: Effect of presynaptic alpha-adrenoceptor blockade on responses to cardiac nerve stimulation in anesthetized dog. Eur. J. Pharmacol. 40: 183-186 (1976).

LOKHANDWALA, M.F., COATS, J.T. and BUCKLEY, J.P.: Effects of several catecholamines on sympathetic transmission to the myocardium: role of presynaptic alpha-adrenoceptors. Eur. J. Pharmacol. 42: 257-265 (1977).

LORENZ, R.R., VANHOUTTE, P.M. and SHEPHERD, J.T.: Interaction between neuronal amine uptake and prejunctional alpha-adrenergic receptor activation in smooth muscle from canine blood vessels and spleen. Blood Vessels 16: I13-125 (1979).

LOWE, M.C., REICHENBACH, D.D. and HORITA, A.: Biochemical and electron microscopic localization of an extraneuronal monoamine oxidase in chemically sympathectomized rat heart. Fed. Proc. 30: 223 (1971).

McCULLOCH, M.W., BEVAN, J. and SU, C.: Effects of phenoxybenzamine and norepinephrine on transmitter release in the pulmonary artery of the rabbit. Blood Vessels 12: 122-133 (1975).

McCULLOCH, M.W., RAND, M.J. and STORY, D.F.: Evidence for a dopaminergic mechanism for modulation of adrenergic transmission in the rabbit ear artery. Br. J. Pharmacol. 49: 141-142p (1973).

McCULLOCH, M.W., RAND, M.J. and STORY, D.F.: Inhibition of ^{3}H-noradrenaline release from sympathetic nerves of guinea-pig atrai by a presynaptic alpha-adrenoceptor mechanism. Br. J. Pharmacol. 46: 523-524p (1972).

McGRATH, M.A.: 5-Hydroxytryptamine and neurotransmitter release in canine blood vessels: Inhibition by low and augmentation by high concentrations. Cir. Res. 41: 428-435 (1977).

NAGATSU, T., RUST, L.A. and DeQUATTRO, V.: The activity of tyrosine hydroxylase and related enzymes of catecholamine biosynthesis and metabolism in dog kidney - effects of denervation. Biochem. Pharmacol. 18: 1441-1446 (1969).

NEDERGAARD, O.A. and SCHROLD, J.: Release of ^{3}H-noradrenaline from incubated and superfused rabbit pulmonary artery. Acta Physiol. Scand. 89: 296-305 (1973).

NICKERSON, M. and COLLIER, B.: Drugs inhibiting adrenergic nerves and structures innervated by them. In "The pharmacological basis of therapeutics," (Eds. Goodman, L.S. and Gilman, A.), MacMillan Publishing Co., New York, pp. 533-564 (1975).

NICKERSON, M. and GUMP, W.S.: The chemical basis for adrenergic blocking activity in compounds related to dibenamine. J. Pharmacol. Expt. Ther. 97: 25-47 (1949).

NOON, J.P. and ROTH, R.H.: Some physiological and pharmacological characteristics of the stimulus induced release of norepinephrine from the rabbit superior cervical ganglion. Arch. Pharmacol. 291: 163-174 (1975).

O'CONNOR, P.C. and SLATER, P.: Effect of cocaine on contractile responses to noradrenaline of canine isolated mesenteric vein. J. Pharm. Pharmacol. 33: 55-58 (1981).

OHLIN, P. and STRÖMBLAD, B.C.R.: Observations on the isolated vas deferens. Br. J. Pharmacol. 20: 299-306 (1963).

PATON, W.D.M.: Discussion of the article presented by Brown, G.L. "Release of sympathetic transmitter by nerve stimulation." In "Adrenergic mechanisms," (Eds. Vane, J.R., Wolstenholm, G.E.W. and O'Connor, M.), J. & A. Churchill Ltd., London, pp. 116-130 (1960).

PATON, W.D.M. and VIZI, E.S.: The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. Br. J. Pharmacol. 35: 10-28 (1969).

POTTER, L.T., COOPER, T., WILLIAM, V.L. and WOLFE, D.E.: Synthesis binding, release, and metabolism of norepinephrine in normal and transplanted dog hearts. Cir. Res. 16: 468-481 (1965).

PUECH, A.J., LECRUBIER, Y. and SIMON, P.: Are alpha and beta presynaptic receptors involved in mood regulation? Pharmacological and clinical data. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.), Pergamon Press, Oxford, pp. 359-362 (1979).

RAND, M.J., McCULLOCH, M.W. and STORY, D.F.: Prejunctional modulation of noradrenergic transmission by noradrenaline, dopamine and acetylcholine. In "Central action of drugs in blood pressure regulation," (Eds. Davies, D.S. and Reid, L.J.), University Park Press, Baltimore, pp. 94-132 (1975).

RAND, M.J., STORY, D.F., ALLEN, G.S., GLOVER, A.B. and McCULLOCH, M.W.: Pulse-to-pulse modulation of noradrenaline release through a prejunctional alpha-receptor autoinhibitory mechanism. In "Frontiers in catecholamine research," (Eds. Usdin, E. and Snyder, S.H.), Pergamon Press, Oxford, pp. 579-581 (1973).

ROACH, A.G., DENNIS, T., SCATTON, B. and CAVERO, I.: Contribution of cardiac presynaptic alpha-adrenoceptors to the clonidine-induced bradycardia in dogs. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.), Pergamon Press, Oxford, pp. 53-58 (1979).

ROBIE, N.W.: Physiological evaluation of presynaptic alpha-receptor function in the canine renal vasculature. Fed. Proc. 39: 615 (1980a).

ROBIE, N.W.: Evaluation of presynaptic alpha-receptor function in the canine renal vascular bed. Am. J. Physiol. 239: H422-H426 (1980b).

ROBINSON, J.: Amine oxidase in the iris and nictitating membrane of the cat and the rabbit. Br. J. Pharmacol. 7: 99-102 (1952).

ROBSON, R.D. and ANTONACCIO, M.J.: Effect of clonidine on responses to cardiac nerve stimulation as a function of impulse frequency and stimulus duration in vagotomized dogs. Eur. J. Pharmacol. 29: 182-186 (1974).

ROSELL, S., KOPIN, I.J. and AXELROD, J.: Fate of ^{3}H-noradrenaline in skeletal muscle before and following sympathetic stimulation. Am. J. Physiol. 205: 317-321 (1963).

ROSENBLUETH, A. and CANNON, W.B.: Studies on conditions of activity in endocrine organs: some effects of sympathin on the nictitating membrane. Am. J. Physiol. 99: 398-407 (1932).

SALZMANN, R., PACHA, W., TAESCHLER, M. and WEIDMANN, H.: The effect of ergotamine on humoral and neuronal actions in the nictitating membrane and the spleen of the cat. Arch. Pharmacol. Expt. Pathol. 261: 360-378 (1968).

SCHWARTZ, J.C., DE LA BAUME, S., LORENS, C., PATEY, G. and POLLARD, H.: Presynaptic opiate receptors on catecholamine neurones in brain: possible implication in tolerance and dependence to opioids. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.), Pergamon Press, Oxford, pp. 137-144 (1979).

SCRIABINE, A., STAVORSKI, J., WENGER, H.C., TORCHIANA, M.L. and STONE, C.A.: Cardiac slowing effects of clonidine (ST-155) in dogs. J. Pharmacol. Expt. Ther. 171: 256-264 (1970).

SCRIABINE, A. and STAVORSKI, J.M.: Effect of clonidine on cardiac acceleration in vagotomized dogs. Eur. J. Pharmacol. 24: 101-104 (1973).

SMITH, A.D. and WINKLER, H.: Fundamental mechanisms in the release of catecholamines. In "Catecholamines," (Eds. Blaschko, H. and Muscholl, E.), Springer-Verlag, Berlin, pp. 538-617 (1972).

SNYDER, S.H., FISCHER, J. and AXELROD, J.: Evidence for the presence of monoamine oxidase in sympathetic nerve endings. Biochem. Pharmacol. 14: 363-365 (1965).

STARKE, K.: Influence of alpha-receptor stimulants on noradrenaline release. Naturwissenschaften 58: 420 (1971).

STARKE, K.: Influence of extracellular noradrenaline on the stimulation-evoked secretion of noradrenaline from sympathetic nerves. Evidence for an alpha-receptor-mediated feedback inhibition of noradrenaline release. Arch. Pharmacol. 275: 11-23 (1972a).

STARKE, K.: Alpha sympathomimetic inhibition of adrenergic and cholinergic transmission in the rabbit heart. Arch. Pharmacol. 274: 18-45 (1972b).

STARKE, K.: Regulation of catecholamine release: alpha-receptor mediated feedback control in peripheral and central neurones. In "Frontiers in catecholamine research," (Eds. Usdin, E. and Snyder, S.H.), Pergamon Press, Oxford, pp. 561-565 (1973).

STARKE, K.: Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol. Biochem. Pharmacol. 77: 1-124 (1977).

STARKE, K. and ALTMAN, K.P.: Inhibition of adrenergic neurotransmission by clonidine: An action on prejunctional alpha-receptors. Neuropharmacology 12: 339-347 (1973).

STARKE, K., BOROWSKI, E. and ENDO, T.: Preferential blockade of presynaptic alpha-adrenoceptors by yohimbine. Eur. J. Pharmacol. 34: 385-388 (1975).

STARKE, K., ENDO, T. and TAUBE, H.D.: Relative pre- and postsynaptic potencies of alpha-adrenoceptor agonists in the rabbit pulmonary artery. Arch. Pharmacol. 291: 55-78 (1975).

STARKE, K. and MONTEL, H.: Alpha-receptor mediated modulation of transmitter release from central noradrenergic neurones. Arch. Pharmacol. 279: 53-60 (1973a).

STARKE, K. and MONTEL, H.: Sympathomimetic inhibition of noradrenaline release: mediated by prostaglandins? Arch. Pharmacol. 278: 111-116 (1973b).

STARKE, K. and MONTEL, H.: Influence of drugs with affinity for alpha-adrenoceptors on noradrenaline release by potassium, tyramine and dimethylphenylpiperazinium. Eur. J. Pharmacol. 27: 273-280 (1974).

STARKE, K., MONTEL, H., GAYK, W. and MERKER, R.: Comparison of the effects of clonidine on pre- and postsynaptic adrenoceptors in the rabbit pulmonary artery. Arch. Pharmacol. 285: 133-150 (1974).

STARKE, K., MONTEL, H. and SCHUMANN, J.J.: Influence of cocaine and phenoxybenzamine on noradrenaline uptake and release. Arch. Pharmacol. 270: 210-214 (1971).

STARKE, K., MONTEL, H. and WAGNER, J.: Effect of phentolamine on noradrenaline uptake and release. Arch. Pharmacol. 271: 181-192 (1971).

STEINSLAND, O.S., FURCHGOTT, R.F. and KIRPEKAR, S.M.: Inhibition of adrenergic neurotransmission by parasympathomimetics in rabbit ear artery. J. Pharmacol. Expt. Ther. 184: 346-356 (1973).

STEINSLAND, O.S. and NELSON, S.H.: 'Alpha-adrenergic' inhibition of the response of the isolated rabbit ear artery to brief intermittent sympathetic nerve stimulation. Blood Vessels 12: 378-379 (1975).

STEPPELER, A., TANAKA, T. and STARKE, K.: A comparison of pre- and postsynaptic alpha-adrenergic effects of phenylephrine and tramazoline on blood vessels of the rabbit in vivo. Arch. Pharmacol. 304: 223-230 (1978).

STINSON, R.H.: Electrical stimulation of the sympathetic nerves of isolated rabbit ear and the fate of the neurohormone released. Can. J. Biochem. Physiol. 39: 309-316 (1961).

STJÄRNE, L.: Alpha-adrenoceptor mediated feedback control of sympathetic neurotransmitter secretion in guinea-pig vas deferens. Nature New Biol. (Lond.) 241: 190-191 (1973a)

STJÄRNE, L.: Mechanisms of catecholamine secretion. Dual feedback control of sympathetic neurotransmitter secretion; role of calcium. In "Frontiers in catecholamine research," (Eds. Usdin, E., Snyder, S.H.), Pergamon Press, New York, pp. 491-496 (1973b).

STJÄRNE, L.: Michaelis-Menten kinetics of secretion of sympathetic neurotransmitter as a function of external calcium: effect of graded alpha-adrenoceptor blockade. Arch. Pharmacol. 278: 323-327 (1973c).

STJÄRNE, L.: Lack of correlation between profiles of transmitter efflux and of muscular contraction in response to nerve stimulation in isolated guinea-pig vas deferens. Acta Physiol. Scand. 88: 137-144 (1973d).

STJÄRNE, L.: Stereoselectivity of presynaptic alpha-adrenoceptors involved in feedback control of sympathetic neurotransmitter secretion. Acta Physiol. Scand. 90: 286-288 (1974).

STJÄRNE, L.: Selectivity of catecholamines of presynaptic alpha-receptors involved in feedback control of sympathetic neurotransmitter secretion in guinea-pig vas deferens. Arch. Pharmacol. 288: 296-303 (1975).

STJÄRNE, L.: Role of prostaglandins and cyclic adenosine monophate in release. In "The release of catecholamines from adrenergic neurones," (Ed. Paton, D.M.), Pergamon Press, Oxford, pp. 111-142 (1979).

STJÄRNE, L. and BRUNDIN, J.: Dual adrenoceptor mediated control of noradrenaline secretion from human vasoconstrictor nerves: facilitation by beta-receptors and inhibition by alpha-receptors. Acta Physiol. Scand. 94: 139-141 (1975).

STJÄRNE, L. and BRUNDIN, J.: Frequency-dependence of ^{3}H-noradrenaline secretion from human vasoconstrictor nerves: modification by factors interfering with alpha- or beta-adrenoceptor or prostaglandin E_2 mediated control. Acta Physiol. Scand. 101: 199-210 (1977).

STJÄRNE, L. and GRIPE, K.: Prostaglandin-dependent and -independent feedback control of noradrenaline secretion in vasoconstrictor nerves of normotensive humans subjects. Arch. Pharmacol. 280: 441-446 (1973).

STRÖMBLAD, B.C.R. and NICKERSON, M.: Accumulation of epinephrine and norepinephrine by some rat tissues. J. Pharmacol. Expt. Ther. 134: 154-159 (1961).

STRÖMBOM, U.: On the functional role of pre- and postsynaptic catecholamine receptors in brain. Acta Physiol. Scand. Suppl. 431: 1-42 (1975).

SU, C. and BEVAN, J.A.: The release of ^{3}H-norepinephrine in arterial strips studied by the technique of superfusion and transmural stimulation. J. Pharmacol. Expt. Ther. 172: 62-68 (1970).

SU, C. and BEVAN, J.A.: Adrenergic transmitter release and distribution in blood vessels. In "Physiology and pharmacology of vascular neuroeffector systems," (Eds. Bevan, J.A., Furchgott, R.F., Maxwell, R.A. and Somlyo, A.P.), S. Karger, Basel, pp. 13-21 (1971).

SULLIVAN, A.T. and DREW, G.M.: Pharmacological characterization of pre- and postsynaptic alpha-adrenoceptors in dog saphenous vein. Arch. Pharmacol. 314: 249-258 (1980).

SUMMERS, R.J. and TILLMAN, J.: Investigation of the role of calcium in the supersensitivity produced by cocaine in cat spleen strips. Br. J. Pharmacol. 65: 689-699 (1979).

TAUBE, H.D., ENDO, T., BANGERTER, A. and STARKE, K.: Presynaptic receptor systems on the noradrenergic nerves of the rabbit pulmonary artery. Arch. Pharmacol. 293: R2 (1976).

TAUBE, H.D., STARKE, K. and BOROWSKI, E.: Presynaptic receptor systems on the noradrenergic neurones of rat brain. Arch. Pharmacol. 299: 123-141 (1977).

THOENEN, H., HAFFELY, W., GEY, K.F. and HÜERLIMANN, A.: Quantitative aspects of the replacement of norepinephrine by dopamine as a sympathetic transmitter after inhibition of dopamine-beta-hydroxylase by disulfiram. J. Pharmacol. Expt. Ther. 156: 246-251 (1967).

THOENEN, H., HÜERLIMANN, A. and HAEFELY, W.: Dual site of action of phenoxybenzamine in the cat's spleen. Blockade of alpha-adrenergic receptors and inhibition of re-uptake of neurally released norepinephrine. Experientia 20: 272-273 (1964a).

THOENEN, H., HÜERLIMANN, A. and HAEFELY, W.: The effect of sympathetic nerve stimulation on volume, vascular resistance, and norepinephrine output in the isolated perfused spleen of the cat, and its modification by cocaine. J. Pharmacol. Expt. Ther. 143: 57-63 (1964b).

THOENEN, H., HÜERLIMANN, A. and HAEFELY, W.: Cation dependence of the noradrenaline-releasing action of tyramine. Eur. J. Pharmacol. 6: 29-37 (1969).

THOMPSON, R.H.S. and TICKNER, A.: The occurrence and distribution of monoamine oxidase in blood vessels. J. Physiol. (Lond.) 115: 34-40 (1951).

TRENDELENBURG, U.: The supersensitivity caused by cocaine. J. Pharmacol. Expt. Ther. 125: 55-65 (1959).

TRENDELENBURG, U.: The relaxation of rabbit aortic strips after a preceding exposure to sympathomimetic amines. Arch. Pharmacol. 281: 13-46 (1974).

TRENDELENBURG, U. and HENSELING, M.: Factors determining the rate of relaxation of rabbit aortic strips after an exposure to noradrenaline. Arch. Pharmacol. 293: 235-244 (1976).

TRENDELENBURG, U., MAXWELL, R.A. and PLUCHINO, S.: Methoxamine as a tool to assess the importance of intraneuronal uptake of l-norepinephrine in the cat's nictitating membrane. J. Pharmacol. Expt. Ther. 172: 91-99 (1970).

VAN ZWIETEN, P.A.: Characterization of the control alpha-adrenoceptors involved in the hypotensive action of clonidine by yohimbine corynanthine and rauwolscine. Arch. Pharmacol. 311: R59 (1980).

VARMA, D.R. and McCULLOUGH, H.N.: Dissociation of the supersensitivity to norepinephrine caused by cocaine from inhibition of ^{3}H-norepinephrine uptake in cold-stored smooth muscle. J. Pharmacol. Expt. Ther. 166: 26-34 (1969).

VIZI, E.S., SOMOGYI, G.T., HADHÁZY, P. and KNOLL, J.: Effect of duration and frequency of stimulation on the presynaptic inhibition by alpha-adrenoceptor stimulation of the adrenergic transmission. Arch. Pharmacol. 280: 79-91 (1973).

VOGEL, S.A., SIBERSTEIN, S.D., BERV, K.R. and KOPIN, I.J.: Stimulation-induced release of norepinephrine from rat superior cervical ganglion in vitro. Eur. J. Pharmacol. 20: 308-311 (1972).

VON EULER, U.S.: General views on the relevance of presynaptic receptor systems. In "Presynaptic receptors," (Eds. Langer, S.Z., Starke, K. and Dubocovich, M.L.), Pergamon Press, Oxford, pp. 5-9 (1979).

WALTMAN, S. and SEARS, M.: Catechol-O-methyl transferase and monoamine oxidase activity in the ocular tissues of albino rabbits. Invest. Ophthalmol. 3: 601-605 (1964).

WATERSON, J.G. and SMALE, D.E.: Location of noradrenergic structures in the control artery of the rabbit ear. Aust. J. Expt. Biol. Med. Sci. 45: 301-308 (1967).

WEITZELL, R., TANAKA, T. and STARKE, K.: Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in th pulmonary artery of the rabbit. Arch. Pharmacol. 308: 127-136 (1979).

WERNER, U., STARKE, K. and SCHÜMANN, H.J.: Actions of clonidine and 2-(2-methyl-6-ethyl-cyclohexylamino)-2-oxazoline on postganaglionic autonomic nerves. Arch. Int. Pharmacodyn. 195: 282-290 (1972).

WESTFALL, T.C.: Local regulation of adrenergic neurotransmission. Physiol. Rev. 57: 659-728 (1977).

WESTFALL, T.C. and LEIGHTON, H.J.: Effect of decentralization on presynaptic receptor regulation of NE release. Pharmacologist 18: 208 (1976).

WHITBY, L.G., AXELROD, J. and WEIL-MALHERBE, H.: The fate of ^{3}H-norepinephrine in animals. J. Pharmacol. Expt. Ther. 132: 193-201 (1961).

WOLFE, D.E., POTTER, L.T., RICHARDSON, K.C. and AXELROD, J.: Localizing tritiated norepinephrine in sympathetic axons by electron micrograph autoradiography. Science 138: 440 (1962).

YAMAGUCHI, N., DE CHAMPLAIN, J. and NADEAU, R.A.: Regulation of norepinephrine release from cardiac sympathetic fibres in the dog by presynaptic alpha- and beta-receptors. Cir. Res. 41: 108-117 (1977).

YONG, M.S. and CHEN, P.C.: The effect of cocaine, reserpine, and 6-hydroxydopamine on the response of the perfused central artery of the rabbit ear to sympathomimetic amines. Can. J. Physiol. Pharmacol. 53: 38-46 (1975).

ZIMMERMAN, B.G., LIAO, J.C. and GISSLEN, J.: Effect of phenoxybenzamine and combined administration of iproniazid and tropolone on catecholamine release elicited by renal sympathetic nerve stimulation. J. Pharmacol. Expt. Ther. 176: 603-610 (1971).

ZUPANCIC, A.O.: The mode of action of acetylcholine. Acta Physiol. Scand. 29: 63-71 (1953).