UNIVERSITÉ D'OTTAWA / UNIVERSITY OF OTTAWA
École des études supérieures/School of Graduate Studies

Title of thesis STUDIES ON THE INFLUENCE OF SOME PSYCHOACTIVE DRUGS ON BRAIN HISTAMINE

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Date of defence June 10, 1977

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STUDIES ON THE INFLUENCE OF SOME PSYCHOACTIVE DRUGS ON BRAIN HISTAMINE

A Thesis

Presented to

The School of Graduate Studies

of

The University of Ottawa

by

R. W. Henwood, B.Sc., M.Sc.

In partial fulfillment of requirements

for the degree of

Doctor of Philosophy

April 1977

To: My parents

ACKNOWLEDGMENTS

First of all, I would like to thank my wife Beverley for her sacrifices and encouragement throughout my studies. Without her strong support these studies would not have been possible.

The financial support of the Ontario Mental Health Foundation, in the form of a Research Studentship to the author and the research support from the OMHF Grant 379-71D is gratefully acknowledged.

Mr. Ross Layberry's valuable assistance in computer programming was greatly appreciated as was the generosity of the Department of Physiology for the use of their computing facilities.

The conscientious workmanship of the staff of the animal care department deserves considerable thanks.

Finally, I am grateful to Mrs. Marie Gagné of Ottawa and Mrs. Mary-Ann Barr of Saskatoon for their expert typing of this manuscript, and to Mr. S. Klosevych and the staff of Medical Communications Services for the preparation of the figures.

ABSTRACT

A major emphasis of research in mental health and neuropharmacology has been placed upon putative neurotransmitters. Histamine has received less attention until now partly due to difficulties encountered in measuring its concentration and turnover rates in discrete brain regions of rats. The enzymatic-isotopic assay procedures currently employed now afford accurate and reliable estimates in various brain regions of rats.

Acute treatment with morphine, methadone, or naloxone did not induce any significant changes in the endogenous histamine concentrations in the hypothalamus, midbrain or cortex.

Endogenous brain histamine concentrations have been found to be reduced in the hypothalamus, midbrain, and cortex following chronic morphine administration and these results are exacerbated by withdrawal. The metabolizing enzyme, histamine methyltransferase, was discovered to have unaltered activity after such treatments and the activity of the synthesizing enzyme histidine decarboxylase has been reported to be enhanced. These findings leave interference with precursor availability, impairment of histamine storage, increased release, or enhancement of an alternative metabolic pathway as explanations for the decreases noted in endogenous histamine concentrations.

The decreases in brain histamine observed following chronic morphine administration could be prevented or reversed by simultaneous chronic administration of histidine.

Acute and chronic pentazocine administration have been found to produce changes in brain histamine concentrations in a fashion parallel

to the effects of morphine although qualitatively reduced.

Mandrax and its compoments methaqualone and diphenhydramine were found to induce decreases in endogenous brain histamine concentrations in the brain regions investigated but these changes were less severe and shorter-lived following cessation of treatment thatn the changes noted following chronic morphine treatment.

Chronic amphetamine administration produced a qualitatively different pattern of changes in brain histamine levels from that seen following the sedatives. Hypothalamic hsitamine was reduced and mid-brain and cortical histamine levels were increased significantly.

Tetrahydrocannabinol, either acutely or chronically, did not induce any detectable changes in brain histamine levels in the brain regions investigated.

The sedative agents clearly depressed and the central stimulants significantly elevated the locomotor activities of the rats in these studies in a dose-dependent manner. The relationships between endogenous brain histamine concentrations or changes therein and spontaneous locomotor activities have been discussed.

The current studies illustrate that histamine may be involved in the abnormal behavioral states induced by the psychoactive drugs used in these studies as has been proposed for other biogenic animes.

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LIST OF ABBREVIATIONS

Ach	— Acetylcholine
AMPT	— Alpha-methyl-p-tyrosine
CA	— Catecholamines
CNS	— Central nervous system
Cyclic AMP	— Cyclic-3',5'-adenosine monophosphate
Cyclic GMP	— Cyclic-3',5'-guanosine monophosphate
DA	— Dopamine
DIPH	— Diphenhydramine
L-DOPA	— L-3,4-dihydroxyphenylalanine
GABA	— Gamma-aminobutyric acid
HA	— Histamine
HD	— Histidine Decarboxylase
HMT	— Histamine-N-methyltransferase
5-HT	— 5-hydroxytryptamine
5-HTP	— 5-hydroxytryptophan
MAOI	— Monoamine oxidase inhibitor
MQ	— Methaqualone
MX	— Mandrax
NA	— Noradrenaline
SLA	— Spontaneous locomotor activity
Δ^9-THC	— Δ^9-tetrahydrocannabinol

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INTRODUCTION

Histamine the Autacoid

Histamine (HA) like other autacoids possesses an astonishingly wide range of pharmacological activities in vanishingly small amounts. No clear pattern has yet emerged which would explain the probable biological significance of this amine in the mammalian central nervous system (CNS). Recently the possible involvement of HA in brain function has been proposed. Histamine in the CNS may or may not function independently of the HA in the periphery and indeed many of the peripheral effects of HA may influence its effects within the CNS. To date there have been observed very large variations in HA levels in various brain regions even within the same species. Technical difficulties in assaying procedures and the extremely low levels of HA found in the mammalian CNS also serve to further complicate matters.

It has been proposed that some of the HA present in the brain is acting as a neurotransmitter analogous to other monoamines and that it may be involved in such basic functions as wakefulness, thermoregularion, cardiovascular regulation, thirst, and hunger. The current studies are intended to investigate a possible involvement of HA in the pharmacological actions of various psychoactive drugs.

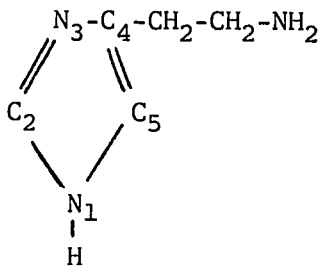
Before considering the evidence indicating some possible roles for HA in the abnormal mental states induced by some current drugs of abuse, it is important to discuss its physiological properties, localization, and metabolism within the brain.

LITERATURE REVIEW

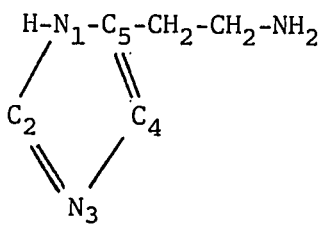
Physical Properties of Histamine

Histamine exists in two tautomeric forms as seen below. Form "A" seems to predominate but the presence of "B" can also be inferred from studies of its metabolites in urine (1, 2).

"A"



"B"



The molecular characteristics of HA could prompt numerous guesses on how it might interact with tissues. Histamine forms chelates in which the primary amino group on each side chain and the pyridine-like nitrogen on the ring bind the cation; complexes of 1:1 or 2:1 are possible. Salt formation occurs through the primary amino group (3) which is protonated at physiological pH. Imidazole forms hydrogen bonds through both the pyridine-like and pyrrole nitrogens, and can form oligomers by intermolecular hydrogen bonding; HA should have a similar capacity for hydrogen bonding. The C_5 carbon in tautomer "A" is strongly nucleophilic and the ease of cyclization of HA rests on this property. The C_2 carbon is electron deficient and could attract nucleophilic groups. The calculated energies of the highest occupied and lowest empty molecular orbitals

suggests that the imidazole ring does not engage in charge transfer reactions.

Histamine is a strong base with a pKa of about 9.7 for the side chain amino group and of about 5.8 for the imidazole group. Consequently, within the physiological pH range, HA will be present almost exclusively as the univalent cation, the ratio of ionized to unionized molecules being more than 100:1. Finally, the interactions of HA with its sites of action have been found to be pH dependent (4-6).

Localization of Histamine in Mammalian Central Nervous System

The cerebral concentrations of HA are considerably lower than those of serotonin (5-HT) and noradrenaline (NA) (7, 8), and do not differ too significantly among mammals with the exception of the frog. It has been pointed out that HA may be of significance as a neurotransmitter in other species such as the Octopoda (9). In the Octopoda HA levels have been found to be higher than either 5-HT or NA and perhaps it plays a more pronounced role.

Knowledge of the cerebral regional distribution of some biogenic chemicals has aided considerably in achieving an understanding of their function. The following table shows the regional distribution of HA in the brains of several species (10). The analytical methods employed are indicated by the following: (a) bioassay, (b) fluorometric, and (c) enzymatic-isotopic.

As can be seen, the concentration of HA is highest in the hypothalamus, less in the thalamus and midbrain, and least in the cerebral

DISTRIBUTION OF HISTAMINE IN THE CNS OF SEVERAL MAMMALS

Species	Cat(a)	Dog(a)	Monkey(b)	Rabbit(a)	Frog(c)
	Means expressed as ng/g wet weight				
Structures					
Spinal cord	<100	< 40	63	100	
Cerebellum	< 20	< 50	15	60	60
Thalamus			196		
Medial thalamus	250	260		270	
Ventrolateral	75				
Dorsolateral	350	220			
Medial geniculate	370	270			
Lateral geniculate	180	140			
Hypothalamus		610	1850	700	760
Mammilary body	1150	740			
Ventral hypothalamus	800	900			
Dorsal hypothalamus	480	460			
Preoptic area	430				
Median eminance	12500	15100			
Cerebral Cortex	90			110	290
Caudate nucleus	50	40	103	150	
Hippocampus	60	80	81	90	
Hypophysis	2400	900	580		
Anterior lobe	2400	750		650	
Posterior lobe	1700	1060		400	
References	11	12	*	*	13

* Personal communication in Ref. 7.

cortex. These are similar to the regional distributions of 5-HT and NA. Much higher concentrations of HA (higher than 5-HT or NA) are found in all three regions of the hypophysis, especially the hypopyseal stalk. This is a highly vascular region and the wide variation in the HA concentration might be explained by variations in the mast cell population. It might also be noted that the bioassay methods gave much higher values than are now found with the enzymatic isotopic assay. This might also lead to some difficulties of comparison.

With respect to the biogenic amines, 5-HT and NA, their localization within the hypothalamus and limbic forebrain enabled investigators to link them to the regulation of emotional behavior long before there was strong evidence that they might be neurotransmitters.

Recently, Snyder developed a micro version of the enzymatic-isotopic assay for HA and was able to explore the regional localization of HA in monkey brain focussing on individual hypothalamic nuclei and nuclei of other brain regions (14). As much as a 25-fold variation of HA levels was found among the various nuclei of the hypothalamus. The mammillary bodies had the highest levels of HA which was 250 times greater than the lowest found in the brain, in the cerebellum, and 5 times higher than the concentration found in the infundibulum. While there was excellent consistency in values for most hypothalamic nuclei, HA concentrations in the supraoptic nucleus varied as much as 10-fold in different animals.

Subcellular fractionation is a valuable tool for determining the intracellular locale and possible function of numerous chemicals in the brain. The subcellular distribution of HA in mammalian brain has been

repeatedly investigated (15-20). In 1963, Carlini and Green (21) found the microsomal fraction of guinea pig whole brain to be especially rich in HA. Michaelson and Coffman (22) reported the majority of brain HA to be confined to the crude mitochondrial pellet. The difference between these results may be related to the conditions of centrifugation used for preparing the crude mitochondrial fractions.

Histamine was localized to an area of the gradient corresponding to the maximal potassium and lactic acid dehydrogenase activity. The highest levels of HA occurred in a portion of the gradient less dense than the peak activity for monoamine oxidase, a marker for mitochondria. Hypotonic shock released HA into the supernatant fluid. Since potassium and lactic acid dehydrogenase are markers for cytoplasm occluded within synaptosomes, and since synaptosomes and not mitochondria, can be lysed by hypotonic shock, these data indicate that the HA was localized to synaptosomes (14).

From this work it appears that the true distribution of HA resembles that of the other putative neurotransmitter NA. In order to verify this conclusion direct positive identification techniques would need to be employed. These techniques might include an electronmicroscopic histochemical method and perhaps mass spectrometric determination of the contents of these tissues.

Although redistribution of HA during homogenization interferes with studies of its intracellular localization, it is evident that a major portion of brain HA is localized in nerve terminals which is an important piece of evidence in support of the case for HA as a possible neurotransmitter in the brain.

Unlike the situation of the minor peaks of HA in sucrose gradients of adult rat brain, there is no evidence of redistribution of neonatal brain HA during homogenization.

Mast Cell and Non Mast Cell Histamine

Before the physiological functions of brain HA may be understood, its precise identification in specific cells along with details of its synthesis and storage are required. Although there is a gross correlation between the concentration of HA and the number of mast cells in the three portions of the hypophysis, there is reason to think that HA in the anterior lobe of the hypophysis is retained in cells different from mast cells, whereas HA in the posterior lobe and stalk is found at least in part in mast cells (11). It appears relevant that the rabbit, which is deficient in mast cells, possessed less HA in the posterior lobe of its hypophysis than is the case in other species (23).

From studies on the adult rat brain, there is extensive evidence of HA in a neuronal system developing after birth other than mast cells (24). In conjunction with this hypothesis of a dual localization of HA in neurons and mast cells, it has been found that slices of mouse brain (25) or rat hypothalamus (26) incubated in a physiological medium, released both HA and endogenously synthesized ^{3}H-HA (27) when depolarized. This HA is believed to arise from neurons and not mast cells. Unfortunately, the mechanism of HA release from mast cells is similar in many ways to the transmitter release mechanisms from nerve endings (28).

The importance of mast cells as a potential storage site for HA has been overlooked for a long time. Owing to the low levels of HA in

the brain, a relatively small number of mast cells containing HA in amounts similar to typical peritoneal mast cells could account for the entire amine content. Recently several authors have reported the occurrence of mast cells in the mammalian brain (29-32) and it appears that there is some regional variation in their occurrence which might to a certain extent parallel the variations in HA levels. These studies do not however, give any idea as to the percentage of cerebral HA in mast cells or closely related cells. In the neonatal rat brain, mast cells could well be a major storage site for HA (33). In neonatal animals, neurons actively proliferate and most neurotransmitters increase in concentration with age (34-36) but in contrast HA levels in the brain of neonatal rats is at least five times higher than in adults (37, 38) yet the function of this HA is not yet certain. Perhaps it plays some role in the rapid growth processes of the brain and is involved with functions of the nucleus in regulating growth. Histamine is a very basic compound as are the polyamines and perhaps HA may play an analogous role to that proposed for the polyamines which have been proposed as regulators of nucleic acid function in rapidly growing tissues.

Histamine Formation and Metabolism

White (39) and Adam (40) have reported that the hypothalamus of cats did not concentrate HA but was capable of synthesizing it. In 1959, White (41) showed that the rate of HA formation was by far the greatest in hypothalamic tissue, the area postrema was less, and the cerebral cortex showed slight but detectable activity.

The substrate of HA formation is L-histidine which contains an imidazole ring that animals are incapable of synthesizing. Histamine is

then formed by decarboxylation of histidine and although this is not a major degradative pathway for histidine it is perhaps the only pathway for HA synthesis within the body. As it does not easily pass the blood brain barrier, almost all HA in the brain must be formed intracerebrally (37, 42, 43).

The greatest histidine decarboxylase (HD) activity among nervous tissue was found in the hypothalamus of cats, dogs (39-41), pigs (41), humans (42, 43) and the rat fetus (44, 45). High levels of HD were found in the hypothalamus, cerebral cortex, and hippocampus of rats, but not in the cerebellum and cervical ganglia (44). The regional differences were more distinct for HD than for HA. In contrast to HA ontogenesis, HD shows an increase from birth to adulthood parallelling synaptogenesis.

There are at least two histidine decarboxylases, both of which require pyridoxal-5'-phosphate (46) and are found in the soluble portion of the cell. Histamine can be formed by the specific HD and by the non-specific HD (aromatic amino acid decarboxylase) and probably both types were measured in the homogenates in earlier experiments. Due to the large amount of HA formed daily by rat tissues, it is difficult to suppose that it is formed only by the specific HD. The specific HD has a high affinity for histidine and does not decarboxylate dihydroxyphenylalanine (DOPA) or 5-hydroxytryptophan (5-HTP). It is not inhibited by alpha-methyldopa whereas the non-specific decarboxylase is (47). The activities of both decarboxylases are very temperature and pH dependent (48). The specific HD can be inhibited by alpha-hydrazinohistidine or NSD-1055; however, the lowering of brain HA by these treatments could represent displacement or some other effect rather

than inhibition of synthesis. The rate of decline of HA may reflect the turnover rate of at least a portion of brain HA and if this is so, it appears to be turning over much more rapidly than 5-HT or NA.

Histamine Formation from Exogenously Administered Histidine

Histamine formation in vivo can be studied after intraventricular administration of ^{3}H- or ^{14}C-histidine (14, 49). Schayer and Reilly (50) reported ^{14}C-histamine formation in whole mouse brain but such data only reveal the sum of all the changes occurring after treatment and possible opposite effects of drugs on HA concentrations in various brain regions cannot be detected. The advantage of the intraventricular method lies in the very small quantities of precursor that must be administered in order to detect the radioactivity of the ^{3}H- or ^{14}C-histamine formed in different brain regions of the rat. Intravenous radiolabeled HA formation requires prohibitive amounts of expensive precursor. Several serious objections have been raised against this method considering the nonphysiological route and whether the distribution would be the same as the naturally occurring substance. In addition, this procedure itself induces considerable stress in unanesthetized animals or the anesthetics themselves may introduce changes in normal brain function and hence neurotransmitter levels, or distribution.

It appears that the mean concentration of free histidine in brain tissues [around 5×10^{-5} according to Levi *et al.* (51)] is not sufficient to saturate the HD (Km 10^{-4}M) which is a similar situation to that of central serotonergic neurons in which the enzyme tryptophan hydroxylase is not saturated by its substrate. The assumption that the biosynthesis of HA might be influenced by the level of L-histidine in the plasma

appears to be confirmed by the effects of histidine loads. Such treatment markedly increased the cerebral HA levels in various brain regions and subcellular fractions (52, 53).

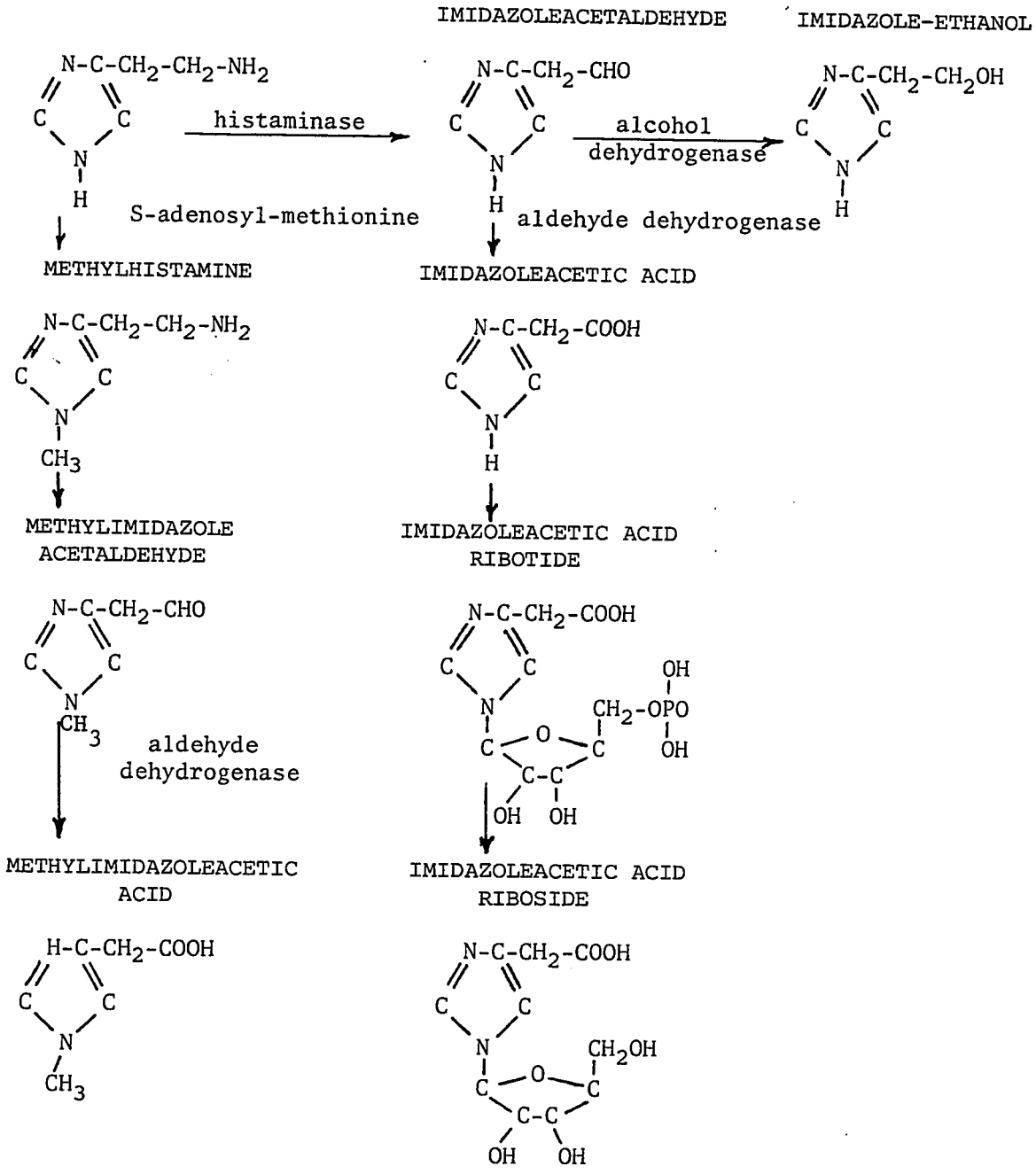
Following acute histidine administration, HA levels have been found to be increased 2-3 times in all brain regions except the medulla oblongata-pons where there was no alteration in the HA content (14, 54-56). The greatest formation of HA from exogenously administered histidine in the hypothalamus coincides with the highest brain concentrations and the rapid turnover of HA found in this brain region (14).

After HA is formed in the soluble portion of the cell it must be protected in order to escape degradation by the catabolizing enzymes present (57). It is possible that in the soluble cytoplasm HA is in complex with acidic material which prevents its catabolism or possibly the decarboxylation occurs at or near the surface of the particulate material to which HA attaches. These problems are not peculiar to HA but they exist as well for the other biogenic amines.

Histamine Catabolism

Some possible routes of HA metabolism (58) are shown in the diagram on the following page and it can be seen that HA may be metabolized by either oxidative deamination or methylation.

In most species including man, there is some direct oxidative deamination of HA by histaminase to produce imidazoleacetaldehyde. Almost all of the aldehyde is converted by aldehyde dehydrogenase to imidazoleacetic acid but a small portion is reduced by alcohol dehydrogenase to imidazole ethanol (histidol). In the brain, however, histaminase activity is low or absent in some regions (59-61) and this



supports the view that the pathway of HA degradation in the brain under physiological conditions may not involve oxidative deamination. One cannot exclude the possibility that histaminase may be localized in very specialized cerebral structures and the examination of the whole brain

so dilutes the activity that it is impossible to detect. Further examinations are necessary using more sensitive and more specific methods on certain structures especially nerves and nerve terminals in order to be certain.

The most important route of catabolism of HA is via methylation to methylhistamine with S-adenosylmethionine as the methyl donor and histamine methyltransferase (HMT) as the converting enzyme. The methylhistamine is then oxidatively deaminated to form methylimidazole acetaldehyde and from this by the action of aldehyde dehydrogenase the major urinary product of HA catabolism results (methylimidazoleacetic acid). Methylation plays a significant role in brain (62, 63) being found in the cat (39, 64), mouse (65, 50), rat (66) and guinea pig (67). In the rat brain, the predominance of methylation as the major metabolic pathway (68, 69) is indirectly emphasized by finding very low diamine oxidase activity (70, 71). There are however, considerable differences in HA metabolism in other species and even in various organs within the same species (72, 73).

Methylhistamine is the HA methylation product (62, 63, 74, 75), but it is also possible that methylhistamine may be formed by a decarboxylation of methylhistidine. This metabolic pathway described by Schwartz (76) is five times slower than the pathway methylating HA, however, we cannot overlook the significance of this pathway in those species or tissues in which HMT activity is very low. The existence of this pathway has not been demonstrated in the CNS .

Quantitative Importance of Histamine as a Neurotransmitter

The great volume of research published in recent years on biogenic amines has led to the assumption that they are major neurotransmitters

in the CNS. While they undoubtedly play an important role in brain function, the biogenic amines probably account for transmission at only a small proportion of central nervous synapses (77). For instance, Hokfelt (78) has estimated that in the corpus striatum, the brain region richest in DA, only about 15% of the nerve terminals appear to contain CA and in the hypothalamus, which contains the brain's highest levels of NA, only 5% of the nerve terminals are catecholaminergic. Serotonin probably accounts for a smaller proportion of brain synapses than NA. Estimates suggest that acetylcholine (Ach)-containing nerve terminals account for no more than about 10% of brain synapses. Histamine may also be a transmitter in the brain but its levels are only about 10% those of 5-HT. The important factor to consider here is that the turnover rate of HA is many times faster than that of the CA and is comparable to Ach and hence the functional significance of HA may be quite comparable to the other amines (79).

Direct Effects of Histamine on Neurons

The microelectrophoretical application of HA into the immediate vicinity of single neurons has been reported to alter resting potentials as well as firing rates (80-84). In a number of cases however, the significance of the responses remains unclear since the strict conditions required for this kind of procedure were barely met. Additionally, most of the studies have been performed before the discovery of specific agonists and antagonists of H_1 and H_2 receptors (85).

Direct HA application depressed the firing rate of many interneurons and hyperpolarized motoneurons of the cat spinal cord (86). A depressant, generally weak, action was also recorded after application

of HA on neurons of the cuneate nucleus (87), brain stem reticular formation (88), motor cortex (89) or of the cerebellar Purkinje cells (90). In contrast, the great majority of the cells in the hypothalamus were excited by HA and when it was applied in high dosages on cerebrocortical neurons excitation was also recorded (91). In contrast to the depressant effect of HA on the excitability of neurons in the cerebral cortex, and brain stem, HA demonstrates predominantly excitatory action on supraoptic and other unidentified hypothalamic neurons (81, 92). Recent studies by L. Renaud (93) on identified hypothalamic neurons in the centromedial nucleus of rats imply that there are differences not only in the nature of HA sensitive neurons within individual hypothalamic nuclei but also regional differences within the hypothalamus. Microelectrophoretically applied HA has a relative potency similar to that of other biogenic amines (94).

The actions of HA on cerebral neurons appears to result from the activation of specific receptors either H_1, H_2 or both. As H_1 receptor antagonist (mepyramine) partially blocks these effects and the addition of the H_2 blocker (metiamide) totally blocked these effects. The data with H_1 antagonists should be considered with caution in view of their potent membrane stabilizing action which results in a non-specific blockade of the actions of a variety of neurotransmitters. The new H_2 receptor antagonist metiamide is more selective than the H_1 blockers (95-97) and should permit studies on those excitant actions which do not involve H_2-receptors, but its effects may be complicated by partial agonist activity (98).

Histaminergic Pathways and Receptors

If HA is a neurotransmitter then it arises to elucidate the locations of the neuronal pathways and the cell bodies. Studies using selective brain lesions in different mesencephalic or diencephalic structures (99) failed to produce any significant reduction of HA in various brain regions. In fact, large diencephalic lesions were found to cause a slow but marked elevation in hypothalamic HA and this was interpreted as due to a process of heterotypic re-innervation (100, 101). These processes may, however, be due only to increased numbers of mast cells as found in the sheaths of injured peripheral nerves. Perhaps even HA levels in brain regions are not specific markers for putative histaminergic neurons and instead HD activity measurements may be more reliable.

Starting from the observations of very similar patterns in the regional distribution of HA and the monoamines NA and 5-HT, it was postulated that there are histaminergic fibers in the medial forebrain bundle, the ascending pathway containing the bulk of the monoaminergic neurons. Diencephalic lesions performed unilaterally at the level of the lateral hypothalamic area were found to lower HD activity in the ipsilateral cerebral cortex (91, 102, 103) with a time course compatible with Wallerian degeneration. The enzyme activity in the contralateral side was not significantly altered, indicating that the telencephalic degeneration was restricted to the lesioned side. The maximal reduction in HD (50-60%) was observed after 10 days but the HA content was decreased by only 20-30%.

The existence of a non-neuronal compartment of HA representing

about 50% of the total amine in rat cerebral cortex and characterized, like mast cells, by a low HD activity could explain why the lesions affected the synthesizing enzyme more than the level of the amine. This view is supported by experiments in the cat in which surgical interruption of both the ascending and lateral afferent neurons resulted in an almost complete disappearance of HD activity whereas HA levels were only reduced by 50% (104).

Although lesions affecting HA in the telencephalon also interrupted the monoaminergic ascending pathways, their effects cannot be ascribed to an indirect consequence of the degeneration of the latter since neither 6-hydroxydopamine nor 5,6-dihydroxytryptamine affected HD activity in the rat telencephalon (102, 103).

Schwartz (105) concludes there is a distinct histaminergic pathway emanating from the midbrain or brain stem, ascending throughout the entire ipsilateral telencephalon. Recently a histaminergic pathway to the cerebral cortex has been evidenced with the help of microelectrode techniques (106). This system may set the overall excitability of the telencephalon, perhaps in relay from the reticular formation and this might explain its apparent role in states of sleep and wakefulness. A variety of data indirectly support this hypothesis. First, the fact that reserpine is able to release HA (26, 107) suggests that the mechanism of storage of the imidazolamine might bear some similarity with that of the monoamines. In addition, several behavioral studies are in agreement with this hypothesis. The application of HA as well as that of its precursor amino acid histidine, in the lateral hypothalamus, has been shown to modify self-stimulation (108) a behavior whose anatomical substrate is believed to be the medial forebrain bundle (109). The

participation of HA in cortical arousal mechanisms has also been evoked in view of the desynchronized electroencephalographic patterns recorded after its intraventricular administration (110). The desynchronization has recently been reported to be reversed by low doses of peripherally administered mepyramine. The almost immediate decrease of cerebral HA turnover in rats receiving barbiturates (111, 112), as well as the marked sedative properties of most antihistamines used in therapeutics, are also consistent with the possible involvement of ascending histaminergic neurons in arousal mechanisms.

Increasing evidence supports an important role for cyclic-3',5'-adenosine monophosphate (cyclic AMP) in the CNS. Several putative neurotransmitters such as catecholamines (CA), 5-HT and HA, have been found capable of increasing cyclic AMP concentrations in rat brain slices from various brain regions (113-115). From these studies it might be concluded that there are distinguishable adenylate cyclase receptors for these biogenic amines.

As early as 1968, Kakiuchi and Rall (116, 117) found that HA increased cyclic AMP in rabbit brain slices and that the increase was 10 times that produced by NA or 5-HT. A series of experiments was performed to determine whether cyclic AMP may play a role in regulating the activities of the cells of the CNS as an intracellular mediator of responses to transmitters and hormone-like compounds. The results found in chicks, suggest that the behavioral responses to HA may be mediated by an increased rate of formation of cyclic AMP. Responses of cyclic AMP generating systems to HA have now been reported in slices from a variety of species and brain regions (118). In guinea pig, HA evokes the largest responses in slices from the hippocampus, cerebral cortex, and thalamus.

The effect of narcotic drugs on the cyclic nucleotides in brain that are involved in adrenergic transmission have mainly been examined indirectly. Intravenous cyclic AMP antagonozed the analgetic response of morphine in both tolerant and non-tolerant mice and accelerated the development of tolerance to and physical dependence upon morphine (119). These authors also suggested that cyclic AMP administered intracerebrally might reverse the depressant effect of morphine. Gullis et.al. have reported that morphine increases the content of cyclic-3',5'-guanosine monophosphate (cyclic GMP) and decreases that of cyclic AMP in neuroblastoma cell lines (120) but they have now retracted these findings amid serious controversy. Since in brain the opiate receptor may exist in two conformational states, sodium-dependent and sodium-free (121), it was suggested that each was linked in its function with either cyclic AMP or cyclic GMP. Morphine in very high doses increases the cyclic AMP content of the striatum (122), although since the doses of morphine required were high, perhaps they were unrelated to a specific binding of morphine to the opiate receptor. Other reports have shown that morphine increases cyclic AMP content and decreases cyclic GMP content in various brain structures including the striatum (123). It has been reported that morphine enhances the cyclic GMP content of the striatum when brain enzymes are inactivated within 2 seconds (124). This work should illustrate some of the complexities involved in unravelling the truth with regard to the physiological involvement of HA and these nucleotides.

It is clear that the effect of HA is mediated by receptors that are distinct from those mediating the response of the cyclic AMP generating system to NA. This is indicated by the large regional differences in responsiveness to the two biogenic amines during brain maturation (125) and by the lack of effect of alpha-adrenergic and beta-adrenergic

receptor blockers on the HA-induced stimulation (126). On the other hand, classical antihistamines (H_1 antagonists) have been repeatedly found to inhibit the HA-induced stimulation but in most cases, the blockade was either a partial one or was complete only with high drug concentrations. Recently, Baudry *et al.* (127) have demonstrated that the HA-induced accumulation of cyclic AMP in slices from guinea pig cerebral cortex, was in fact, mediated by the activation of the two classes of receptors: it could be partially inhibited in a dose-dependent fashion by either an H_1-antagonist (mepyramine) or an H_2-antagonist (metiamide), and totally blocked in the presence of both agents. This is in contrast to the predominant H_2-receptor involvement peripherally (128-131). Furthermore, a specific H_2-receptor agonist, 4-methylhistamine, induced a partial activation which in this case could be totally prevented by metiamide. In hippocampal slices, the results were quite different and H_1 and H_2-receptor linked cyclases appeared to be of nearly equal importance. It has been shown more recently that a classical H_1-receptor is associated with the major portion of the response of HA-sensitive cyclic AMP generating systems in guinea pig neocortical slices, and that a small portion of the responses may be due to activation of H_2-receptors (132). In rats, HA has only marginal effects on cyclic AMP levels in cerebral cortical slices except in the presence of a phosphodiesterase inhibitor (133-135) or adenosine (133, 136). Recent results suggest that the HA response in rat cortical slices has primarily H_2 character (135) leading to accumulation of adenosine-3′,5′-monophosphate.

The cellular localizations and functional roles of the two classes of cerebral HA receptors, as evidenced by the stimulation of the cyclic AMP generating system, is still a matter of conjecture, however, from

their presence in the cerebral cortex and hippocampus (regions where lesion studies indicate that HA-containing neurons might be ending) it is tempting to speculate that such receptors, or at least one kind of them, are localized post-synaptically and that cyclic AMP is playing the same role of "second messenger" at histaminergic synapses as hypothesized at noradrenergic synapses of the cerebellum or hippocampus (83). Consistent with this view are the recently reported similar actions of cyclic AMP and HA (137) which were microiontophoretically applied on cells of the cat medullary reticular formation: the great majority of cells depressed by cyclic AMP were also depressed by HA. The finding of HA receptors linked with the cyclic AMP generating system on isolated nerve endings would also be consistent with this hypothesis. On the other hand, the responsiveness of cultured glioma cells (138) and, glia being the major morphological site of glycogen deposition, the finding of an H_1 receptor-mediated stimulation of glycogenolysis in brain slices (139) would be in favor of a non-neuronal localization of these receptors.

The mechanism and physiological significance of the synergistic interaction between HA and adenosine is unknown. The results with combinations of H_1 or H_2 agonists with adenosine however, provide some interesting insights. In both cerebro-cortical and hippocampal slices, the synergism between HA and adenosine appears to involve primarily H_1 receptors. The characteristics of these receptors are, however, apparently altered by the presence of adenosine. Thus although the affinity of the H_1 receptor for HA is not significantly changed, the affinity for the H_1-agonist 2-aminoethylthiazole, is increased 100-fold and even the H_2-agonist, 4-methylhistamine, now appears to activate the

H_1-receptor. Such results suggest that adenosine, upon interaction with its receptor, not only activates an adenylate cyclase but also profoundly alters the properties of associated amine-sensitive receptor-cyclase complexes (140).

Although not exactly comparable, it is still worth mentioning that in a recent study (141) it was suggested that intracellular cyclic AMP regulates the calcium gating mechanism which is supposed to control HA secretion from rat peritoneal mast cells. This idea however, remains to be substantiated by measuring intracellular cyclic AMP and calcium uptake in the CNS.

Possible Physiological Roles for Histamine

Histamine may be involved as a substrate for some reaction, as a mediator of certain CNS functions, or it may directly influence the CNS. Studies have employed drugs, lesions, phychological stresses, and physiological influences in order to alter the levels and turnover of brain HA. Histamine has been reported to be involved in drug dependence, wakefulness, hunger-satiety, aggression, respiration, cardiovascular control, temperature regulation, and schizophrenia either directly or via interactions with the other biogenic amines present in the CNS. The studies performed to elucidate the role of HA in brain function include clinical studies, animal studies, and in-vitro studies. Together, these studies are beginning to assign significant roles to HA in brain function.

Histaminoceptive neurones with distinct functions exist in the hypothalamus where HA excites many neurones including neurosecretory cells of the supraoptic nucleus of the cat and neurons in the rat

ventromedial hypothalamus producing releasing factors (142). These results strongly suggest an involvement of HA in the secretion of the posterior pituitary hormones and of releasing factors for the adeno-hypophyseal hormones on the synaptic level in the hypothalamus.

The hypothalamus acts as a "final common pathway" for the integration of all neural activity that affects anterior pituitary activity, and it is the principal structure through which analgesics exert their effects on the pituitary. In nearly all cases chronic morphine administration inhibits pituitary function (143). The mechanism by which narcotic analgesics might influence hypothalamic-pituitary activity focuses mainly on the presence and role of cholinergic and monoaminergic pathways in the CNS (144, 145). If one assumes that the neurosecretory cells which contain the releasing and inhibiting factors are in a postsynaptic position, then any alteration in the synthesis, release, or degradation of transmitter substances in the presynaptic neurons innervating these cells could influence the synthesis and/or release of the contents of the cells. Changes in turnover rates and concentrations of the transmitter substances in brain have been associated with alterations in anterior pituitary function, with particular emphasis on adreno-corticotrophic hormone and leutinizing hormone function (146-152).

The supraoptic nucleus is thought to be mainly concerned with making neurosecretory granules containing antidiuretic hormone. A marked antidiuresis followed the injection of HA into the cat supraoptic nucleus (153). Experiments are being performed to examine the effects of water restriction or hypertonic saline on HA synthesis in this area of the hypothalamus. Intrahypothalamic or intraventricular HA

consistently elicited a water intake which was blocked or reversed by H_1 antagonists (154, 155).

The ventromedial region of the hypothalamus, among other functions, is involved in the regulation of eating behavior and perhaps HA neurons may play a relevant role similar to, or complementary to the role described for CA (156, 157).

The possible role of brain HA in behavioral performance was studied in rats using thirst-induced water consumption, continuous avoidance, and reinforcement withdrawal test systems (155). Parenteral administration of a variety of antihistaminics to the rats decreased the thirst-induced water consumption, and this effect could be antagonized by intraventricular administration of HA. When intraventricular doses of HA were given to rats at weekly intervals, an adaptation was seen in the effects of the amine on continuous avoidance behavior. With succeeding doses the initial period of depression of avoidance responding was shortened and the subsequent rebound stimulation disappeared.

The reported depletion of brain HA by 4-thiazolylmethoxyamine was accompanied by a significant reduction of spontaneous locomotor activity (SLA), total suppression of REM sleep, and a reduction of slow wave sleep (158). In contrast, histidine loading has also been shown to be accompanied by a significant decrease in SLA (159). Histidine loading also impaired amphetamine-induced hyperactivity and shortened the duration of the ipsilateral rotatory behavior induced by amphetamine in rats whose nigrostriatal tract was unilaterally destroyed. Histidine loading however, did not modify prochlorperazine-induced catalepsy, ptotic and cataleptic actions of reserpine, tremorigenic, analgesic, and hypothermic effects of oxotremorine, mepyramine, and pentetrazol-

induced seizures and the hypnotic activity of pentobarbital and chloral hydrate (160). In fact HA potentiates reserpine or tetrabenazine-induced catatonia (161).

In the conscious rabbit, intravenous infusion of HA diminished electroencephalogram slow wave activity and increased arousal (162). Larger amounts of HA have been found in the dialysates of blood from waking than from sleeping rabbits (163).

In addition to the work done directly on the effects of HA. little is known of the mechanism responsible for the sedative effects of antihistaminics. Perhaps their CNS effects are related to changes in brain HA as was suggested by Crossland (164). There have been confusing observations on the effects of the antihistamines on brain HA. While low doses of diphenhydramine have been reported to lower HA levels, higher doses have not (165).

Histamine induces vomiting by stimulating the vomiting center, and it augments the electrical activity of the cerebellum either directly or by way of an action on midbrain neurons. The emetic response elicited in the dog by stimulation of the chemoreceptor trigger zone in the area postrema is mediated both by H_1 and H_2 receptor mechanisms (166, 167). The anti-emetic properties of antihistamines provides one of their most beneficial pharmacological properties.

Crossland (168) reported that the activity of some neurons in the CNS is inhibited when HA is applied directly to them. Histamine inhibited electrical self-stimulation at the injection site when injected into the lateral hypothalamus, but it did not affect self-stimulation of the contralateral location. This effect was found to be blocked by pretreatment with antihistaminics such as diphenhydramine or chlor-

pheniramine.

Finch and Hicks (169) have reported that bilateral injections of HA into the posterior hypothalamus can elicit short-lasting rises in resting blood pressure and heart rate in rats. This action appeared to be mediated by H_1 receptors. Similar responses were also demonstrated after anterior hypothalamic injections of HA and these authors stated that α-adrenoreceptors or cholinergic receptors do not appear to be involved in these responses. Sastry and Phillis (170) have reported that clonidine-induced hypotension in rats may be mediated through H_2 receptor activation. Clonidine produced some effects that were not blocked by metiamide and this raises doubt about the specificity and significance of these results.

The ability of the biogenic amines to alter body temperature when injected into the cerebral ventricles of cats led to the hypothesis that they serve as neurochemical mediators in the CNS regulation of body temperature. From their findings, Feldberg and Myers (171) put forward the hypothesis that temperature regulation is achieved by a balance of opposing actions due to the relative rates of release of CA and 5-HT. The results with cats were confirmed on dogs and rhesus monkeys; however, different responses were found when these substances were tested in other species. A summary of some of the results up to the present has been compiled by Bligh, Cottle, and Maskrey (172).

In 1949, Fabinyi and Szebehelyi (173) showed that HA caused a fall in body temperature in mice and rats maintained at an environmental temperature of 18-20°C. When the animals were kept at 30°C, HA produced a slight rise in core temperature. The hyperthermic effect was abolished by adrenalectomy from which it was concluded that release of adrenaline

increased heat production in the intact animals at 30°C. The hyper- and hypothermic effects could be prevented by prior administration of antihistamines (174).

In 1967 Borison and Clark (175) found that HA induces hypothermia in guinea pigs by an action on the hypothalamus. In rats and mice, injection of HA directly into the rostral hypothalamus to avoid the blood brain barrier, caused a dose-dependent fall in body temperature (176) which could be prevented by systemic administration of an H_1-antagonist such as pyrilamine (177). The dose dependence was more apparent on the duration than on the amplitude of the hypothermia.

Alternative approaches to the study of the central effects of HA include increasing brain levels by systemic injections of high doses of its immediate precursor histidine, or the depletion of HA levels using inhibitors of the enzyme HD.

Histidine loading intraperitoneally did not produce any effect on colonic temperature in mice (178) but it did produce a hypothermic effect in rats (177, 179) and the response can be blocked by prior intraventricular injection of an H_2-receptor antagonist such as burimamide (180). These experiments indicate the involvement of both H_1 and H_2 receptors for HA in central thermoregulatory pathways. These results (179) are not conclusive however, as it has recently been shown that high plasma and brain levels of histidine can affect the concentrations of other amino acids which may then in turn exert influences upon thermoregulatory mechanisms.

The temperature changes resulting from central injection of HA are not large when compared to those caused by some other hypothermic agents. In part, this results from compensatory mechanisms which come

into play when effector pathways are activated and also because temperature variations are an insensitive measure of thermoregulatory mechanisms due to the time required to bring about changes in the heat content of the body mass (182).

Along the lines of the second approach to these studies, as mentioned above, namely the inhibition of HD, Taylor *et al.* (183) have reported that 4-imidazolyl-3-amino-2-butanone (McN-A-1293), a new and specific inhibitor of HD, although less potent than other L-aromatic amino acid decarboxylase inhibitors, is more specific and induces hypothermia. In addition it has been observed that the HD inhibitors, alphahydrazino-histidine and NSD 1055, induce marked hypothermia (184).

Recently much attention has been paid to the identification of neurohormonal substances involved in the hypothalamic regulation of body temperature. The finding that the highest concentration of HA in the brain is present in the hypothalamus is in itself justification of a closer examination of the candidature of HA for such a role. Central thermoregulatory mechanisms constitute one neuronal system on which an effect and hence a neurophysiological role for HA may be demonstrated.

Morphine can cause hypothermia or hyperthermia depending on the species, dosage, route of administration, and the development of tolerance (185). Histamine has been demonstrated to be involved in morphine tolerance and dependence (186) and hence, the interrelationship of morphine's known thermoregulatory actions (187) and the possible involvement of HA should be investigated.

Another group of observations that has stimulated the idea that HA may play a role in brain function is the relatively low incidence of allergy in schizophrenic patients and their abnormal blood HA levels

(188). High blood HA levels have been associated with suicidal depression but HA metabolism is unknown in these patients. The effects of some of the major psychoactive drugs administered to these patients such as the phenothiazines or butyrophenones may bear some relationship to their ability to interact with the histaminergic as well as other neurotransmitter systems (190, 191). Attempts at using HA tolerance as a diagnostic procedure for schizophrenia have so far been inconclusive. Theories implicating noradrenergic fibers (192), serotonergic fibers (193) as well as dopaminergic fibers (194-196) have been proposed to explain the symptoms of this disease. It is becoming obvious that several biogenic amines may be involved in this complex illness concurrently.

Influence of Dependence Inducing Agents on Brain Biogenic Amines

In recent years considerable attention has been focused upon the relationship between the biogenic amines and certain pharmacological actions of the narcotic analgesics and other dependence-producing agents. Tolerance and dependence develop concurrently and disappear upon abrupt withdrawal and are therefore believed to be inseparable parts of a common mechanism (197, 198). Evidence relating either phenomenon to biogenic amines as indicative of a role for that amine are therefore combined.

Recently Pert and Snyder demonstrated specific binding of opiate agonists and antagonists to the pharmacologically relevant opiate receptor in the brains of several mammalian species (199, 200). There are dramatic regional variations in opiate receptor binding in the brains of rat, monkey, and man with the highest levels occurring in the anterior amygdala, the periaquaductal gray, thalamus, hypothalamus, and caudate head. Much work remains to be performed in order to assign roles and establish the validity of the peptides termed enkephalins which may be part of the opiate receptors themselves.

Herz *et al.* (201) postulated that structures in the caudal brain stem, most probably in the floor of the fourth ventricle, are important substrates for the development of dependence on morphine. Unfortunately, the regional distribution of opiate receptor sites throughout the mammalian brain, fails to perfectly parallel that of any known neurotransmitter substance.

Iwamoto, in 1972 (202), reported his findings in mice and rats with respect to the possible involvement of the biogenic amines, 5-HT, NA, and DA with the action of the opiates. In the rat, NA and 5-HT concentrations in the brain were found to decrease, increase, or to remain unaltered after acute morphine administration while NA turnover was increased (122, 203-207). These conflicting results were reported possibly due to differences in the dosages employed, the time of sampling, and the species used in these studies.

Acute administration of morphine reduces the brain CA content of rats, mice, and cats (208) and increases the release and turnover of CA from the brain. During the primary phase of addiction to morphine in man, the urinary excretion of adrenaline, NA, DA, and their metabolites was increased (209). These observations indicate that morphine also releases adrenal CA in man. In 1971, Rethy (210) found a good correlation between the ability of a narcotic analgesic to increase the locomotor activity of the mouse and its ability to deplete brain CA.

Until relatively recently, DA was known only as the percursor of NA but now it commands at least as much attention (211). Dopamine has been linked to such diverse activities as growth, vomiting, and neurotransmission, particularly in the basal ganglia and mesolimbic forebrain (212-216). There is now evidence that abnormalities of dopaminergic

neurotransmission in the brain may be of clinical importance (217-219) in Parkinsonism and perhaps even schizophrenia. More relevant to our studies, however, are the reports of interactions between narcotics and dopaminergic mechanisms in the CNS.

Eidelberg and Erspamer (220) proposed that morphine exerts some of its central nervous actions by first interfering with DA-mediated synaptic transmission and then initiating compensatory changes that superficially resemble denervation supersensitivity. They felt that these compensatory changes may underlie the excitatory actions of morphine. More recently Lee and Fennessey (221) have reported an inverse relationship between the morphine-induced changes in brain HA and locomotor activity in mice. These reports indicate the possibility of a DA-HA interaction.

Laverty and Sharman (222) have reported that in the cat, large doses of morphine (30-50 mg/kg) increase the homovanillic acid concentration in the caudate nucleus without affecting the concentration of DA. Takagi and Nakama (223) have shown that morphine (20 mg/kg) causes a slight decrease in the concentration of DA in the mouse brain which is followed by a reduction in the concentration of NA. Still others (224) using the rat showed that acute administration of morphine caused an accelerated depletion of brain DA after inhibition of CA synthesis and decreased dopaminergic transmission (225-227). These effects are competitively inhibited by naloxone but naloxone appears to be ineffective on the increased rate of DA synthesis and on catalepsy induced by chlorpromazine (226).

In addition to the above findings, DA given peripherally, has been found to decrease the analgesic activity of morphine while the

intracerebroventricular injection of DA is reported to potentiate the antinociceptive activity of morphine (228, 229).

Kuschinski and Hornykiewicz (230) concluded that the main site of morphine action may be presynaptic. They proposed that morphine administration may lead to a diversion of newly synthesized DA from storage sites to sites of catabolism. This diversion could result in a deficiency of the readily available DA for release onto the receptors, with catalepsy as its pharmacological correlate and an increase in DA synthesis as a compensatory biochemical measure. This hypothesis is in agreement with the observation that the striatum preferentially uses newly synthesized DA for synaptic release (231).

It is well established that morphine and other opioids have different effects upon locomotor activity in mice and rats. In mice, acute morphine induces a dose and time-dependent increase-decrease (179) in running activity, whereas in rats the locomotor activity is mostly decreased. Van Der Wende (232) using mice, found evidence that a dopaminergic system mediates morphine-induced motor activity and this can be modified by interrupting either the noradrenergic or cholinergic systems.

Slaughter and Nunsell (233) and Schneider and McArthur (234) have reported that the cholinesterase inhibitors neostigmine and ibogaine have synergistic effects on morphine analgesia. The naloxone-precipitated withdrawal jumping response in morphine-dependent animals appears to be associated with an elevation of brain DA levels and subject to cholinergic regulation as well.

Brain Ach levels were reported to increase following acute morphine administration (204, 205) and central administration of

physostigmine potentiated morphine analgesia. This potentiation of the antinociceptive effects of morphine was completely blocked by 6-hydroxydopamine or AMPT treatment. These observations suggest that there may exist an adrenergic-cholinergic interaction in the mediation of morphine analgesia.

As an inhibitory transmitter in the CNS, GABA could conceivably play a role in the process of development of morphine tolerance and physical dependence. It has been demonstrated that in rats rendered tolerant to morphine, levels of GABA are found in the subcortical and hypothalamic areas (235) showing a certain similarity to the distribution of the opiate receptor (236). In addition it has been reported (237) that elevating tissue levels of GABA by administering the compound exogenously, or slowing its destruction with aminooxyacetic acid, antagonizes morphine antiociception and enhances tolerance and physical dependence.

Morphine has been demonstrated to produce increased gamma-hydroxybutyrate levels in subcortical structures and the time course of the increase is roughly parallel to the development and loss of analgesia (238). Gamma-hydroxybutyrate is a naturally occurring metabolite of GABA with analgesic/anesthetic characteristics (239). It appears now, however, that the excellent neurochemical fit to the experimental time course is correlational rather than functional (240).

Cardenas and Ross (241) have recently reported that morphine, in vivo, decreases the regional calcium levels in 8 discrete brain regions in rats in a linear dose-dependent, time-dependent manner. This effect of morphine was blocked by naloxone and exhibited a high degree of stereospecificity. Assuming the amount of morphine to be constant

throughout the brain regions investigated, finding a quantitively identical response to calcium in each brain region, changes in the levels of the neurotransmitters could be visualized as a secondary reflection of the degree of calcium depletion in any particular brain region. Loss of membrane-bound calcium could therefore be responsible for the elevation of one or another of the putative neurotransmitters through alteration of the calcium-dependent release mechanism, and in fact, the increase in Ach levels seen following naloxone administration, occurs on the same time course as the maximal depletion of calcium.

It is becoming clear that any agent capable of significantly influencing one of the monoaminergic systems in the brain is likely to exert an influence on most other systems. If HA is to play a role as a neurotransmitter substance it might be expected to show some involvement in the pharmacological actions of morphine as do the other biogenic amines.

Pentazocine and Biogenic Amines

Pentazocine is a nonopioid benzmorphan derivative chemically related to morphine. Use of pentazocine can lead to dependence and tolerance to its analgesic effects, although more slowly than with the other narcotics (242, 243). Pentazocine given parenterally is approximately one third as potent as morphine, however oral pentazocine is a much less effective analgesic (244). Up to 50% of patients receiving oral pentazocine (245) have reported adverse effects including dizziness, drowsiness, increased sweating, nausea, vomiting. constipation, and hallucinations.

It is now becoming apparent that narcotic-antagonist analgesics

can also affect the disposition of brain monoamines and pentazocine is one such agent. It lowers the total brain content of both NA and DA in the rat but has only a slight effect of 5-HT levels (246). The depletion of brain CA is closely associated with a stimulation of locomotor activity. With the possible exception of the mouse, it is difficult to establish a major role for 5-HT in the actions of pentazocine and a significant depletion of medullary 5-HT was observed only in rats pretreated with naloxone (247, 248).

Naloxone, a potent narcotic antagonist that is virtually devoid of agonistic activity (249, 250) blocks neither the depletion of brain monoamines nor the stimulation of locomotor activity produced by pentazocine. In contrast, most of the other agonistic actions of pentazocine such as analgesia and respiratory depression, are blocked by naloxone (251, 252). Pretreatment with naloxone antagonized the pentazocine-induced depletion of brain DA and NA in the cerebral cortex and midbrain, but failed to affect the depletion of NA in the medulla and actually enhanced the depletion of hypothalamic NA. The failure of naloxone to block some of the effects of pentazocine suggests that pentazocine's agonistic component of action is mediated by at least two distinct mechanisms: one which is blocked by naloxone and one which is not.

Sugrue (253) recently reported that both morphine and pentazocine increase the turnover of DA in the rat corpus striatum thus indicating a morphine and pentazocine-induced increase in striatal DA neuronal activity. In the same paper, it was also reported that they observed a morphine-induced increase in NA neuronal activity in the medulla-pons, an ability not possessed by pentazocine.

The mechanism by which pentazocine can markedly reduce the content of brain DA has not been established. It is clear that differences in the biologic disposition of the d- and l- isomers of pentazocine cannot explain their different potency because their brain concentration and regional distribution within the brain are fairly similar (254). It is also unlikely that differences between the isomers are secondary to the respiratory depressant activity which resides primarily in the d-isomer (255) because morphine, which is an even stronger respiratory depressant, does not reduce brain DA content (256). Berkowitz proposed that not only does pentazocine facilitate increased release and utilization of brain DA, but also that this action is direct (257). Dopamine may therefore be an important mediator of the central actions of pentazocine and possibly others of the narcotic agonist/antagonist types of analgesics.

Any studies on the possible involvement of brain HA with the effects of pentazocine appear to be lacking to date. As there have been changes observed in HA metabolism following chronic morphine administration and withdrawal (184) and some involvement of other putative neurotransmitters, it would seem worthwhile to look for corresponding changes in HA levels following pentazocine administration.

Pharmacology of Mandrax, Methaqualone, and Diphenhydramine

The need for sedative-hypnotic agents in patients with disorders complicated, aggravated, or initiated by tension and insomnia is considerable. Because of the ability of these agents to reduce tension, induce sleep, and generally calm the patient, they are often helpful in effecting therapeutic gain when used judiciously. Indiscriminate use

and accidental overdosing create problems not only for the physician and patient but for society as well. The involvement of the monoamines in the mechanisms of action of these agents remains to be determined.

Ever since the barbiturates were found to possess a very high dependence liability, investigators have been searching for a sedative hypnotic which is not potentially addictive. With the introduction of methaqualone (MQ) to the North American market many pharmacologists and clinicians thought that this was such a product. Methaqualone abuse has become a widespread problem in the United States (258), West Germany (259), Sweden (260), and also in Japan (261). Methaqualone abuse is often associated with the simultaneous ingestion of alcohol (262) and indeed the combined effect has been reported to be stronger than alcohol alone (263). Methaqualone and tetrahydrocannabinol use have also been observed in the same individuals (258) and narcotic addicts have been known to take MQ when they cannot get heroin (262, 264, 265). The only true indications of psychological dependence developing with MQ come from clinical reports and these consist of ill-defined reports concerning MQ-users who have a craving or strong desire to keep taking the drug after prolonged use (266, 267).

Methaqualone is readily absorbed from the stomach and upper small intestine, distributed mainly to fat, liver, kidney, and brain. It is metabolized almost completely in the liver, undergoes enterohepatic circulation, and is excreted in the urine and feces (268).

Although several clincians would like to correlate the degree of toxicity observed upon overdose with the plasma levels of MQ, this does not seem to be a straightforward task. This might suggest the occurrence of tolerance development in certain patients. Nyak and his coworkers

(269) reported a study of MQ pharmacokinetics in man and they extrapolated that there was no self-induction of MQ metabolism. The incidence of multiple drug use among MQ-users often makes it difficult to attribute the toxic signs noted with MQ overdose in the case reports or clinical studies purely to MQ.

Signs of MQ overdosage have been well documented by several clinicians and usually include: dizziness (270), numbness and/or paraesthesias in the limbs (271), hypertonia and muscular rigidity (271-274), hyperreflexia (272-274), tremor and muscle twitching (271, 273, 274), muscular weakness (275), unconsciousness (271-273), depressed body temperature (274), increased vascular premeability (276), and respiratory depression (270-277).

Piepho and O'Connor (278) observed that a significant degree of tolerance to the hypnotic and hypothermic actions of MQ can be produced in rats although the actual mechanisms behind its development, remain unidentified. It is quite likely in their estimation that the tolerance noted was at least in part of a dispositional nature; however, they stress the fact that functional tolerance may still be found in the presence of dispositional tolerance.

Kohli and others (246, 279-283) investigated what they termed the dependence liability of MQ in rats by studying the body weights, group food intake, rectal temperatures, spontaneous motor activity and changes in gross behavior at certain times throughout their experimental period. The conclusion of these experiments was that tolerance developed during the first and second weeks of the study, however the distinction between metabolic and functional tolerance was not made in this study.

Indications of physical dependence upon withdrawal of MQ after

chronic use are very common and include headaches, severe cramps, convulsions (261, 284), delirium, tremor and visual hallucinations (280). Additional symptoms reported following chronic MQ use or overdosage in persons known to be previously dependent on barbiturates are: weakness, tachycardia, vomiting, nausea, nervousness, and insomnia (265, 285). Similar withdrawal symptoms have been reported following mandrax (MX) overdosage.

Mandrax, a combination of MQ (250 mg) and diphenhydramine (DIPH) (25 mg), is widely used as a nonbarbiturate hypnotic and as an alternative to MQ alone. Concern has also arisen over the abuse of MX by the young population (267, 286-288).

Experimental work has shown that MQ can be potentiated by a number of drugs, and of these the incidence of side effects is lowest for DIPH and it was thus selected for use in MX. Because MQ is the main constituent of MX, similar dangers can be expected after overdosage with the latter as were described for MQ.

Considering the presence of both an agent reported to produce tolerance and dependence and a potent antihistaminic in MX, it appears worthwhile to investigate the possible influences on brain HA levels of acute and chronic administration of mandrax and its components.

Amphetamine and Biogenic Amines

In Sweden there has been a long lasting epidemic of amphetamine abuse. Since 1969 there has been a relative increase in the number of opiate addicts but amphetamine abuse is still a major problem of concern. Amphetamine addiction is a serious condition with a relapse rate comparable to that of heroin hence the development of a pharmacological

antagonist may be of great value (289). In order to achieve this goal, a great deal more must be known about the mechanism of action of amphetamine.

One of the first investigators to point out the dangers of amphetamine addiction was Gutman (290). Administered orally to humans a 10-30 mg dose generally induces alertness, mood elevation, and euphoria. Large doses over extended periods of time are usually accompanied by complaints of depression, fatigue, and headaches.

The metabolism of d-amphetamine to p-hydroxyamphetamine and p-hydroxynorephedrine has been known since 1954 (291). These hydroxylated metabolites have been implicated in some of the pharmacological effects of the parent compound (292, 293). The ratio of the individual detoxification steps varies from species to species. In rats, ring hydroxylation in the para position is the main metabolic pathway, whereas deamination is usual in man and rabbits. Some of the amphetamine is excreted via the kidneys, with more being excreted unchanged under acidic conditions (294). It has been found that amphetamine induces a stage of euphoria noticeable for 10-12 hours (with a maximum 15 minutes after administration) followed by signs of dysphoria which reached a maximum 14 hours after the drug injection and which remained for 48 hours (295). The amphetamine was largely eliminated within this time.

Neurochemical and pharmacological investigations have indicated that amphetamine is an indirectly acting amine and that an uninterrupted synthesis of CA is required for its central stimulant effects (296, 297). The central action of amphetamine is generally attributed to an increase in the concentration of CA in the synaptic cleft which may be achieved by an increase in the release of the amine from presynaptic terminals

and/or inhibition of the reuptake of the amine released. There is no agreement yet concerning which of the two mechanisms is predominant. Although most authors consider amphetamine as a direct releaser of CA (298-306), several studies also give various degrees of importance to the inhibitory effect of the drug on CA reuptake (307-309) which is sometimes considered to be predominant (310-312).

Since amphetamine has been shown to be an indirectly acting amine (297) and it exerts its excitatory action only in the presence of releasable CA in the brain, various attempts to block this action have been directed toward the neuronal CA stores. Reserpine does not inhibit the stimulating effect of amphetamine probably because it does not interfere with the rapid resynthesis of CA. On the contrary, reserpine potentiates amphetamine, possibly by denervation hypersensitivity induced by the pharmacological "sympathectomy" (313). Inhibition of the rate limiting enzyme tyrosine hydroxylase, on the other hand, was shown to eliminate various behavioral effects of amphetamine in rats (296, 314). A corresponding degree of inhibition of amphetamine-induced excitation and stereotyped behavior had earlier been demonstrated with various neuroleptic drugs particularly chlorpromazine (315).

The behavioral pharmacology of amphetamine is rather complex. D-amphetamine has been reported to produce an initial response characterized by enhanced locomotor activity for 15-20 minutes followed by stereotyped behavior consisting of continuous sniffing, licking, and biting (316). This dual response has been explained as a time bound phenomenon of amphetamine; it first releases NA and then DA (317, 318) which corresponds to horizontal and then vertical stereotyped behavior respectively.

Amphetamine is known to be a potent anorexic agent in both animals and man. The results of studies on rats have demonstrated that lesions in the lateral portion of the hypothalamus attenuate or abolish the anorexic effect of peripherally administered amphetamine (319-322), an interesting result considering the effects of this same lesion on brain HA levels. It has also been discovered that the anorexia induced by lateral hypothalamic injection of amphetamine could be blocked by locally administered beta-adrenergic and dopaminergic receptor blockers.

Tolerance to the repeated administration of d-amphetamine appears to be dependent upon the behavioral or psychological measures under investigation. In particular, tolerance has been observed to the toxic, hyperthermic, and anorexigenic effects of d-amphetamine, as well as to the drug-induced response patterns in time-based schedules of reinforcement (323-329). In contrast, tolerance has not been observed to develop to the locomotor stimulating properties of amphetamine nor to amphetamine-induced stereotypes (329, 330).

One mechanism which may account for the biochemical distinctions regarding tolerance may involve the metabolic fate of d-amphetamine (324, 331). Specifically, in mice, which exhibit moderate aromatic hydroxylation of amphetamine (332), the metabolite, p-hydroxynorephedrine, may be taken up and stored in noradrenergic terminals thus displacing NA from storage granules and acting as a false neurotransmitter (324). Accordingly, those behaviors mediated by NA should be subject to tolerance whereas behaviors dependent on DA mediation should be unaffected by chronic amphetamine treatment (331). An alternative possibility is that other transmitter systems are responsible for the tolerance to d-amphetamine. Cholinergic and serotonergic manipulations may influence

alternation behavior (333-336). Indeed, treatment with physostigmine effectively antagonizes amphetamine-induced perseveration (335), thus the possibility cannot be dismissed that chronic d-amphetamine treatment produced modifications of cholinergic activity (337).

There have been numerous reports on the possible physiological roles of brain HA and amphetamine has been shown to profoundly influence most of these same physiological functions. In view of the existence of extensive adrenergic-cholinergic interactions in the actions of most psychoactive agents studied to date, and in view of the probability of interactions of these transmitter systems with a histaminergic system, if one exists, the role of HA in the pharmacological actions of amphetamine warrants investigation.

Tetrahydrocannabinol and Biogenic Amines

Δ^9-tetrahydrocannabinol, (Δ^9-THC) is now generally accepted to be the major psychoactive ingredient of marihuana and hashish (338, 339). After absorption, Δ^9-THC is quickly metabolized, first to several hydroxyl derivatives and then to carboxylic acids which are excreted partly conjugated (340, 341). Among these metabolites at least one, the 11-hydroxy THC, seems to be equally active in terms of producing the characteristic acute psychological and heart rate accelerating effects of marihuana and Δ^9-THC (342).

Most of the early reports concerning the effects of Δ^9-THC on the putative neurotransmitter amines in the CNS used impure extracts of varying origin (343). Even since the indentification of Δ^9-THC as the major psychoactive component there have been many contradictory reports about the effects of this substance on both endogenous levels and

turnover of brain NA, DA, and 5-HT. In experiments where rats were used, some authors have reported no change in endogenous concentrations of DA (344-346) or NA (344-347) but either an increase (345, 348) or no change (345, 349) in 5-HT concentrations. Studies with mice have shown that there are species differences (350). It now appears that behavioral effects produced in rats after acute Δ^9-THC administration are not a result of any marked effect on brain function mediated by NA or DA.

In man and animals, many of the effects of Δ^9-THC parallel those of anticholinergic agents. It has been reported to cause dry mouth and impairment of short-term memory in man (351-353). In animals, these effects and other behavioral effects on performance, habituation, and conditioned fear, have been associated with altered cholinergic function (354). More recently Δ^9-THC was found to decrease the synthesis of Ach in various brain regions of rats (355). These results suggested that the inhibition of Ach synthesis observed in Δ^9-THC-treated rats may be related to interference with the propagated action potential or with the depolarization process in cholinergic neurons.

Because of the interrelationship between cholinergic and histaminergic systems referred to previously, it may be of value to look for any changes in HA metabolism following Δ^9-THC administration.

REASONS FOR INITIATION OF THE PRESENT STUDY

"At present, basic information about the action of drugs at multicellular, cellular, subcellular, microchemical, and molecular levels is approaching the point where we can soon expect a coherent and highly specific analysis of the mode of action of almost every major drug of interest to psychiatry. These promising developments will hopefully lead to a transition from a 'cookbook' use of drugs to a rational pharmacotherapy in the future." (G. K. Aghajanian Ch. 19 in: American Handbook of Psychiatry 1974)

A major emphasis of research in neuropharmacology has been upon presumptive transmitter substances. Of the amines likely to possess a transmitter role in the CNS, HA has received rather less attention than Ach, CA, and 5-HT. A number of factors have contributed to the apparent lack of interest in HA in the brain including the difficulties encountered in measuring the content of this amine in various brain regions in a reliable and accurate manner.

Histamine is present in the brain, its distribution being uneven. The enzymes involved in its synthesis and degradation are distributed in approximately the same pattern as the amine itself. The subcellular localization of the amine and its associated enzymes is consistent with its having a transmitter function. The turnover rate of at least a portion of brain HA is extremely rapid and can be modified. Interference with the normal functioning state of HA in the CNS has been shown to result in changes in CNS function and stimulation of central HA receptors

has been shown to have effects on a number of parameters of CNS function. It appears that direct HA application to neurons is capable of modifying their normal firing rate. There is thus a large body of evidence to support the suggestion that HA has a role as a synaptic transmitter in the brain and it would therefore appear worthwhile to further explore its involvement in brain function.

If HA plays a significant role as a neurotransmitter in the CNS, then abnormal mental states may be associated with changes in the endogenous content and/or turnover of HA in analogy to the changes noted for the other biogenic amines. These alterations in HA levels and/or turnover may also show behavioral manifestations. In order to test such hypotheses it is necessary to study the role of HA in drug-induced abnormal mental states as was previously done to elucidate the roles of the other biogenic amines in brain function.

Experiments were selected and designed with the hope of correlating any changes observed in the HA levels or turnover or behavioral changes to those changes reported to occur with the other biogenic amines. The relevant literature has been covered in the literature review and in the discussion any obvious correlations will be described.

These studies were intended to further explore whether sedatives, both narcotic and non-narcotic, CNS stimulants, or other psychoactive drugs could be differentiated on the basis of their effects on endogenous brain HA concentrations and/or turnover. It was also of interest to explore whether any of these changes might be associated with behavioral alterations as determined by measuring the spontaneous locomotor activities of all animals just prior to sacrifice. If there were any changes in the brain HA levels, it would be of interest to determine

whether they are reversible and to see if this reversal is reflected in behavioral measurements. Whether such treatment could result in an attenuation of tolerance development to these drugs of dependence would be of considerable clinical importance.

These studies should contribute to a more complete understanding of the pharmacological effects of the drugs studied and perhaps contribute to the treatment and/or prevention of drug abuse in the future.

PROCEDURES

Male Sprague Dawley rats obtained from Canadian Biobreeding Laboratories, Ottawa, were used in all of our studies. The rats were housed four to a cage and kept in a room at 22°C, 60% humidity, maintained on 12 hr. light/dark cycles. All animals were fed Purina Laboratory Chow. Body weight records were kept for all rats undergoing chronic treatment. All injections of drugs or saline were administered intraperitoneally in a volume of 0.1 mg/100 g body weight unless otherwise specified.

A. Biochemical Studies

All animals were sacrificed by decapitation and their brains rapidly removed, washed with ice cold saline, blotted free of excess saline and placed on special glass plates kept on ice. The hypothalamus, midbrain, and cortex were dissected out according to the method of Glowinski and Iversen (346).

(i) Labware

Sterile disposable polypropylene labware was used exclusively in order to minimize losses of histamine or methylhistamine due to adsorption on glass. The tissues were homogenized in 1 ml. of 0.01 M phosphate buffer (pH 7.9) using a Brinkman Polytron Homogenizer. The homogenates were boiled for 5 minutes, cooled on ice, and centrifuged in an international ultracentrifuge model B at 50,000 G. An aliquot of the sample supernatant was removed for histamine analysis and another sample (0.1 ml) for the determination of the histamine methyltransferase

activity. All pipetting was performed with Brinkman fixed volume pipets with disposable polypropylene tips.

(ii) Histamine Assay

Tissues were assayed for their histamine contents according to a modification of the double isotope technique of Taylor and Snyder (54). This procedure depends on the methylation of endogenous histamine in the tissues by added histamine methyltransferase using ^{14}C-S-adenosyl-L-methionine (56 mCi/m mole, New England Nuclear) as the ^{14}C-methyl donor. A tracer amount of ^{3}H-histamine (5-10 Ci/m mole, New England Nuclear) was added to correct for any variation of histamine methylation in different samples. Endogenous S-adenosylmethionine was destroyed by boiling the tissue, a procedure which also served to precipitate protein. ^{14}C-^{3}H-methylhistamine and ^{14}C-methylhistamine were separated from ^{14}C-S-adenosyl-L-methionine and ^{3}H-histamine by extracting into chloroform from a salt-saturated alkaline solution. The chloroform was evaporated and the residue taken up into scintillation fluid and counted, in a Beckman Ambient Temperature Liquid Scintillation Counter, 'Model LS-150'.

(iii) Histamine Methyltransferase Assay

Histamine methyltransferase (HMT) was determined according to a modification of Taylor and Snyder's method (54). This procedure depends on the determination of methylhistamine formed by HMT. An excess of histamine was added to the aliquot of sample supernatant and optimal conditions for the enzyme HMT were maintained. ^{14}C-S-adenosyl-L-methionine was used as the ^{14}C-methyl donor. Methylhistamine (^{14}C-labelled) was extracted into a toluence: isoamyl alcohol solution, centrifuged, and an aliquot of the supernatant added to premixed scintillation fluid

(Econofluor, New England Nuclear) and counted in a Beckman ambient temperature liquid scintillation counter.

(iv) Protein Assay

Protein determinations were performed according to the method of Lowry *et al.* (357). The final colour is a result of: the biuret reaction of protein with copper ion in alkali and the reduction of the phosphomolybdic-phosphotungstic reagent by the tyrosine and tryptophan present in the treated protein. The absorbance was measured using a Klett Spectrophotometer and bovine serum albumin (fraction V) as standard.

B. Spontaneous Locomotor Activity Studies

Spontaneous locomotor activities were determined by the use of a Selective Activity Meter 'Model S' (Columbus Instruments, Ohio). This instrument registered the animal's activity in arbitrary units and printed the cummulative total each minute. The measurements were carried out on individual animals placed in a regular cage located in an isolated room in order to minimize external influences. The results were calculated in terms of activity counts/minute for consecutive 5 minute periods, 20-60 minutes before sacrifice.

C. Statistical Analysis

The results were subjected to statistical evaluation using a Student's "t-test". Significant differences between the means are shown when the P value was <0.05.

D. Drugs and Chemicals

All reagents and drugs used in these studies were of the purest grade available and were dissolved in glass distilled water unless otherwise stated.

Histamine-^{3}H, S-adenosyl-L-methionine (^{14}-methyl), and premixed scintillation fluid (Econofluor) were purchased from New England Nuclear (Boston, Mass.). Phenol reagent, chloroform, sodium borate, cupric sulface, sodium phosphate, and sodium chloride were purchased from Fisher Scientific (Ottawa, Ont.). Bovine serum albumin, fraction V, and L(+)-histidine monohydrochloride were purchased from J. T. Baker Co. (Phillipsburg, N.J.).

Morphine sulfate (Allen and Hanbury's, Toronto, Ont.), methadone hydrochloride (National Health and Welfare, Ottawa), naloxone hydrochloride (Endo Laboratories, Garden City, N.J.) and amphetamine sulfate (Smith, Kline, and French Canada Ltd., Montreal, Qué.), were dissolved in physiological saline. Pentazocine lactate (Talwin) was purchased from Winthrop Laboratories (Aurora, Ont.).

Mandrax (Roussel Co., Montreal, Qué.), methaqualone hydrochloride (C. E. Frosst Co., Dorval, Qué.) and diphenhydramine hydrochloride (Parke Davis & Co. Ltd., Toronto, Ont.), were suspended in an aqueous preparation of 1% tragacanth gum (Matheson, Coleman and Bell, Norwood, Ohio) and administered orally in a total volume of 1 ml. Control animals always received an equal volume of 1% tragacanth gum orally.

(-)-trans Δ^{9}-tetrahydrocannabinol (93% in dehydrated alcohol) (National Health and Welfare, Ottawa) was suspended in corn oil and administered orally in a final volume of 1 ml. Control animals received an equal volume of corn oil.

E. Experimental Procedures

(i) Acute Morphine, Methadone, or Naloxone Treatment

In these acute studies, experimental rats (200-225 g) were injected intraperitoneally (i.p.) with a single dose of morphine (30 mg/kg), methadone (15 mg/kg), or naloxone (0.4 mg/kg). Control rats were administered 0.9% saline (0.1 ml/100 g i.p.) for the same time periods as experimental animals. The animals were sacrificed one hour after morphine or methadone and 20 minutes following naloxone administration.

(ii) Chronic (9 days) Morphine Treatment

In "short term" studies, experimental rats (starting weights 175-200 g) were injected with morphine (30 mg/kg i.p. once daily) for 9 days. Control rats received 0.9% saline (0.1 ml/100 g i.p. once daily) for 9 days. All animals were sacrificed 18 hours after the last treatment.

(iii) Chronic (5 days) Methadone Treatment

In "short term" studies, experimental rats (starting weights 175-200 g) were administered methadone at one of the following doses each successive day: 5,5, 10,10, and 15 mg/kg i.p. Control rats were injected with 0.9% saline (0.1 ml/100 g i.p. once daily). The animals were sacrificed 18 hours after the last treatment.

(iv) Chronic Morphine Treatment and Withdrawal

In "long term" chronic studies, rats (starting weights 150-165 g) were treated with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days according to the schedule of

Takemori (358). Control rats in this study were administered identical volumes of 0.9% saline (0.1 mg/100 g i.p. once daily).

After 21 days of chronic morphine treatment, the rats were divided into four groups: one group was administered naloxone (0.4 mg/kg i.p.), while a second group was withdrawn from morphine treatment and was injected with 0.9% saline (0.1 ml/100 g i.p.) for two consecutive days; a third group of rats was treated with methadone (15 mg/kg i.p. once daily) for two days, and a fourth group was maintained on morphine (90 mg/kg i.p. once daily) for two days. Control animals were injected with saline (0.1 ml/100 g i.p. once daily) for 21 ("C_1") or we ("C_2") days.

The naloxone-induced withdrawal syndrome in rats treated chronically with increasing doses of morphine was confirmed by observing sniffing, rearing, "wet shakes", teeth chattering, writhing, defecation, urination, salivation, and a decrease in body temperature. The latter was measured by the use of a rectal probe YSI telethermometer (Yellow Springs Instrument Co.).

Following these different treatments, the rats were sacrificed 18 hours after the last injection of the drug or saline, except for the rats treated with naloxone, which were sacrificed after 20 minutes.

(v) Chronic Histidine with Chronic Morphine Treatment

In these studies, rats with starting weights 150-165 g were used. The rats were divided into four groups. The first group served as controls and was injected with 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The second group was treated chronically with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for

21 days. The third group of rats received histidine (500 mg/kg i.p. once daily) for 21 days. The fourth group was treated chronically with morphine in a fashion identical to the rats in group two but in addition these animals were treated simultaneously with histidine (500 mg/kg i.p. once daily) for 21 days. All animals were sacrificed 18 hours after their last drug or saline administration and the endogenous histamine concentrations in various brain regions determined.

(vi) Chronic Histidine after Morphine Withdrawal from Morphine Dependent Rats

These chronic studies were performed on rats with starting weights 150-165 g. The animals were divided into four groups. The first group served as control and was treated with 0.9% saline (0.1 ml/100 g i.p. once daily) for 23 days. The second group was administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and the treatment was then withdrawn for two days. The third group of rats was treated with histidine (500 mg/kg i.p. once daily) for 23 days. The fourth group was treated chronically (21 days) with increasing doses of morphine in the same fashion as group two, but in addition, histidine (500 mg/kg i.p. once daily) was administered simultaneously. The histidine treatment was continued for 23 days while morphine was withdrawn after 21 days of chronic administration. All animals were sacrificed 10 hours after the last drug or saline administration.

(vii) Histamine Formation in Rat Brain Following Chronic Morphine

The first group of rats in this study (starting weights 150-165 g)

served as controls and were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The second group (200-225 g) was treated acutely with histidine (500 mg/kg i.p.). The third group of rats was treated chronically with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by acute treatment with histidine (500 mg/kg i.p.) 18 hours after the last morphine administration. The fourth group (150-165 g) was administered the same doses of morphine as the third group and they were then treated acutely with histidine (500 mg/kg i.p.) 66 hours after the last morphine injection. All animals in this study were sacrificed one hour after saline or histidine administration.

(viii) Histamine Formation in Rat Brain Following Chronic Histidine or Chronic Histidine with Morphine

Histamine formation from exogenously administered histidine was investigated in four groups of rats. These rats all had a starting weight of 150-165 g, and they all were injected with histidine (500 mg/kg i.p. once daily) for 21 days. The first group of rats, administered only histidine, were sacrificed one hour after their last treatment. The second group was administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and then sacrificed one hour later. The third group of animals was treated with histidine for three weeks and they were then administered histidine (500 mg/kg i.p.) 18 hours after the three weeks treatment and they were sacrificed one hour later. The fourth group of rats in this study were treated in a fashion identical to the second group with regard to the morphine and histidine injections, and then 18 hours after the last treatments, they were administered histidine (500 mg/kg i.p.) and sacrificed one hour later.

(ix) Effect of an Acute Histidine Load to Morphine-Dependent Rats

The rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) or weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days. Histidine (500 mg/kg i.p.) was administered at the time of the last morphine treatment and the rats were sacrificed 18 hours later.

(x) Acute and Chronic Pentazocine Treatment

In acute studies, experimental rats (200-225 g) were injected with pentazocine (30 mg/kg i.p.) and control animals were administered 0.9% saline (0.1 ml/100 g i.p.). All animals were sacrificed one hour after treatment.

In chronic studies, rats (starting weights 150-165 g) were administered increasing doses of pentazocine (15, 30 and 45 mg/kg i.p. twice daily) for 21 days. Control animals were treated with the same volume of 0.9% saline (0.1 ml/100 g i.p. twice daily) for 21 days. All animals were sacrificed 18 hours after the last drug or saline administration.

(xi) Acute Mandrax, Methaqualone, or Diphenhydramine Treatment

In acute studies, the first group of rats (200-225 g) were treated with mandrax (82.5 mg/kg p.o.) and sacrificed one hour later. Mandrax was suspended in freshly prepared aqueous solution of 1% tragacanth gum. The administered dose was adjusted so that it would always be contained in 1 ml of the suspension. The second group of animals was treated acutely with a corresponding dose of methaqualone (75.0 mg/kg p.o.) and

the third group was administered diphenhydramine (7.5 mg/kg p.o.). Control rats were treated with 1 ml of the vehicle orally. All animals were sacrificed one hour after the drug or vehicle administration.

(xii) Chronic Treatment with Mandrax, Methaqualone, or Diphenhydramine for 18 Days

In chronic studies, rats (starting weights 160-175 g) were treated orally for 18 days with the same doses of mandrax, methaqualone or diphenhydramine used in the acute studies. These animals were sacrificed 18 hours after the last drug or vehicle administration.

(xiii) Chronic Mandrax Treatment and Withdrawal

In another group of chronic studies, rats (starting weights 150-165 g) were treated for 21 days with increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o.) for the first, second and third weeks respectively. One group was sacrificed 18 hours after the last mandrax administration. The second group was withdrawn from mandrax treatment and was administered the vehicle for an additional two-day period. These rats were sacrificed 18 hours after the last vehicle administration.

(xiv) Acute and Chronic Amphetamine Treatment

In acute studies, rats (200-225 g) were injected with d-amphetamine (5 mg/kg or 15 mg/kg i.p.) and sacrificed one hour later. Controls received the same volume of 0.9% saline (0.1 ml/100 g) and were also sacrificed one hour later.

In chronic studies, rats (starting weights 150-165 g) were administered increasing doses of d-amphetamine (5, 10 and 15 mg/kg i.p.) for the first, second and third weeks respectively. All animals were

sacrificed 18 hours after the last drug or saline injection.

(xv) Acute and Chronic Δ^9-Tetrahydrocannabinol Treatment

In acute studies, rats (200-225 g) were administered Δ^9-tetrahydrocannabinol (Δ^9-THC) (30 mg/kg p.o.). The Δ^9-THC was suspended in 1 ml of corn oil, which was also administered to controls. The animals were sacrificed one hour after the drug or vehicle administration.

In chronic studies, rats (starting weights 150-165 g) were administered Δ^9-THC (30 mg/kg p.o.) daily for 7 days. Controls were treated with the same volume of vehicle for the same period of time. All animals in this study were sacrificed 18 hours after the last drug or vehicle administration.

RESULTS

I. Acute Morphine, Methadone or Naloxone Treatment

Acute administration of morphine (30 mg/kg i.p.) for one hour as indicated in Table 1 did not result in any significant changes in the endogenous histamine (HA) concentrations in the hypothalamus, midbrain or cerebral cortex. This treatment, however, did produce statistically significant decreases in the spontaneous locomotor activity (SLA) of these rats as illustrated in Fig. 1. The morphine-treated animals were less active within the first 5 minute recording period and remained sedated until sacrifice 60 minutes later.

Acute methadone administration (5 mg/kg i.p.) for one hour as indicated in Table 1 did not result in any significant changes in the endogenous HA concentrations in the hypothalamus, midbrain or cerebral cortex. This treatment did however, produce statistically significant decreases in SLA of these rats as indicated in Fig. 2. The methadone-treated animals displayed depressed activities within the first 5 minute recording period and they remained sedated until sacrifice.

Acute administration of naloxone (0.4 mg/kg i.p.) for 20 minutes as indicated in Table 1 did not produce any significant changes in the endogenous HA concentrations in the hypothalamus, midbrain or cerebral cortex. Naloxone administration also failed to produce any significant changes in the SLA of these rats as illustrated in Fig. 3.

TABLE 1

ENDOGENOUS HISTAMINE CONCENTRATIONS IN VARIOUS BRAIN REGIONS OF RATS AFTER ACUTE TREATMENTS WITH MORPHINE, METHADONE, OR NALOXONE

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL (SALINE)	256.8 ± 31.7 (11)	45.7 ± 10.1 (11)	26.6 ± 4.8 (10)
MORPHINE (30 mg/kg)	285.5 ± 35.2 (11)	45.3 ± 6.0 (10)	25.2 ± 2.6 (10)
CONTROL (SALINE)	270.7 ± 25.3 (8)	48.6 ± 8.3 (8)	22.6 ± 1.4 (6)
METHADONE (5 mg/kg)	258.5 ± 42.4 (6)	45.0 ± 7.4 (6)	28.6 ± 2.8 (4)
CONTROL (SALINE)	295.4 ± 17.1 (7)	42.7 ± 7.8 (7)	23.0 ± 2.0 (7)
NALOXONE (0.4 mg/kg)	291.3 ± 29.1 (7)	47.5 ± 5.3 (7)	24.0 ± 2.4 (8)

Experimental rats were treated with morphine (30 mg/kg i.p.), methadone (5 mg/kg i.p.), or naloxone (0.4 mg/kg i.p.). Control rats were administered 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed 20 minutes after naloxone administration and one hour after the other treatments. Values represent the Mean ± S.E.M. The number of experiments is shown in parentheses.

TABLE 1P

ENGOGENOUS HISTAMINE CONCENTRATIONS IN VARIOUS BRAIN REGIONS OR RATS AFTER ACUTE TREATMENTS WITH MORPHINE, METHADONE, OR NALOXONE

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (% of Control)		
CONTROL (SALINE)	100.0 ± 12.3 (11)	100.0 ± 22.1 (11)	100.0 ± 18.0 (10)
MORPHINE (30 mg/kg)	111.2 ± 13.7 (11)	99.1 ± 13.1 (10)	94.7 ± 9.8 (10)
CONTROL (SALINE)	100.0 ± 9.3 (8)	100.0 ± 17.1 (8)	100.0 ± 6.2 (6)
METHADONE	95.5 ± 15.7 (6)	92.6 ± 15.2 (6)	126.5 ± 12.4 (4)
CONTROL (SALINE)	100.0 ± 5.8 (7)	100.0 ± 18.3 (7)	100.0 ± 8.7 (7)
NALOXONE	98.6 ± 9.9 (7)	111.2 ± 12.4 (7)	104.3 ± 10.4 (8)

Experimental rats were treated with morphine (30 mg/kg i.p.), methadone (5 mg/kg i.p.), or naloxone (0.4 mg/kg i.p.). Control rats were administered 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed 20 minutes after naloxone administration and one hour after the other treatments. Values represent the Mean ± S.E.M. The results are expressed as percent of control values. The number of experiments is shown in parentheses.

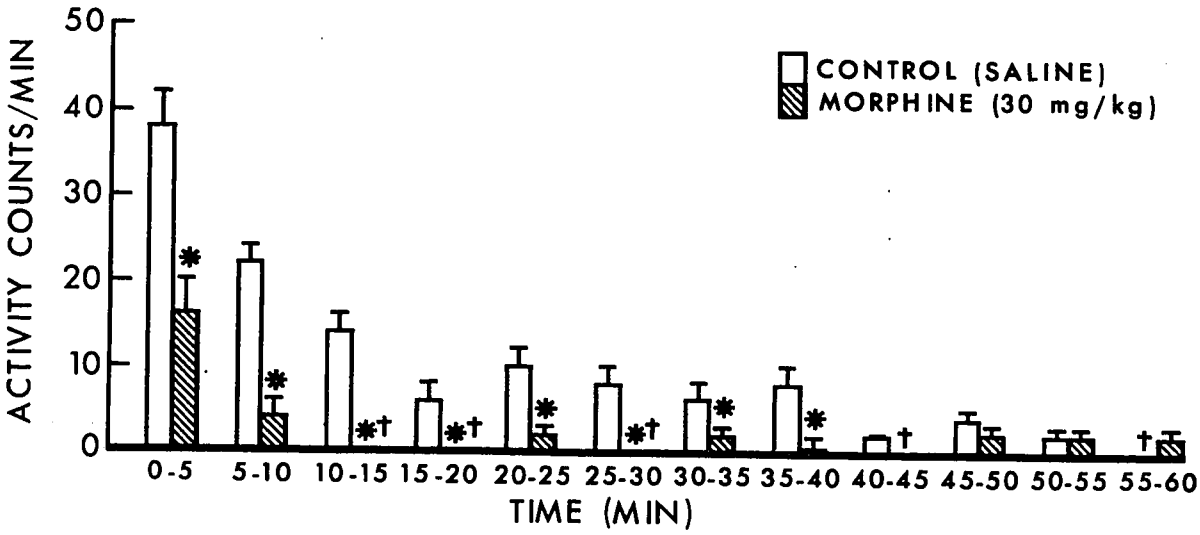


Fig. 1. Effect of acute morphine administration on spontaneous locomotor activity of rats. Experimental animals were injected with morphine (30 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods. Values represent the Mean ± S.E.M. of 7 control and 8 experimental animals.

† The activity of the animals was below detectable limits.
* Statistically significant from control at $P < 0.01$.

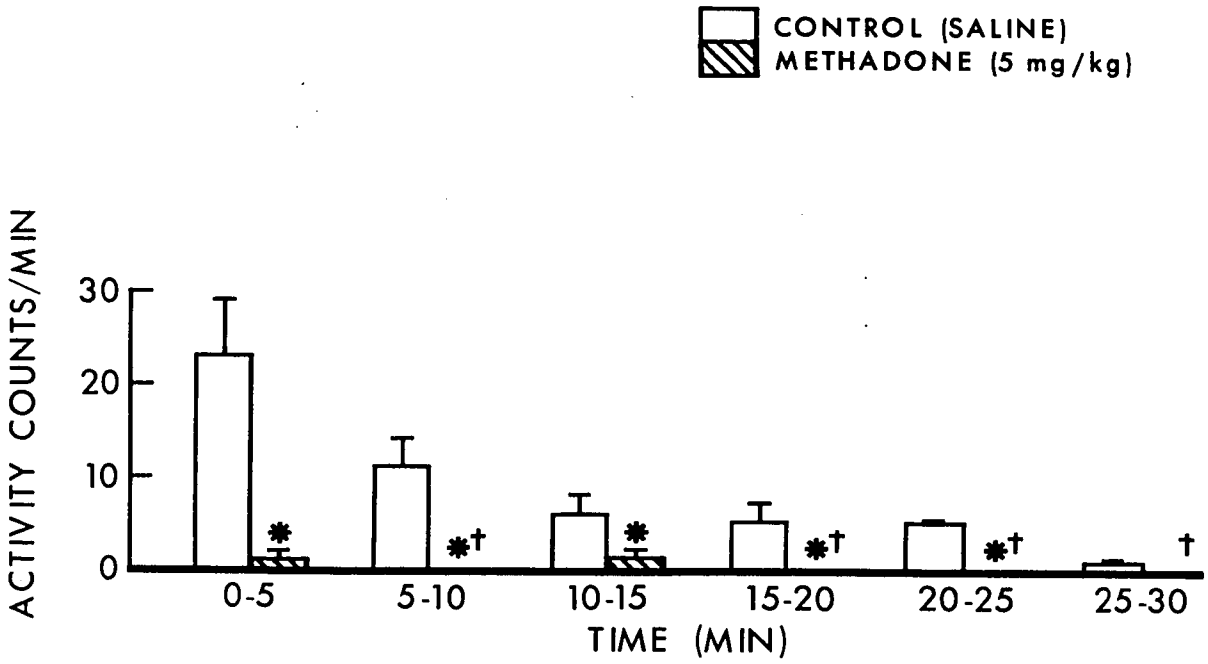


Fig. 2. Effect of acute methadone administration on spontaneous locomotor activity of rats. Experimental animals were injected with methadone (5 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 4 control and 4 experimental animals.

† The activity of the animals was below detectable limits.
* Statistically significant from control at $P < 0.005$.

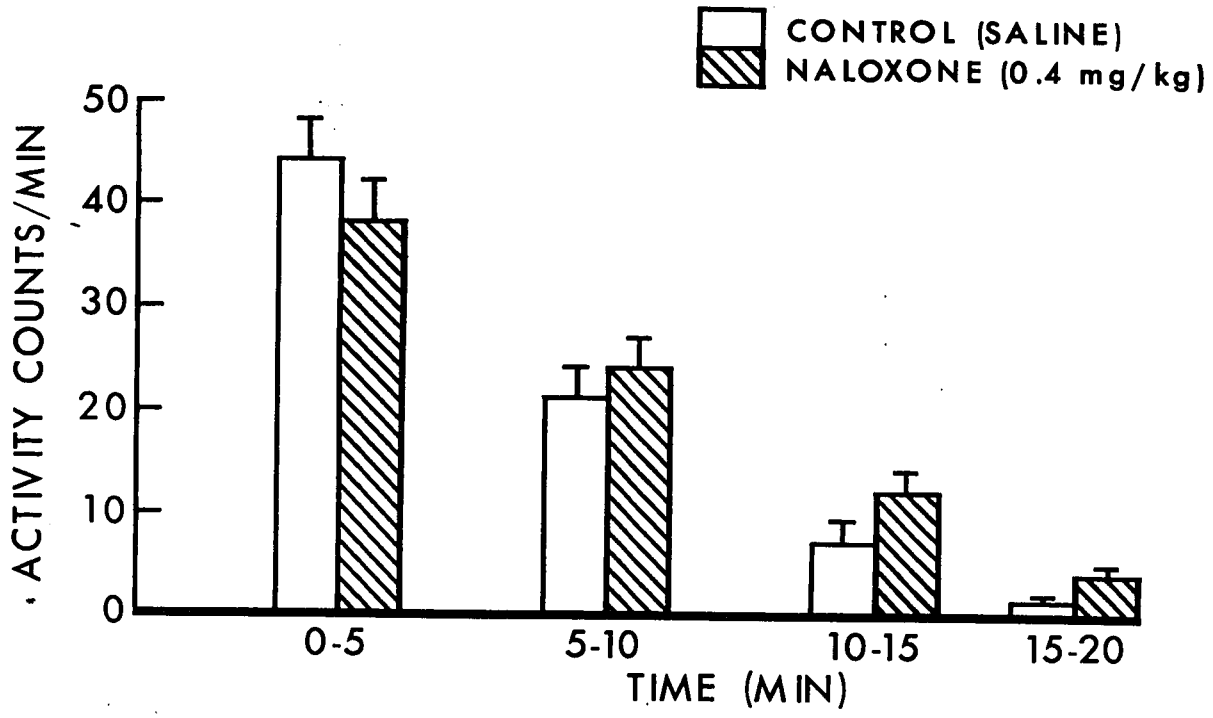


Fig. 3. Effect of acute naloxone administration on spontaneous locomotor activity of rats. Experimental animals were injected with naloxone (0.4 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed 20 minutes after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods. Values represent the Mean ± S.E.M. of 7 control and 8 experimental animals.

II. Chronic (9 Days) Morphine Treatment

Chronic treatment with morphine (30 mg/kg i.p. once daily) for 9 days resulted in a small but significant decrease in the HA concentration in the hypothalamus (84.6 ± 3.0% of control), while the HA levels in the midbrain and cerebral cortex were not significantly affected as indicated in Table 2.

III. Chronic (5 Days) Methadone Treatment

Chronic treatment with increasing doses of methadone (5 mg/kg i.p. once daily for 2 days, 10 mg/kg i.p. once daily for 2 days, and 15 mg/kg i.p. once) as indicated in Table 3, did not result in any statistically significant changes in the HA concentrations in the hypothalamus, midbrain or cerebral cortex.

IV. Chronic Morphine Treatment and Withdrawal

Chronic treatment with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days resulted in a significant decrease in the hypothalamic HA concentration. This treatment also resulted in decreases in the midbrain and cerebral cortex as indicated in Table 4 and Figs. 4-6.

Chronic treatment with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed 18 hours after the last morphine treatment by acute naloxone (0.4 mg/kg i.p.) 20 minutes before sacrifice, resulted in statistically significant decreases in hypothalamic and cerebro-cortical HA as indicated in Table 4 and Figs. 4-6. This treatment also resulted in a decrease in the HA concentration in the midbrain although this change was not statistically significant

from control values.

Chronic treatment with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by 0.9% saline (0.1 ml/100 g i.p. once daily) for 2 days resulted in statistically significant decreases in HA in all three brain regions investigated as indicated in Table 4 and Figs. 4-6.

Chronic treatment with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by methadone (15 mg/kg i.p. once daily) for 2 days resulted in statistically significant decreases in hypothalamic and cerebro-cortical HA concentrations when compared to the values for saline-treated controls. The midbrain HA levels failed to show any significant decreases following this treatment as indicated in Table 4 and Figs. 4-6.

Chronic treatment with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by morphine (90 mg/kg i.p. once daily) for 2 days resulted in statistically significant decreases in hypothalamic and cerebro-cortical HA concentrations, although no significant decrease was observed in the midbrain as indicated in Table 4 and Figs. 4-6. The pattern of changes in HA concentrations is the same for the 21 or 23 day morphine treatments in the brain regions investigated.

The body weights of the rats treated chronically with weekly increasing doses of morphine were significantly decreased by day 7 (Fig. 7) and this decrease became progressively more evident throughout the experimental period. The naloxone-precipitated withdrawal syndrome caused a further drop in body weights as indicated by arrow 1. Withdrawal of morphine and replacement with saline for 2 days also resulted in a

significant decrease in body weights (77% of morphine-treated rats) as indicated by arrow 2. The body weights of rats withdrawn from morphine and administered methadone (15 mg/kg i.p. once daily) for 2 days, did not differ significantly from those treated with morphine alone (81% of morphine-treated rats). It should be noted that there was evidence of considerable growth in both control and experimental animals and in spite of the statistically significant decreases in body weights of experimental animals, when compared to control rats, the tissue weights of the brain regions did not differ significantly from those of the control animals. This is particularly important because the HA concentrations in these studies are all reported in nanograms per gram of tissue (wet weight).

The SLA of rats receiving the various chronic treatments just described are illustrated in Fig. 8. It can be seen that only the animals treated with morphine for 3 weeks and withdrawn for 2 days showed any statistically significant change in their activities. These rats appeared sedated during both the first and second 5 minute recording periods.

The naloxone-treated rats were lower than controls during both time periods but the decreases were not statistically significant perhaps due to the restricted number of experimental animals in this study.

Another experiment was performed to better determine the effects of naloxone-precipitated withdrawal on SLA of rats. These data are illustrated in Fig. 9. The rats undergoing naloxone-precipitated withdrawal were statistically significantly less active than the saline-treated controls.

In continuation of the studies just described, the time course of the changes observed following chronic morphine treatment in endogenous

brain HA levels has been investigated. In these experiments, rats were treated with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and then withdrawn for various periods of time.

Table 5 and Fig. 10 illustrate the time course of the changes observed in the HA concentrations in the hypothalamus. Histamine was significantly decreased at 1, 6, 18 and 66 hours following the last injection of morphine. It can be seen that the HA level returned toward normal within 1 week and that it was fully recovered to control value 3 weeks after cessation of treatment.

Table 5 and Fig. 11 illustrate the time course of the changes observed in the HA concentrations in the midbrain. In this brain region, the HA concentration was not significantly different from control values at one hour, 6 hours or 18 hours after the last morphine treatment. It took 66 hours after the last morphine treatment for a statistically significant change to appear in midbrain HA levels. This decrease was less pronounced one week following the end of treatment, however, a notable decrease was still present in the midbrain after 3 weeks.

Table 5 and Fig. 12 illustrate the time course of the changes observed in the HA concentration in the cortex after morphine withdrawal from morphine-dependent rats. There is no change in the HA levels when measured one hour or 6 hours after the last morphine injection; however, by 18 hours, and at 66 hours, there were statistically significant decreases. The HA levels in the cortex returned to control values within one week as in the hypothalamus, and were fully restored following a 3 week period.

TABLE 2

ENDOGENOUS HISTAMINE CONCENTRATIONS IN VARIOUS BRAIN REGIONS OF RATS AFTER CHRONIC (9 DAYS) MORPHINE TREATMENT

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL (SALINE)	294.5 ± 5.7 (7) 100.0 ± 1.9%	43.0 ± 4.4 (6) 100.0 ± 10.2%	26.8 ± 1.8 (8) 100.0 ± 6.7%
MORPHINE (9 days) (30 mg/kg)	249.2 ± 8.9* (5) 84.6 ± 3.0%*	40.7 ± 3.5 (6) 94.7 ± 8.1%	27.2 ± 2.4 (7) 101.5 ± 9.0%

Experimental animals were treated with morphine (30 mg/kg i.p. once daily) for 9 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 9 days. The animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

TABLE 3

ENDOGENOUS HISTAMINE CONCENTRATIONS IN VARIOUS BRAIN REGIONS OF RATS AFTER CHRONIC (5 DAYS) METHADONE TREATMENT

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL (SALINE)	273.7 ± 21.8 (8) 100.0 ± 8.0%	31.2 ± 3.4 (8) 100.0 ± 10.9%	20.8 ± 1.0 (7) 100.0 ± 4.8%
METHADONE (5 days)	270.5 ± 30.2 (5) 98.8 ± 11.0%	27.9 ± 3.0 (6) 89.4 ± 0.6%	25.8 ± 2.7 (5) 124.0 ± 13.0%

Experimental animals were administered increasing doses of methadone for 5 days (5 mg/kg i.p. once daily for 2 days, 10 mg/kg i.p. once daily for 2 days, and 15 mg/kg i.p. once), and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily for 5 days). The animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

TABLE 4

ENDOGENOUS HISTAMINE CONCENTRATIONS IN BRAIN REGIONS OF RATS AFTER VARIOUS CHRONIC TREATMENTS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
"C_1" SALINE (21 days)	293.3 ± 15.3 (23)	45.3 ± 3.5 (21)	18.4 ± 2.0 (21)
MORPHINE (Mo) (21 days)	209.2 ± 12.5* (25)	39.8 ± 2.8 (25)	12.7 ± 1.7 (24)
Mo (21 days) and NALOXONE (20 minutes)	209.8 ± 17.9* (7)	37.5 ± 4.6 (9)	10.4 ± 0.8* (9)
Mo (21 days) and SALINE (2 days)	175.4 ± 9.6† (8)	30.0 ± 4.3† (8)	11.5 ± 1.0† (7)
Mo (21 days) and METHADONE (2 days)	223.0 ± 19.9† (7)	39.3 ± 6.6 (7)	10.1 ± 1.5† (6)
MORPHINE (23 days)	234.5 ± 14.3† (7)	44.6 ± 4.7 (6)	10.7 ± 2.2† (8)
"C_2" SALINE (23 days)	322.1 ± 17.6 (12)	45.3 ± 4.0 (13)	18.2 ± 3.0 (11)

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by naloxone (0.4 mg/kg i.p.), 0.9% saline (0.1 ml/100 g i.p.), methadone (15 mg/kg i.p.) or morphine (90 mg/kg i.p.). Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days "C_1" or 23 days "C_2". The animals were sacrificed 20 minutes after naloxone administration and 18 hours after the other treatments. Values represent the Mean ± S.E.M. The number of experiments is shown in parentheses.

* Statistically significant from control "C_1" at $P < 0.05$
† Statistically significant from control "C_2" at $P < 0.05$

TABLE 4P

ENDOGENOUS HISTAMINE CONCENTRATIONS IN BRAIN REGIONS OF RATS
AFTER VARIOUS CHRONIC TREATMENTS

Treatment	Hypothalmus	Midbrain	Cortex
	Histamine (% of control)		
"C_1" SALINE (21 days)	100.0 ± 5.2 (23)	100.0 ± 7.7 (21)	100.0 ± 10.9 (21)
MORPHINE (Mo) (21 days)	71.3 ± 4.3* (25)	87.9 ± 6.2 (25)	69.0 ± 9.2 (24)
Mo (21 days) and NALOXONE (20 minutes)	71.5 ± 6.1* (7)	82.8 ± 10.2 (9)	56.5 ± 4.3* (9)
Mo (21 days) and SALINE (2 days)	54.5 ± 3.0† (8)	66.2 ± 9.5† (8)	63.2 ± 5.5† (7)
Mo (21 days) and METHADONE (2 days)	69.2 ± 6.2† (7)	86.8 ± 14.6 (7)	55.5 ± 8.2† (6)
MORPHINE (23 days)	72.8 ± 4.4† (7)	98.5 ± 10.4 (6)	58.8 ± 12.1† (8)
"C_2" SALINE (23 days)	100.0 ± 5.5 (12)	100.0 ± 8.8 (13)	100.0 ± 16.5 (11)

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by naloxone (0.4 mg/kg i.p.), 0.9% saline (0.1 ml/1-0 g i.p.), methadone (15 mg/kg i.p.) or morphine (90 mg/kg i.p.). Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days "C_1" or 23 days "C_2". The animals were sacrificed 20 minutes after naloxone administration and 18 hours after the other treatments. Values represent the Mean ± S.E.M. The results are expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control "C_1" at $P < 0.05$
† Statistically significant from control "C_2" at $P < 0.05$

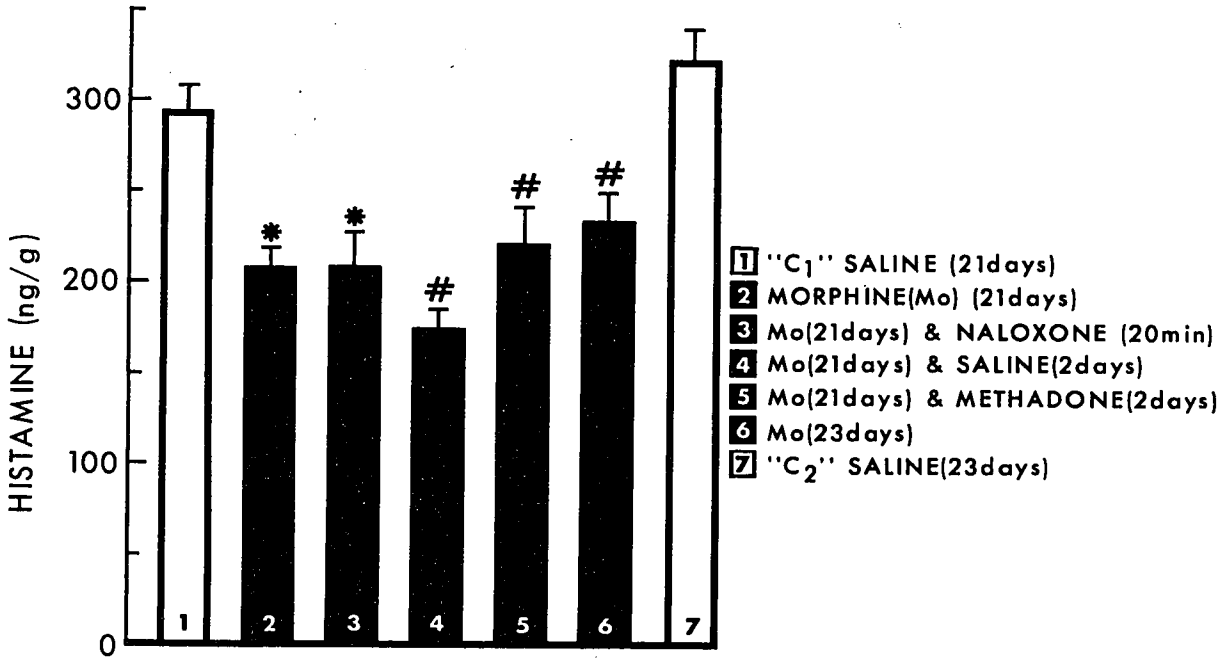


Fig. 4. Histamine concentrations in the rat hypothalamus following various treatments. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by naloxone (0.4 mg/kg i.p.), 0.9% saline (0.1 ml/100 g i.p. once daily), methadone (15 mg/kg i.p. once daily), or morphine (90 mg/kg i.p. once daily) as indicated. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days "C_1" or 23 days "C_2". The animals were sacrificed 20 minutes after naloxone administration and 18 hours following the other treatments. Values represent the Mean ± S.E.M. of at least 7 animals in each group.

* Statistically significant from Control "C_1" at $P < 0.05$
† Statistically significant from Control "C_2" at $P < 0.05$

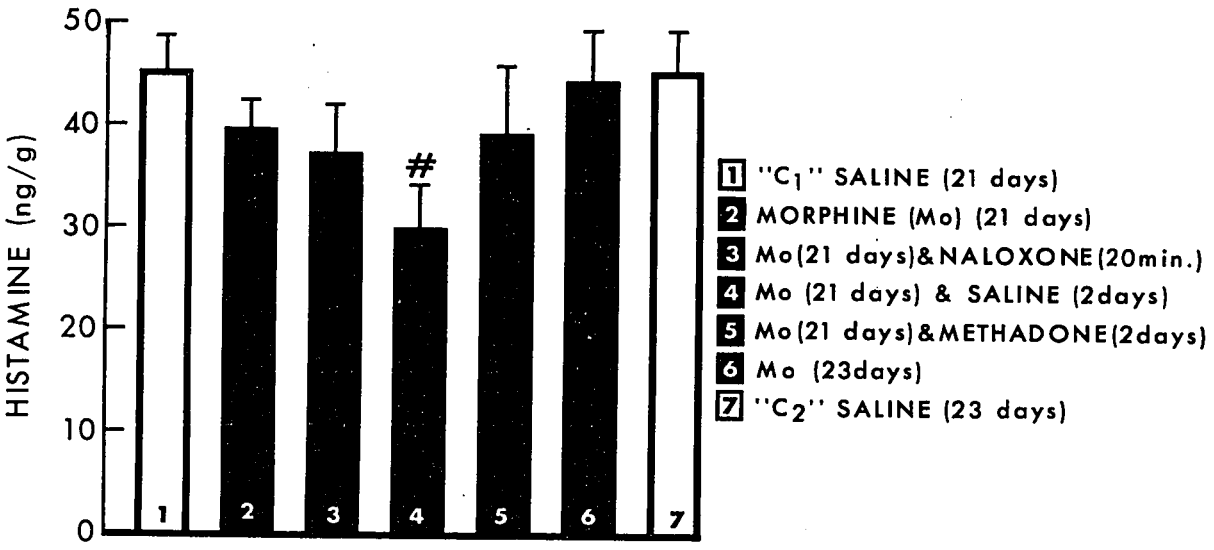


Fig. 5. Histamine concentrations in the rat midbrain following various treatments. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by naloxone (0.4 mg/kg i.p.), 0.9% saline (0.1 ml/100 g i.p. once daily), methadone (15 mg/kg i.p. once daily), or morphine (90 mg/kg i.p. once daily) as indicated. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days "C_1" or 23 days "C_2". The animals were sacrificed 20 minutes after naloxone administration and 18 hours following the other treatments. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

Statistically significant from Control "C_2" at $P < 0.05$

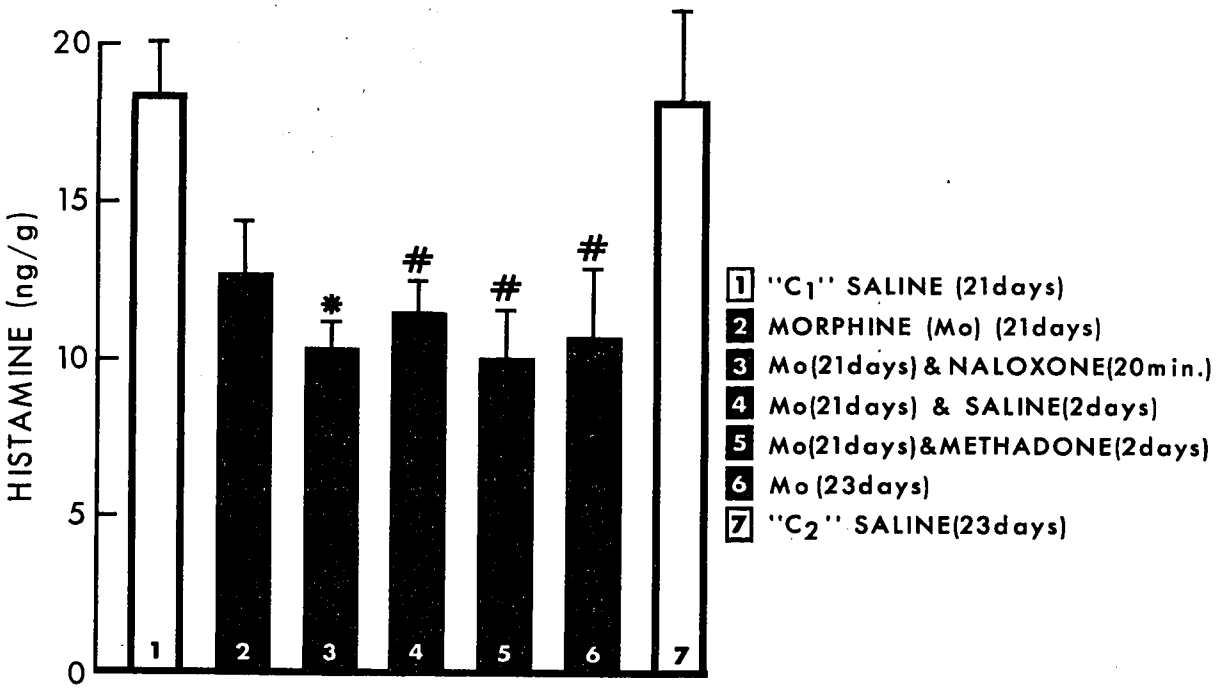


Fig. 6. Histamine concentrations in the rat cortex following various treatments. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by naloxone (0.4 mg/kg i.p.), 0.9% saline (0.1 ml/100 g i.p. once daily), methadone (15 mg/kg i.p. once daily), or morphine (90 mg/kg i.p. once daily) as indicated. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days "C_1" or 23 days "C_2". The animals were sacrificed 20 minutes after naloxone administration and 18 hours following the other treatments. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

* Statistically significant from Control "C_1" at $P < 0.05$
# Statistically significant from Control "C_2" at $P < 0.05$

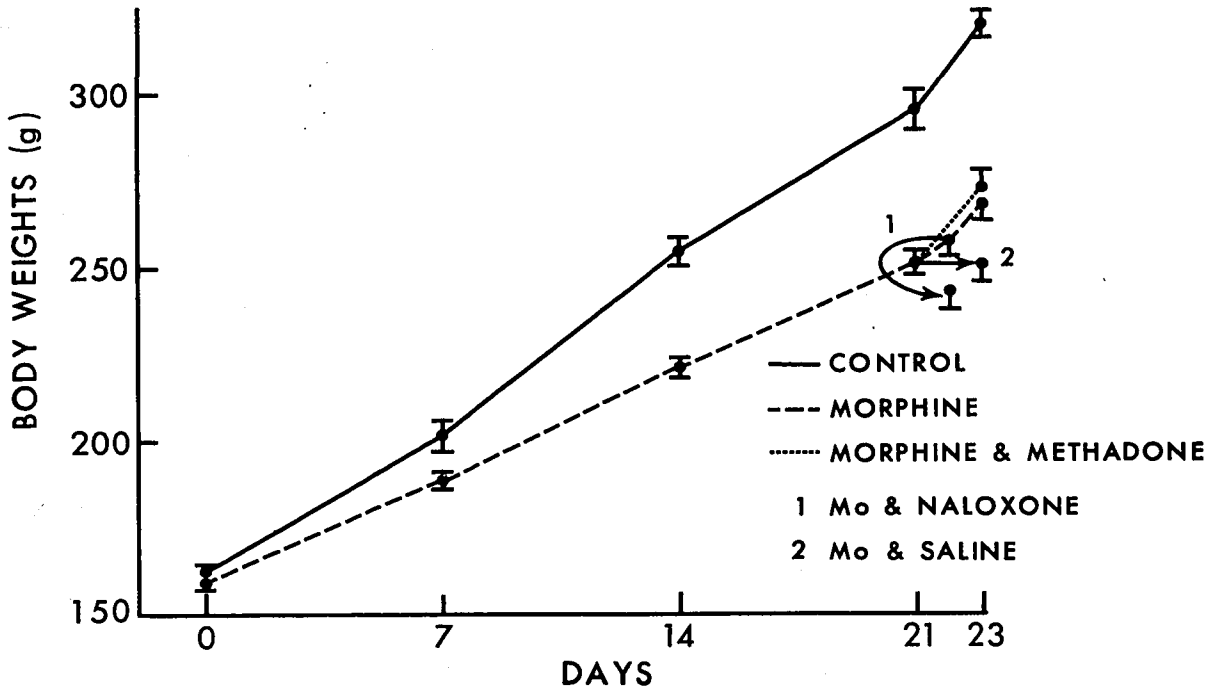


Fig. 7. Influence of various chronic treatments on body weight changes. Experimental animals were treated with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by naloxone (0.4 mg/kg i.p.) for 20 minutes or 0.9% saline (0.1 ml/100 g i.p. once daily), methadone (15 mg/kg i.p. once daily), or morphine (90 mg/kg i.p. once daily) for 2 days as indicated. Control animals were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 23 days. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

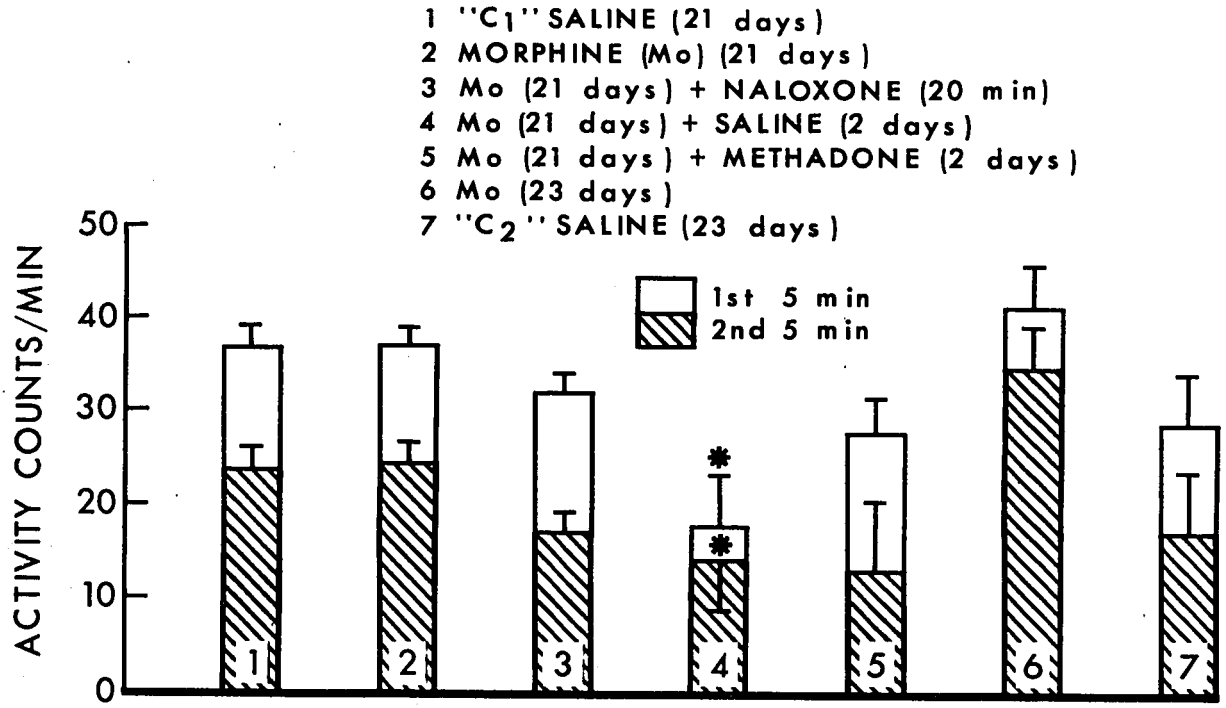


Fig. 8. Spontaneous locomotor activity of rats after various chronic treatments. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p.), 0.9% saline (0.1 ml/100 g i.p. once daily), methadone (15 mg/kg i.p. once daily), or morphine (90 mg/kg i.p. once daily) for 2 days. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days "C_1" or 23 days "C_2". The animals were sacrificed 20 minutes after naloxone administration and 18 hours after the other treatments. The data indicate the mean activity counts/minute determined for two consecutive 5 minute periods. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

* Statistically significant from control at $P < 0.05$

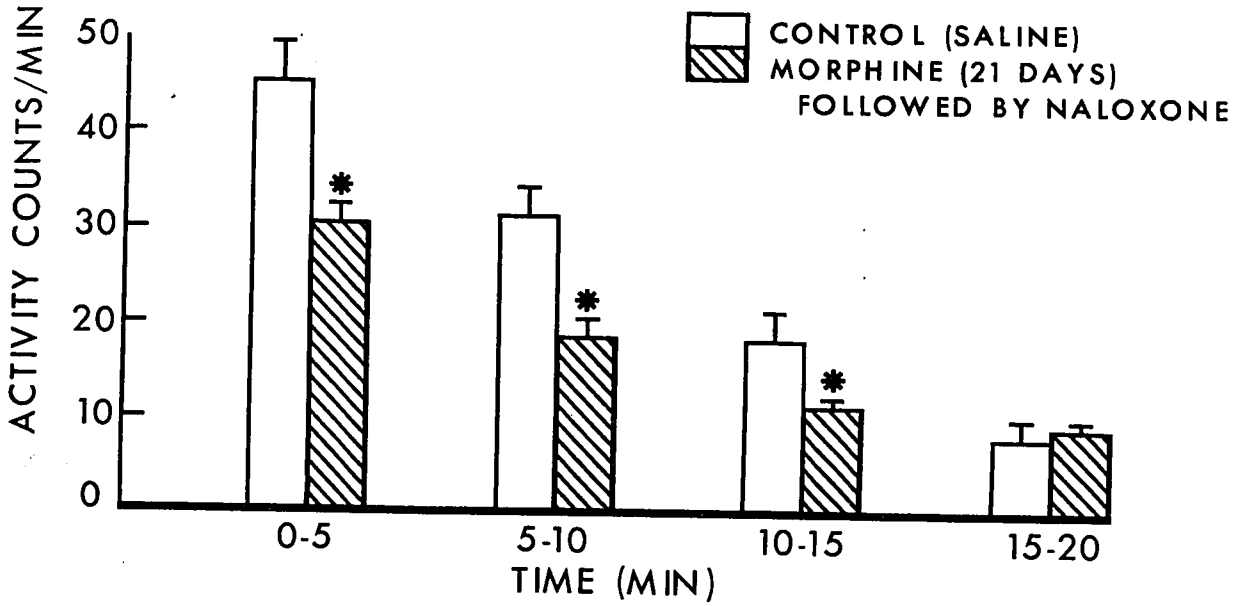


Fig. 9. Effect of naloxone precipitated withdrawal on spontaneous locomotor activity of morphine dependent rats. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and injected with naloxone (0.4 mg/kg i.p.) 18 hours after the last morphine injection. Control animals were administered 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed 20 minutes after naloxone administration. The data represent the mean activity counts/minute determined for consecutive 5 minute periods. Values represent the Mean ± S.E.M. of 6 control and 10 experimental animals.

* Statistically significant from control at $P < 0.01$

TABLE 5

EFFECT OF CHRONIC MORPHINE ADMINISTRATION ON ENDOGENOUS HISTAMINE CONCENTRATIONS IN VARIOUS BRAIN REGIONS OF RATS

Time of Sacrifice After Treatment	Treatment	Hypothalamus	Midbrain	Cortex
		Histamine (ng/g)		
1 hr.	SALINE	370.2 ± 26.0 (10)	62.2 ± 4.3 (12)	17.6 ± 1.5 (10)
	MORPHINE	262.5 ± 31.4* (11)	51.0 ± 3.4 (11)	19.7 ± 1.6 (9)
6 hr.	SALINE	339.5 ± 28.4 (7)	55.4 ± 6.9 (6)	19.0 ± 3.6 (6)
	MORPHINE	218.3 ± 25.2* (8)	58.9 ± 6.6 (6)	23.3 ± 2.6 (6)
18 hr.	SALINE	293.3 ± 15.3 (23)	45.3 ± 3.5 (21)	18.4 ± 2.0 (21)
	MORPHINE	209.2 ± 12.5* (25)	39.8 ± 2.8 (25)	12.7 ± 1.7* (24)
66 hr.	SALINE	322.1 ± 17.6 (12)	45.3 ± 4.0 (13)	18.2 ± 3.0 (12)
	MORPHINE	175.4 ± 9.6* (8)	30.0 ± 4.3* (8)	11.5 ± 1.0* (7)
1 week	SALINE	302.7 ± 38.6 (6)	43.8 ± 5.7 (6)	16.1 ± 2.4 (6)
	MORPHINE	254.5 ± 30.7 (6)	38.0 ± 5.8 (6)	12.8 ± 2.9 (6)
3 weeks	SALINE	247.8 ± 46.2 (7)	45.0 ± 7.3 (7)	16.0 ± 3.4 (7)
	MORPHINE	248.3 ± 39.4 (7)	34.8 ± 4.1 (7)	16.3 ± 3.2 (8)

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed at the time indicated after the last treatment. Values represent the Mean ± S.E.M. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

TABLE 5P

EFFECT OF CHRONIC MORPHINE ADMINISTRATION ON ENDOGENOUS HISTAMINE CONCENTRATIONS IN VARIOUS BRAIN REGIONS OF RATS

Time of Sacrifice After Treatment	Treatment	Hypothalamus	Midbrain	Cortex
		Histamine (% of control)		
1 hr.	SALINE	100.0 ± 7.0 (10)	100.0 ± 6.9 (12)	100.0 ± 8.5 (10)
	MORPHINE	70.9 ± 8.5* (11)	82.0 ± 5.5 (11)	111.9 ± 9.1 (9)
6 hr.	SALINE	100.0 ± 8.4 (7)	100.0 ± 12.5 (6)	100.0 ± 18.9 (6)
	MORPHINE	64.3 ± 7.4* (8)	106.3 ± 11.9 (6)	122.6 ± 13.7 (6)
18 hr.	SALINE	100.0 ± 5.2 (23)	100.0 ± 7.7 (21)	100.0 ± 10.9 (21)
	MORPHINE	71.3 ± 4.3* (25)	87.9 ± 6.2 (25)	69.0 ± 9.2 (24)
66 hr.	SALINE	100.0 ± 5.5 (12)	100.0 ± 8.8 (13)	100.0 ± 16.5 (12)
	MORPHINE	54.4 ± 3.0* (8)	66.2 ± 9.5* (8)	63.2 ± 5.5* (7)
1 week	SALINE	100.0 ± 12.8 (6)	100.0 ± 13.0 (6)	100.0 ± 14.9 (6)
	MORPHINE	84.1 ± 10.1 (6)	86.8 ± 13.2 (6)	79.5 ± 18.0 (6)
3 weeks	SALINE	100.0 ± 18.6 (7)	100.0 ± 16.2 (7)	100.0 ± 21.3 (7)
	MORPHINE	100.2 ± 15.6 (7)	77.3 ± 9.1 (7)	101.9 ± 20.0 (8)

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed at the times indicated after the last experiment. Values represent the Mean ± S.E.M. The results are expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

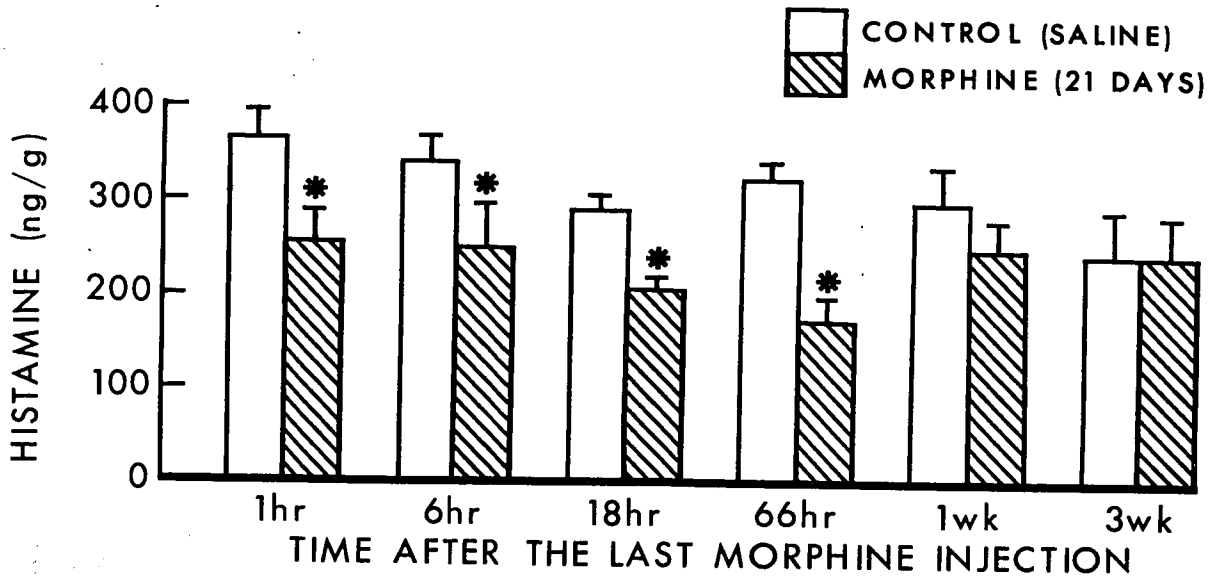


Fig. 10. The effect of chronic morphine administration on endogenous brain histamine concentrations in the rat hypothalamus. Experimental animals were adminstered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed at the times indicated after the last treatment. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

* Statistically significant from control at $P < 0.05$

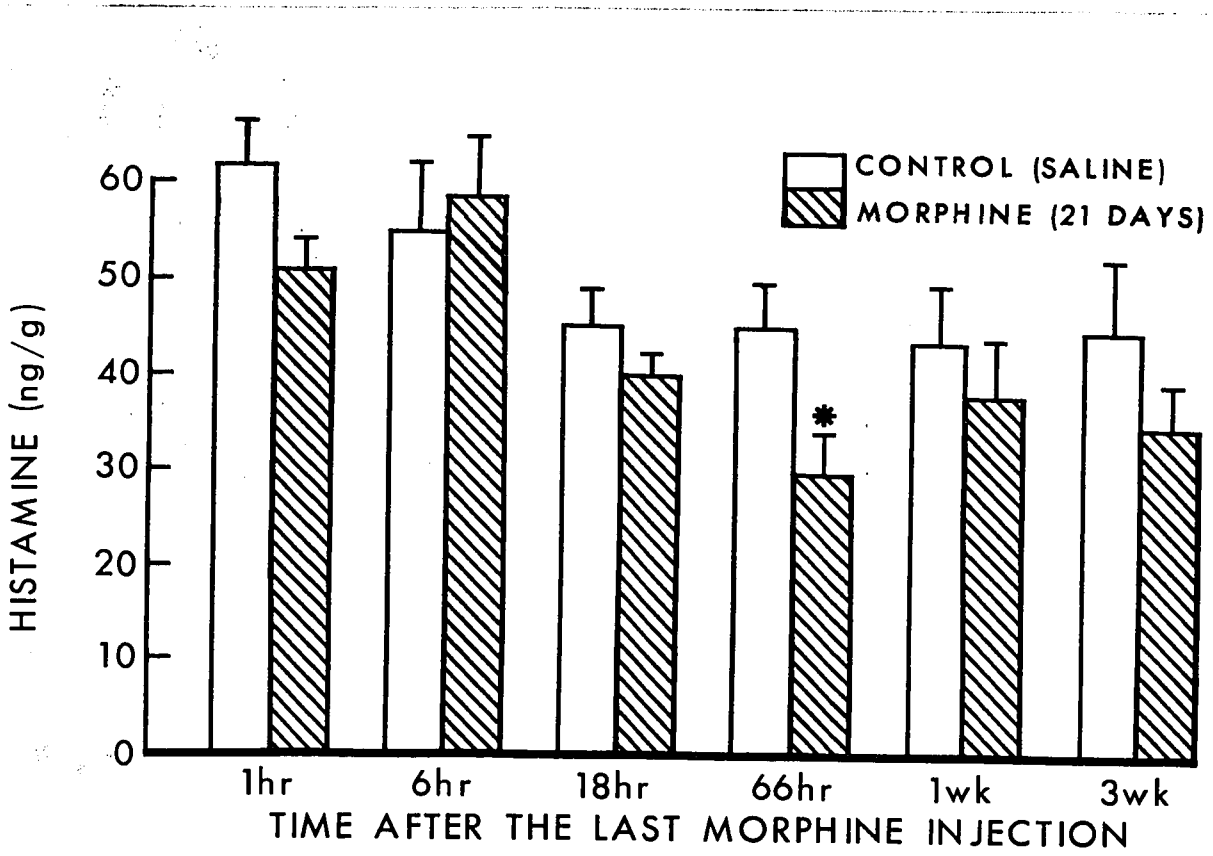


Fig. 11. The effect of chronic morphine administration on endogenous brain histamine concentrations in the rat midbrain. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed at the times indicated after the last treatment. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

* Statistically significant from control at $P < 0.05$

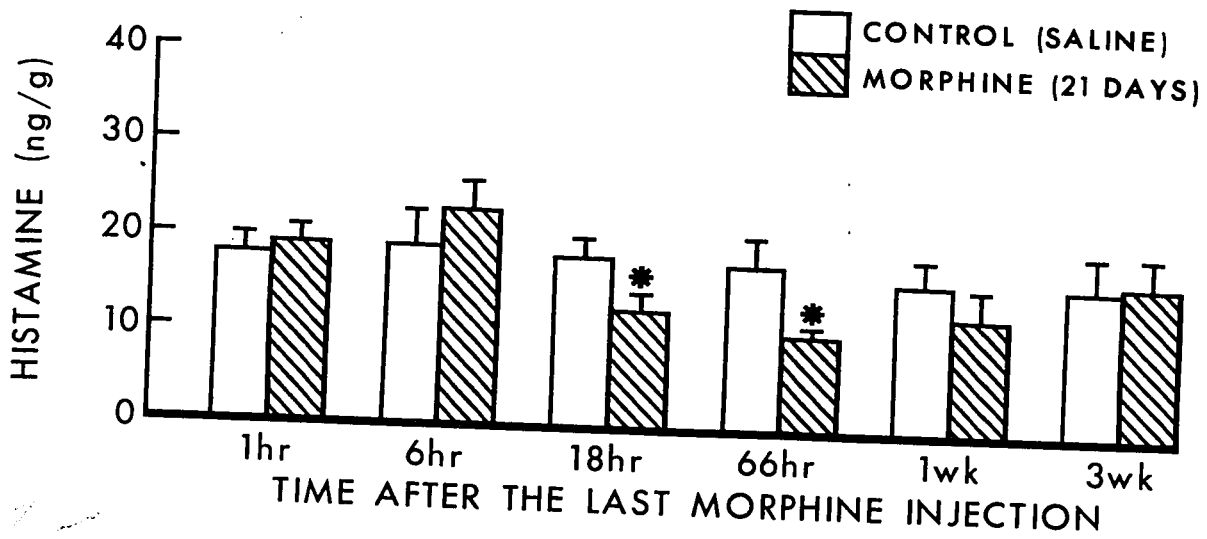


Fig. 12. The effect of chronic morphine administration on endogenous brain histamine concentrations in the rat cortex. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed at the times indicated after the last treatment. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

* Statistically significant from control at $P < 0.05$

Figs. 13 to 19 illustrate the data for the SLA of rats, treated with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days, measured at various times after the last treatment.

Within the first hour following the administration of the last chronic dose of morphine (90 mg/kg i.p.) the rats were clearly sedated as illustrated in Fig. 13. Three hours following the last morphine treatment as illustrated in Fig. 14, the SLA of these rats returned to normal although it did not appear to be stabilized. During the first 5 minute period of recording, the morphine-treated rats showed enhanced activity and during the last 15 minutes of recording their locomotor activity appeared depressed. Six hours following the last morphine treatment, the SLA of these rats appear to be elevated above control values. However, the data are only statistically significant during two 5 minute recording periods as illustrated in Fig. 15.

Eighteen hours after the last chronic morphine treatment, the SLA of these rats appeared indistinguishable from control values as illustrated in Fig. 16.

Sixty-six hours after the last chronic morphine treatment, the morphine-treated rats appeared to be sedated as illustrated in Fig. 17. The experimental rats showed statistically significant decreases in their SLA throughout the recording period.

The data in Fig. 18 and Fig. 19 illustrate that one week and three weeks respectively following morphine withdrawal from morphine-dependent rats, their SLA were essentially normal.

In order to attempt an explanation for the observed changes following chronic morphine administration and morphine withdrawal, it was decided to investigate the activity of the major HA metabolizing

enzyme histamine methyltransferase (HMT).

Since the HMT activity is expressed per milligram of protein it was first necessary to determine the effects of administration of weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p.) on the protein concentrations in various brain regions. The data in Table 6, showing the effects of chronic morphine administration on protein concentration in various brain regions, show no significant changes in the hypothalamus, midbrain or cerebral cortex.

Morphine withdrawal for 2 days from morphine-dependent rats also produced no significant changes in the hypothalamus, midbrain or cerebral cortex as indicated in Table 7.

The HMT activities of rats, treated chronically with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and measured 18 hours after the last treatment are indicated in Table 8. There were no significant changes observed in the hypothalamus, midbrain or cerebral cortex.

Chronic morphine treatment followed by 2 days of withdrawal did not produce any significant differences from controls in the brain regions investigated as illustrated in Table 9.

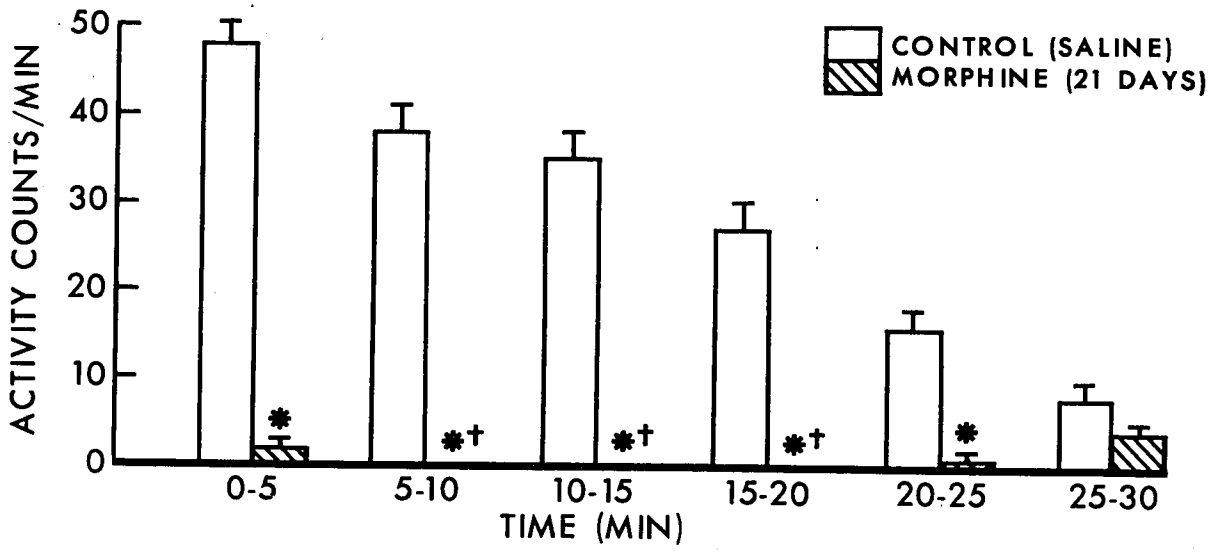


Fig. 13. Effect of chronic morphine administration on spontaneous locomotor activity of rats one hour after the last treatment. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed one hour after the last treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 10 experimental animals.

† The activity of the animals was below detectable limits
* Statistically significant from control at $P < 0.001$

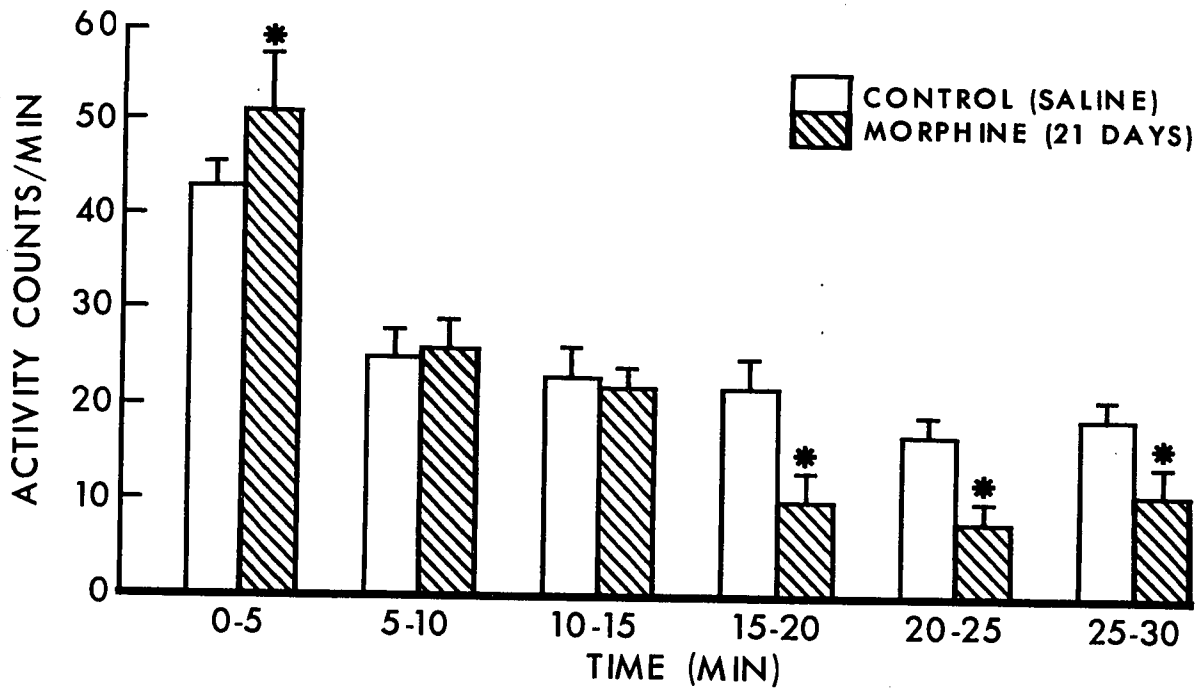


Fig. 14. Effect of chronic morphine administration on spontaneous locomotor activity of rats 3 hours after the last treatment. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed 3 hours after the last treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 7 experimental animals.

* Statistically significant from control at $P < 0.01$

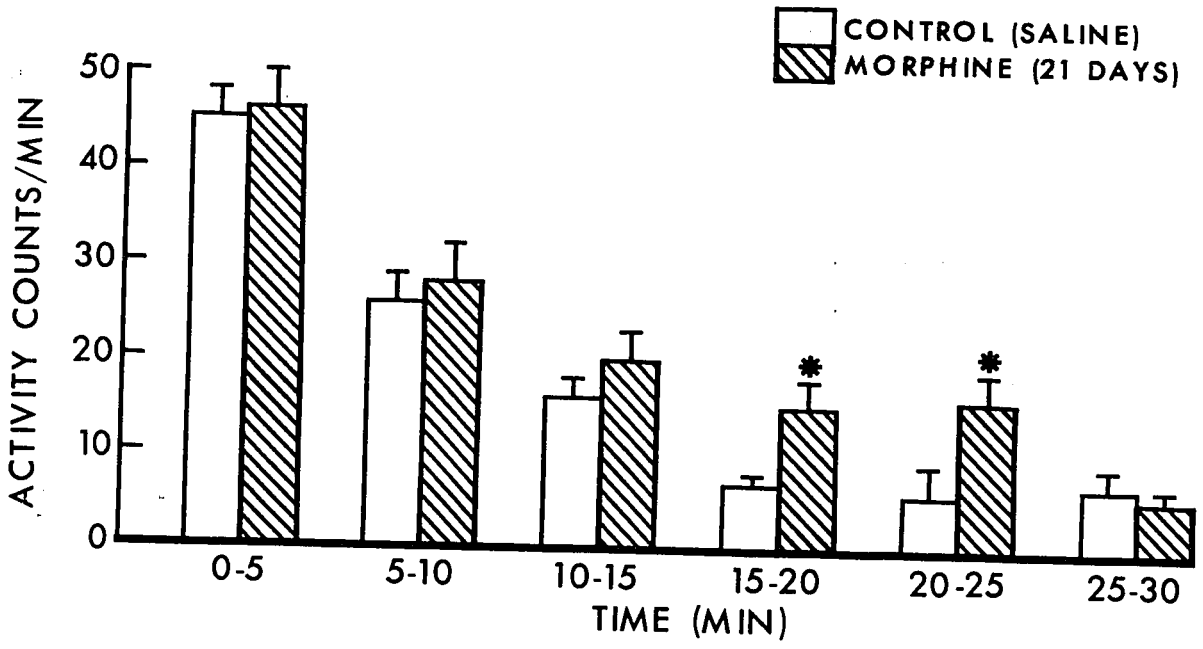


Fig. 15. Effect of chronic morphine administration on spontaneous locomotor activity of rats 6 hours after the last treatment. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed 6 hours after the last treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 10 experimental animals.

* Statistically significant from control at $P < 0.01$

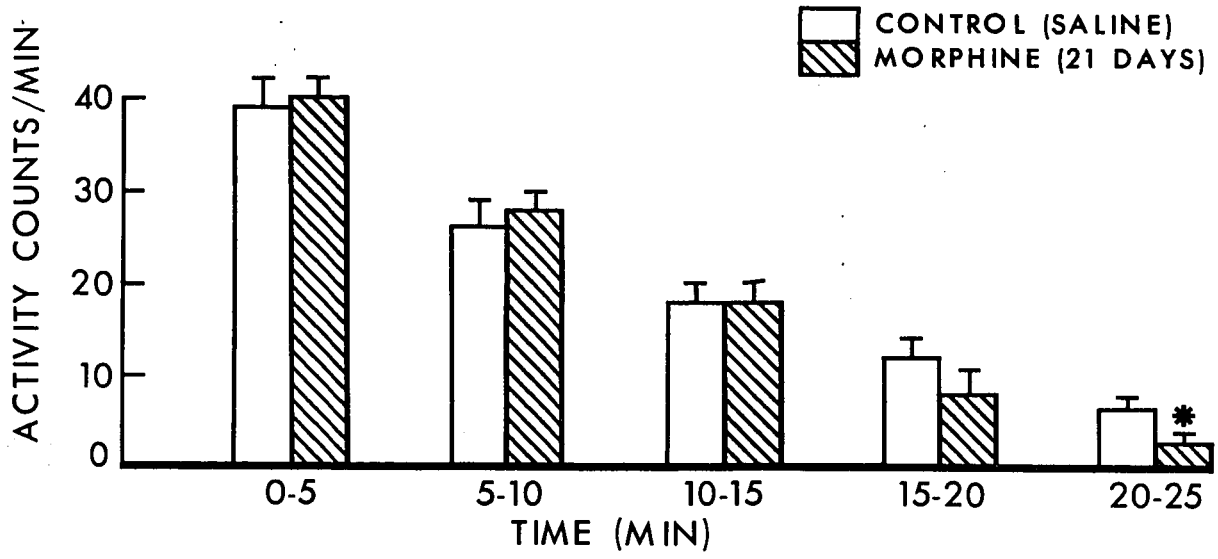


Fig. 16. Effect of chronic morphine administration on spontaneous locomotor activity of rats 18 hours after the last treatment. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed 18 hours after the last treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 25 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 12 control and 12 experimental animals.

* Statistically significant from control at $P < 0.025$

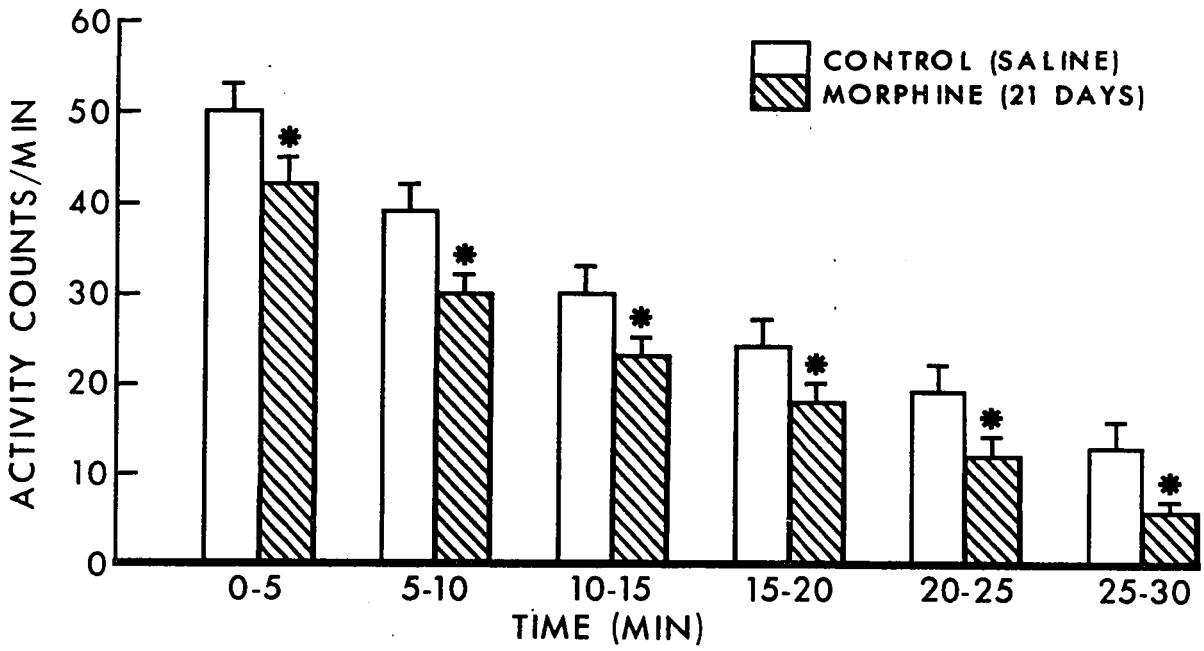


Fig. 17. Effect of chronic morphine administration on spontaneous locomotor activity of rats 66 hours after the last treatment. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed 66 hours after the last treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 10 experimental animals.

* Statistically significant from control at $P < 0.05$

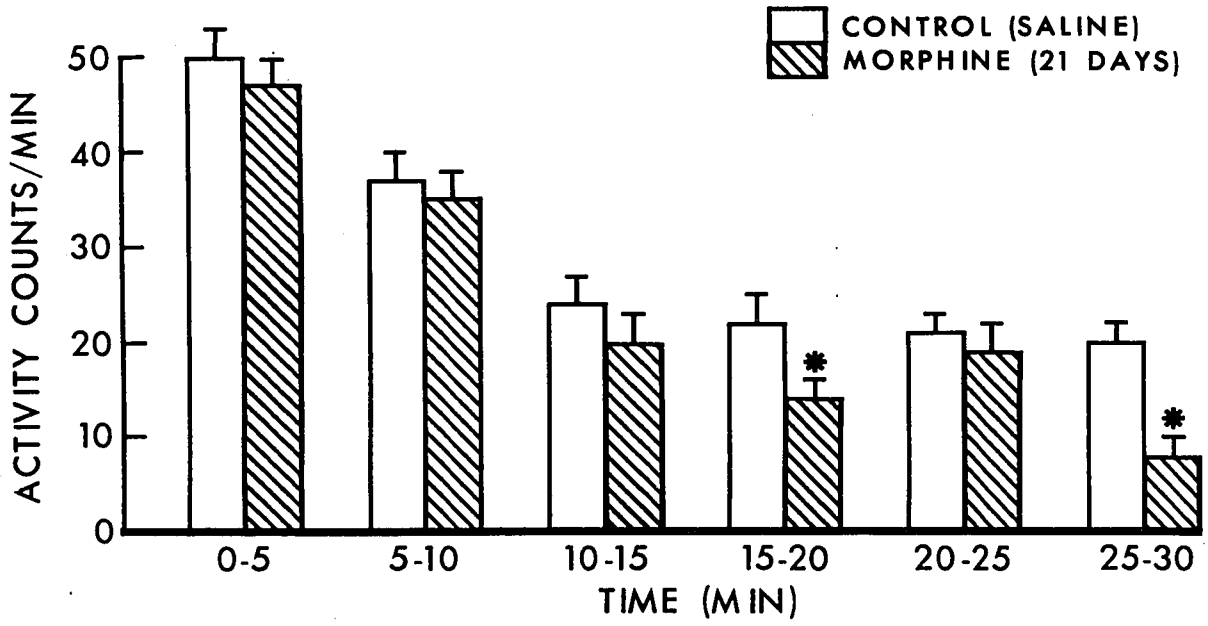


Fig. 18. Effect of chronic morphine administration on spontaneous locomotor activity of rats one week after the last treatment. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed one week after the last treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 7 experimental animals.

* Statistically significant from control at $P < 0.01$

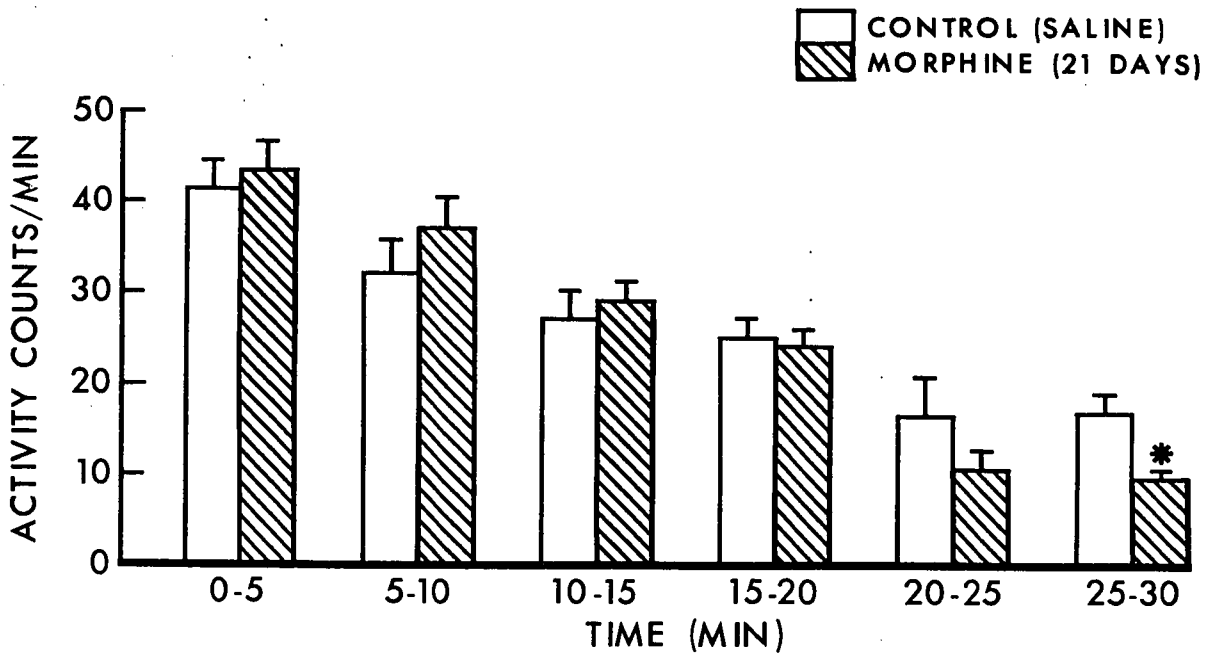


Fig. 19. Effect of chronic morphine administration on spontaneous locomotor activity of rats 3 weeks after the last treatment. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 mk/100 g i.p. once daily) for 21 days. The animals were sacrificed 3 weeks after the last treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 7 control and 8 experimental animals.

* Statistically significant from control at $P < 0.05$

TABLE 6

EFFECT OF CHRONIC MORPHINE ADMINISTRATION ON PROTEIN CONCENTRATION IN VARIOUS REGIONS OF RAT BRAIN

Treatment	Hypothalamus	Midbrain	Cortex
	Protein (mg/g tissue)		
CONTROL (SALINE)	40.6 ± 1.2 (8) 100.0 ± 3.0%	30.9 ± 0.4 (8) 100.0 ± 1.3%	33.2 ± 0.6 (8) 100.0 ± 1.8%
MORPHINE (21 days)	41.4 ± 1.2 (8) 102.0 ± 3.0%	31.6 ± 0.3 (8) 102.3 ± 1.0%	34.6 ± 1.1 (8) 104.2 ± 3.3%

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed 18 hours after the last treatment. The data indicate the mean protein concentrations per gram of tissue. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

TABLE 7

EFFECT OF MORPHINE WITHDRAWAL ON PROTEIN CONCENTRATION IN VARIOUS BRAIN REGIONS OF MORPHINE-DEPENDENT RATS

Treatment	Hypothalamus	Midbrain	Cortex
	Protein (mg/g tissue)		
CONTROL (SALINE	37.4 ± 0.5 (8) 100.0 ± 1.3%	26.2 ± 0.7 (8) 100.0 ± 3.8%	29.1 ± 0.6 (8) 100.0 ± 2.1%
MORPHINE (21 days) SALINE (2 days)	38.3 ± 1.6 (9) 102.4 ± 4.3%	26.1 ± 0.5 (9) 99.6 ± 1.9%	28.8 ± 0.7 (9) 99.0 ± 2.4%

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by 0.9% saline (0.1 ml/100 g i.p. once daily) for 2 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 23 days. The animals were sacrificed 66 hours after the last morphine treatment. The data indicate the mean protein concentration per gram of tissue. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

TABLE 8

EFFECT OF CHRONIC MORPHINE ADMINISTRATION ON HISTAMINE METHYLTRANSFERASE ACTIVITY IN RAT BRAIN

Treatment	Hypothalamus	Midbrain	Cortex
	HMT Activity (μM/hr/g Protein)		
CONTROL (SALINE)	1.30 ± 0.07 (8) 100.0 ± 5.4%	0.95 ± 0.03 (8) 100.0 ± 2.6%	0.84 ± 0.03 (8) 100.0 ± 3.0%
MORPHINE (21 days)	1.22 ± 0.07 (8) 93.8 ± 5.4%	0.96 ± 0.01 (8) 101.1 ± 1.1%	0.82 ± 0.05 (8) 97.0 ± 5.4%

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed 18 hours after the last treatment. The data indicate the mean activity of histamine methyltransferase (HMT), per hour, per gram of tissue protein. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

TABLE 9

HISTAMINE METHYLTRANSFERASE ACTIVITY IN RAT BRAIN AFTER 2 DAYS WITHDRAWAL OR MORPHINE FROM MORPHINE-DEPENDENT RATS

Treatment	Hypothalamus	Midbrain	Cortex
		HMT Activity (μM/hr/g Protein)	
CONTROL (SALINE)	1.38 ± 0.12 (8) 100.0 ± 8.7%	1.39 ± 0.08 (8) 100.0 ± 5.8%	1.23 ± 0.07 (8) 100.0 ± 5.3%
MORPHINE (21 days) + SALINE (2 days)	1.37 ± 0.14 (9) 98.9 ± 9.8%	1.35 ± 0.05 (9) 97.1 ± 3.2%	1.21 ± 0.03 (9) 98.0 ± 2.4%

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed by 0.9% saline (0.1 ml/100 g i.p. once daily) for 23 days. The animals were sacrificed 66 hours after the last morphine treatment. The data indicate the mean activity of histamine methyltransferase (HMT) per hour, per gram of tissue protein. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

V. Chronic Histidine with Chronic Morphine Treatment

In order to further explore the mechanism of the changes in endogenous brain HA concentrations occurring after chronic morphine treatment, the effects of administration of the HA precursor histidine were investigated.

The data from Table 5 are repeated here in Table 10 for ease of comparison. Table 10 and Fig. 20 demonstrate that chronic administration of histidine (500 mg/kg i.p. once daily) for 21 days resulted in significantly increased HA concentrations in the hypothalamus and cerebral cortex when determined 18 hours after the last treatment. The midbrain failed to show any significant change at this time.

Table 10 and Fig. 20 demonstrate the effects of simultaneous treatment with histidine (500 mg/kg i.p. once daily) and weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days, on engogenous brain HA concentrations. This treatment resulted in normal values in the hypothalamus, midbrain and cerebral cortex when determined 18 hours after the last treatment.

The SLA of rats receiving simultaneous treatment with histidine and weekly increasing doses of morphine for 21 days is compared with saline-treated controls in Fig. 21. Eighteen hours after the last morphine with histidine treatment these animals showed statistically significantly decreased activity during all but one recording period.

The effect of chronic histidine or histidine with morphine treatment on SLA of rats determined 18 hours after the last treatment is illustrated in Fig. 22. The rats receiving morphine plus histidine appeared less active throughout the period of measurement with several intervals showing statistically significant decreases.

VI. Chronic Histidine after Morphine Withdrawal from Morphine-Dependent Rats

The next set of investigations was performed in order to determine the effect of chronic histidine administration on brain HA concentrations after morphine withdrawal from morphine-dependent rats. The results are shown in Table 11 and Fig. 23. The data of the saline-treated control and morphine-withdrawn rats from Table 5 are repeated for ease of comparison. Chronic histidine administration (500 mg/kg i.p. once daily) for 23 days, resulted in statistically significantly elevated HA levels in the hypothalamus and cerebral cortex when determined 18 hours after the last treatment. The midbrain failed to show any significant increase when determined 18 hours after the last treatment.

The changes observed during morphine withdrawal from rats treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and histidine (500 mg/kg i.p. once daily) for 21 days followed by histidine alone (500 mg/kg i.p. once daily) for 2 days as indicated in Table 11 and Fig. 23, resulted in a significant decrease in the hypothalamic HA concentration. In the midbrain and the cerebral cortex the effects of morphine withdrawal appear to be prevented by the histidine administration.

Chronic histidine with morphine treatment significantly reduced the rate of body weight changes (growth) when compared to saline-treated controls as illustrated in Fig. 24.

Fig. 25 illustrates the effects on SLA of chronic morphine with histidine treatment for 21 days followed by either 2 days of histidine alone or withdrawal of both agents. These groups of animals did not show any significant differences during the period of measurement although

those animals continued on histidine (500 mg/kg i.p. once daily) for 2 days (controls) appeared somewhat less active than the experimental rats.

In order to determine the influence histidine withdrawal by itself would have on SLA of rats treated with histidine for 3 weeks, the next experiment was carried out. Fig. 26 clearly illustrates that chronic histidine administration followed by 2 days withdrawal does not produce any significant alterations of SLA when compared to rats receiving chronic histidine.

TABLE 10

EFFECT OF CHRONIC HISTIDINE ADMINISTRATION ON MORPHINE-INDUCED CHANGES IN BRAIN HISTAMINE CONCENTRATIONS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
SALINE (21 days)	293.3 ± 15.3 (23) 100.0 ± 5.2%	45.3 ± 3.5 (21) 100.0 ± 7.7%	18.4 ± 2.0 (21) 100.0 ± 10.9%
MORPHINE (21 days)	209.2 ± 12.5* (25) 71.3 ± 4.3%*	39.8 ± 2.8 (25) 87.9 ± 6.2%	12.7 ± 1.7* (24) 69.0 ± 9.2%*
HISTIDINE (21 days)	366.3 ± 44.5* (7) 124.9 ± 15.2%*	52.2 ± 6.8 (8) 115.2 ± 15.0%	26.0 ± 1.3* (8) 141.3 ± 7.1%*
Mo + HD (21 days)	337.6 ± 48.0 (11) 115.1 ± 16.4%	44.1 ± 1.6 (13) 97.4 ± 3.5%	17.7 ± 2.3 (10) 96.2 ± 12.5%

Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. Morphine (Mo) was administered in weekly increasing doses (30, 60 and 90 mg/kg i.p. once daily) for 21 days to the second group of rats. The third group of rats was administered histidine (500 mg/kg i.p. once daily) for 21 days. The fourth group of rats was treated simultaneously with weekly increasing doses of morphine (as above) and histidine (as above) for 21 days. All animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

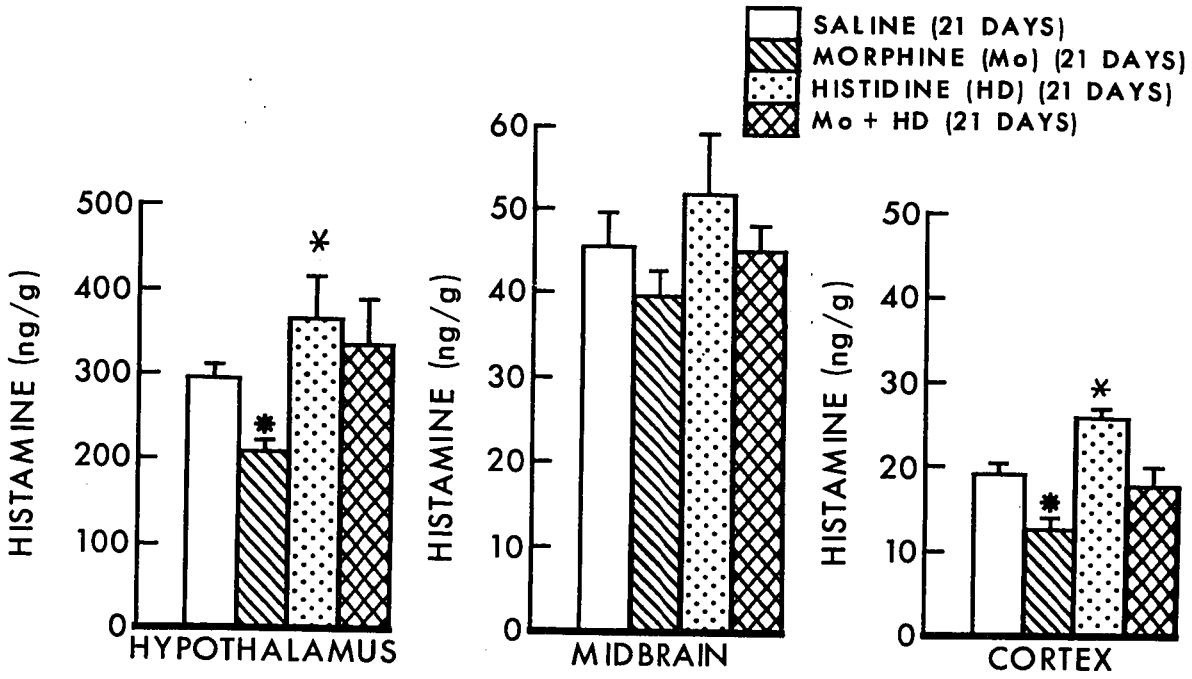


Fig. 20. Effect of chronic histidine administration on morphine-induced changes in brain histamine concentrations. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. Morphine (Mo) was administered in weekly increasing doses (30, 60 and 90 mg/kg i.p. once daily) for 21 days to the second group of rats. The third group of rats was administered histidine (500 mg/kg i.p. once daily) for 21 days. The fourth group of rats was treated simultaneously with weekly increasing doses of morphine (as above) and histidine (as above) for 21 days. Animals were sacrificed 18 hours after the last drug or saline administration. Values represent the Mean ± S.E.M. of at least 7 animals in each group.

* Statistically significant from control at $P < 0.05$

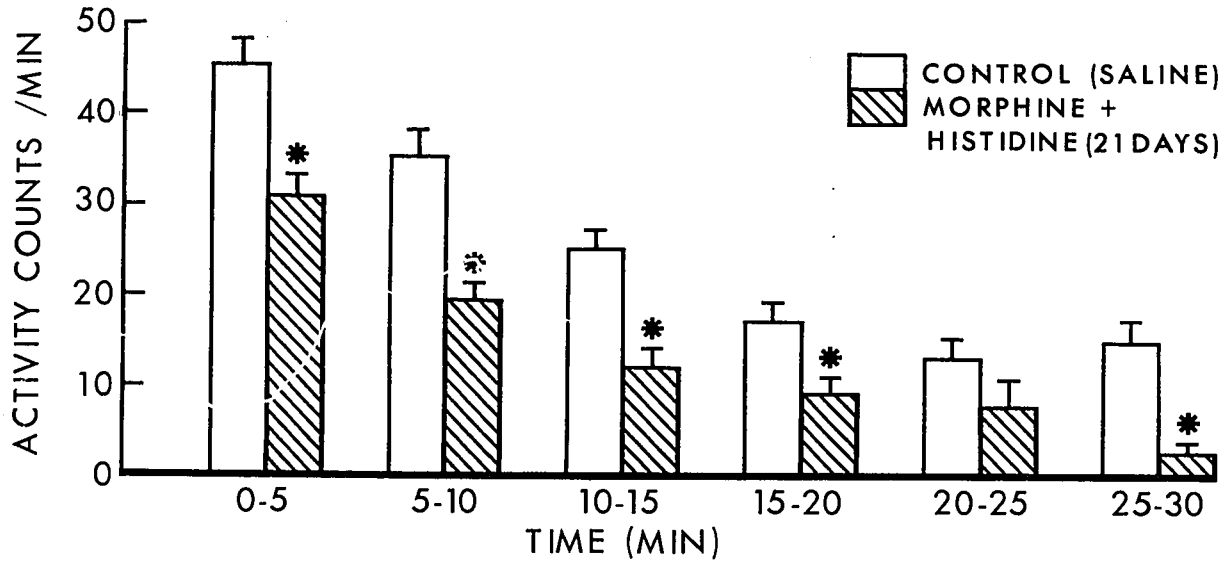


Fig. 21. Effect of chronic histidine with morphine treatment on spontaneous locomotor activity of rats. Experimental animals were treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and histidine (500 mg/kg i.p. once daily) for 21 days. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. of 7 control and 8 experimental animals.

* Statistically significant from control at $P < 0.005$

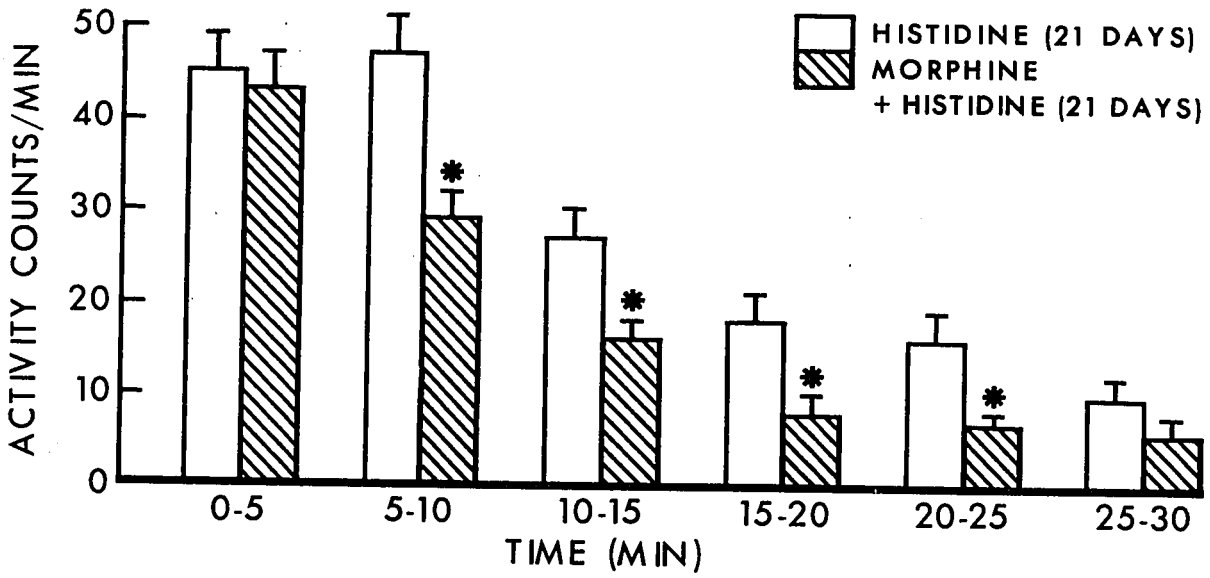


Fig. 22. Effect of chronic histidine or histidine with morphine treatment on spontaneous locomotor activity of rats 18 hours after the last drug administration. Experimental animals were administered histidine (500 mg/kg i.p. once daily) for 21 days or they were treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and histidine (500 mg/kg i.p. once daily) for 21 days. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. of 8 control and 8 experimental animals.

* Statistically significant from control at $P < 0.01$

TABLE 11

EFFECT OF CHRONIC HISTIDINE ADMINISTRATION ON BRAIN HISTAMINE CONCENTRATIONS AFTER MORPHINR WITHDRAWAL FROM MORPHINE-DEPENDENT RATS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
SALINE (23 days)	322.1 ± 17.6 (12) 100.0 ± 5.5%	45.3 ± 4.0 (13) 100.0 ± 8.8%	18.2 ± 3.0 (11) 100.0 ± 16.5%
Mo (21 days) + SALINE (2 days)	175.4 ± 9.6* (8) 54.5 ± 3.0%*	30.0 ± 4.3* (8) 66.2 ± 9.5%*	11.5 ± 1.0* (7) 63.2 ± 5.5%*
HISTIDINE (23 days)	419.7 ± 24.8* (8) 130.3 ± 7.7%*	49.5 ± 2.8 (8) 109.3 ± 6.2%	29.4 ± 2.6* (7) 161.5 ± 14.3
Mo (21 days) + HD (23 days)	237.8 ± 22.5* (7) 73.8 ± 7.0%*	47.7 ± 7.8 (7) 105.3 ± 17.2%	17.6 ± 1.8 (6) 96.7 ± 9.9

Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 23 days. The second group of animals was administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed with saline administration for 2 days. The third group of rats was administered histidine (500 mg/kg i.p. once daily) for 23 days. The fourth group of rats was treated simultaneously with weekly increasing doses of morphine (as above) for 21 days and histidine (as above) for 23 days. The animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

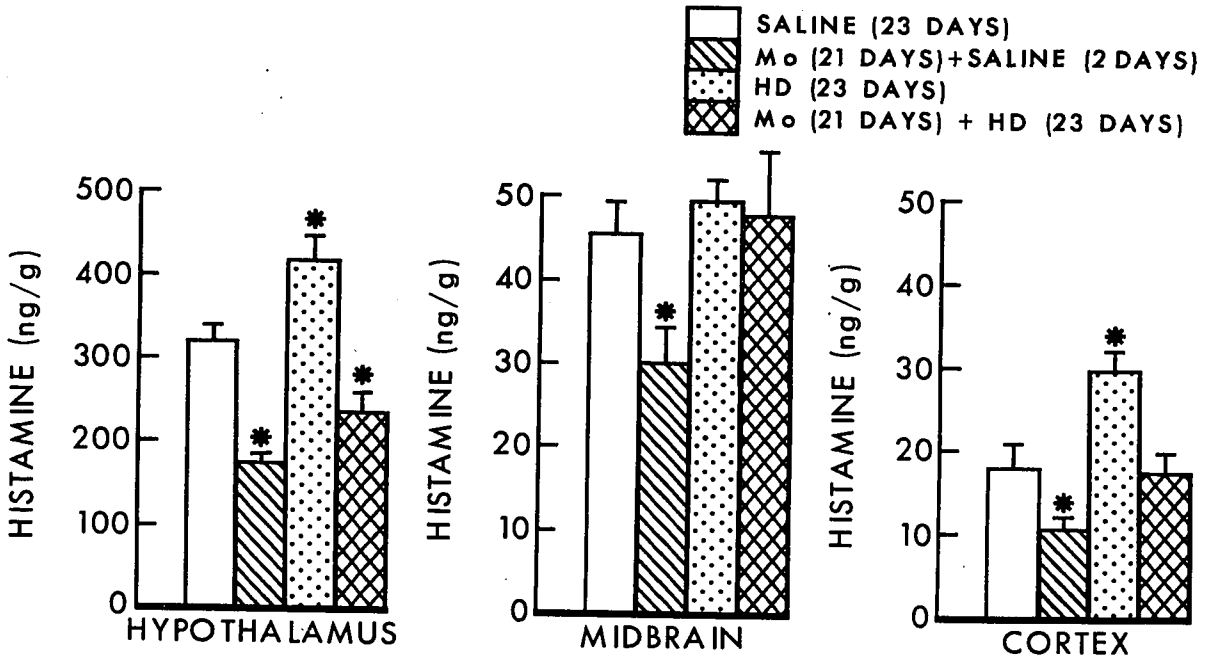


Fig. 23. Effect of chronic histidine administration on brain histamine concentrations after morphine withdrawal from morphine dependent rats. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 23 days. The second group of animals was administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days followed with saline administration for 2 days. The third group of rats was administered histidine (500 mg/kg i.p. once daily) for 23 days. The fourth group of rats was treated simultaneously with weekly increasing doses of morphine (as above) for 21 days and histidine (as above) for 23 days. The animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. of at least 5 animals in each group.

* Statistically significant from control at $P < 0.05$

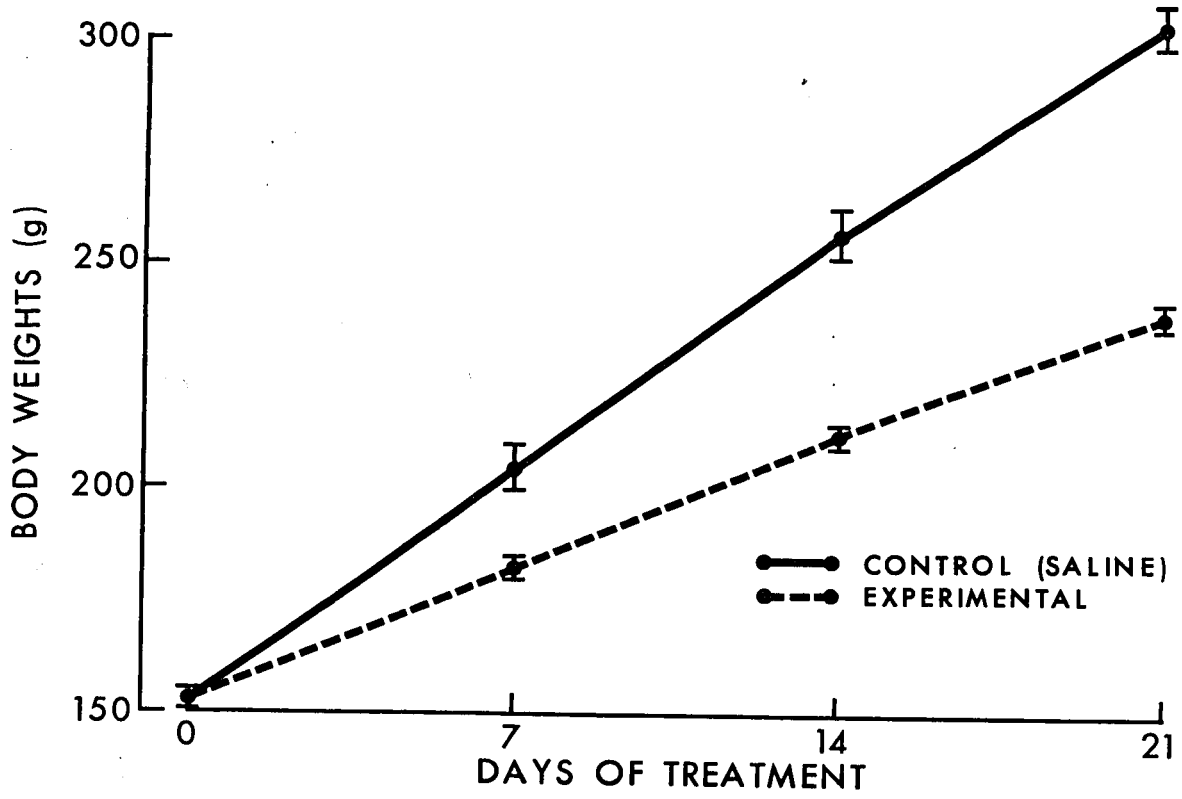


Fig. 24. Influence of chronic histidine with morphine treatment on body weight changes. Experimental animals were treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and histidine (500 mg/kg i.p. once daily) for 21 days. Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. Values represent the Mean ± S.E.M. of 7 control and 8 experimental rats.

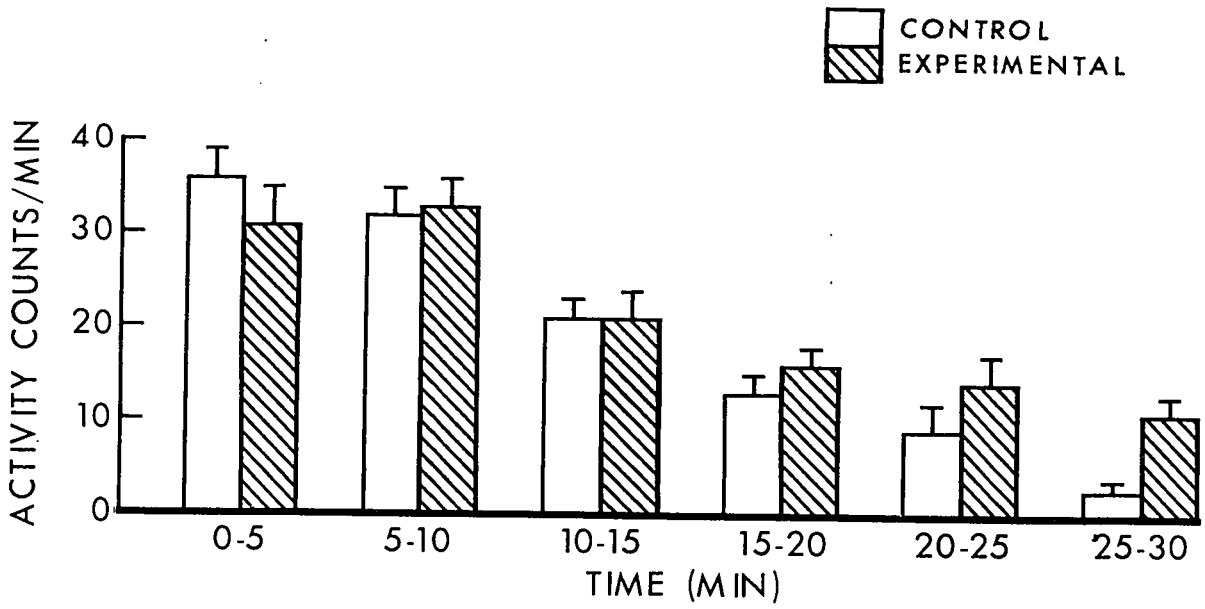


Fig. 25. Spontaneous locomotor activity of rats administered chronic morphine and chronic histidine followed for 2 days by histidine or withdrawal. All animals were treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and histidine (500 mg/kg i.p. once daily) for 21 days. The animals were sacrificed 66 hours after the last morphine administration. The data represent the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes before sacrifice. Values represent the Mean ± S.E.M. of 7 control and 7 experimental animals.

* Statistically significant from control at $P < 0.001$

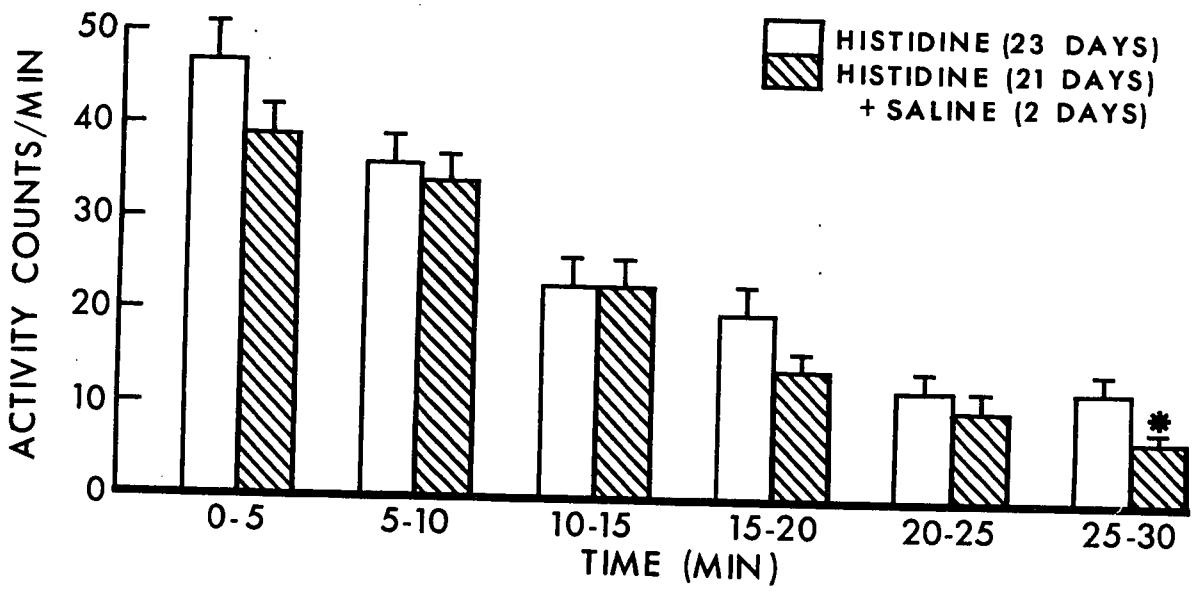


Fig. 26. Spontaneous locomotor activity of rats administered histidine for 21 days followed by 2 days of withdrawal. All animals were injected with histidine (500 mg/kg i.p. once daily) for 21 days. Experimental animals were withdrawn for 2 days and control animals were continued on histidine for 2 days. The animals were sacrificed 18 hours after the last histidine administration. The data represent the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 10 experimental animals.

* Statistically significant from control at $P < 0.01$

VII. Histamine Formation in Rat Brain Following Chronic Morphine

Since we were able to induce changes in the HA concentrations in various brain regions of rats by administering morphine in weekly increasing doses and these changes could be prevented or reversed by concurrent histidine administration, it was of interest to study the effects of these treatments on HA formation.

Table 12 and Fig. 27 indicate the effects of acute histidine (500 mg/kg i.p.) administration to naive animals and acute histidine administered to rats treated with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days.

Acute histidine administration produced a statistically significant increase in the hypothalamic HA concentration, in the midbrain, and in cerebro-cortical HA, when compared to saline-treated control values.

Acute histidine administration to rats 18 hours after the last morphine treatment following 21 days of weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily), produced HA concentrations that were closely comparable to saline treated control values.

Acute histidine administration to rats 66 hours after the last morphine treatment following 21 days of weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily), produced HA levels that were closely comparable to saline-treated control values in all three brain regions investigated.

TABLE 12

EFFECT OF CHRONIC MORPHINE ADMINISTRATION ON HISTAMINE FORMATION IN RAT BRAIN

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
SALINE (21 days)	327.7 ± 21.9 (12) 100.0 ± 6.7%	44.3 ± 5.4 (11) 100.0 ± 12.2%	15.6 ± 1.8 (11) 100.0 ± 11.5%
ACUTE HD	446.3 ± 28.7* (15) 136.2 ± 8.8%*	60.5 ± 5.7* (15) 136.6 ± 12.9%*	30.6 ± 2.4* (16) 196.2 ± 15.4%*
ACUTE HD 18 hours after CHRONIC Mo	317.6 ± 52.7 (8) 96.9 ± 16.1%	54.5 ± 3.6 (9) 123.0 ± 8.1%	21.1 ± 3.5 (8) 135.3 ± 22.4%
ACUTE HD 66 hours after CHRONIC Mo	293.3 ± 18.1 (7) 89.5 ± 5.5%	47.3 ± 5.1 (6) 106.8 ± 11.5%	21.3 ± 3.8 (7) 136.5 ± 24.4%

Control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. The second group of rats was administered histidine (500 mg/kg i.p.) once. The third group of rats was administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and 18 hours after the last morphine treatment they were administered a histidine load (500 mg/kg i.p.). The fourth group was treated in a fashion identical to the third group except that the histidine load was administered 66 hours after the last morphine treatment. All animals were sacrificed one hour after the last treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

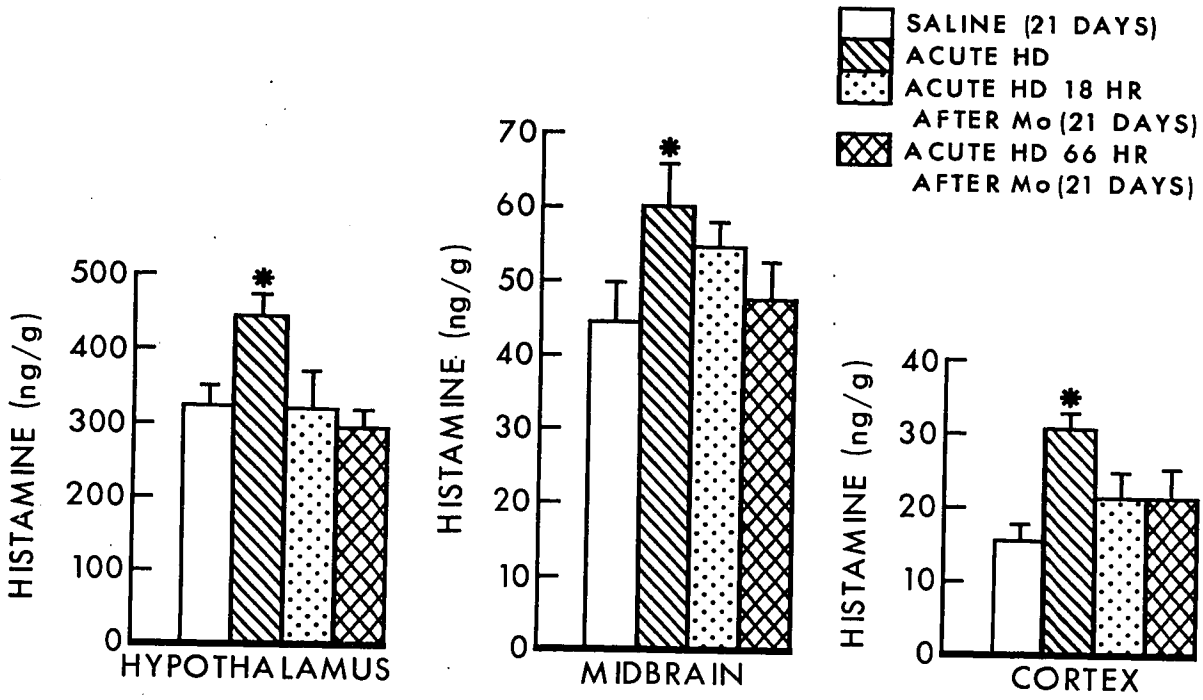


Fig. 27. Effect of chronic morphine administration on histamine formation in rat brain. Morphine was administered in weekly increasing doses (30, 60 and 90 mg/kg i.p. once daily) for 21 days then histidine (500 mg/kg i.p.) was administered 18 or 66 hours after the last morphine treatment. Control animals were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. All animals were sacrificed one hour after the last treatment. Values represent the Mean ± S.E.M. of at least 7 animals in each group.

* Statistically significant from control at $P < 0.05$

The effect of acute histidine administration (500 mg/kg i.p.) for one hour on SLA rats is illustrated in Fig. 28. The histidine-treated rats showed less activity than the saline-treated controls, although statistically significant differences were only achieved during two periods of measurement.

Fig. 29 illustrated the effects of chronic histidine or chronic histidine with morphine treatment on SLA of rats determined within one hour following the last treatment. Those animals receiving morphine plus histidine are significantly more active than those administered chronic histidine by itself.

The SLA of rats receiving an acute histidine load either 18 or 66 hours after morphine withdrawal from morphine-dependent animals appears to be the same as the activity of the saline-treated controls receiving an acute histidine load as shown in Fig. 30 and Fig. 31.

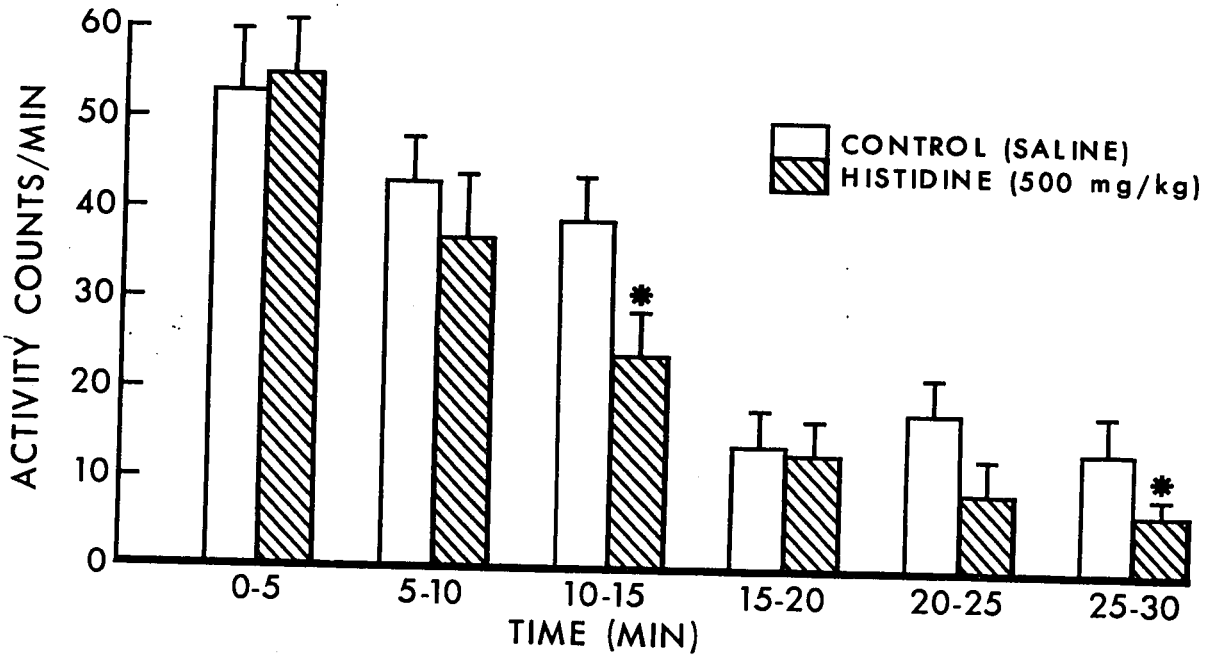


Fig. 28. Effect of acute histidine administration on spontaneous locomotor activity of rats. Experimental animals were administered histidine (500 mg/kg i.p.) and controls were injected with 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 4 control and 4 experimental animals.

* Statistically significant from control at $P < 0.05$

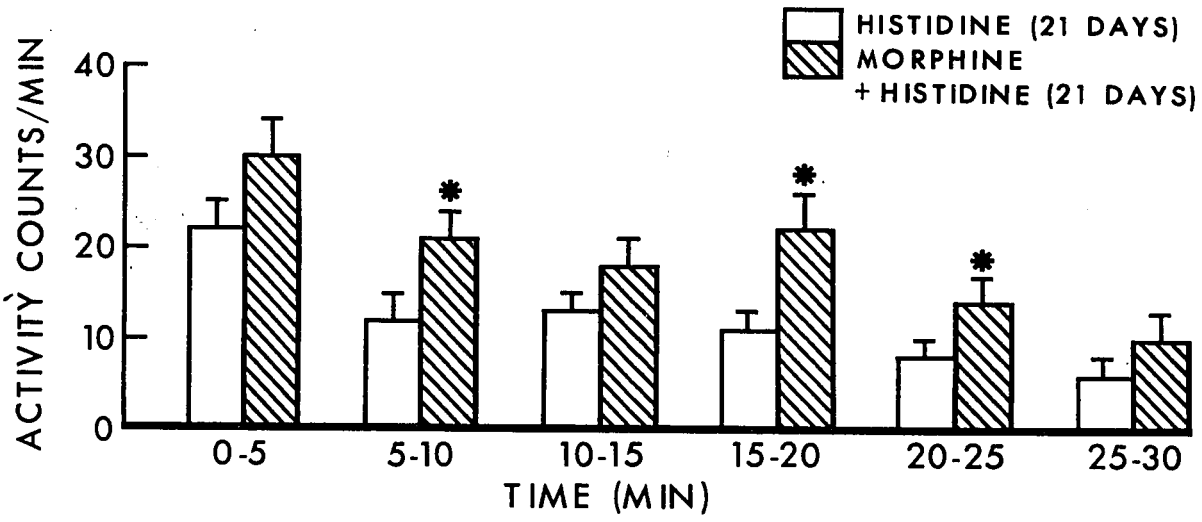


Fig. 29. Effect of chronic histidine or histidine with morphine treatment on spontaneous locomotor activity of rats one hour after the last drug administration. Experimental animals were administered histidine (500 mg/kg i.p. once daily) for 21 days or they were treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and histidine (500 mg/kg i.p. once daily) for 21 days. Control rats were administered 0.9% saline (0.1 ml/100 g i.p.). The animals were sacrificed one hour after the last injection. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 8 experimental animals.

* Statistically significant from control at $P < 0.01$

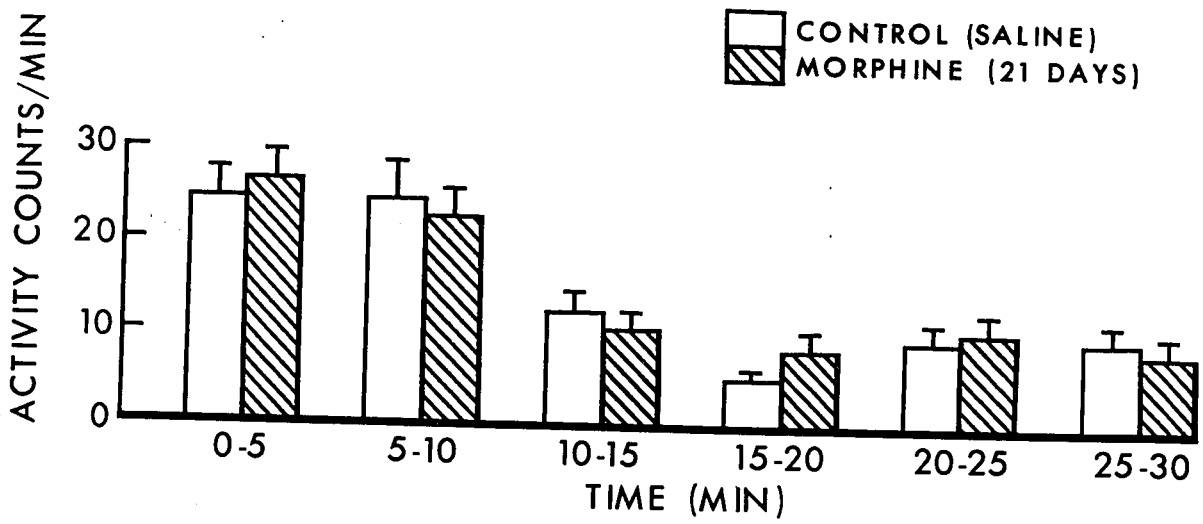


Fig. 30. Effect of an acute histidine load on spontaneous locomotor activity of morphine-dependent rats 18 hours after morphine withdrawal. Experimental animals were injected with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. Histidine (500 mg/kg i.p.) was administered 18 hours after the last treatment and all animals were sacrificed one hour later. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 9 experimental animals.

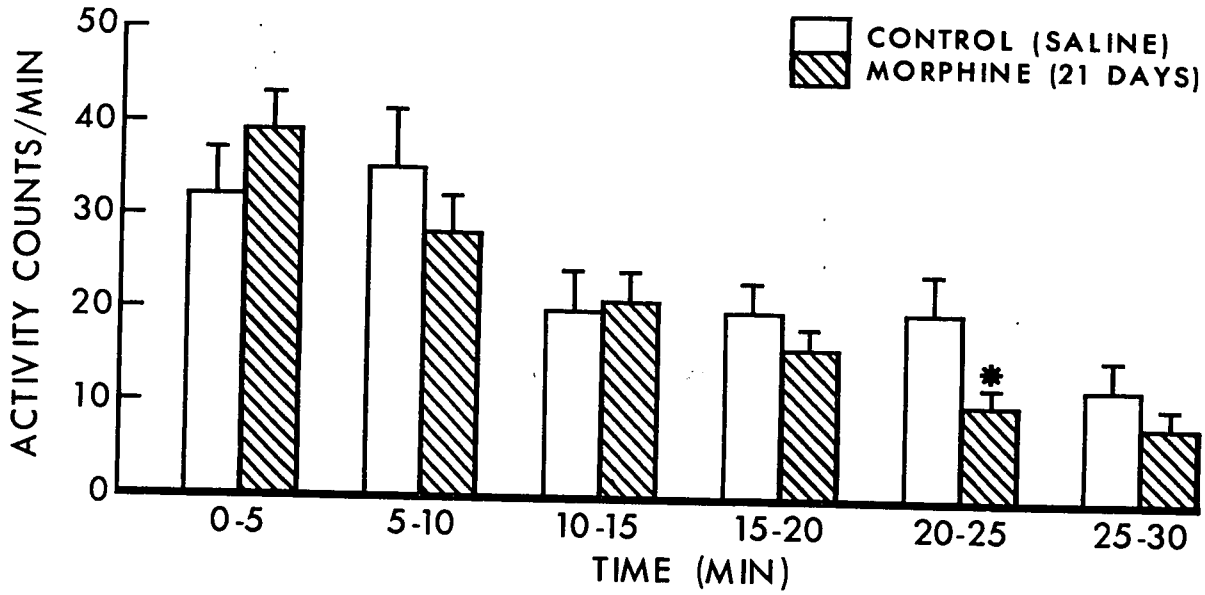


Fig. 31. Effect of an acute histidine load on spontaneous locomotor activity of morphine-dependent rats 66 hours after morphine withdrawal. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. Histidine (500 mg/kg i.p.) was administered 66 hours after the last treatment and all animals were sacrificed one hour later. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 4 control and 7 experimental animals.

* Statistically significant from control at $P < 0.01$

VIII. Histamine Formation in Rat Brain Following Chronic Histidine or Chronic Histidine with Morphine

Table 13 and Fig. 32 demonstrate the effects of chronic histidine or chronic histidine (500 mg/kg i.p. once daily) with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days. The animals in these experiments were sacrificed one hour after the last treatment. Chronic histidine treatment resulted in very high levels of HA in the hypothalamus, midbrain and cerebral cortex when determined one hour after the last injection. Simultaneous treatment with histidine and morphine resulted in value different from those reported for determinations performed 18 hours after the last treatment (Table 10). In these experiments, HA levels were the same in the hypothalamus, elevated in the midbrain and again in the cerebral cortex when compared to the 18 hour values.

Table 13 and Fig. 32 illustrate the effects on brain HA of acute histidine loads (500 mg/kg i.p.) 18 hours after chronic histidine (500 mg/kg i.p. once daily) for 21 days or chronic histidine administered simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days. Acute histidine (18 hours after the last chronic histidine treatment), resulted in significantly elevated HA levels in the hypothalamus, midbrain and cerebral cortex. These levels were not as high as those observed one hour after the last chronic injection as described earlier.

Acute histidine 18 hours after the last simultaneous treatment with histidine and morphine resulted in HA levels that were just less in each brain region than the levels seen one hour after the chronic histidine treatment as described earlier.

Simultaneous administration of histidine and morphine for 21 days resulted in significant decreases in body weight changes when compared with chronic histidine as illustrated in Fig. 33.

TABLE 13

EFFECT OF CHRONIC HISTIDINE OR CHRONIC HISTIDINE WITH MORPHINE TREATMENT ON HISTAMINE FORMATION IN RAT BRAIN

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
HISTIDINE (HD) (21 days)	799.2 ± 109.7 (7)	61.9 ± 7.8 (6)	33.7 ± 5.2 (6)
Mo and HD (21 days)	358.7 ± 39.2 (6)	62.6 ± 6.7 (7)	35.4 ± 6.4 (8)
ACUTE HD 18 hours after HD (21 days)	569.9 ± 58.8 (7)	60.7 ± 7.3 (6)	28.0 ± 3.0 (6)
ACUTE HD 18 hours after Mo and HD (21 days)	357.7 ± 25.9 (7)	55.6 ± 4.5 (7)	26.7 ± 2.7 (5)

The first group of rats was administered histidine (HD) (500 mg/kg i.p. once daily) for 21 days. The second group of rats was treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and HD (500 mg/kg i.p. once daily) for 21 days. The third group of rats was administered histidine (500 mg/kg i.p. once daily) for 21 days and 18 hours after the last chronic treatment, acute HD (500 mg/kg i.p.) was administered. The fourth group of rats was treated simultaneously with morphine and HD (as for group two) and 18 hours after the last treatment, acute HD (500 mg/kg i.p.) was administered. All animals were sacrificed one hour after the last treatment. Values represent the Mean ± S.E.M. The number of experiments is shown in parentheses.

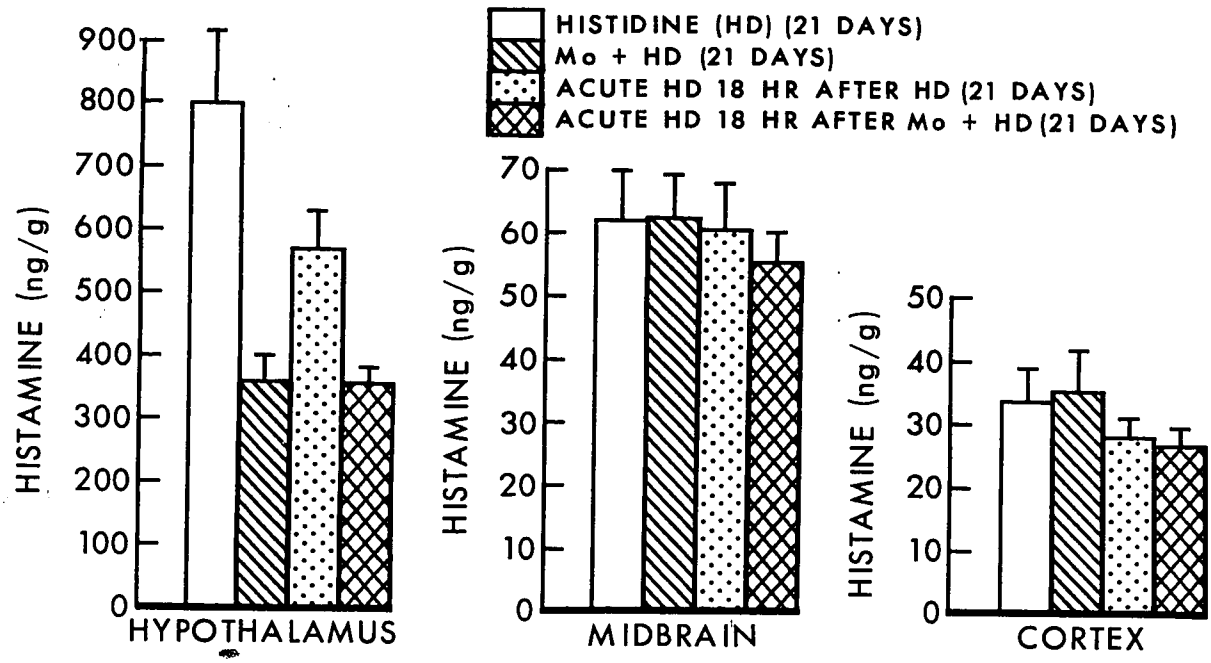


Fig. 32. Effect of chronic histidine or histidine with morphine treatment on histamine formation in rat brain. The first group of rats was administered histidine (HD) (500 mg/kg i.p. once daily) for 21 days. The second group was treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and HD (500 mg/kg i.p. once daily) for 21 days. The third group of animals was treated in a fashion identical to the first group and 18 hours after the last HD treatment they were administered HD (500 mg/kg i.p.) and sacrificed one hour later. The fourth group of animals was treated simultaneously with morphine and HD (as for group two) and 18 hours after the last treatment, acute HD (500 mg/kg i.p.) was administered. All animals were sacrificed one hour after the last treatment. Values represent the Mean ± S.E.M. of at least 5 animals in each group.

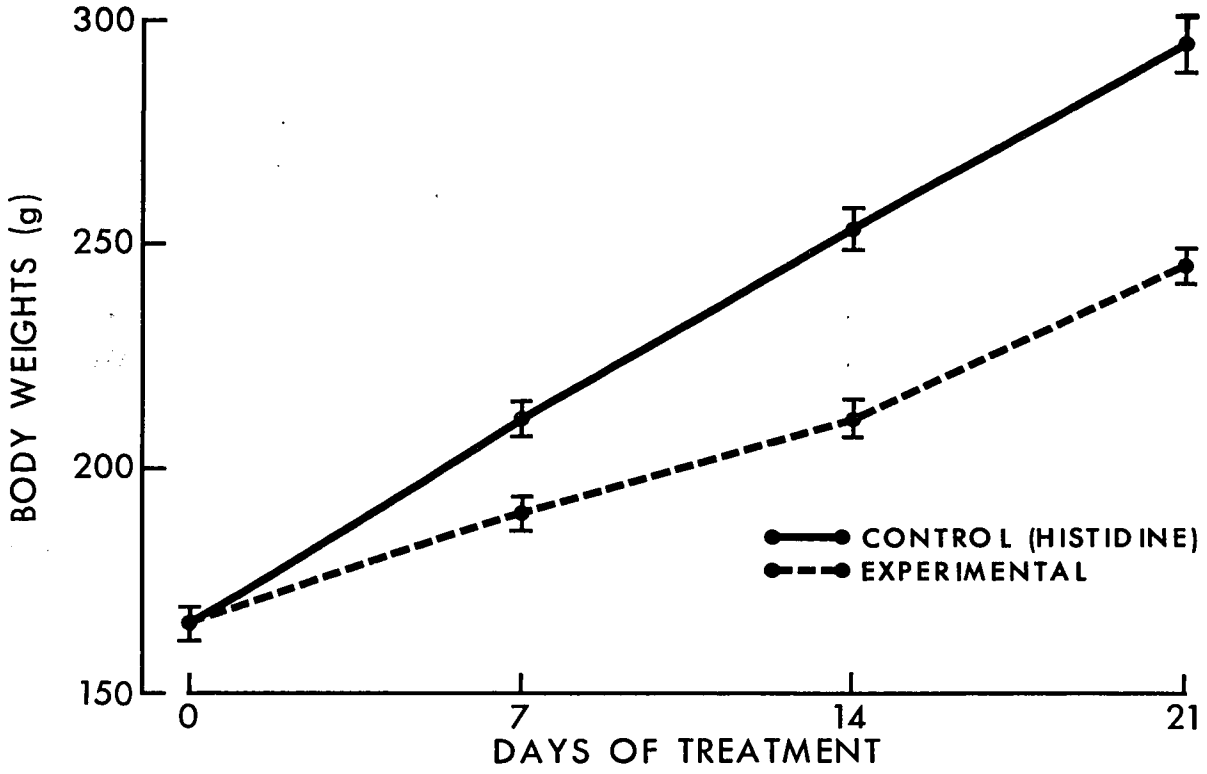


Fig. 33. Influence of chronic histidine or histidine with morphine treatment on body weight changes. Control rats were administered histidine (500 mg/kg i.p. once daily) for 21 days. Experimental animals were treated simultaneously with weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) and histidine (500 mg/kg i.p. once daily) for 21 days. Values represent the Mean ± S.E.M. of 8 control and 8 experimental rats.

The data in Fig. 34 illustrate that an acute histidine administration to rats chronically treated with morphine resulted in lower SLA than those found in saline-treated controls receiving a similar histidine load.

Rats treated chronically with histidine showed higher activities than morphine-treated rats when both groups were administered an acute histidine load 18 hours after the last chronic treatment. These data are depicted in Fig. 35.

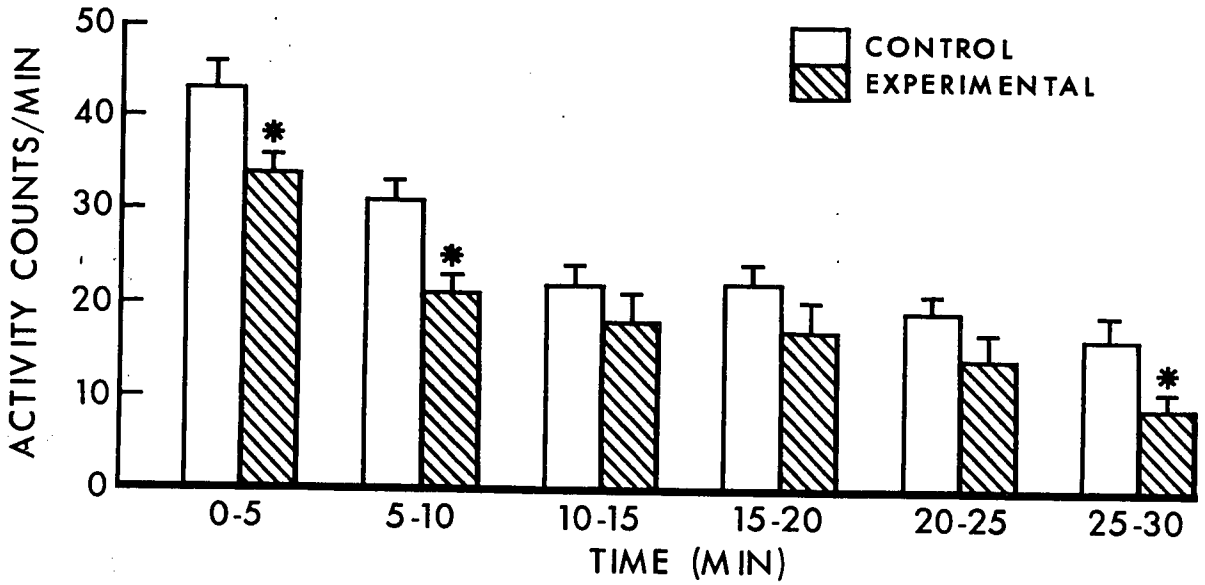


Fig. 34. Effect of acute histidine administration on spontaneous locomotor activity of rats treated chronically with morphine. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. Acute histidine (500 mg/kg i.p.) was administered to all rats 18 hours after the last treatment and the animals were sacrificed one hour later. The data represent the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 6 experimental animals.

* Statistically significant from control at $P < 0.05$

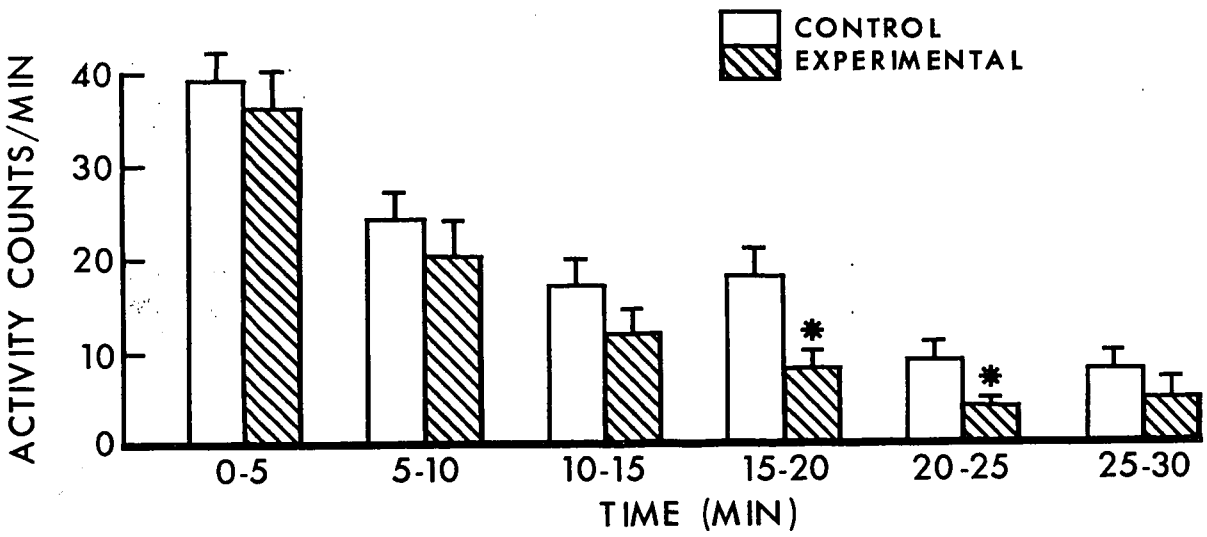


Fig. 35. Effect of an acute histidine load on spontaneous locomotor activity of rats treated chronically with histidine or histidine with morphine. Experimental animals were treated simultaneously with histidine (500 mg/kg i.p. once daily) and weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered histidine (500 mg/kg i.p. once daily) for 21 days. Acute histidine (500 mg/kg i.p.) was administered and all animals were sacrificed one hour later. The data represent the mean activity count/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 7 experimental animals.

* Statistically significant from control at $P < 0.01$

IX. Effect of an Acute Histidine Load to Morphine-Dependent Rats

Since chronic morphine administration produced changes in brain HA concentrations and since chronic histidine administration seemed to prevent or reverse these changes it was of interest to determine whether acute histidine administration might also reverse these changes. The data in Table 14 indicate that when histidine (500 mg/kg i.p.) was administered at the time of the last morphine treatment and the HA levels assayed 18 hours later, the morphine-induced changes were not prevented. The decreases in HA on the hypothalamus, midbrain and cerebral cortex indicate that morphine is still producing as much effect as seen in Table 5.

The SLA of rats 18 hours after an acute histidine load to morphine-dependent rats is illustrated in Fig. 36. The morphine-treated rats are clearly more sedated than the control rats that received chronic saline injections and one histidine load.

TABLE 14

ENDOGENOUS BRAIN HISTAMINE CONCENTRATION OF MORPHINE-DEPENDENT RATS 18 HOURS AFTER AN ACUTE HISTIDINE LOAD

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL (SALINE)	314.5 ± 30.7 (4) 100.0 ± 9.8%	47.6 ± 6.6 (4) 100.0 ± 13.9%	19.2 ± 3.2 (4) 100.0 ± 16.7%
MORPHINE (21 days)	193.1 ± 20.8* (5) 61.4 ± 6.6%*	32.8 ± 6.0 (5) 68.9 ± 12.6%	17.2 ± 2.4 (5) 89.6 ± 12.5%

Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. Histidine (500 mg/kg i.p.) was administered at the time of the last chronic treatment and all rats were sacrificed 18 hours later. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

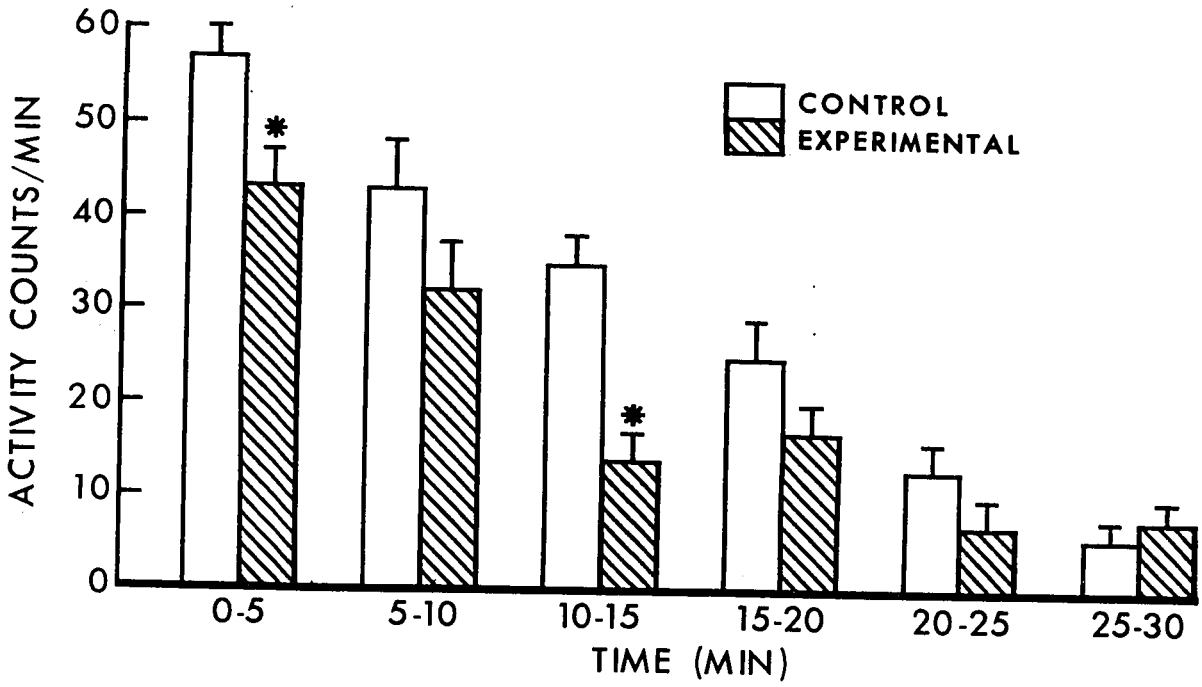


Fig. 36. Spontaneous locomotor activity 18 hours after an acute histidine load to morphine-dependent rats. Experimental animals were administered weekly increasing doses of morphine (30, 60 and 90 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. Histidine (500 mg/kg i.p.) was administered at the time of the last morphine treatment and the animals were sacrificed 18 hours later. The data represent the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 4 control and 5 experimental animals.

* Statistically significant from control at $P < 0.01$

X. Acute and Chronic Pentazocine Treatment

The effect of acute pentazocine administration (30 mg/kg i.p.) for one hour on endogenous brain HA concentrations is illustrated in Table 15 and Fig. 37. There were no significant changes observed in the hypothalamus, midbrain or cerebral cortex.

The effect of acute pentazocine administration on SLA of rats is illustrated in Fig. 38. These rats displayed significantly decreased activities when compared to saline-treated controls, during the first 5 minute recording period. The pentazocine-treated rats showed elevated activities during the remainder of the recording periods.

Chronic administration of weekly increasing doses of pentazocine (15, 30 and 45 mg/kg i.p. twice daily) for 21 days, as seen in Table 16 and Fig. 39, did result in statistically significant decreases in HA in the hypothalamus and cerebral cortex. The midbrain HA levels, however, were also lower but this was not a statistically significant difference.

Chronic treatment with weekly increasing doses of pentazocine did not significantly influence body weight changes (growth) as can be seen in Fig. 40.

The SLA of rats treated chronically with weekly increasing doses of pentazocine for 21 days, and determined 18 hours after the last treatment is illustrated in Fig. 41. The pentazocine-treated animals are significantly less active than the saline-treated controls especially during the last half of the recording period.

TABLE 15

EFFECT OF ACUTE PENTAZOCINE ADMINISTRATION ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL (SALINE)	301.0 ± 26.2 (5) 100.0 ± 8.7%	34.9 ± 3.1 (6) 100.0 ± 8.9%	22.7 ± 0.8 (5) 100.0 ± 3.5%
PENTAZOCINE (30 mg/kg)	279.9 ± 9.3 (6) 93.0 ± 3.1%	42.2 ± 3.2 (5) 120.9 ± 9.2%	20.5 ± 4.7 (5) 90.3 ± 20.7%

Experimental animals were injected with pentazocine (30 mg/kg i.p.), and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). All animals were sacrificed one hour after treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

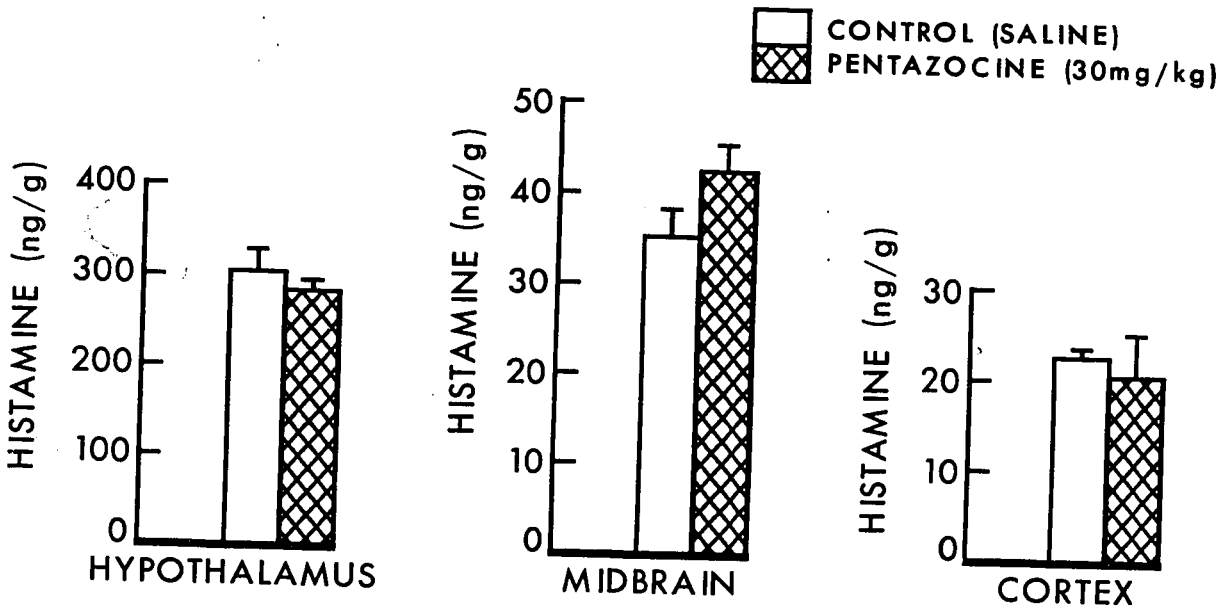


Fig. 37. Effect of acute pentazocine administration on endogenous brain histamine concentrations. Experimental animals were injected with pentazocine (30 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). All animals were sacrificed one hour after treatment. Values represent the Mean ± S.E.M. of 5 or 6 determinations.

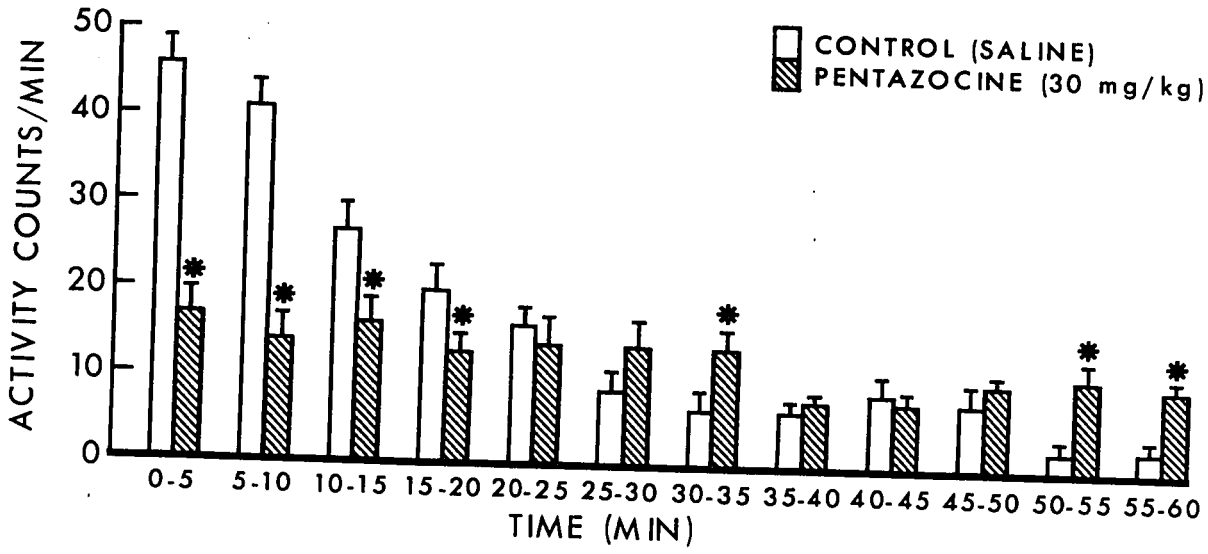


Fig. 38. Effect of acute pentazocine administration on spontaneous locomotor activity of rats. Experimental animals were injected with pentazocine (30 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). All animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods. Values represent the Mean ± S.E.M. of 12 control and 12 experimental animals.

* Statistically significant from control at $P < 0.01$

TABLE 16

EFFECT OF CHRONIC PENTAZOCINE ADMINISTRATION ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL (SALINE)	237.7 ± 15.9 (7) 100.0 ± 6.7%	35.3 ± 4.0 (7) 100.0 ± 11.3%	15.0 ± 0.8 (8) 100.0 ± 5.3%
PENTAZOCINE (21 days)	159.1 ± 13.5* (6) 66.9 ± 5.7%*	31.5 ± 4.1 (8) 89.2 ± 11.6%	11.8 ± 1.0* (7) 78.7 ± 6.7%*

Experimental animals were administered weekly increasing doses of pentazocine (15, 30 and 45 mg/kg i.p. twice daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. twice daily) for 21 days. All animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

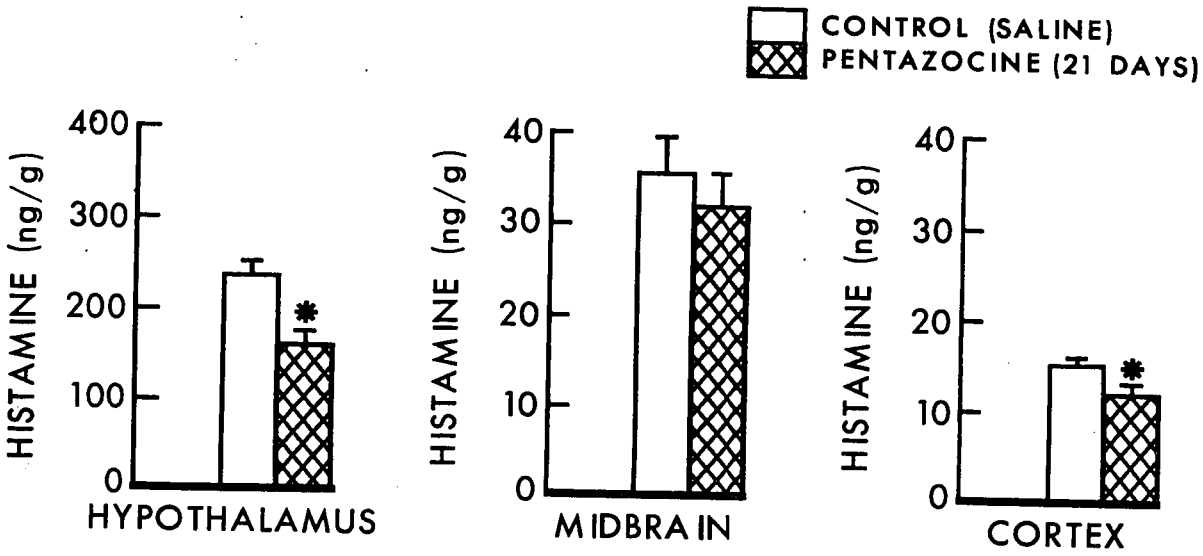


Fig. 39. Effect of chronic pentazocine administration on endogenous brain histamine concentrations. Experimental animals were administered weekly increasing doses of pentazocine (15, 30 and 45 mg/kg i.p. twice daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. twice daily) for 21 days. All animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. of at least 8 animals in each group.

* Statistically significant from control at $P < 0.05$

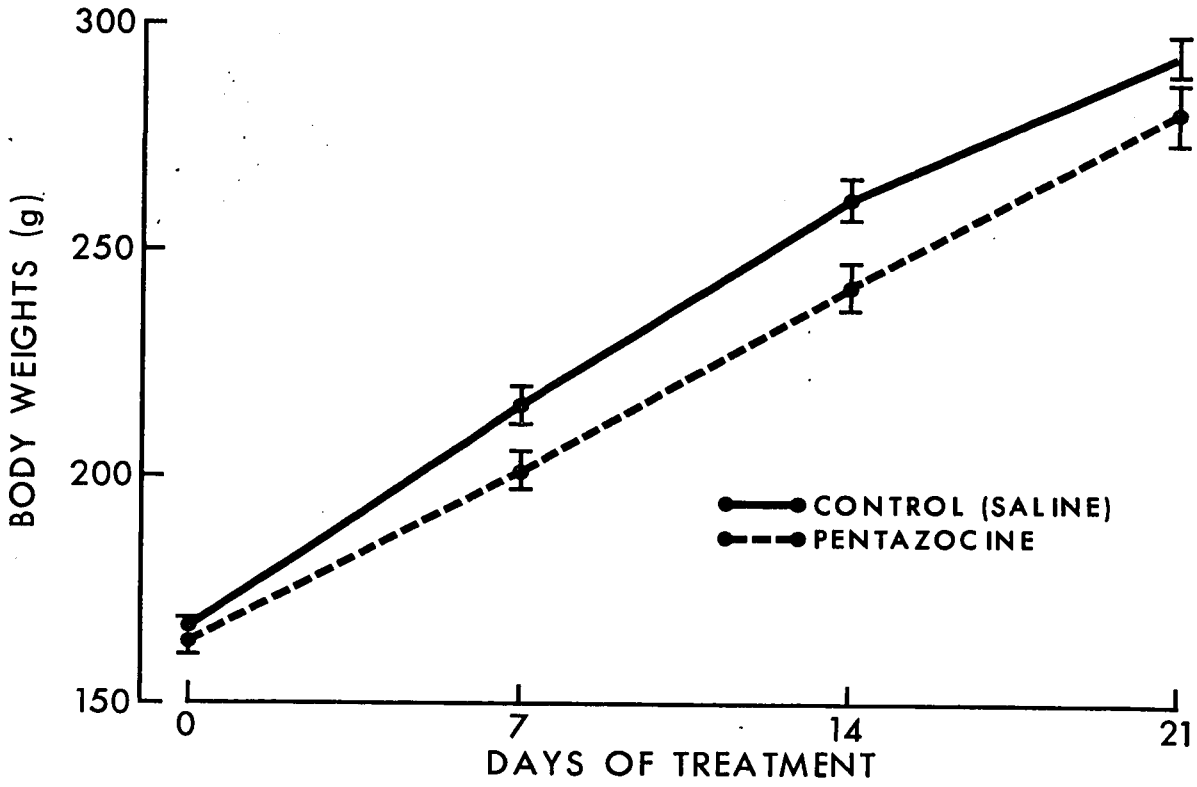


Fig. 40. Influence of chronic pentazocine administration on body weight changes. Experimental animals were administered weekly increasing doses of pentazocine (15, 30 and 45 mg/kg i.p. twice daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. twice daily) for 21 days. All animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. of 8 control and 8 experimental animals.

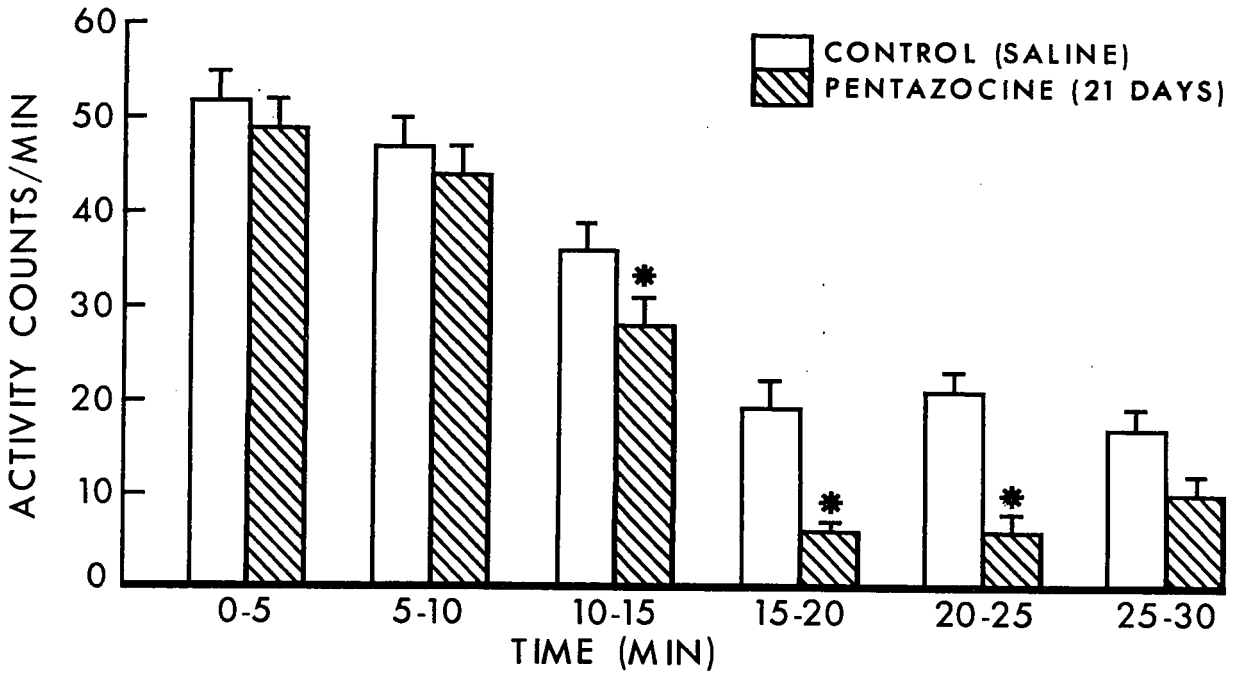


Fig. 41. Effect of chronic pentazocine administration on spontaneous locomotor activity of rats. Experimental animals were administered weekly increasing doses of pentazocine (15, 30 and 45 mg/kg i.p. twice daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. twice daily) for 21 days. All animals were sacrificed 18 hours after the last treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 8 experimental animals.

* Statistically significant from control at $P < 0.05$

XI. Acute Mandrax, Methaqualone, or Diphenhydramine Treatment

Acute treatment with MX (82.5 mg/kg i.p.), MQ (75.0 mg/kg p.o.), or DIPH (7.5 mg/kg p.o.) resulted in a significant decrease in the endogenous HA concentration in the hypothalamus as indicated in Table 17 and Fig. 42. In the midbrain, some decrease in HA was noted after MX and MQ, but the only significant decrease in this brain region was noted after DIPH. In the cerebral cortex, the only significant decrease in HA was observed after MX while the changes found after MQ and DIPH although quite pronounced, did not reach the level of significance.

Acute treatment with MX, MQ, or DIPH depicted in Figs. 43, 44, and 45 respectively, all produced significant sedation when compared with the SLA of the vehicle-treated controls.

TABLE 17

EFFECTS OF ACUTE TREATMENT WITH MANDRAX, METHAQUALONE, OR DIPHENHYDRAMINE ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL	265.1 ± 23.3 (10)	38.1 ± 3.0 (15)	14.6 ± 1.3 (14)
MANDRAX 82.5 mg/kg)	204.3 ± 15.5* (12)	31.4 ± 2.8 (15)	9.6 ± 1.1** (13)
CONTROL	230.6 ± 25.1 (11)	48.6 ± 5.5 (15)	15.5 ± 2.4 (15)
METHAQUALONE (75.0 mg/kg)	167.8 ± 14.0* (8)	40.4 ± 6.0 (10)	10.7 ± 1.6* (9)
CONTROL	240.7 ± 26.0 (10)	50.0 ± 6.4 (13)	15.0 ± 1.5 (11)
DIPHENHYDRAMINE (7.5 mg/kg)	161.5 ± 13.9** (8)	33.0 ± 7.0 (9)	10.7 ± 2.2 (6)

Experimental animals were administered mandrax (82.5 mg/kg p.o.), methaqualone (75.0 mg/kg p.o.), or diphenhydramine (7.5 mg/kg p.o.), suspended in 1 ml of vehicle and control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed one hour after administration of the drugs or vehicle. Values represent the Mean ± S.E.M. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$
** Statistically significant from control at $P < 0.025$

TABLE 17P

EFFECTS OF ACUTE TREATMENT WITH MANDRAX, METHAQUALONE, OR DIPHENHYDRAMINE ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (% of control)		
CONTROL	100.0 ± 8.8 (10)	100.0 ± 7.9 (15)	100.0 ± 8.9 (14)
MANDRAX (82.5 mg/kg)	77.1 ± 5.8* (12)	82.4 ± 7.3 (15)	65.8 ± 7.5** (13)
CONTROL	100.0 ± 10.9 (11)	100.0 ± 11.3 (15)	100.0 ± 15.4 (15)
METHAQUALONE (75.0 mg/kg	72.8 ± 6.1* (8)	83.1 ± 12.3 (10)	69.0 ± 10.3* (9)
CONTROL	100.0 ± 10.8 (10)	100.0 ± 12.8 (13)	100.0 ± 10.0 (11)
DIPHENHYDRAMINE (7.5 mg/kg)	67.1 ± 5.8** (8)	66.0 ± 14.0 (9)	71.2 ± 14.7 (6)

Experimental rats were administered mandrax (82.5 mg/kg p.o.), methaqualone (75.0 mg/kg p.o.), or diphenhydramine (7.5 mg/kg p.o.) suspended in 1 ml of vehicle and control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed one hour after administration of the drugs or vehicle. Values represent the Mean ± S.E.M. The results are expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$
** Statistically significant from control at $P < 0.025$

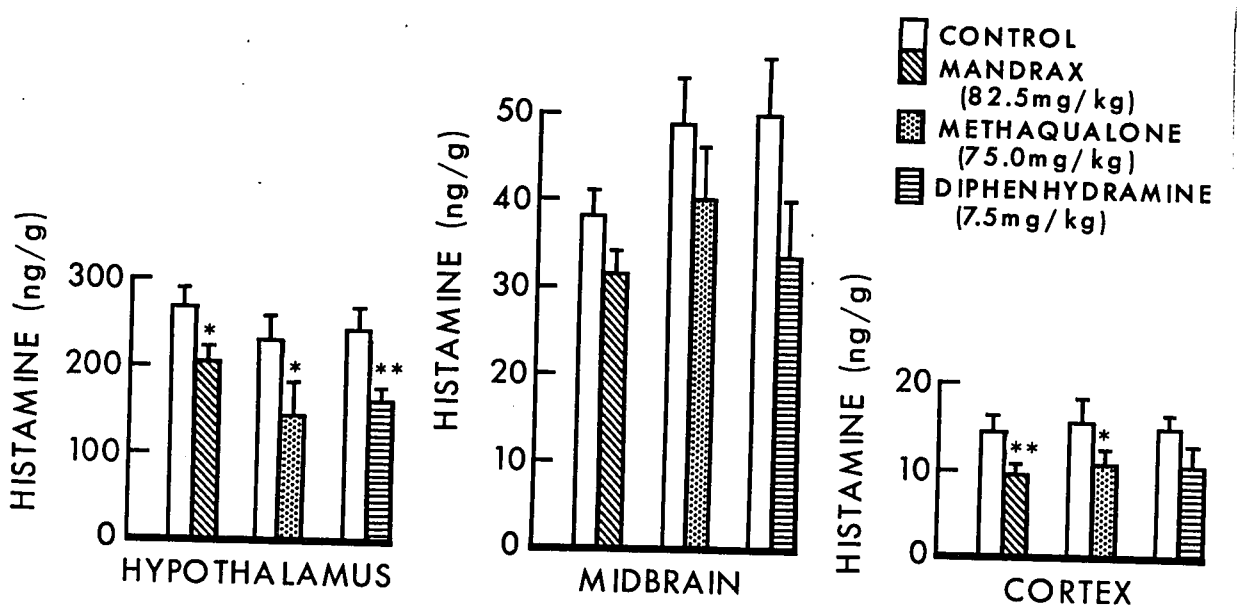


Fig. 42. Effects of acute treatment with mandrax, methaqualone or diphenhydramine on endogenous brain histamine concentrations. Experimental rats were administered mandrax (82.5 mg/kg p.o.), methaqualone (75.0 mg/kg p.o.), or diphenhydramine (7.5 mg/kg p.o.) suspended in 1 ml of vehicle. Control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed one hour after administration of the drugs or vehicle. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

* Statistically significant from control at $P < 0.05$
** Statistically significant from control at $P < 0.025$

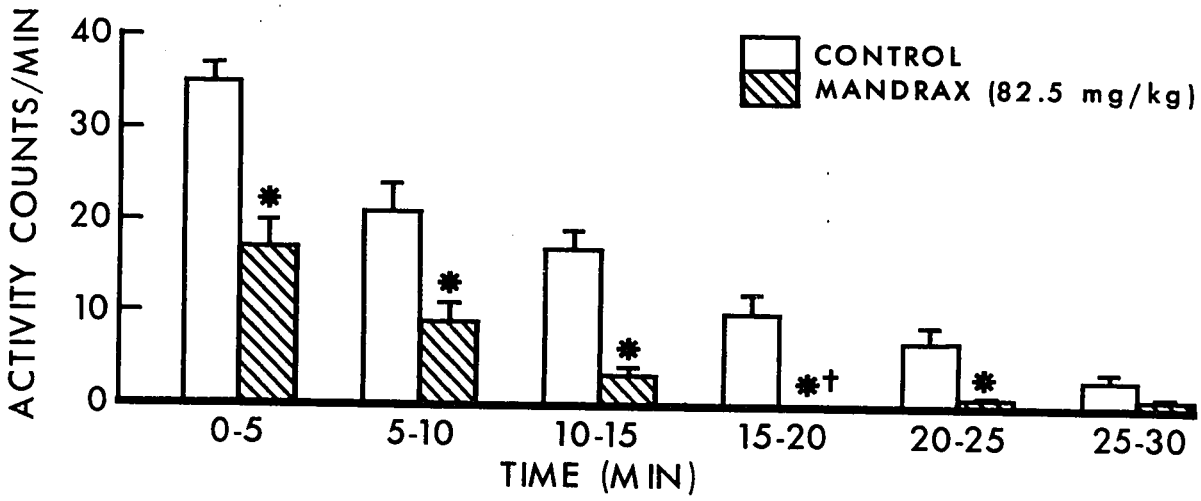


Fig. 43. Effect of acute mandrax administration on spontaneous locomotor activity of rats. Experimental animals were treated with mandrax (82.5 mg/kg p.o.) and control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes before sacrifice. Values represent the Mean ± S.E.M. of 12 control and 12 experimental animals.

† The activity of the animals was below detectable limits
* Statistically significant from control at $P < 0.005$

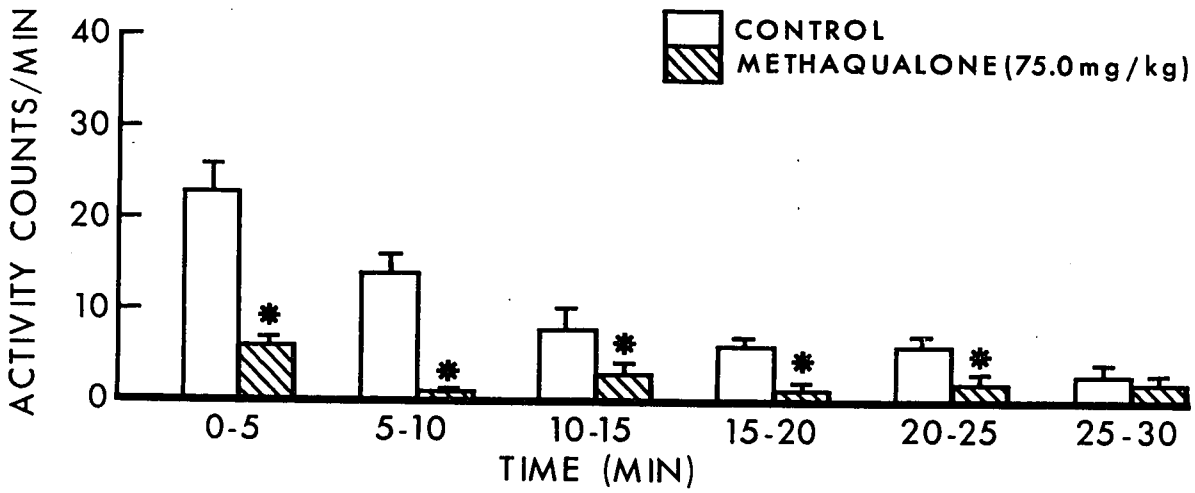


Fig. 44. Effect of acute methaqualone administration on spontaneous locomotor activity of rats. Experimental animals were treated with methaqualone (75 mg/kg p.o.) and control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes before sacrifice. Values represent the Mean ± S.E.M. of 12 control and 12 experimental animals.

* Statistically significant from control at $P < 0.005$

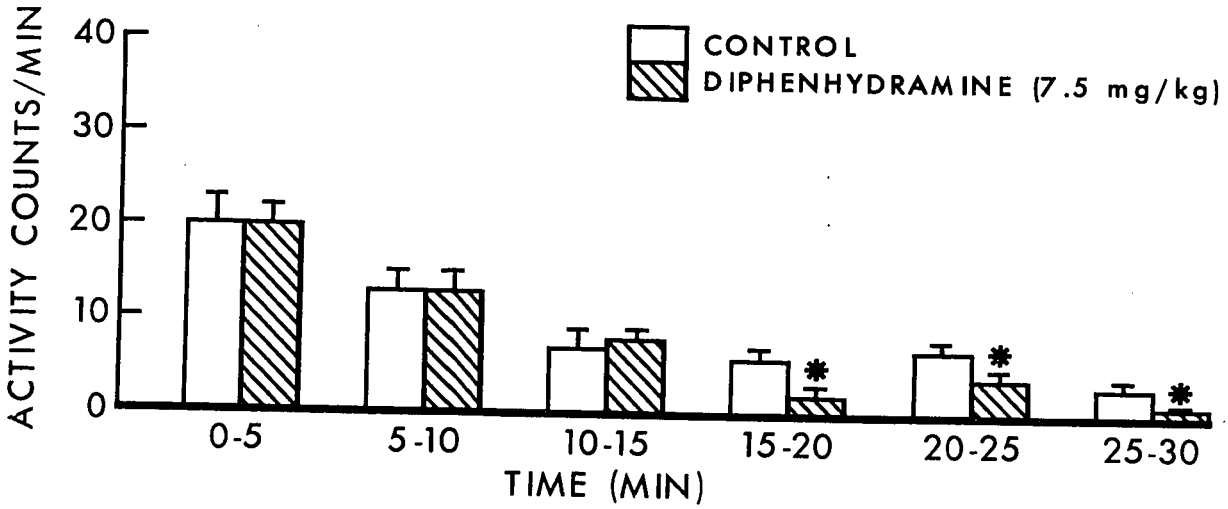


Fig. 45. Effect of acute diphenhydramine administration on spontaneous locomotor activity of rats. Experimental animals were treated with diphenhydramine (7.5 mg/kg p.o.) and control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes before sacrifice. Values represent the Mean ± S.E.M. of 12 control and 12 experimental animals.

* Statistically significant from control at $P < 0.005$

XII. Chronic Treatment with Mandrax, Methaqualone, or Diphenhydramine for 18 Days

Table 18 and Fig. 46 illustrate the effects of chronic treatment for 18 days with MX, MQ, or DIPH on endogenous brain HA concentrations.

Chronic treatment with MX (82.5 mg/kg p.o. once daily) for 18 days resulted in a slight but not statistically significant lowering of hypothalamic HA. Chronic treatment with MQ (75.0 mg/kg p.o. once daily) for 18 days resulted in a slight but not statistically significant lowering of hypothalamic HA. Chronic treatment with DIPH (7.5 mg/kg p.o. once daily) for 18 days induced a significant decrease in the hypothalamic HA level.

In the midbrain, 18 days of MX treatment was observed to induce a slight decrease in the HA concentration and 18 days of DIPH treatment produced a more pronounced decrease. Methaqualone, when administered for 18 days, did not induce any noticeable changes in the midbrain levels of HA.

None of the three drugs investigated in this study altered the endogenous brain HA concentrations in the cerebral cortex to any significant degree.

The SLA of rats treated chronically (18 days) with MX, MQ, or DIPH is illustrated in Fig. 47. Methaqualone or DIPH appeared to produce lower levels of activity in the first 5 minute recording period but successive intervals showed no significant differences among any of the groups of rats when determined 18 hours after the last treatment.

TABLE 18

EFFECTS OF CHRONIC TREATMENT (18 DAYS) WITH MANDRAX, METHAQUALONE, OR DIPHENHYDRAMINE ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL	312.2 ± 35.1 (4) 100.0 ± 11.2%	47.2 ± 12.7 (4) 100.0 ± 26.9%	21.0 ± 4.4 (4) 100.0 ± 21.0%
MANDRAX (82.5 mg/kg)	259.0 ± 46.0 (4) 83.0 ± 14.7%	38.6 ± 10.4 (5) 81.8 ± 22.0%	21.4 ± 2.6 (4) 101.9 ± 12.4%
METHAQUALONE (75.0 mg/kg)	269.5 ± 34.8 (4) 86.3 ± 11.1%	45.5 ± 14.0 (5) 96.4 ± 29.7%	19.6 ± 1.8 (5) 93.3 ± 8.6%
DIPHENHYDRAMINE (7.5 mg/kg)	214.0 ± 33.2* (4) 68.2 ± 10.6%*	30.5 ± 4.0 (5) 64.6 ± 8.5%	20.0 ± 1.4 (5) 95.2 ± 6.7%

Experimental animals were administered mandrax (82.5 mg/kg p.o.), methaqualone (75.0 mg/kg p.o.), or diphenhydramine (7.5 mg/kg p.o.) suspended in 1 ml of vehicle once daily for 18 days and control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed 18 hours after the last drug or vehicle administration. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

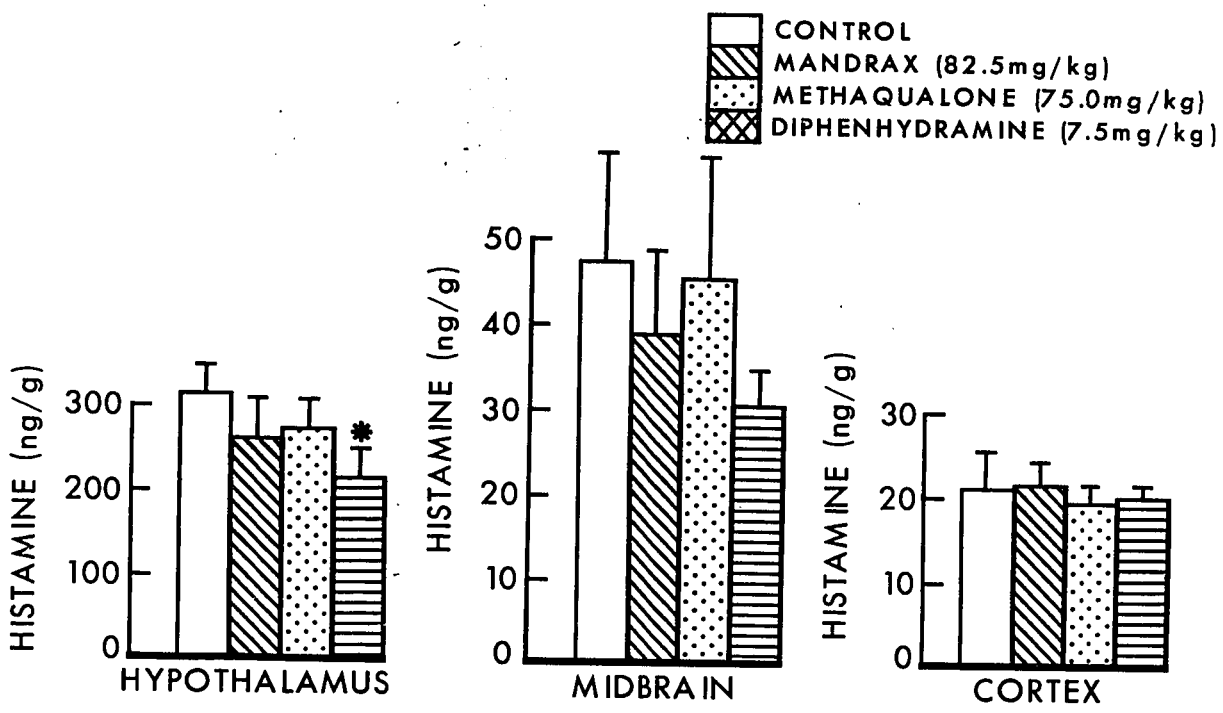


Fig. 46. Effects of chronic treatment (18 days) with mandrax, methaqualone, or diphenhydramine on endogenous brain histamine concentrations. Experimental rats were administered mandrax (82.5 mg/kg p.o.), methaqualone (75.0 mg/kg p.o.), or diphenhydramine (7.5 mg/kg p.o.) suspended in 1 ml of the vehicle once daily for 18 days and control rats were administered the vehicle (1 ml of 1% tragacanth gum p.o. once daily) for 18 days. All animals were sacrificed 18 hours after the last drug or vehicle administration. Values represent the Mean ± S.E.M. of at least 4 animals in each group.

* Statistically significant from control at $P < 0.05$

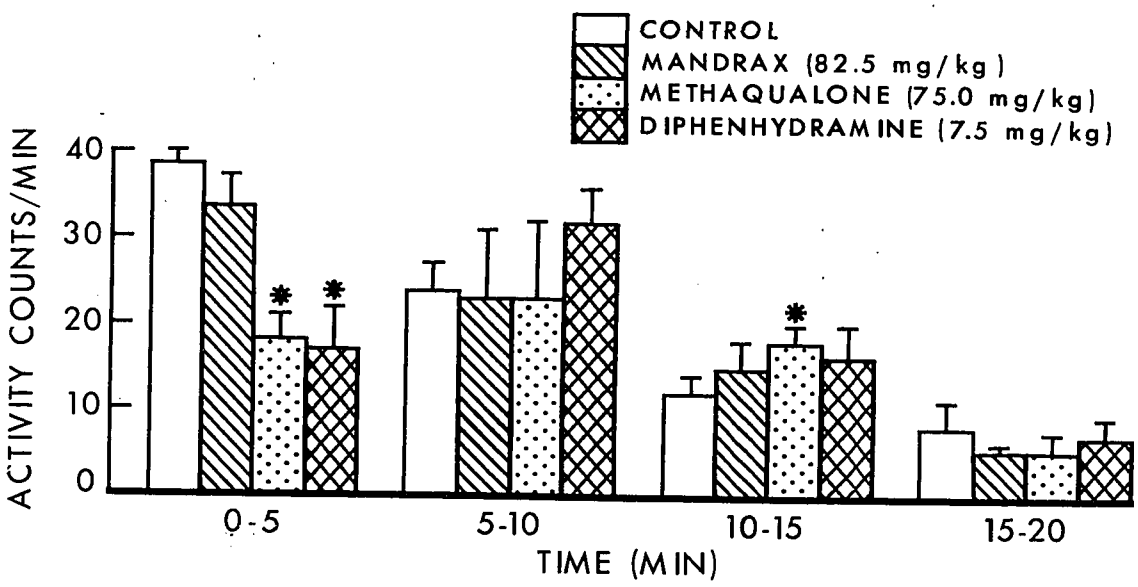


Fig. 47. Effect of chronic treatment (18 days) with mandrax, methaqualone, or diphenhydramine on spontaneous locomotor activity of rats. Experimental animals were administered mandrax (82.5 mg/kg p.o.), methaqualone (75.0 mg/kg p.o.), or diphenhydramine (7.5 mg/kg p.o.) once daily for 18 days and control rats received an equal volume of the vehicle (1.0 ml of 1% tragacanth gum p.o. once daily) for 18 days. All animals were sacrificed 18 hours after the last drug administration. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods 20 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of at least 4 animals in each group.

* Statistically significant from control at $P < 0.05$

XIII. Chronic Mandrax Treatment and Withdrawal

Chronic treatment with weekly increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o. once daily) for 21 days (Table 19 and Fig. 48) induced a significant decrease in the hypothalamic HA concentration, a decrease in the midbrain which was not statistically significant, and no significant change in the cerebral cortex.

Chronic treatment with weekly increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o. once daily) for 21 days followed by 2 days withdrawal resulted in HA concentrations not significantly different from controls as indicated in Table 20 and Fig. 49.

Chronic mandrax administration appears to retard body weight changes (growth) as illustrated in Fig. 50.

The SLA of rats receiving weekly increasing doses of mandrax for 3 weeks shows no significant differences from the vehicle-treated controls when determined either 18 hours (Fig. 51) or 66 hours (Fig. 52) after the last mandrax treatment.

TABLE 19

EFFECTS OF CHRONIC TREATMENT (21 DAYS) WITH MANDRAX ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL	335.1 ± 27.5 (7) 100.0 ± 8.2%	45.3 ± 4.2 (8) 100.0 ± 9.3%	19.4 ± 2.0 (8) 100.0 ± 10.3%
MANDRAX (21 days)	244.0 ± 28.6* (10) 72.8 ± 8.5%*	39.1 ± 2.5 (9) 86.4 ± 5.5%	17.6 ± 3.0 (10) 89.2 ± 15.5%

Experimental animals were treated with weekly increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o. suspended in 1 ml of the vehicle once daily) for 21 days and control rats were administered the vehicle (1 ml of 1% tragacanth gum p.o. once daily) for 21 days. All animals were sacrificed 18 hours after the last oral administration of the drug or vehicle. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

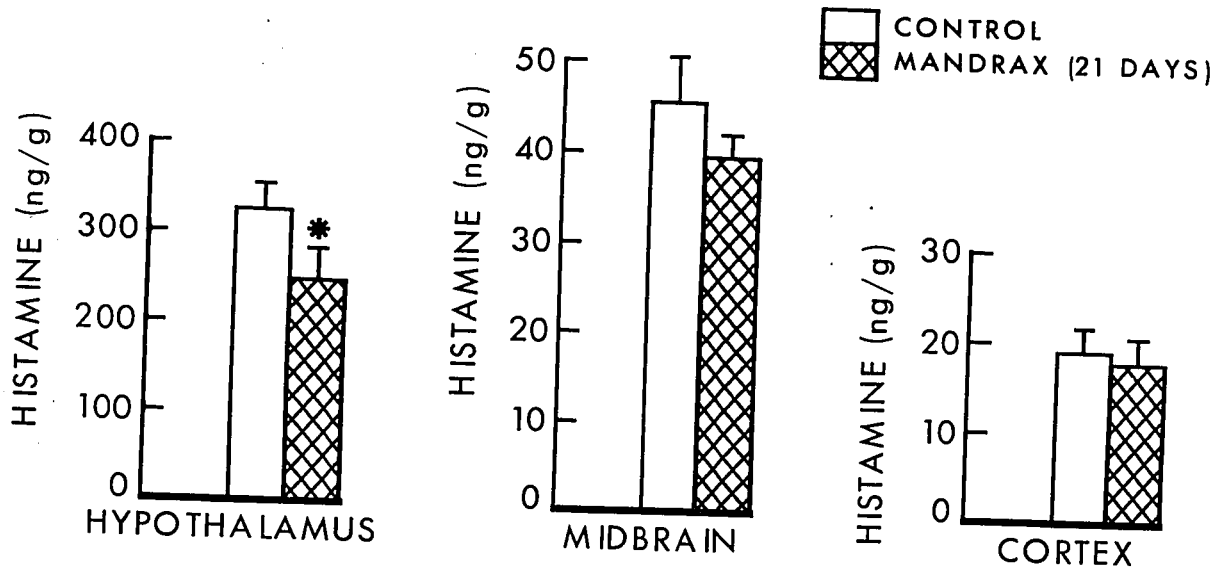


Fig. 48. Effects of chronic treatment (21 days) with mandrax on endogenous brain histamine concentrations. Experimental animals were treated with weekly increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o.) suspended in 1 ml of the vehicle for 21 days and control rats were administered the vehicle (1 ml of 1% tragacanth gum p.o. once daily) for 21 days. All animals were sacrificed 18 hours after the last drug or vehicle administration. Values represent the Mean ± S.E.M. of at least 7 animals in each group.

* Statistically significant from control at $P < 0.05$

TABLE 20

ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS AFTER MANDRAX WITHDRAWAL FROM RATS TREATED CHRONICALLY WITH MANDRAX

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL	256.7 ± 27.1 (6) 100.0 ± 10.6%	38.8 ± 4.4 (7) 100.0 ± 11.3%	22.2 ± 4.6 (6) 100.0 ± 20.7%
EXPERIMENTAL	239.9 ± 21.8 (8) 93.4 ± 8.5%	36.6 ± 3.6 (8) 94.2 ± 9.3%	21.8 ± 3.4 (7) 98.1 ± 15.3%

Experimental animals were treated with weekly increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o. suspended in 1 ml of the vehicle once daily) for 21 days followed by 2 days of withdrawal and control rats were administered the vehicle (1 ml of 1% tragacanth gum p.o. once daily) for 21 days. All animals were sacrificed 66 hours after the last drug or vehicle administration. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

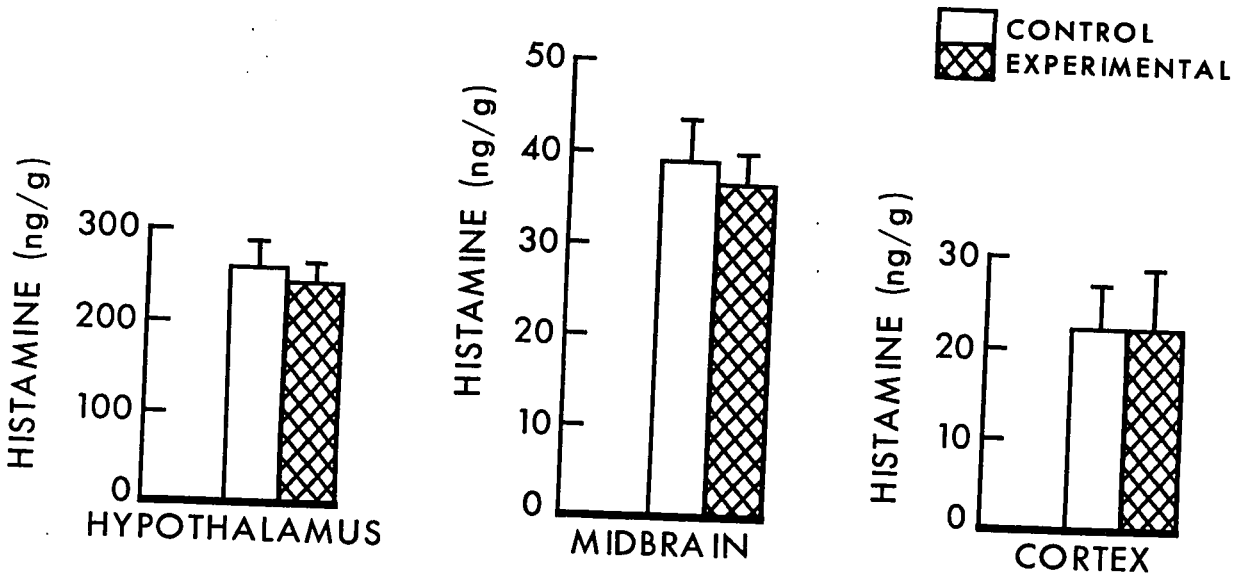


Fig. 49. Endogenous brain histamine concentrations after mandrax withdrawal from rats treated chronically with mandrax. Experimental animals were treated with weekly increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o. suspended in 1 ml of the vehicle) followed by 2 days of withdrawal, and control rats were administered the vehicle (1 ml of 1% tragacanth gum p.o. once daily) for 21 days. All animals were sacrificed 66 hours after the last drug or vehicle administration. Values represent the Mean ± S.E.M. of at least 6 animals in each group.

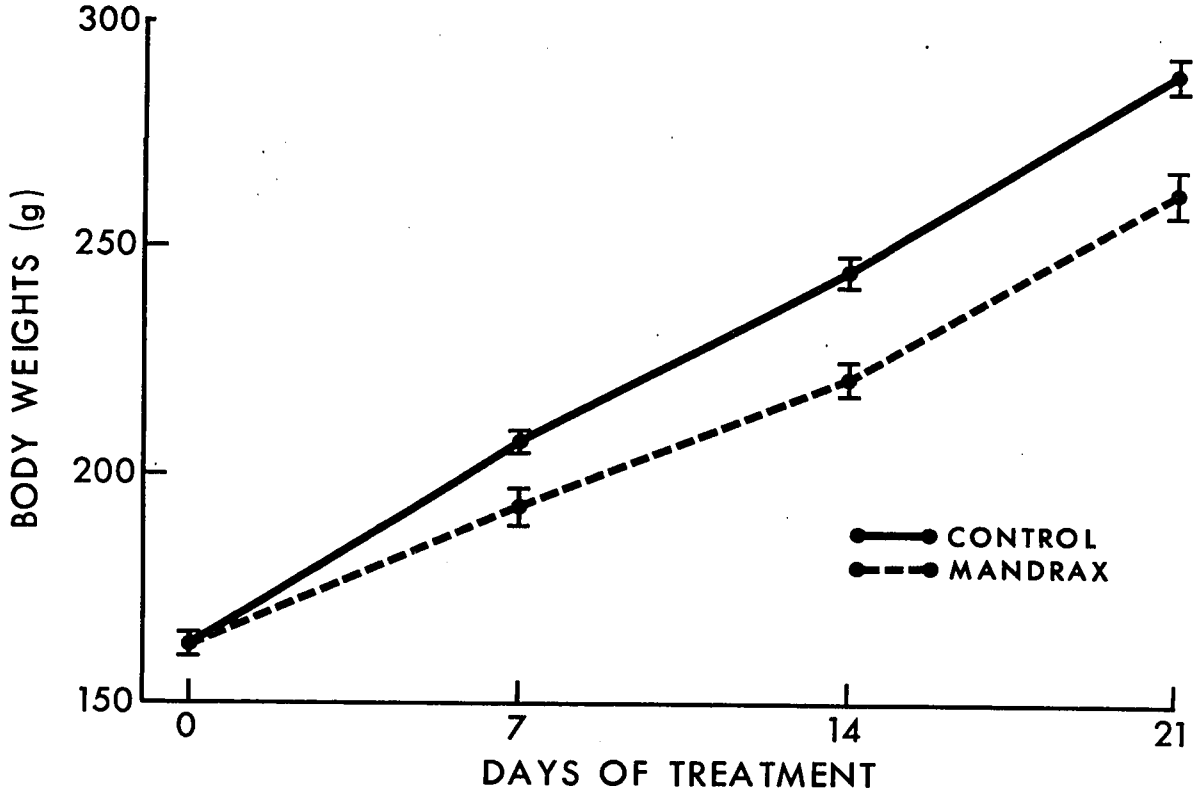


Fig. 50. Influence of chronic mandrax administration on body weight changes. Experimental animals were administered weekly increasing doses of mandrax (82.5, 123.75 and 165.0 kg/kg p.o. once daily) for 21 days and control rats were administered the vehicle (1 ml of 1% tragacanth gum p.o. once daily) for 21 days. All animals were sacrificed 18 hours after the last drug or vehicle administration. Values represent the Mean ± S.E.M. of 8 control and 10 experimental animals.

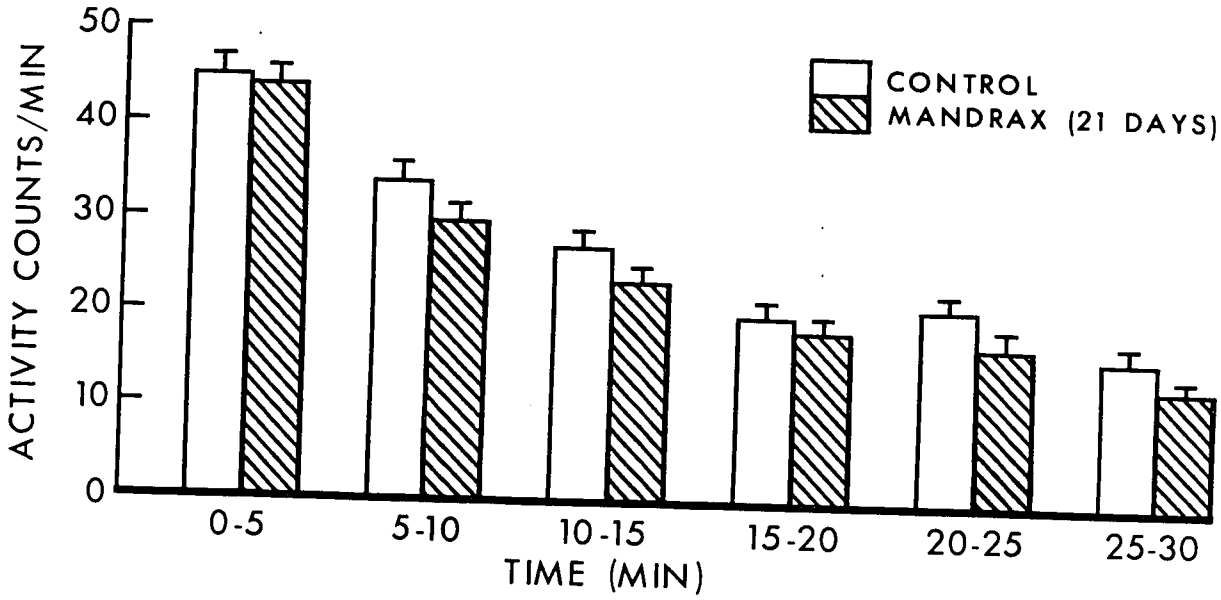


Fig. 51. Effect of chronic treatment (21 days) with mandrax on spontaneous locomotor activity of rats. Experimental animals were administered weekly increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o. once daily) for 21 days and control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed 18 hours after the last mandrax administration. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes before sacrifice. Values represent the Mean ± S.E.M. of 16 control and 16 experimental animals.

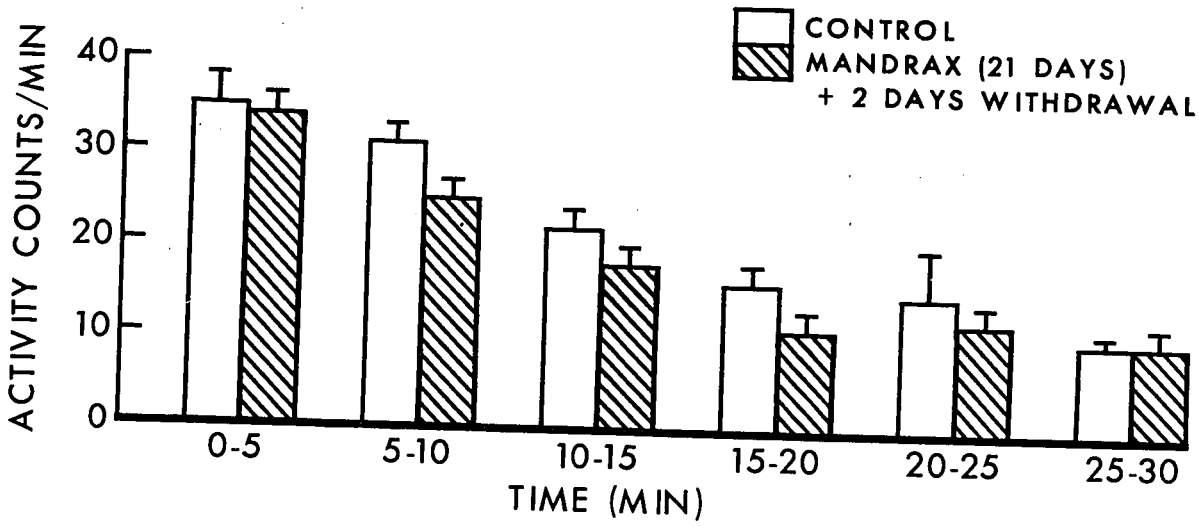


Fig. 52. Effect of chronic mandrax administration on spontaneous locomotor activity of rats 66 hours after the last treatment. Experimental animals were administered weekly increasing doses of mandrax (82.5, 123.75 and 165.0 mg/kg p.o. once daily) for 21 days and withdrawn for 2 days, and control rats were administered the vehicle (1 ml of 1% tragacanth gum) orally. All animals were sacrificed 66 hours after the last mandrax administration. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods for 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 10 experimental animals.

XIV. Acute and Chronic Amphetamine Treatment

Acute treatment with amphetamine (5 mg/kg i.p. or 15 mg/kg i.p.) for one hour did not induce any statistically significant changes in endogenous HA concentrations in any of the brain regions investigated, as indicated in Table 21 and Fig. 53.

Chronic treatment with weekly increasing doses of amphetamine (5, 10 and 15 mg/kg i.p. once daily) for 21 days, resulted in a statistically significant decrease in hypothalamic HA and statistically significant increases in the midbrain and cerebral cortex as indicated in Table 22.

Chronic amphetamine administration with weekly increasing doses of amphetamine produced very slight influences on body weight changes (growth) as illustrated in Fig. 57.

Acute amphetamine administration significantly influenced the SLA of these rats as illustrated in Fig. 54 and Fig. 55 for the 5 mg/kg and 15 mg/kg doses respectively.

The SLA of rats receiving chronic amphetamine and determined 18 hours after the last treatment are illustrated in Fig. 58. These animals appeared to express the same amount of activity as the saline-treated controls.

TABLE 21

EFFECT OF ACUTE AMPHETAMINE ADMINISTRATION ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
SALINE	248.5 ± 20.5 (12) 100.0 ± 8.2%	38.5 ± 3.6 (11) 100.0 ± 9.4%	19.4 ± 2.4 (8) 100.0 ± 12.4%
AMPHETAMINE (5 mg/kg)	213.9 ± 36.2 (6) 86.1 ± 14.6%	32.2 ± 2.7 (7) 83.6 ± 7.0%	20.7 ± 5.5 (4) 106.7 ± 28.4%
AMPHETAMINE (15 mg/kg)	304.6 ± 27.6 (4) 122.5 ± 11.1%	48.0 ± 1.7 (4) 124.8 ± 4.4%	15.6 ± 1.8 (4) 80.5 ± 9.3%

Experimental animals were administered amphetamine (5 mg/kg i.p.) or amphetamine (15 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). All animals were sacrificed one hour after treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

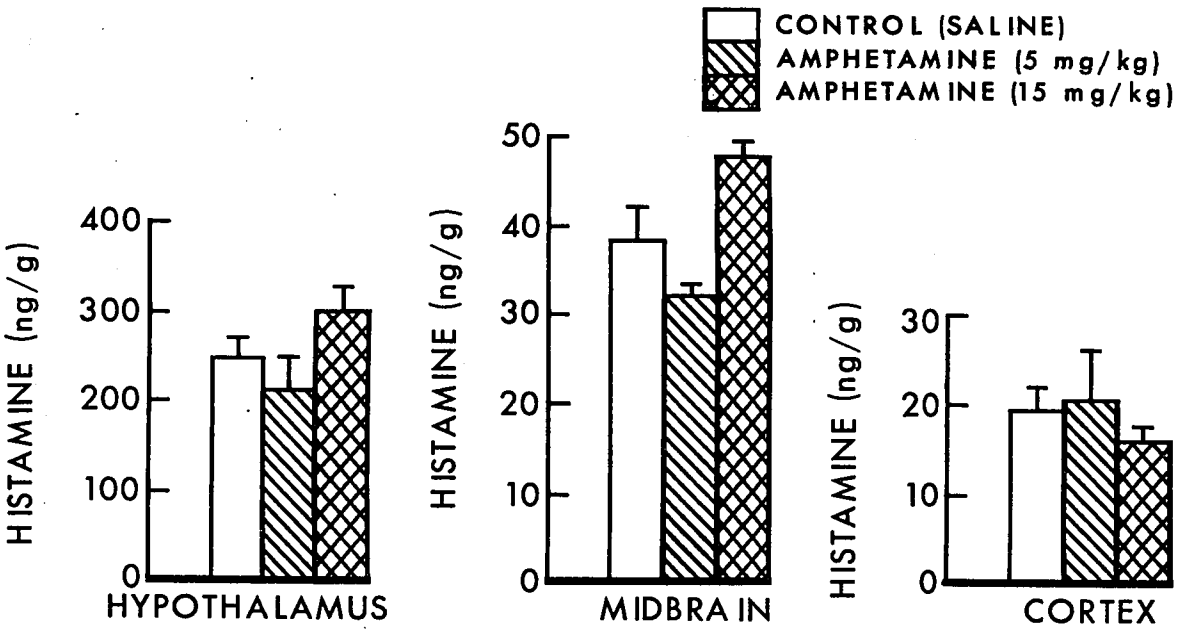


Fig. 53. Effect of acute amphetamine administration on endogenous brain histamine concentrations. Experimental animals were administered amphetamine (5 mg/kg i.p.) or amphetamine (15 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). All animals were sacrificed one hour after treatment. Values represent the Mean ± S.E.M. of at least 4 animals in each group.

TABLE 22

EFFECT OF CHRONIC AMPHETAMINE ADMINISTRATION ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS OF RATS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL (SALINE)	317.9 ± 26.7 (7) 100.0 ± 8.4%	32.0 ± 3.1 (8) 100.0 ± 9.7%	19.2 ± 1.6 (7) 100.0 ± 8.3%
AMPHETAMINE (21 days)	165.8 ± 28.6* (7) 52.2 ± 9.0%	53.0 ± 8.0* (7) 165.5 ± 25.0%	25.2 ± 2.8* (7) 131.2 ± 14.5%

Experimental animals were administered weekly increasing doses of amphetamine (5, 10 and 15 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. All animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. The results are expressed also as percent of control values. The number of experiments is shown in parentheses.

* Statistically significant from control at $P < 0.05$

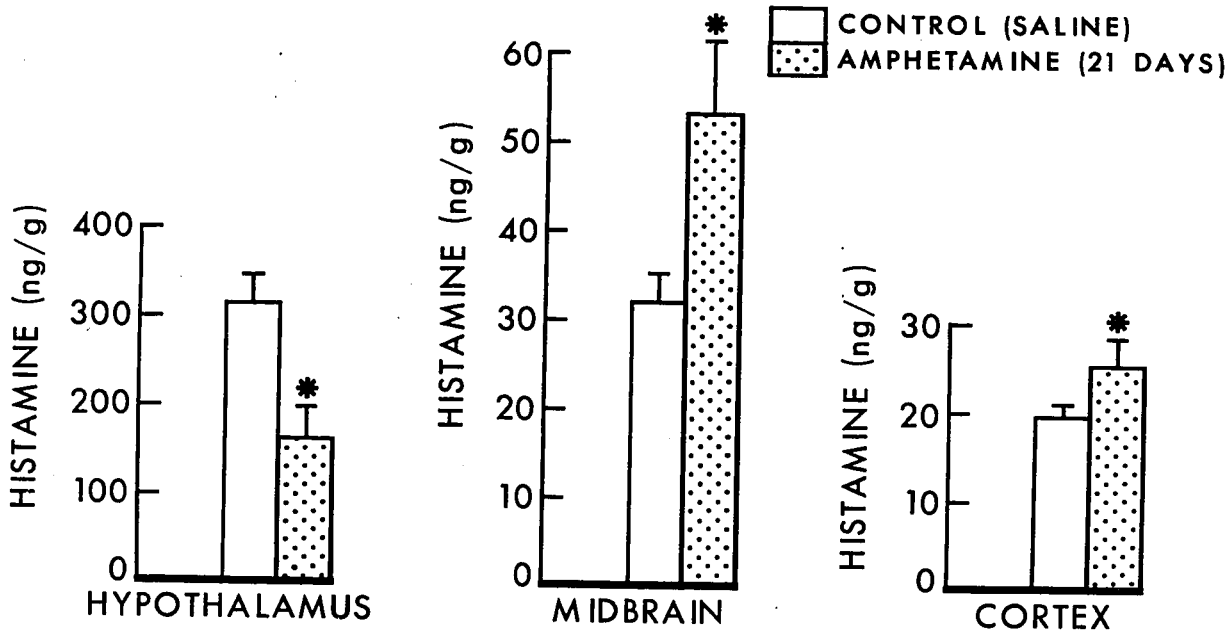


Fig. 54. Effect of chronic amphetamine administration on endogenous brain histamine concentrations. Experimental animals were administered weekly increasing doses of amphetamine (5, 10 and 15 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. All animals were sacrificed 18 hours after the last injection. Values represent the Mean ± S.E.M. of at least 7 animals in each group.

* Statistically significant from control at $P < 0.05$

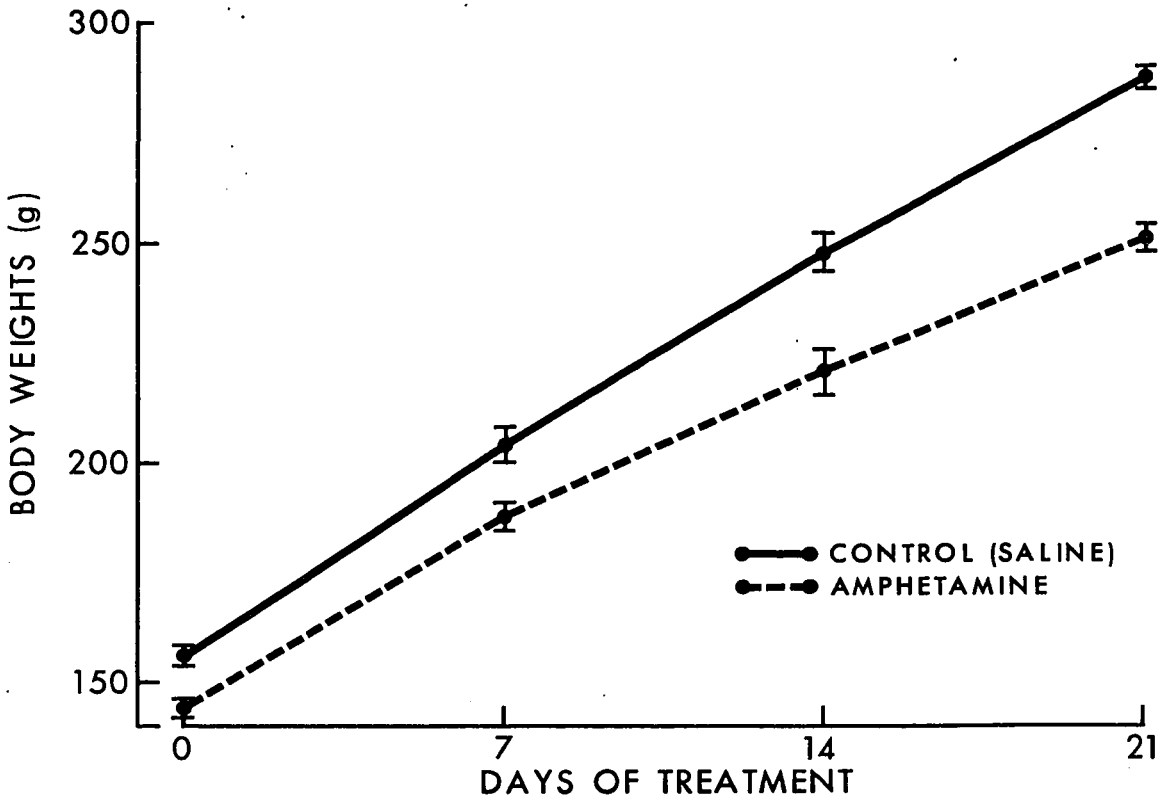


Fig. 55. Influence of chronic amphetamine administration on body weight changes. Experimental animals were treated with weekly increasing doses of amphetamine (5, 10 and 15 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. All animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. of 8 control and 8 experimental rats.

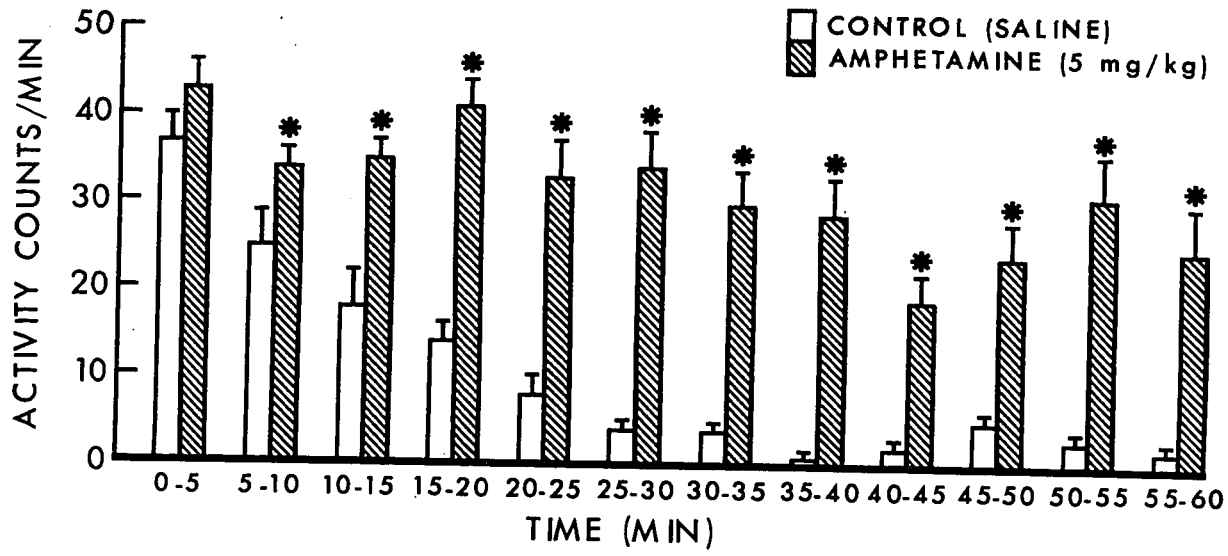


Fig. 56. Effect of acute amphetamine administration on spontaneous locomotor activity of rats. Experimental animals were administered amphetamine (5 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). All animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods. Values represent the Mean ± S.E.M. of 8 control and 6 experimental animals.

* Statistically significant from control at $P < 0.001$

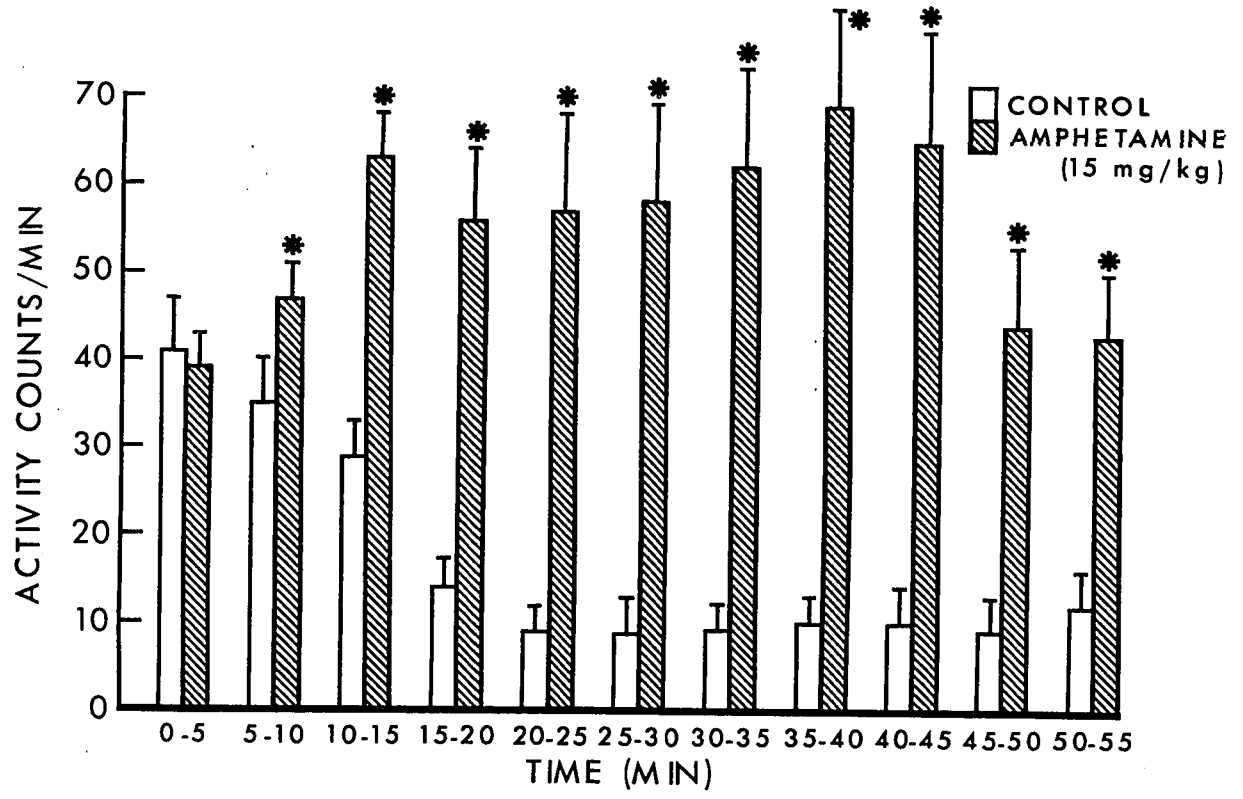


Fig. 57. Effect of acute amphetamine administration on spontaneous locomotor activity of rats. Experimental animals were administered amphetamine (15 mg/kg i.p.) and control rats were administered 0.9% saline (0.1 ml/100 g i.p.). All animals were sacrificed one hour after treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods 55 minutes prior to sacrifice. Values represent the Mean ± of 4 control and 4 experimental animals.

* Statistically significant from control at $P < 0.001$

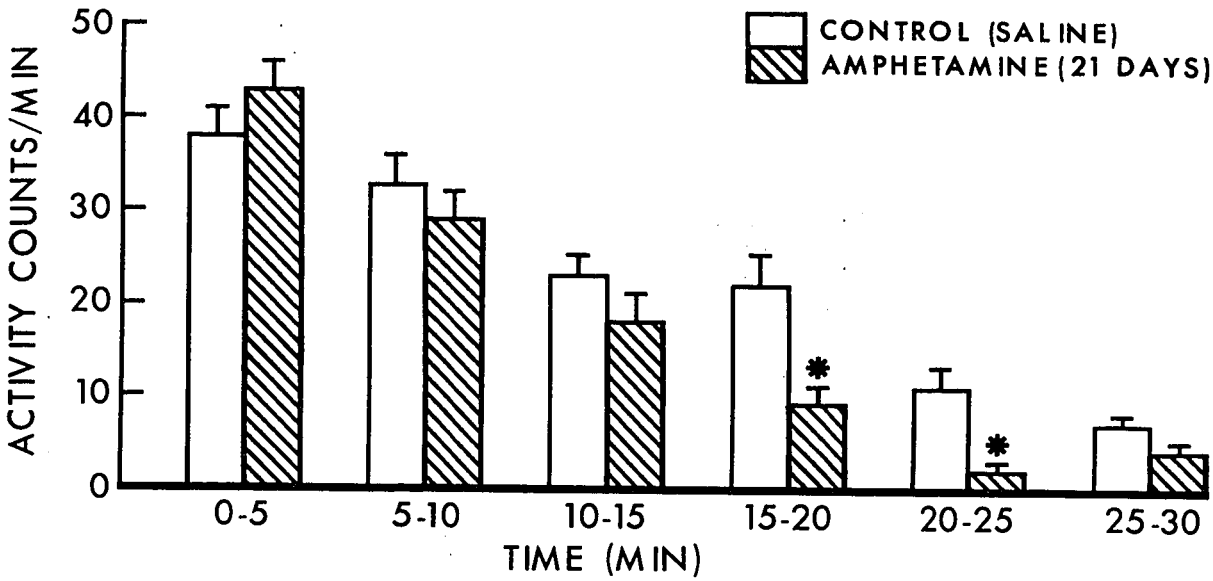


Fig. 58. Effect of chronic amphetamine administration on spontaneous locomotor activity of rats. Experimental animals were administered weekly increasing doses of amphetamine (5, 10 and 15 mg/kg i.p. once daily) for 21 days and control rats were administered 0.9% saline (0.1 ml/100 g i.p. once daily) for 21 days. All animals were sacrificed 18 hours after the last amphetamine treatment. The data indicate the mean activity counts/minute determined for consecutive 5 minute periods, 30 minutes prior to sacrifice. Values represent the Mean ± S.E.M. of 8 control and 8 experimental animals.

* Statistically significant from control at $P < 0.001$

XV. Acute and Chronic Δ^9-Tetrahydrocannabinol Treatment

Acute administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) (30 mg/kg p.o.) for one hour as indicated in Table 23, did not significantly alter the HA levels in the hypothalamus, midbrain or cerebral cortex.

Chronic treatment with Δ^9-THC (30 mg/kg p.o. once daily) for 7 days as indicated in Table 24, did not produce any significant changes in HA levels in the hypothalamus, midbrain or cerebral cortex.

TABLE 23

EFFECT OF ACUTE Δ^9-TETRAHYDROCANNABINOL ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS OF RATS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL	243.7 ± 29.7 (8) 100.0 ± 12.2%	32.7 ± 4.7 (7) 100.0 ± 14.4%	19.9 ± 5.0 (6) 100.0 ± 25.1%
ACUTE Δ^9-THC	284.3 ± 19.6 (7) 116.7 ± 8.0%	37.5 ± 5.2 (7) 114.7 ± 15.9%	18.9 ± 3.0 (6) 95.0 ± 15.1%

Experimental animals were administered Δ^9-tetrahydrocannabinol (Δ^9-THC) (30 mg/kg i.p.) suspended in 1 ml of vehicle and control rats were administered the vehicle (1 ml of corn oil) orally. All animals were sacrificed one hour after treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

TABLE 24

EFFECT OF CHRONIC Δ^9-TETRAHYDROCANNABINOL ON ENDOGENOUS BRAIN HISTAMINE CONCENTRATIONS OF RATS

Treatment	Hypothalamus	Midbrain	Cortex
	Histamine (ng/g)		
CONTROL	260.4 ± 45.3 (4) 100.0 ± 17.4%	29.2 ± 8.8 (4) 100.0 ± 30.1%	14.2 ± 0.9 (4) 100.0 ± 13.5%
EXPERIMENTAL	273.7 ± 43.2 (6) 105.1 ± 16.6%	31.7 ± 1.8 (5) 108.6 ± 6.2%	12.8 ± 1.4 (6) 96.2 ± 10.5%

Experimental rats were administered Δ^9-tetrahydrocannabinol (Δ^9-THC) (30 mg/kg p.o. suspended in 1 ml vehicle once daily) for 7 days and control rats were administered the vehicle (1 ml of corn oil p.o. once daily) for 7 days. All animals were sacrificed 18 hours after the last treatment. Values represent the Mean ± S.E.M. The results are also expressed as percent of control values. The number of experiments is shown in parentheses.

DISCUSSION OF RESULTS

If HA plays a significant role as a neurotransmitter in the mammalian CNS, then the abnormal behavioral states induced by the administration of various psychoactive agents might be expected to result in changes in the endogenous concentration and/or turnover of this amine. This situation would be analogous to those changes reported to occur in the endogenous levels and/or metabolism of the other putative transmitter amines following similar treatments (see Literature Review).

In order to establish that a specific substance such as HA via histaminergic neurones, mediates or modulates the actions of specific psychoactive agents, multiple lines of evidence must be compiled. Experiments in the past few years have shown some of the behavioral and physiological effects of HA administration. A few reports have indicated that certain types of animal behavior are altered or induced by intracerebroventricular HA administration, however, these works have been sharply criticized for being nonphysiological approaches. Other studies as described in the literature review have employed histidine decarboxylase inhibitors, lesions, and more recently iontophoretic applications of HA directly onto neuronal structures. All these studies provide useful and promising information regarding the role of HA in brain function. Another approach to this investigation involves the study of the endogenous concentrations and metabolism of HA following the administration of psychoactive drugs. This procedure permits an analysis by comparison of the effects of these drugs on HA with their influences upon other putative neurotransmitters.

It is clear that the details of the experimental paradigm are particularly important in order to permit a reasonable analysis of the meaning of the resulting data. In the next sections the experimental procedures selected will be discussed along with the corresponding results of the various treatments.

Pilot studies confirmed significant behavioral manifestations following the acute treatments and the dosages selected for the current investigations were based on these studies and the pharmacokinetic literature. Daily body weight records for all chronically-treated rats were maintained since it has previously been demonstrated that protein deficiency may result in elevated brain HA contents (359). Spontaneous locomotor activity measurements were performed in order to demonstrate the abnormal behavioral states induced by the psychoactive agents employed. The times for sacrifice and biochemical determinations were selected to provide an indication of the acute effects of the administered agents, as assessed by stable peak acute behavioral manifestations, or to represent the chronic effects evident three-quarters of the way through the inter-administration intervals. The chronic treatments were designed with the intention of administering a slowly increasing quantity of the drug to a point where by survival and behavioral statistics it became apparent that a significant degree of tolerance and/or dependence had developed.

I. Acute and Chronic Morphine Treatment

Acute treatments with morphine, methadone, naloxone, or even subacute treatments with morphine for 9 days or methadone for 5 days,

as described in the results, failed to produce any significant alterations in endogenous brain HA levels. This apparent lack of changes is perhaps a consequence of the dosages selected, the route of administration, the time of sacrifice, or even the species selected for these studies. The absence of changes in HA levels is in some ways comparable to the lack of consistent effects of similar drug administrations on the other neurotransmitters in the CNS as described in the literature review. There has been considerable confusion over the true effects of morphine or methadone administration on brain biogenic amines.

The behavioral data reported, following acute morphine or methadone treatment clearly illustrates the powerful sedative effects of these agents. These data also demonstrate the absence of any intrinsic effect on SLA from the dose of naloxone employed to precipitate the withdrawal syndrome in the morphine-dependent rats. The fact that the gross behavioral alterations observed after morphine or methadone were not reflected in changes in HA levels in the brain would appear to indicate either that the other biogenic amines may play predominant roles in the immediate or short term effects of these agents or that acute treatments influence the turnover rate but not endogenous levels.

In the chronic studies, the method of rendering the animals tolerant to and dependent upon morphine was patterned after the work of Takemori (358). The existence of a dependent state in our chronically-treated animals was confirmed by the ability of a very

low dose of naloxone to elicit a full blown withdrawal syndrome including urination, defecation, writhing, wet shakes, and a decrease in rectal temperatures shortly after administration. A few dependent animals were studied for extensive time periods following naloxone administration but the withdrawal syndrome was fully established within 20 minutes in agreement with other pharmacokinetic studies (360). It did not appear humane to pursue further study on the effects of longer times of withdrawal since within the time selected significant biochemical, behavioral, and physiological effects were evident.

Chronic morphine treatment for 21 days clearly reduced endogenous brain HA levels of rats (361). It is of interest that the most pronounced and significant decrease was noted in the hypothalamus which coincides with the highest concentration and the rapid turnover rate of HA in this brain region (14). Prolonged morphine administration was needed to induce this decrease which may imply a slowly developing process possibly resulting in a progressive imbalance between the rate of synthesis and metabolism and/or release of this biogenic amine.

Calcium ionophores release HA from mast cells (362) and, as described in the literature review, changes in the levels of the neurotransmitters could be visualized as a secondary reflection on the degree of calcium depletion in any particular brain region (241). This sequential action of morphine on brain HA levels may account for the necessity of chronic treatment in order to observe significant HA depletion. Quantitatively the depletion of brain HA levels observed in the current studies could conceivably have been entirely due to mast cell exocytosis. In order to differentiate mast cell from non mast cell HA depletion, a quantitative determination of the two storage sites for HA in brain would need to be developed. Such a technique is not yet available and only very rough estimates of mast cell HA content are available (363, 364).

Morphine and other drugs are known to release HA from rat mast cells and other peripheral tissues (365). Although most brain HA is believed to be of non mast cell origin, recently mast cells were demonstrated in the rat thalamus and were reported to contribute considerably to the total amount of HA in this tissue (364).

The HA concentration in the brain regions investigated in the present studies remained decreased in tolerant rats when morphine was replaced by methadone for 2 days (361). This may indicate that methadone is capable of maintaining the low HA level resulting from chronic morphine treatment by some analogous mechanism. Since methadone is an agent used to treat narcotic addicts and from which to withdraw them gradually, the biochemical effects of methadone substitution following chronic morphine administration should be investigated. Given acutely to naive rats, 10mg/kg i.p. was found to result in a large

degree of lethality. The fact that 15 mg/kg did not kill or even cause severe respiratory depression in any of the rats treated with this dose of methadone following chronic morphine treatment, indicates the development of a significant degree of cross tolerance.

II. Morphine Withdrawal from Morphine-Dependent Rats

Morphine withdrawal, whether precipitated for 20 minutes by naloxone administration or by cessation for 2 days of chronic morphine treatment, resulted in a significant depletion of brain HA in all three brain regions investigated. The decrease noted under these circumstances was approximately 50% of normal in the hypothalamus and cerebral cortex and 70% of normal in the midbrain. A greater degree of depletion of brain HA is seldom achieved possibly due to the presence of another more stable pool (366). Although naloxone-precipitated withdrawal seems to be more stressful to rats than gradual morphine withdrawal, HA depletion was more pronounced under the latter experimental conditions. It seems therefore, that the observed changes in endogenous brain HA concentrations could only in part be accounted for by stress. Stress caused by cold and/or immobilization has been reported to lower hypothalamic HA concentration in rats (49), however others could not confirm these results (364, 367).

It may be important to note that giving larger doses of naloxone for longer periods of time might result in more substantial changes in the parameters determined as has been well established by Blasig (359). In addition these authors have demonstrated that the behavioral expressions of the dependent animals is directly linked to the degree of dependence which is a function of the administration pattern of the drugs.

The direction of the biochemical changes observed in the present studies is in marked contrast to the effects of withdrawal on most of the other well known neurotransmitters. Cholinergic-dopaminergic interactions have been implicated in morphine abstinence (368). Morphine withdrawal or naloxone precipitated withdrawal in mice and rats was reported to decrease the brain Ach concentration and to increase the brain DA concentration in the corpus striatum while NA and 5-HT remained unchanged. It is of interest that the brain Ach concentration was found to be decreased during the morphine withdrawal syndrome as both Ach and HA are known to have very rapid turnover rates (369).

The animals used in the current studies could have been given much larger doses of morphine over 3 weeks and this may have enhanced some of the findings, but it was felt to be important to administer a reasonable dosage to the animals in order that they maintain a reasonable growth rate. The daily records of the body weights confirm that those animals receiving morphine grew significantly less than the control animals, but they did continue to gain weight (see Fig. 7 Results).

Naloxone administration to morphine-dependent animals which resulted in further decreases in endogenous brain HA levels within 20 minutes also resulted in significant decreases in SLA when compared to saline-treated control rats (see Fig. 9 Results). These findings parallel the observed changes in the endogenous brain HA levels and may indicate some correlation. Perhaps the HA released by withdrawal led to sedation as observed in the SLA measurements. This would agree with the central sedative effects of HA as described in the literature review.

III. Time Course for Recovery from Morphine-Induced Changes in Endogenous Brain Histamine Concentrations

Since chronic morphine administration resulted in a significant depletion of HA in the brain regions investigated and since 2 days of withdrawal following chronic morphine treatment produced a further depletion of HA, it was decided to investigate the time course for recovery to normal HA levels. The significance of these studies lies in the fact that alterations in brain HA required considerable time to develop and they also took a matter of weeks to return to normal values upon cessation of treatment. This time course of events would appear to correlate with the slow development of tolerance to the effects of morphine in man and with the prolonged effects of morphine withdrawal.

The data for the SLA of rats one hour following the last chronic morphine administration (Fig. 13 Results) clearly showed that the dose of morphine used still produced a significant degree of depression of SLA and also that considerable tolerance to these large doses had developed. The successive activity studies taken together showed a tendency for the SLA of the rats to fluctuate particularly in the first two days following morphine withdrawal from morphine dependent animals. Lee and Fennessey (370) reported that morphine produces a biphasic effect on brain HA levels of the mouse and that these modifications appear to be closely but inversely related to the overt behavioral effects produced by morphine in the mouse; that is locomotor activity. The results of the present studies do not support or refute these studies on mice as the dosages used, the species and brain regions studied, and the time course following the changes were all different. Perhaps many of the results reported by these investigators were due to acute handling stresses most

evident within the first hour of manipulation.

Morphine may cause changes in locomotor activity in various species by a mechanism not directly involving HA and as a consequence, this alteration in activity may in turn alter HA levels. The evidence to date only supports the statement of coincidence between changes induced by morphine in endogenous brain HA levels and SLA.

IV. Histamine Methyltransferase Activity Following Chronic Morphine Treatment and Withdrawal

In order to better understand and explain the changes observed following chronic morphine treatment and withdrawal, the activity of the major HA metabolizing enzyme HMT was investigated. There were no detectable changes in either brain protein concentration or HMT activity following morphine treatment or following morphine withdrawal from morphine dependent rats. These findings necessitated a consideration of the activity of the major synthesizing enzyme HD following these treatments.

It has been reported (371) that when the HA concentration is decreased, the HD activity is increased. Histidine decarboxylase is not saturated by the endogenous level of its substrate (51) and hence a decrease in HA may stimulate HD as compensation. Depending on the availability of the precursor histidine more HA may be formed. In light of the finding of reduced HA levels occurring in rats treated chronically with morphine, HD activity being increased, and HMT activity being unchanged, there must be limited precursor availability, impairment of HA storage, increased HA release, or an enhancement of an alternative metabolic pathway. The current findings certainly indicate the need for these possibilities to be pursued further.

V. Chronic Treatment with Histidine or Histidine with Morphine

In order to further explore the mechanism of the changes in endogenous brain HA concentrations occurring after chronic morphine treatment, the effects of administration of the HA precursor histidine were investigated. The present investigation revealed that chronic daily treatment with histidine along with chronic morphine administration resulted in a restoration of the low hypothalamic and cerebro-cortical HA values toward the control levels. It is possible that in these animals morphine may still be reducing the levels of HA in the brain regions studied but now from the elevated levels produced by chronic histidine administration and therefore the brain HA concentration stays within the normal range. Histidine treatment was however, less effective in preventing the decreases in hypothalamic HA concentration observed during morphine withdrawal (Fig. 23 Results). The inability of chronic histidine pretreatment to completely replenish the hypothalamic HA in morphine withdrawn rats could have been due to the very significant depletion of HA in this brain region. The histidine-induced restoration of the low HA values in different brain regions noted here is in direct correlation with the reported elevation of HA concentrations in different brain regions of rats and mice (52, 53) following acute administration of histidine in a dose similar to the one used presently. In acute studies in rats (52) the return of the elevated hypothalamic HA concentration toward the control level was noted within 10 hours. In the chronic studies presented here the midbrain HA concentration was not significantly increased by a histidine load when tested 18 hours after the last drug treatment, however significant increases in the hypothalamic

(30%) and cerebro-cortical (55%) HA were still apparent 18 hours after the last histidine administration. It is quite possible that the HA metabolizing enzymes could not cope with the chronic daily increased level of this biogenic amine or that chronic administration of histidine may have led to an increase in HA concentration not only in the neuronal but also in the non-neuronal storage sites; the latter consisting most likely of mast cell stores. Mast cell HA is known to have a slow turnover rate (372). Indeed, multiple compartmentation of HA in the brain has been advocated recently (373) and may account for some of the current observations.

The present results may very well indicate that the magnitude of the stresses imposed by morphine withdrawal are significantly greater than those resulting from chronic morphine treatment. On the other hand, it is plausible that although the changes in brain HA levels are in the same direction during withdrawal as during treatment, they may have entirely different mechanisms. This possibility is supported by the observation of significantly different effects produced by chronic morphine treatment and withdrawal on endogenous brain Ach levels.

Simultaneous treatment with morphine and histidine for 21 days resulted in a reduced rate of growth (Figs. 24 and 33 Results) similar to that observed in rats treated with morphine alone. Therefore, although chronic histidine when administered simultaneously with morphine, does prevent the decreases in brain HA, it does not alter the depression of the growth rate. This result may be explained perhaps by considering the sedated behavior of these animals and hence the inhibition of their normal eating behavior. It may also be that HA may be involved in feeding behavior, a role that has been proposed for several putative

neurotransmitters (374).

The simultaneous treatment of rats with morphine and histidine for 21 days did produce significant decreases in their SLA when compared with rats receiving either saline or histidine alone (Figs. 21 and 22 Results). This finding is in marked contrast with the lack of effect of chronic morphine treatment on SLA when measured at the same time (Fig. 16 Results). These observations are very possibly indicative of the effects of altered HA metabolism in the rat brain and not just increased or decreased HA concentrations.

Since withdrawal of morphine from morphine-dependent rats for 2 days appeared to have truly pronounced effects on endogenous HA concentrations and SLA, studies were performed to observe the SLA of rats treated simultaneously with morphine and histidine for 21 days and then withdrawn for 2 days. The studies from these two groups of animals (Fig. 25 Results) showed that chronic histidine administration along with morphine treatment prevented the significant decreases in SLA noted when rats were withdrawn for 2 days following chronic morphine treatment. The fact that withdrawal from histidine itself did not cause any changes in SLA of the rats (Fig. 26 Results) substantiates the improvement in behavior as assessed by SLA in rats treated simultaneously with histidine and morphine.

VI. Histamine Formation Studies

It was felt to be mandatory to study the effects of chronic morphine, chronic histidine or a combination of these treatments on HA formation in the rat brain after our results had clearly shown the effects of these treatments on endogenous HA levels. The cold precursor

L-histidine was employed for these studies as was previously used by Snyder and Taylor (49) and Schwartz (52) and the HA formation 1 hour following histidine administration was determined. The choice of 1 hour was based on studies performed to determine the time of maximal effect of exogenously administered histidine on brain HA levels (375).

In agreement with previous reports (49) it was noted that histidine loading significantly elevated brain HA concentrations in naive rats and in addition the brain HA levels were also significantly elevated in the morphine dependent rats. Acute histidine administration resulted in somewhat reduced SLA when compared to that seen in saline-treated rats. This finding is in agreement with a previous report by Maslinski (376) who reported that higher doses of histidine can even produce a state of catalepsy. The lack of a clear-cut depression of activity at all time intervals here is believed to result from the limited number of animals used for this study. It is important to note however, the greatly increased sensitivity of the SLA measurements employed in the current studies when compared to the use of a stabilimeter (377) which failed to detect the histidine-induced reduction in activity.

In rats administered histidine alone or treated simultaneously with morphine and histidine, it is most important to note the higher level of activity seen 1 hour after simultaneous treatment with morphine and histidine when compared with animals administered histidine alone. The animals treated with histidine alone appear to be strongly sedated within the first hour following the drug administration. Rats treated with morphine and histidine do not show such depressed activity which may indicate some impairment of access of the histidine to its normal sites of action or less synthesis of HA occurring at these sites. The

former indication is supported by the enhanced HD activity reported in those rats treated chronically with morphine (371) which would result in HA formation from any histidine available.

The SLA of rats receiving an acute histidine load either 18 or 66 hours after morphine withdrawal from morphine dependent rats appeared to be the same as the activity of the saline-treated controls receiving the same acute dose of histidine (Figs. 30, 31 Results). This may be accounted for by the absence of the acute effects of morphine on the uptake or synthesis of HA when the two are administered simultaneously.

Acute histidine administration either 1 or 18 hours after the last chronic histidine administration produced even greater increases in hypothalamic HA (Fig. 32 Results) than the increases observed with acute histidine administration to saline-treated control rats. These results might indicate a form of enzyme induction or possibly increased accessibility of the precursor to the site of synthesis. The data for the midbrain seem to indicate much more stable yet elevated HA levels following chronic histidine treatment or chronic simultaneous treatment with morphine and histidine. The results for the cerebral cortex showed a pattern similar to the midbrain.

Acute histidine loading depressed the SLA of rats treated with morphine more than saline-treated controls (Fig. 34 Results), and it depressed the activity of rats treated chronically with morphine and histidine to a greater degree than the activity of those animals receiving only chronic histidine (Fig. 35 Results). These findings could be interpreted to result from the prevention of HA uptake intraneuronally by morphine or from the enhanced release of newly formed HA.

Since chronic histidine administration appears to prevent or reverse the decreases in brain HA concentration observed following chronic morphine treatment for at least 18 hours, it was of interest to determine whether the normal levels of HA observed 1 hour following acute histidine administration to rats treated chronically with morphine might also remain normal for 18 hours. The data from this study (Table 14 Results) showed that the rise to normal values had returned to the decreased levels noted especially in the hypothalamus 18 hours following the last morphine injection. The SLA of these rats was slightly depressed 18 hours after the acute histidine load (Fig. 36 Results) and these rats were now probably feeling the first effects of withdrawal. Perhaps this decreased activity and the sudden drop in HA concentration may be related. Many of the changes recorded in brain HA levels and the subsequent changes in synthesis may result from a cholinergic triggering mechanism. This concept has been proposed earlier (378) and the present evidence lends considerable support. This mechanism would also explain the increased depletion of brain HA during morphine withdrawal as the release of Ach under these conditions is well documented (204, 205).

VII. Acute and Chronic Pentazocine Treatment

Pentazocine, a partial narcotic agonist and partial antagonist is widely used as an analgesic hence further knowledge about neurochemical alterations induced by this agent would be of prime importance.

The experimental design selected for the pentazocine studies was based on that used in the morphine studies. It was felt that using the same dosages and times of sacrifice would allow a reasonable basis for comparison of any results. In the acute studies the time of sacrifice

agrees well with the maximal time-action curves for the effects of pentazocine observed in humans (242). Considering the shorter duration of action of pentazocine than morphine, it was decided to inject the animals twice daily in the chronic studies.

The data from the present studies (Figs. 37 and 39 Results) indicate that the changes in endogenous brain histamine concentrations, observed after acute and chronic pentazocine administration, parallel those seen following similar morphine treatments. Perhaps as has been suggested previously (370), analgesia may be associated with the biosynthesis of HA.

Unlike the depressing effect of chronic morphine treatment on the growth rate of rats, chronic pentazocine treatment showed no such strong influence on the rate of body weight changes (Fig. 40 Results). These rats gained weight at a rate parallel to the saline-treated controls. This might be explained by the fact that pentazocine, in the doses employed in these studies, did not suppress SLA of the rats to the same extent as the morphine treatments. This may have permitted the rats a more normal eating activity and hence weight gain. This is not to say that pentazocine did not influence the SLA in the current studies because it did significantly depress activities even within the first 5 minutes of recording following acute pentazocine administration as can be seen in the results (Fig. 38 Results). It may also be noteworthy that the rats receiving acute pentazocine failed to settle down in their cages to the same degree that the controls did once they became accustomed to their surroundings. The significance of these observations is not yet clear and further studies would be required to more fully characterize these findings.

Rats treated chronically with pentazocine for 21 days, showed somewhat depressed SLA 18 hours after the last pentazocine injection when compared to saline-treated controls (Fig. 41 Results). This observation is probably not of significance however because the control rats in this study, displayed higher than usual SLA particularly at the later recording intervals when compared to other studies. This may have been due to some extraneous disturbing influence on the day of investigation. The lack of effect of chronic pentazocine treatment on SLA 18 hours after the last injection correlates with the normal activity of chronic morphine-treated rats examined at the same time.

There now appears to be evidence of an influence of pentazocine on brain HA similar to that observed following morphine treatment. This may be indicative of the similarity in the pharmacological actions of these two agents upon a putative monoaminergic system. Whether the actions of pentazocine are primarily mediated via adrenergic, cholinergic, or histaminergic pathways is not yet certain. The current studies do add evidence to support the importance of considering HA as a neurotransmitter when attempting to ascertain the pharmacological mechanisms of action of pentazocine.

VIII. Acute Mandrax, Methaqualone, or Diphenhydramine Treatment

Within the past few years concern has arisen over the abuse by the young population of MX (267, 286-288). This drug is a combination of a nonbarbiturate hypnotic, MQ, and a potent antihistaminic, DIPH. Methaqualone has also been reported to possess considerable addiction liability (265, 280-283). Considering the possible involvement of HA

with narcotic addiction and the presence of both an agent reported to produce tolerance and dependence and an antihistaminic, it was considered probable that HA may show some involvement with the pharmacological effect of MX.

In acute studies, the rats were sacrificed one hour after oral administration of MX, MQ, or DIPH. Although it is difficult to extrapolate from clinical to animal studies, the time of sacrifice was selected on the basis of clinical studies (379, 380) and studies of MQ absorption and distribution following oral administration to mice (381).

The data from the present acute studies (see Fig. 42 Results) show that the most significant changes in brain HA concentrations occurred in the hypothalamus, although some significant decreases were also observed in the cortex after MX or MQ but not after DIPH administration. The decreases noted after acute MX or MQ are in accord with the decrease in brain HA turnover in rats acutely treated with other hypnotics such as barbiturates (382). It is possible that the significant decreases observed after MX administration may have been due in part to the DIPH component in the compound; however, MQ also lowered the hypothalamic HA concentration. It has been claimed on clinical grounds that DIPH improves the hypnotic potency of MQ (383, 384); however, DIPH does not alter the rate of absorption of the distribution of MQ in blood (385) and therefore a synergistic effect of these two drugs has been suggested (386, 387). The decrease in the HA concentration noted in the midbrain after MX administration, did not reach the level of statistical significance.

In the cortex, the story is similar to the lowering effects noted in the hypothalamus although due to a larger variation in the sample, DIPH failed to produce a statistically significant degree of HA depletion.

Acute MX or MQ administration in the dosages employed in these studies, resulted in significantly depressed SLA of experimental rats when compared to vehicle-treated controls. Diphenhydramine also had some sedating effect by itself and perhaps the result noted with MX was partly due to DIPH and partly due to MQ.

IX. Mandrax, Methaqualone, or Diphenhydramine Treatment for 18 Days

The effects of treatment with MX, MQ, or DIPH for 18 days were less significant than the acute effects (see Fig. 46 Results). Here it would appear that some tolerance had developed to the effects of MX and MQ and only those rats treated with DIPH showed any significant decreases in their brain HA levels. The hypothalamic and midbrain HA concentrations were decreased considerably, although the midbrain value was not statistically significant due to the large standard error of the control values. The various treatments failed to alter the cerebrocortical HA levels to any noticeable extent again suggesting tolerance development.

The SLA of rats treated with the same dose of MX, MQ, or DIPH for 18 days and measured for a 20 minute period 18 hours after the last drug administration did not provide any clear cut suggestions due to the variability of the animals' behavior. Although there were some statistically significant differences in some recording intervals, these are of little practical value.

X. Chronic Mandrax Treatment and Withdrawal

Chronic treatment with weekly increasing doses of MX for 21 days resulted in significantly lower hypothalamic HA levels when compared to vehicle-treated controls (see Fig. 48 Results). The HA concentrations in the midbrain and cortex were not significantly decreased from vehicle-treated controls. These data again show the hypothalamus to be the most vulnerable area for the influence of MX perhaps because of the high concentrations here and perhaps because of the important life functions this area controls. The effects noted in the hypothalamic HA concentration 18 hours after the last chronic treatment with weekly increasing doses of MX had disappeared within 66 hours following the last drug administration. This may hopefully signify somewhat less severe or at least somewhat less prolonged withdrawal effects in subjects made tolerant to MX. In addition to this, the effects of chronic MX administration at least in the doses selected for the current studies, on body weight changes appears to be much less serious (see Fig. 50 Results) than the pronounced effects seen in the chronic morphine studies. This difference may only reflect the lesser degree of sedation induced in the rats by the doses of MX administered.

Chronic MX treatment for 21 days using weekly increasing doses, did not induce any significant changes in SLA of the rats when determined 18 hours or 66 hours after the last drug administration. This also supports the concept of less severe withdrawal from this agent than from morphine.

Although a significant decrease was noted in the hypothalamic HA concentration after acute MX, MQ, or DIPH treatment, these changes have

not been associated with an increase in histidine decarboxylase (HD) activity in this brain region (371). This lack of any observed changes in the activity of HD in these acute studies may be due to the time of sampling used. It is possible that any enzyme activity alterations may take longer or the peak of the activity of this enzyme may have occurred before 1 hour after treatment, in connection with the very rapid rate of turnover of HA in this brain region (79). Alternatively the decrease in the HA concentration may not have been sufficient to stimulate the HD activity noticeably. The decrease in HA concentration in the cortex previously following acute MX or MQ treatment was found to be associated with a slight but significant increase in HD activity indicating increased HA synthesis in this brain region.

Chronic treatment with weekly increasing doses of MX for 21 days resulted in an increase in HD activity in the hypothalamus and cortex which correlates with the decreased HA concentration and again increased synthesis in these brain regions. These results appear suggestive of a feedback induction system.

XI. Acute and Chronic Amphetamine Treatment

The doses and times of sacrifice selected for amphetamine were based on the literature (389) and on a screening test. The behavioral results (see Figs. 56 and 57 Results) clearly indicate significant effects on the SLA of our rats. The increased SLA of these animals was clearly dose dependent and sustained throughout the acute time period studied.

The fact that chronic amphetamine treatment produced significant alterations in the endogenous HA concentrations in the brain regions studied, and yet failed to produce any noteworthy effects on SLA of these

animals immediately prior to sacrifice, argues against any direct and immediate correlation of SLA with brain HA concentrations. Tolerance development to the stimulant effects of amphetamine, as determined by SLA measurements, was looked for but was not apparent in the present studies.

Acute amphetamine administration did not produce any clear cut changes in brain HA levels (see Fig. 53 Results); however, chronic studies were more rewarding. Rats treated with weekly increasing doses of amphetamine for 21 days showed decreased hypothalamic and increased midbrain and cortical HA concentrations (see Fig. 54 Results). The pattern of these changes is distinctly different from that seen following drugs with central sedative properties, and may perhaps lead to some basis of differentiation of these agents.

XII. Acute and Chronic Δ^9-Tetrahydrocannabinol Treatment

In view of the existing uncertainties in the literature with regard to the pharmacological effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the levels and turnover rates of the biogenic amines (390), it remains to be established which if any of the putative neurotransmitters currently of interest, play any role in its actions. The choice of 1 hours as the time of sacrifice in the current studies was based on reports that 30 minutes to 2 hours is the time during which maximum behavioral changes occur following acute Δ^9-THC administration to rats and mice (391-394).

An investigation of the possibility of a role for brain HA in the mechanism of action of Δ^9-THC in rats has now been attempted.

Neither acute (1 hour) nor short term (7 days) oral treatment produced any significant changes in endogenous HA concentrations in the brain regions investigated. These results are in line with the reported lack of changes in the endogenous concentrations of other biogenic amines following Δ^9-THC administration (395). It is still possible however that changes may be induced in other species and/or even in rats if the Δ^9-THC was given in higher doses, for longer periods, or via alternative routes such as inhalation.

From the data currently available it appears unlikely that behavioral effects produced in rats after acute or short term Δ^9-THC administration are a result of any marked effect on brain function mediated by HA.

It appears that the administration of potent psychoactive agents to rats leading to the development of tolerance and dependence results in changes in endogenous brain HA concentrations and/or turnover. Some degree of correlation between brain HA levels and SLA has been demonstrated. These same treatments have been reported to alter the endogenous levels of several other putative neurotransmitters in rat brain. It may be stated that there is now pharmacological evidence supportive of an involvement of brain HA in the action of these psychoactive agents. Whether the changes in HA levels are direct or indirect results of treatments with the agents currently under investigation remains to be elucidated. Histamine may indeed play a significant role as a neurotransmitter in the mammalian CNS since, as expected, the abnormal behavioral states induced by the administration of various psychoactive agents did result in changes in the endogenous concentration and turnover of this amine.

SUMMARY

Experiments have been performed in order to investigate the influences of some psychoactive drugs that are often abused upon endogenous brain HA concentrations. The agents used in these acute and chronic studies included the narcotic analgesic morphine, the partial narcotic agonist partial antagonist pentazocine, the non-narcotic sedative hypnotic mandrax, and its components methaqualone and diphenhydramine, a central stimulant amphetamine, and another drug of abuse tetrahydrocannabinol. It is difficult to determine conclusively whether the changes in brain biogenic amine levels observed after treatment with these various drugs correlates with any of the pharmacological effects of these agents or if they are merely secondary. The principal aim of these studies was to search for any influence these agents might exert on a brain histaminergic system and to determine whether under some conditions these drugs are reversible or can be prevented.

Histamine concentrations were determined in various brain regions of rats by an enzymatic-isotopic assay immediately after sacrifice. The activity of the HA metabolizing enzyme, HMT, was determined in those animals showing considerable changes in their histamine levels following chronic morphine treatment or chronic morphine treatment followed by withdrawal. Spontaneous locomotor activity measurements were performed on the rats prior to sacrifice in order to correlate the behavioral manifestations of the various treatments with the biochemical changes observed. Growth records were

maintained on all chronically treated animals to ensure that grossly inadequate nutritional intake was not a contributing factor to the biochemical changes discovered.

The current studies have shown that chronic but not acute treatments with morphine resulted in significant decreases in endogenous brain HA concentrations (361, 396, 397). These changes were exacerbated by withdrawal of the animals from morphine whether precipitated by naloxone administration or by cessation of treatment. The brain region most influenced by these treatments was the hypothalamus, the region with the highest endogenous HA levels. The decreases in HA in the hypothalamus and cerebral cortex induced by chronic morphine administration were restored to normal values within one week of the end of treatment. Midbrain HA levels were slower to recover and remained slightly reduced even 3 weeks after the last morphine injection.

The development of significant cross tolerance to methadone was demonstrated by the absence of withdrawal symptoms or severe respiratory depression and death following substitution with methadone for 2 days following chronic morphine treatment.

The activity of the histamine methyltransferase was found to be unaltered following either chronic morphine treatment or chronic morphine treatment followed by withdrawal for 2 days. Similar treatments have been reported to result in increases in the HA synthesizing enzyme HD.

In experiments designed to prevent the decreases in endogenous brain HA observed following chronic morphine administration, it has been found that simultaneous administration of histidine with the daily injections of morphine prevented the decreases seen after morphine treatment alone.

Histamine formation in rats treated chronically with morphine was found comparable to that observed in saline treated controls. The reduced levels of HA observed in animals chronically treated with morphine were raised to normal control values within 1 hour following acute administration of histidine. The hypothalamic level returned to the reduced value within 18 hours, however the midbrain and cerebrocortical HA levels did not fully return to the reduced values. These changes may reflect the slow turnover and storage of HA in mast cells. HA formation was found to be enhanced in rats treated chronically with histidine, perhaps indicating induction of the enzyme HD.

Spontaneous locomotor activity recordings for all animals prior to sacrifice clearly indicated the overt behavioral effects of the drugs administered. These studies demonstrated that the psychoactive agents used actually reached the central nervous system to produce their behavioral effects however the changes in activity following acute treatments were not reflected in changes in endogenous HA levels. Acute morphine or methadone resulted in significant depression of the rats' activities. Naloxone-precipitated withdrawal in morphine dependent rats induced a significant reduction in SLA when compared to saline treated controls receiving the same dose of naloxone. The naloxone administration itself produced no apparent alteration in SLA of the rats. Chronic treatments with morphine followed by withdrawal of morphine or precipitation of the withdrawal syndrome by naloxone administration did produce significant decreases in activity and in endogenous HA concentrations. These results support the argument for a correlation between the presence of free brain HA and sedation.

Chronic histidine administration along with morphine treatment prevented the significant decreases in spontaneous locomotor activity noted when rats were withdrawn for 2 days following chronic morphine treatment. The fact that withdrawal from histidine itself did not cause any changes in the activity of the rats substantiates the behavioral improvement as assessed by spontaneous locomotor activities in these animals.

Studies on the SLA of rats administered histidine alone compared with the activity of rats treated simultaneously with morphine and histidine indicated the possibility of some impairment of access of histidine to its normally sedating sites of action.

Morphine treated rats were found to gain weight more slowly than their saline-treated controls which is considered to be due to their generally reduced locomotor activity but this may indicate some involvement of HA in feeding behavior in analogy to the roles proposed for several other putative neurotransmitters. The weight records provided evidence of growth which eliminated the necessity of considering the influence of protein deficiency on brain HA levels.

The changes in endogenous brain HA concentrations seen after acute and chronic pentazocine administration parallelled those observed after similar morphine treatments. The activity measurements following acute pentazocine treatment show a distinctly different pattern from that seen following acute morphine treatment. Rats treated acutely with pentazocine displayed somewhat reduced SLA when compared to saline-treated controls within the first 5 minute recording period. This constant level of activity continued throughout the recording period in contrast to the severe depression of activity noted following

acute morphine treatment.

Chronic pentazocine administration did not appear to induce any significant alterations in the activity of the experimental rats at the time investigated which was comparable to the effects of chronic morphine treatment at the same time. In addition, chronic pentazocine treatment did not influence the weight gain of the rats in these studies.

Acute and chronic studies with mandrax, methaqualone, and diphenhydramine showed the hypothalamus to be the area most vulnerable for inducing decreases in brain HA. Upon cessation of chronic treatment with mandrax, the decreases in brain HA noted were returned to normal values within 2 days.

The SLA of the rats receiving acute treatments of mandrax, methaqualone or diphenhydramine were all significantly depressed when compared to saline-treated controls. Chronic mandrax treatment failed to produce any significant alterations in activity of the rats used in these studies. This demonstrated the lesser stressful effects of mandrax withdrawal when compared to narcotic withdrawal. The body weight records of rats treated chronically with mandrax showed the treated rats gaining weight more slowly than controls.

Acute amphetamine administration did not produce any significant changes in brain HA levels in the brain regions investigated but it did produce significant dose-dependent elevations of the SLA of these animals.

Chronic amphetamine administration resulted in significantly decreased hypothalamic and significantly increased midbrain and cerebro-cortical HA levels. The activity of these rats appeared normal when determined 18 hours after the last amphetamine administration.

Chronic treatment with increasing doses of amphetamine produced negligible effects on the weight gain of the rats.

Studies using either acute or chronic tetrahydrocannabinol treatments resulted in no significant changes in brain HA in any of the brain regions investigated.

PROPOSALS FOR FUTURE RESEARCH

In spite of extensive studies on the involvement of several biogenic amines in brain function, until recently there has been very little information available concerning the possible roles of brain HA. It seems now to be accepted that no single neurotransmitter but rather an interaction between several of them may better explain the complicated processes linking receptor interactions with them.

We need to know the changes occurring in the content, synthesis, and/or release of various transmitters simultaneously. Techniques must be developed to determine whether there are changes in metabolic pathways reflecting a shift from intra- to interneuronal metabolism and also what changes are occurring in pre- and postsynaptic receptors to affect sensitivity. In addition, the constraints imposed on these mechanisms by genetic and environmental factors should be taken into consideration. The assumption that identical monoaminergic systems to those described in the rat are also present in higher species including primates, must be approached with some caution. Further studies in other species and brain regions should provide invaluable data on the pharmacological profiles for HA receptors in the mammalian CNS.

Considering the striking differences in HA levels noted in studies using CNS depressants versus CNS stimulants, it would be of considerable value to further investigate the time course relationships involved. The effects of administration of chronic amphetamine followed by withdrawal and subsequent recovery times bears investigation as well as the effects on the synthesizing and metabolizing enzymes. Whether

these changes are the cause or consequence of changes in other biogenic amines at other sites remains to be elucidated. Future studies must focus on the dynamic changes associated with the regulatory mechanisms of the transmitters.

The possibility that some of the changes in HA levels may result from stress could well be investigated by monitoring plasma corticosteroids at various times during and after treatments. Preliminary results (Ontario Mental Health Final Report of R. Henwood) have indicated stress-induced changes in brain HA concentrations.

Further studies at shorter and longer time intervals following treatment with the various drugs of interest may reveal changes in brain HA occurring at these times. Since the acute studies reported herein induced significant behavioral changes but not changes in brain HA, it is unlikely that smaller doses would cause changes. It is also possible that using larger doses of the drugs currently employed may lead to changes in brain HA but these changes may only reflect toxicologic effects.

The effects of drugs used in the treatment of narcotic dependence such as methadone or cyclazocine on brain HA should be studied. In addition, the effects of other potent drugs with considerable dependence liability with CNS stimulant properties, such as cocaine, could be compared using similar experimental paradigms.

The work to elucidate the functional roles of HA in the mammalian CNS has just begun. Former speculations are now being researched for their factual basis and I believe that several of the findings reported in this thesis have opened promising new avenues for further work.

REFERENCES

1. J. P. Green
Uptake and Binding of Histamine.
Fed. Proc. 26, 211-218 (1967).

2. J. P. Green
Pp 125-145 in: Mechanism of Release of Biogenic Amines.
Edited by U. S. Von Euler, S. Rosell, and B. Uvnas.
Pergamon Press, London (1966).

3. F. Holmes, F. Jones
Metal Complexes of Histamine and Some Structural Analogs. Part I.
J. Chem. Soc. Perkin 1, Part II, 2398-2401 (1960).

4. M. Levy
A Note on the Titration Constants of Imidazole Derivatives.
J. Biol. Chem. 109, 361-363 (1935).

5. M. Roche, E. Silva
Influence of pH on the Interaction of Histamine with its Receptors in the Guinea Pig Ileum.
Arch. Int. Pharmacodyn. 128, 355-374 (1960).

6. E. J. Ariens, A. M. Simonis
pH and Drug Action
Arch. Int. Pharmacodyn. 141, 309-330 (1963).

7. A. H. Amin, T. B. B. Crawford, J. H. Gaddum
The Distribution of Substance P and Serotonin in the Central Nervous System of the Dog.
J. Physiol. 126, 596-618 (1954).

8. A. Bertler, E. Rosengren
Occurrence and Distribution of Catecholamines in Brain.
Acta. Physiol. Scand. 47, 350-361 (1959).

9. G. Bertaccini
Pp 305-306 Discussion in: Regional Neurochemistry.
Edited by S. S. Kety and J. Elkes.
Pergamon Press, Oxford (1961).

10. J. P. Green
Pp 221-250 in: Handbook of Neurochemistry. IV. Ch. 10.
Histamine. Editied by A. Lajtha.
Plenum Press, New York (1970).

11. H. M. Adam, H. K. A. Hye
Concentration of Histamine in Different Parts of the Brain and Hypophysis of Cat and Its Modification by Drugs.
Br. J. Pharmacol. 28, 137-152 (1966).

12. H. M. Adam
Histamine in the Central Nervous System and Hypophisis of the Dog.
Pp. 293-306 in: Regional Neurochemistry. Edited by S. S. Kety and J. Elkes.
Pergamon Press, London (1961).

13. S. H. Snyder, J. R. Baldessarini, J. Axelrod
A Sensitive and Specific Enzymatic Isotopic Assay for Tissue Histamine.
J. Pharmacol. Exp. Ther. 153, 544-549 (1966).

14. S. H. Snyder, K. M. Taylor
Histamine in the Brain: a Neurotransmitter?
Pp 43-74 in: Perspectives in Neuropharmacology. Edited by S. H. Snyder.
Oxford University Press, New York (1972).

15. I. A. Michaelson, P. Z. Coffman
An Improved Ion-Exchange Purification Procedure for the Fluorometric Assay of Histamine.
Anal. Biochem. 27, 257-261 (1969).

16. E. A. Carlini, J. P. Green
The Subcellular Distribution of Histamine, Slow-Reacting Substance and 5-Hydroxytryptamine in the Brain of the Rat.
Br. J. Pharmacol. 20, 264-277 (1963).

17. L. A. Loizou
The Postnatal Ontogeny of Monoamine-Containing Neurons in the Central Nervous System of the Albino Rat.
Brain Res. 40, 395-418 (1972).

18. M. J. Kuhar, K. M. Taylor, S. H. Snyder
The Subcellular Localization of Histamine and Histamine Methyltransferase in Rat Brain.
J. Neurochem. 18, 1515-1527 (1971).

19. S. H. Snyder, B. Brown, M. J. Kuhar
The Subsynaptosomal Localization of Histamine, Histidine Decarboxylase and Histamine Methyltransferase in Rat Hypothalamus.
J. Neurochem. 23, 37-45 (1974).

20. J. C. Schwartz, G. Barbin, S. Bischoff, M. Garbarg, H. Pollard, C. Rose, M. Verdiere.
Histamine in an Ascending Pathway in the Brain: Localization, Turnover and Effects of Psychopharmacological Agents. Pp 575-583 in: Proceedings of the IX Congress of the Collegium Internationale Neuropsychopharmacologicum (Paris) 1974. Edited by J. R. Boissier, H. Hippius and P. Pichot. American Elsevier Pub. Co. Inc., New York (1975).

21. E. A. Carlini, J. P. Green
The Measurement of Histamine in Brain and its Distribution.
Biochem. Pharmacol. 12, 1448-1449 (1963).

22. A. Michaelson, P. Z. Coffman
The Subcellular Localization of Histamine in Guinea Pig Brain: A Re-evaluation.
Biochem. Pharmacol. 16, 2085-2090 (1967).

23. Y. Z. Aboy, H. M. Adam, W. R. G. Stephen
Concentration of Histamine in Different Parts of the Brain and Hypophysis of Rabbit: Effect of Treatment with Histidine, Certain other Amino Acids and Histamine.
Br. J. Pharmacol. 48, 577-589 (1973).

24. A. L. Ronnberg, J. C. Schwartz
Repartition Regional de l'Histamine dans le Cerveau de Rat.
C. R. Acad. Sci. (D) (Paris) 268, 2376-2379 (1968).

25. C. Atack, A. Carlsson
In Vitro Release of Endogenous Histamine. Together with Noradrenaline and 5-Hydroxytryptamine, from Slices of Mouse Cerebral Hemispheres.
J. Pharm. Pharmacol. 24, 990-991 (1972).

26. K. M. Taylor, S. H. Snyder
The Release of Histamine from Tissue Slices of Rat Hypothalamus.
J. Neurochem. 21, 1215-1223 (1973).

27. M. Verdiere, C. Rose, J. C. Schwartz
Synthesis and Release of ^{3}H-Histamine in Slices from Rat Brain.
Agents Actions 4, 184-185 (1974).

28. B. Uvnas
The Molecular Basis for the Storage and Release of Histamine in Rat Mast Cell Granules.
Life Sci. 14, 2355-2366 (1974).

29. M. A. M. Ibrahim
The Mast Cells of the Mammalian Central Nervous System. Part 1. Morphology, Distribution and Histochemistry.
J. Neurol. Sci. 21, 431-478 (1974).

30. J. J. Dropp
Mast Cells in the Central Nervous System of Several Rodents.
Anat. Rec. 174, 227-237 (1972).

31. A. L. Ronnberg, L. Edvinsson, L. J. Larsson, K. C. Neilson, C. Owman
Regional Variation in the Presence of Mast Cells in the Mammalian Brain.
Agents Actions 3, 191 (1973).

32. T. M. El-Ackad, M. J. Brody
Fluorescence Histochemical Localization of Non-mast Cell Histamine.
Pp 551-559 in: Proceedings of the IX Congress of the Collegium Internationale Neuropsychopharmacologicum (Paris) 1974. Edited by J. R. Bossier, H. Hippius and P. Pichot. American Elsevier Publ. Co. Inc., New York (1975).

33. M. P. Martres, M. Baudry, J. C. Schwartz
Histamine Synthesis in the Developing Rat Brain: Evidence for a Multiple Compartmentation.
Brain Res. 83, 261-275 (1975).

34. G. R. Breese, T. D. Traylor
Developmental Characteristics of Brain Catecholamines and Tyrosine Hydroxylase in the Rat: Effects of 6-Hydroxydopamine.
Br. J. Pharmacol. 44, 210-222 (1972).

35. H. C. Agrawal, S. N. Glisson, W. A. Himwich
Changes in Monoamines in Rat Brain during Postnatal Ontogeny.
Biochem. Biophys. Acta 130, 511-513 (1966).

36. J. T. Coyle, J. Axelrod
Development of the Uptake and Storage of L-(^{3}H)-Norepinephrine in the Rat Brain.
J. Neurochem. 18, 2061-2075 (1971).

37. J. C. Schwartz, C. Lampart, C. Rose, M. C. Rehault, S. Bischoff, H. Pollard
Histamine and Spermidine Content in Brain during Development.
J. Neurochem. 18, 1787-1789 (1971).

38. L. A. Pearce, S. M. Schanberg
Histamine and Spermidine Content in Brain during Development.
Science 166, 1301-1303 (1969).

39. T. White
Formation and Catabolism of Histamine in Cat Brain In Vivo.
J. Physiol. 152, 299-308 (1960).

40. H. M. Adam, H. K. A. Hye, N. G. Watson
Studies on Uptake and Formation of Histamine by Hypophysis and Hypothalamus in the Cat.
J. Physiol. 175, 70-71 (1964).

41. T. White
Formation and Catabolism of Histamine in Brain Tissue In Vitro.
J. Physiol. 149, 34-42 (1959).

42. S. Lindberg, S. E. Lindell, H. Westling
Formation and Inactivation of Histamine by Human Fetal Tissue In Vitro.
Acta Obstet. Gynecol. Scand. 42, Suppl. 1, 49-58 (1963).

43. S. E. Lindell, K. Nilsson, R. W. Schayer, H. Westling
Metabolism of Injected ^{14}C-Histamine in Pregnant Women.
J. Physiol. 143, 62 (1958).

44. J. C. Schwartz, A. L. Ronnberg, Y. Cohen, G. Valette
Histamine Formation in Rat Stomach: Study of Regulation Mechanisms.
Eur. J. Pharmacol. 5, 272-278 (1969).

45. S. H. Snyder, J. Axelrod, H. Bauer
The Fate of ^{14}C-Histamine in Animal Tissues.
J. Pharmacol. Exp. Ther. 144, 373-379 (1964).

46. R. W. Schayer
Histamine Metabolism in the Mammalian Organism. Mechanism of Hypersensitivity. Pp 227-233 in: Henry Ford Hospital Intern. Symposium. Little, Brown, Boston (1959).

47. J. C. Schwartz, C. Lampart, C. Rose
Properties and Regional Distribution of Histidine Decarboxylase in Rat Brain.
J. Neurochem. 17, 1527-1534 (1970).

48. C. Maslinski
Histamine and Its Metabolism in Mammals. Part 1. Chemistry and Formation of Histamine.
Agents Actions 5, 89-107 (1975).

49. K. M. Taylor, S. H. Snyder
Brain Histamine: Rapid Apparent Turnover Altered by Restraint and Cold Stress.
Science 172, 1037-1039 (1971).

50. R. W. Schayer, M. A. Reilly
In Vivo Formation and Catabolism of ^{14}C-Histamine in Mouse Brain.
J. Neurochem. 17, 1649-1655 (1970).

51. G. Levi, J. Kandera, A. Lajtha
Control of Cerebral Metabolite Levels I. Amino Acid Uptake and Levels in Various Species.
Arch. Biochem. Biophys. 119, 303-311 (1967).

52. J. C. Schwartz, C. Lampart, C. Rose
Histamine Formation in the Rat Brain In Vivo: Effects of Histidine Loads.
J. Neurochem. 19, 801-810 (1972).

53. K. M. Taylor, S. H. Snyder
Dynamics of the Regulation of Histamine Levels in Mouse Brain.
J. Neurochem. 19, 341-354 (1972).

54. K. M. Taylor, S. H. Snyder
Isotopic Microassay of Histamine, Histidine, Histidine-Decarboxylase and Histamine Methyltransferase on Brain Tissue.
J. Neurochem. 19, 1343-1358 (1972).

55. M. K. Menon, D. Aures, W. G. Clark
Effect of Thiazol-4-ylmethoxyamine, an Inhibitor of Histamine Biosynthesis.
Life Sci. 10, 1097-1109 (1971).

56. R. W. Schayer
Evidence of a Specific Function for Histamine.
J. Pharmacol. Suppl. 5, 71-72 (1974).

57. D. D. Brown, R. Tomchick, J. Axelrod
The Distribution and Properties of a Histamine-Methylating Enzyme.
J. Biol. Chem. 234, 2948-2950 (1959).

58. C. Maslinski
Histamine and its Metabolism in Mammals II. Catabolism of Histamine and Histamine Liberation.
Agents Actions 5, 183-225 (1975).

59. R. S. Alphin, T. M. Lin
Effect of Histamine Metabolites and Histaminase Inhibitor on Gastric Secretion in Dog.
Fed. Proc. 19, 192 (1960).

60. G. C. Cotzias, V. P. Dole
The Activity of Histaminase in Tissues.
J. Biol. Chem. 196, 235-242 (1952).

61. R. W. Schayer, M. A. Reilly
Metabolism of ^{14}C-Histamine in Brain.
J. Pharmacol. Exp. Ther. 187, 34-39 (1973).

62. D. H. Fram, J. P. Green
Methylhistamine in Urine and Brain.
Fed. Proc. 24, 778 (1965).

63. J. P. Green, D. H. Fram
Methylhistamine in Urine and Brain.
Fed. Proc. 25, 559 (1966).

64. T. White
Histamine and Methylhistamine in Cat Brain and Other Tissues.
Br. J. Pharmacol. 26, 494-501 (1966).

65. M. A. Reilly, R. W. Schayer
Histamine Formation and Catabolism in the Mouse Brain.
Fed. Proc. 29, Abs. 2083 (1970).

66. J. C. Schwartz, H. Pollard, S. Bischoff, M. C. Rehault, M. Verdiere-Sahuque
Catabolism of ^{3}H-Histamine in the Rat Brain after Intracisternal Administration.
Eur. J. Pharmacol. 16, 326-335 (1971).

67. D. H. Fram, J. P. Green
Methylhistamine in Guinea-Pig Brain.
J. Neurochem. 15, 597-602 (1968).

68. M. A. Reilly, R. W. Schayer
In Vivo Studies on Histamine Catabolism and Its Inhibition.
Br. J. Pharmacol. 38, 478-489 (1970).

69. M. A. Reilly, R. W. Schayer
Techniques for Measuring Histamine Formation in Mice.
Br. J. Pharmacol. 42, 375-282 (1971).

70. W. P. Burkard, K. F. Gey, A. Pletscher
Uber das Vorkommen von Diaminoxydase im Gehirn von Vertebraten.
Helv. Physiol. Pharmacol. Acta 19, C61-63 (1961).

71. W. P. Burkard, K. F. Gey, A. Pletscher
Diamine Oxidase in the Brain of Vertebrates.
J. Neurochem. 10, 183-186 (1963).

72. S. A. Karjala, B. W. Turnquest
The Characterization of the Two Methylimidazolacetic Acids as Radioactive Histamine Metabolites.
J. Amer. Chem. Soc. 77, 6358-6359 (1955).

73. R. W. Schayer, M. A. Reilly
Histamine Metabolism in Guinea Pigs, Rats and Mice.
Eur. J. Pharmacol. 25, 101-107 (1974).

74. J. W. Fowler, F. C. Lowell
The Accumulation of Eosinophils as an Allergic Response to Allergen Applied to the Denuded Skin Surface.
J. Allergy Clin. Immunol. 37, 19-28 (1966).

75. R. W. Schayer
Catabolism of Physiological Quantities of Histamine in Vivo.
Physiol. Rev 39, 116-126 (1959).

76. J. C. Schwartz, H. Cailters, C. Rose
An Anternative Route for Biosynthesis of Methylhistamine: In Vitro and In Vivo Formation Through Decarboxylation of L-3-Methylhistidine.
Experientia 28, 904-905 (1972).

77. S. H. Snyder, A. B. Young, J. P. Bennett, A. H. Mulder
Synaptic Biochemistry of Amino Acids.
Fed. Proc. 32, 2039-2047 (1973).

78. T. Hokfelt
Morphological Contributions to Monoamine Pharmacology.
Fed. Proc. 33, 2177-2186 (1974).

79. K. Dismukes, S. H. Snyder
Histamine Turnover in Rat Brain.
Brain Res. 78, 467-481 (1974).

80. K. Krnjevic, J. W. Phillis
Actions of Certain Amines on Cerebral Cortical Neurones.
Br. J. Pharmacol. Chemother. 20, 471-490 (1963).

81. H. L. Haas
Histamine: Action on Single Hypothalamic Neurones.
Brain Res. 76, 363-366 (1974).

82. L. Hosli, H. C. Haas
Effects of Histamine, Histidine, and Imidazole Acetic Acid on Neurones of the Medulla Oblongata of the Cat.
Experientia 27, 1311-1312 (1971).

83. L. Hosli, H. L. Haas, E. G. Anderson
The Action of Histamine and Metabolites on Single Neurones of the Mammalian Central Nervous System.
J. Pharmacol. 5 (Suppl. 1), 72-73 (1974).

84. E. G. Anderson, H. L. Haas, L. Hosli
Comparison of Effects of Noradrenaline and Histamine with Cyclic AMP on Brain Stem Neurones.
Brain Res. 49, 471-475 (1973).

85. J. W. Black, W. A. M. Duncan, C. J. Durant, G. R. Ganellin, E. M. Parsons
Definition and Antagonism of Histamine H_2-Receptors.
Nature 236, 385-390 (1972).

86. J. W. Phyllis, A. K. Tebecis, D. H. York
Depression of Spinal Motoneurones by Noradrenaline, 5-Hydroxytryptamine and Histamine.
Eur. J. Pharmacol. 4, 471-475 (1968).

87. A. Galindo, K. Krnjevic, S. Schwartz
Micro-Iontophorotic Studies on Neurons in the Cuneate Nucleus.
J. Physiol. (London) 192, 359-377 (1967).

88. H. L. Haas, E. G. Anderson, L. Hosli
Histamine and Metabolites; Their Effects and Interactions with Convulsants on Brain Stem Neurons.
Brain Res. 51, 269-278 (1973).

89. M. Verdiere, C. Rose, J. C. Schwartz
Synthesis and Release of Histamine Studied on Slices from Rat Hypothalamus.
Eur. J. Pharmacol. 34, 157-168 (1975).

90. G. R. Siggins, B. J. Hoffer, F. E. Bloom
Studies on Norepinephrine-Containing Afferents to Purkinje Cells of Rat Cerebellum. (3) Evidence for Mediation of Norepinephrine Effects by Cyclic 3',5'-Adenosine Monophosphate.
Brain Res. 25, 535-553 (1971).

91. J. W. Phillis, A. K. Tebecis, D. H. York
Histamine and Some Antihistamines: Their Actions on Cerebral Cortical Neurons.
Br. J. Pharnacol. 33, 426-440 (1968).

92. H. L. Haas, P. Wolf, J. C. Nussbaumer
Histamine: Action on Supraoptic and Other Hypothalamic Neurones of the Cat.
Brain Res. 88, 166-170 (1975).

93. L. P. Renaud
Histamine Microiontophoresis on Identified Hypothalamic Neurons: 3 Patterns of Response in the Ventromedial Nucleus of the Rat.
Brain Res. 115, 339-344 (1976).

94. H. L. Haas, P. Wolf
Central Actions of Histamine: Microelectrophoretic Studies.
Brain Res. 122, 269-279 (1977).

95. B. S. R. Sastry, J. W. Phillis
Depression of Rat Cerebral Cortical Neurons by H_1 and H_2 Histamine Receptor Agonists.
Eur. J. Pharmacol. 38, 269-273 (1976).

96. H. L. Haas, U. M. Bucher
Histamine H_2-receptors on Single Neurones.
Nature 255, 634-635 (1975).

97. J. W. Phillis, G. K. Kostopoulos, A. Odutola
On the Specificity of Histamine H_2-receptor Antagonists in the Cat Cerebral Cortex.
Can. J. Physiol. Parmacol. 53, 1205-1209 (1975).

98. J. Satayavivad, E. B. Kirsten
Iontophoretic Studies of Histamine and Histamine Antagonists in the Feline Vestibular Nuclei.
Eur. J. Pharmacol. 41, 17-26 (1977).

99. M. Garbarg, M. S. Krishnamoorthy, J. Feger, J. S. Schwartz
Effects of Mesencephalic and Hypothalamic Lesions on Histamine Levels in Rat Brain.
Brain Res. 50, 361-367 (1973).

100. M. S. Krishnamoorthy, M. Garbarg, J. Feger, J. C. Schwartz
Augmentation in Hypothalamic Histamine Induced by Diencephalic Lesions in Rats.
Agents Actions 3, 181 (1973).

101. K. Dismukes, M. J. Kuhar, S. H. Snyder
Brain Histamine Alterations After Hypothalamic Isolation.
Brain. Res. 78, 144-151 (1974).

102. M. Garbarg, G. Barbin, S. Bischoff, H. Pollard, J. C. Schwartz
Evidence for a Specific Decarboxylase Involved in Histamine Synthesis in an Ascending Pathway in Rat Brain.
Agents Actions 4(3), 181-182 (1974).

103. M. Garbarg, G. Barbin, J. Feger, J. C. Schwartz
Histaminergic Pathway in Rat Brain Evidenced by Lesions of the Medial Forebrain Bundle.
Science 186, 833-834 (1974).

104. G. Barbin, J. Hirsch, M. Garbarg, J. C. Schwartz
Decrease in Histamine Content and Decarboxylase Activities in an Isolated Area of the Cerebral Cortex of the Cat.
Brain Res. 92, 170-174 (1975).

105. J. C. Schwartz
Histamine as a Transmitter in Brain.
Life Sci. 17, 503-518 (1975).

106. B. S. R. Sastry, J. W. Phillis
Evidence for an Inhibitory Histaminergic Pathway to Rat Cerebral Cortex.
The Pharmacologist 18, 204 (1976).

107. H. Pollard, S. Bischoff, J. C. Schwartz
Increased Synthesis and Release of ^{3}H-Histamine in Rat Brain by Reserpine.
Eur. J. Pharmacol. 24, 399-401 (1973).

108. M. Huang, H. Shimizu, J. W. Daly
Regulation of Adenosine 3',5'-Phosphate Formation in Cerebral Cortical Slices Interaction Among Norepinephrine, Histamine, Serotonin.
Mol. Pharmacol. 7, 155-162 (1970).

109. P. Wolf, M. Monnier
Electroencephalographic, Behavioral and Visceral Effects of Intraventricular Infusion of Histamine in the Rabbit.
Agents Actions 3, 196 (1973).

110. M. Monnier, R. Sauer, A. M. Hatt
The Activating Effect of Histamine on the Central Nervous System.
Pp. 265-305 (Vol. 12) in: Int. Rev. Neurobiol. Edited by C. C. Pfeiffer and J. R. Smythies.
Academic Press, New York (1970).

111. H. Pollard, S. Bischoff, J. C. Schwartz
Turnover of Histamine in Rat Brain and Its Decrease Under Barbiturate Anesthesis.
J. Pharmacol. Exp. Ther. 190, 88-99 (1974).

112. H. Pollard, S. Bischoff, J. C. Schwartz
Decreased Histamine Synthesis in the Rat Brain by Hypnotics and Anesthetics.
J. Pharm. Pharmacol. 25, 920-922 (1973).

113. G. C. Palmer, F. Sulser, G. A. Robinson
Effects of Neurohumoral and Adrenergic Agents on Cyclic AMP Levels in Various Areas of the Rat Brain In Vitro.
Neuropharmacol. 12, 327-337 (1973).

114. K. von Hugen, S. Roberts, D. F. Hill
Serotonin-Sensitive Adenylate Cyclase Activity in Immature Rat Brain.
Brain Res. 84, 257-267 (1975).

115. J. Schultz
Inhibition of Phosphodiesterase Activity in Brain Cortical Slices from Guinea Pig and Rat.
Res. Commun. Chem. Pathol. Pharmacol. 6, 341-355 (1974).

116. S. Kakiuchi, T. W. Rall
The Influence of Chemical Agents on the Accumulation of Adenosine 3',5'-Phosphate in Slices of Rabbit Cerebellum.
Mol. Pharmacol. 4, 267-278 (1968).

117. S. Kakiuchi, T. W. Rall
Studies on Adenosine 3',5'-Phosphate in Rabbit Cerebral Cortex.
Mol. Pharmacol. 4, 379-388 (1968).

118. J. W. Daly
Pp. 47-130 in: Handbook of Psychopharmacology (Vol. 5).
Edited by L. L. Iverson, S. D. Iverson and S. H. Snyder.
Plenum Publishing Co., New York (1975).

119. I. K. Ho, H. H. Loh, E. L. Way
Effects of Cyclic 3',5'-Adenosine Monophosphate on Morphine Tolerance and Physical Dependence.
J. Pharmacol. Exp. Ther. 185, 347-357 (1973).

120. R. Gullis, J. Traber, B. Hamprecht
Morphine Elevates Levels of Cyclic GMP in a Neurobastoma and Glioma Hybrid Cell Line.
Nature 253, 563-565 (1975).

121. G. W. Pasternak, S. H. Snyder
Identification of Novel High Affinity Opiate Receptor Binding in Rat Brain.
Nature 256, 57-59 (1975).

122. E. Costa, A. Carenzi, A. Guidotti, A. Levuelta
Pp. 1003-1010 in: Frontiers in Catecholamine Research.
Edited by E. Usdin, S. Snyder.
Pergamon Press, New York (1973).

123. K. A. Bonnet
Regional Alterations in Cyclic Nucleotide Levels with Acute and Chronic Morphine Treatment.
Life Sci. 16, 1877-1882 (1975).

124. C. Racagni, G. Zsilla, A. Guidotti, E. Costa
Accumulation of cGMP in Striatum of Rats Injected with Narcotic Analgesics: Antagonism by Naltrexone.
J. Pharm. Pharmacol. 28, 258-260 (1976).

125. G. C. Palmer, M. J. Schmidt, G. A. Robinson
Development and Characteristics of the Histamine-Induced Accumulation of Cyclic AMP in the Rabbit Cerebral Cortex.
J. Neurochem. 19, 2251-2256 (1972).

126. M. Chasin, F. Mamrak. S. Samaniego, S. M. Hess
Characteristics of the Catecholamine and Histamine Receptor Sites Mediating Accumulation of Cyclic Adenosine 3',5'-Monophosphate in Guinea Pig Brain.
J. Neurochem. 21, 1415-1427 (1973).

127. M. Baudry, M. P. Martres, J. C. Schwartz
H_1 and H_2 Receptors in the Histamine-Induced Accumulation of Cyclic AMP in Guinea Pig Brain Slices.
Nature 253, 362-364 (1975).

128. H. P. Karppanen, E. Westerman
Increased Production of Cyclic AMP in Gastric Tissue by Stimulation of Histamine 2 (H_2)-Receptors.
Naunyn-Schmied. Arch. Pharmacol. 279, 83-87 (1973).

129. G. Poch, W. R. Kukovetz, N. Scholy
Specific Inhibition by Burimamide of Histamine Effects on Myocardial Contraction and Cyclic AMP.
Naunym-Schmied. Arch. Pharmacol. 280, 223-228 (1973).

130. K. Dismukes. M. Rogers, J. W. Daly
Cyclic Adenosine 3',5'-Monophosphate Formation in Guinea Pig Brain Slices: Effects of H_1 and H_2 Histaminergic Agonists.
J. Neurochem. 26, 785 (1976).

131. T. O. Doussa, C. F. Code
Effect of Histamine and Its Methyl Derivatives on Cyclic AMP Metabolism in Gastric Mucosa and Its Blockade by an H_2 Receptor Antagonist.
J. Clin. Invest. 53, 334-337 (1974).

132. J. H. McNeill, S. C. Verma
Blockade by Burimamide of the Effects of Histamine and Histamine Analogs on Cardiac Contractility, Phosphorylase Activation and Cyclic Adenosine Monophosphate.
J. Pharmacol. Exp. Ther. 188, 180-188 (1974).

133. J. Schultz, J. W. Daly
Accumulation of Cyclic Adenosine 3',5'-Monophosphate in Cerebral Cortical Slices from Rat and Mouse: Stimulatory Effect of Alpha and Beta Adrenergic Agents and Adenosine.
J. Neurochem. 21, 1319-1326 (1973).

134. P. Skolnick, M. Huang, J. Daly, B. Hoffer
Accumulation of Adenosine 3',5'-Monophosphate in Incubated Slices from Discrete Regions of Squirrel Monkey Cerebral Cortex. Effect of NE, 5HT and Adenosine.
J. Neurochem. 21, 237-240 (1973).

135. R. K. Dismukes, C. Ghosh, C. R. Creveling, J. W. Daly
Altered Responsiveness of Adenosine 3',5'-Monophosphate Generating Systems in Rat Cortical Slices after Lesions of the Medial Forebrain Bundle.
Exp. Neurol. 49, 725-735 (1975).

136. M. Huang, A. K. S. Ho, J. W. Daly
Stimulatory Effect of Alpha and Beta Adrenergic Agents After Treatment with 6-Hydroxydopamine, 2,3,5-Trihydroxyphenethylamine and Dihydroxytryptamines.
Mol. Pharmacol. 9, 711-717 (1973).

137. H. L. Haas, L. Hosli, E. G. Anderson, P. Wolf
Action of Histamine and Its Metabolites on Single Neurons of the Mammalian Central Nervous System. Pp. 589-596 in: Proceedings of the IX Congress of the Collegium Internationale Neurosychopharmacologicum (Paris), 1974). Edited by J. R. Boissier, H. Hippius and P. Pichot.
American Elsevier Publ. Co. Inc., New York (1975).

138. R. B. Clark, J. P. Perkins
Regulation of Adenosine 3',5'-Cyclic Monophosphate Concentration in Cultured Human Astrocytoma Cells by Catecholamines and Histamine.
Proc. Nat. Acad. Sci. 68, 2757-2760 (1971) (U.S.A.)

139. K. Mrsulja
The Influence of Anistine on Glycogenolytic Effect of Some Biogenic Amines in Rat Brain Slices.
Experientia 29, 76-77 (1973).

140. J. W. Daly
The Nature of Receptors Regulating the Formation of Cyclic AMP in Brain Tissue.
Life Sci. 18, 1349-1358 (1976).

141. J. C. Foreman, J. L. Mongar, B. D. Gomperts, L. G. Garland
A Possible Role for Cyclic AMP in the Regulation of Histamine Secretion and the Action of Cromoglycate.
Biochem. Pharmacol. 24, 538-540 (1975).

142. L. P. Renaud
Response of Identified Ventromedial Hypothalamic Nucleus Neurons to Putative Neurotransmitters Applied by Microiontophorsis.
Br. J. Pharmacol. 55, 227P-278P (1976).

143. R. George
Hypothalamus: Anterior Pituitary Gland. P. 295 in: Narcotic Drugs. Biochem. Pharmacology. Edited by D. H. Clouet.
Plenum Press, New York (1971).

144. C. C. D. Shute, P. R. Lewis
Cholinergic and Monoaminergic Pathways in the Hypothalamus.
Br. Med. Bull. 22, 221-226 (1966).

145. K. Fuxe, T. Hokfelt
Pp. 47-96 in: Frontiers in Neuroendocrinology. Catecholamines in the Hypothalamus and the Pituitary Gland. Edited by W. F. Ganong, L. Martini.
Oxford University Press (1969).

146. E. L. Bliss, J. Ailion, J. Zwanziger
Metabolism of Norepinephrine, Serotonin and Dopamine in Rat Brain with Stress.
J. Pharmacol. Exp. Ther. 164, 122-134 (1968).

147. H. Corrodi, K. Fuxe, T. Hokfelt
The Effect of Immobilization Stress on the Activity of Central Monoamine Neurons.
Life Sci. 7, 107-112 (1968).

148. A. M. Thierry, M. Fekete, J. Glowinski
Effects of Stress on the Metabolism of Noradrenaline, Dopamine, and Serotonin (5HT) in the Central Nervous System of the Rat.
(II) Modifications of Serotonin Metabolism.
Eur. J. Pharmacol. 4, 384-389 (1968).

149. A. De Schaepdryver, P. Preziosi, U. Scapagnini Brain Monoamines and Adrenocortical Activation.
Br. J. Pharmacol. 35, 460-467 (1969).

150. A. B. King
The Effect of Exogenous Dopamine on ACTH Secretion.
Proc. Soc. Exp. Biol. Med. 130, 445-447 (1969).

151. W. Lippman, R. Leonardi, J. Ball, J. A. Coppola
Relationship Between Hypothalamic Catecholamines and Gonadotrophin Secretion in Rats.
J. Pharmacol. Exp. Ther. 156, 258-266 (1967).

152. E. W. Maynert
Some Aspects of the Comparative Pharmacology of Morphine.
Arch. Biol. Y. Med. Exp. 4, 36-41 (1967).

153. C. T. Bennet, A. Pert
Antidiuresis Produced by Injections of Histamine Into the Cat Supraoptic Nucleus.
Brain Res. 78, 151-156 (1974).

154. S. F. Leibowitz
Histamine, a Stimulatory Effect on Drinking Behavior in the Rat.
Brain Res. 63, 440-444 (1973).

155. M. C. Gerald, R. P. Maickel
Studies on the Possible Role of Brain Histamine in Behavior.
Br. J. Pharmacol. 44, 462-471 (1972).

156. R. D. Myers
Brain Mechanisms in the Control of Feeding: A New Neurochemical Profile Theory.
Pharmacol. Biochem. Behav. 3 (Suppl. 1), 75-83 (1975).

157. R. C. Ritter, A. M. Epstein
Control of Meal Size by Central Noradenergic Action.
Proc. Nat. Acad. Sci. 72, 3740-3743 (1975).

158. M. K. Menon, W. G. Clark, D. Aures
Effect of Thiazol-4-ylmethoxyamine, a New Inhibitor of Histamine Biosynthesis on Brain Histamine, Monoamine Levels and Behavior.
Life Sci. 10, 1097-1109 (1971).

159. C. Maslinski, U. Lebrecht, J. Z. Nowak, A. Pilc, Z. Wieczorek-Fila
Catalepsia-like Symptoms Produced by Histidine in Rats.
Agents Actions 3, 185-186 (1973).

160. J. Costentin, J. C. Schwartz, R. Boulu
Histamine et Comportements Effets de Surcharges en L-Histidine.
J. Pharmacol. (Paris) 5, 195-208 (1974).

161. L. C. Cesare, G. R. S. Carlini, E. A. Carlini
Influence of Histamine on the Catatonia Induced in Mice by Tetrabenazone and Reserpine.
Arch. Int. Pharmacodyn. Ther. 169, 26-34 (1963).

162. M. Monnier, M. Fallert, I. C. Bhattacharya
The Waking Action of Histamine.
Experientia (Basil) 23, 21-22 (1967).

163. M. Monnier, R. Sauer
Histamine as a Waking Factor (Validity of Evoked Potentials in Psychopharmacology).
Abst. Electroenceph. Clin. Neurophysiol. 27, 107 (1969).

164. J. Crossland
Chemical Transmission in the Central Nervous System.
J. Pharm. Pharmacol. 12, 1-36 (1960).

165. H. Green, R. W. Erickson
Effect of Some Drugs Upon Rat Brain Histamine Content.
Int. J. Neuropharmacol. 3, 315-320 (1964).

166. K. P. Bhargava, K. S. Dixit
Role of the Chemoreceptor Trigger Zone in Histamine-Induced Emesis.
Br. J. Pharmacol. 34, 508-513 (1968).

167. K. P. Bhargava
Some Neuropharmacological Studies with Histamine. Pp. 597-604 in: Proceedings of the IX Congress of the Collegium Internationale Neuropsychopharmacologicum (Paris) 1974). Edited by J. R. Boissier, H. Hippius and P. Pichot.
American Elsevier Publ. Co. Inc., New York (1975).

168. J. Crossland
Modern Views on Pharmacology of Histamine and 5-Hydroxytryptamine.
Practitioner 207, 382-388 (1971).

169. L. Finch, P. E. Hicks
Involvement of Hypothalamic Histamine Receptors in the Central Cardiovascular Actions of Histamine.
Neuropharmacol. 16, 211-218 (1977).

170. B. S. R. Sastry, J. W. Phillis
Evidence that Clonidine Can Activate Histamine H_2-Receptors in Rat Cerebral Cortex.
Neuropharmacology 16, 223-225 (1977).

171. W. Feldberg, R. D. Myers
Effects on Temperature of Amines Injected Into the Cerebral Ventricles. A New Concept of Temperature Regulation.
J. Physiol. 173, 226-257 (1964).

172. J. Bligh, W. H. Cottle, M. Maskrey
Influence of Ambient Temperature in the Thermoregulatory Responses of 5-Hydroxytryptamine, Noradrenaline and Acetylcholine Injected Into the Lateral Cerebral Ventricles of Sheep, Goats and Rabbits.
J. Physiol. 212, 377-392 (1971).

173. M. Fabinyi, J. Szebehelyi
The Mechanism of Desensitization with Histamine.
Acta Allerg 2, 233-244 (1949).

174. M. Fabinyi, J. Szebehelyi
The Influences of Antihistamines on the Effect of Histamine Upon Body Temperature and Oxygen Consumption in Mice and Rats.
Acta Physiol. Scand. 27, 1-9 (1952).

175. H. L. Borison, W. G. Clark
Drug Actions on Thermoregulatory Mechanisms.
Pharmacol. 5, 129-212 (1967).

176. G. G. Shaw
Hypothermia Produced in Mice by Histamine Acting on the Central Nervous System.
Br. J. Pharmacol. 42, 205-214 (1971).

177. H. E. Brezenoff, P. Lomax
Temperature Changes Following Microinjection of Histamine Into the Thermoregulatory Centres of the Rat.
Experientia 26, 51-52 (1970).

178. J. Costentin, P. Boulu, J. C. Schwartz
Pharmacological Studies on the Role of Histamine in Thermoregulation.
Agents Actions 3, 177 (1973).

179. M. D. Green, M. L. Simon, P. Lomax
Histidine Induced Hyperthermia in the Rat.
Life Sci. 16, 1293-1300 (1975).

180. M. D. Green, B. Cox, P. Lomax
Histamine H_1 and H_2 Receptors in the Central Thermoregulatory Pathways of the Rat.
J. Neuroscience Res. 1, 353-359 (1975).

181. L. A. Tyfield, J. B. Holton
The Effect of High Concentrations of Histidine on the Level of Other Amino Acids in Plasma and Brain of the Mature Rat.
J. Neurochem. 26, 101-105 (1976).

182. P. Lomax, G. V. Knox
The Sites and Mechanisms of Action of Drugs Affecting Thermoregulation. P. 146 in: The Pharmacology of Thermoregulation.
Edited by E. Schonbaum, P. Loax and S. Karger.
Basel (1974).

183. R. J. Taylor, F. J. Leinweber, G. A. Braun
4-Imidazolyl-3-amino-2-butanone (McN-A-1293). A New Specific Inhibitor of Histidine Decarboxylase.
Biochem. Pharmacol. 22, 2299-2310 (1973).

184. J. Costentin, J. M. Andre, J. C. Schwartz, R. Bouler, C. Rendus
Histamine Endogène et Thermorégulation.
C. R. Soc. Biol. (Paris) 166, 1366-1369 (1972).

185. V. J. Lotti
Body Temperature Responses to Morphine. Central Sites and Mechanisms of Action. Pp. 382-394 in: The Pharmacology of Thermoregulation.
Edited by E. Schoenbaum, P. Lomax and S. Karger.
Basel (1973).

186. R. W. Henwood, I. M. Mazurkiewicz-Kwilecki
Possible Role of Brain Histamine in Morphine Tolerance and Morphine Withdrawal.
Fec. Proc. 19, 168 (1976).

187. U. Banerjee
Effect of Body Temperature of Morphine and Ergotamine Injected Into the Cerebral Ventricles of Cats.
Br. J. Pharmacol. 32, 523-538 (1968).

188. J. A. H. Gooszen, J. Donker
A Study of Histamine-Metabolism in Schizophrenic Patients.
Acta Allergol. 10, 149-169 (1956).

189. C. C. Pfeiffer, V. Iliev, L. Goldstein, E. H. Jenny, R. Schultz
Blood Histamine Polyamines and the Schizophrenias Computer Correlations of the Low and High Blood Histamine Types.
Res. Commun. Chem. Pathol. Pharmacol. 1, 247-265 (1970).

190. P. A. J. Janssen
The Pharmacology of the Haloperidol.
Int. J. Neuropsychiat. 3 (Suppl. 1), S10-S18 (1967).

191. J. J. Schildkraut, S. S. Kety
Biogenic Amines and Emotion.
Science 156, 21-30 (1967).

192. L. Stein, C. P. Wise, B. D. Berger
Noradrenergic Mechanisms, Recovery of Function and Schizophrenia.
Pp. 81-103 in: The Chemistry of Mood, Motivation, and Memory.
Edited by J. L. McGaugh.
Plenum Publ. Co., New York (1972).

193. D. W. Wooley, E. A. Shaw
A Biochemical and Pharmacological Suggestion About Certain Mental Disorders.
Proc. Natl. Acad. Sci. (U.S.A.) 40, 228-231 (1954).

194. I. Munkvad, H. Pakkenberg, A. Randrup
Aminergic Systems in Basal Ganglia Associated with Stereotyped Hyperactive Behaviour and Catalepsy.
Brain Behav. Evol. 1, 89-100 (1968).

195. A. Randrup, I. Munkvad
Correlation Between Specific Effects of Amphetamines on the Brain and on Behaviour. Pp. 17-26 in: Current Concepts on Amphetamine Abuse. Edited by E. Ellinwood and S. Cohen.
N.I.M.H. Rockville (1972).

196. S. H. Snyder
Catecholamines in the Brain as Mediators of Amphetamine Psychosis.
Arch. Gen. Psychiatry 27, 169-179 (1972).

197. E. L. Way, H. H. Loh, F. H. Shen
Simultaneous Quantitative Assessment of Morphine Tolerance and Physical Dependence.
J. Pharmacol. Exp. Ther. 167, 1-8 (1969).

198. L. Grumbach, M. Shelofsky, J. E. Boston Jr-
The Kinetics of Physical Dependence to Morphine in the Rat.
Pharmacology 11, 38-51 (1974).

199. C. B. Pert, S. H. Snyder
Properties of Opiate-Receptor Binding in Rat Brain.
Proc. Nat. Acad. Sci. (Washington, U.S.A.) 70, 2243-2247 (1973).

200. C. B. Pert, S. H. Snyder
Opiate-Receptor: As Demonstration in Nervous Tissue.
Science 179, 1011-1014 (1973).

201. A. Herz, J. Blasig, R. Papeschi
Role of Catecholaminergic Mechanisms in the Expression of the Morphine Abstinence Syndrome in Rats.
Psychopharmacologia (Berl.) 39, 121-143 (1974).

202. T. Iwamoto
The Role of Biogenic Amines in Morphine Analgesia, Tolerance, and Physical Dependence.
Ph.D. Thesis, University of California, San Francisco (1972).

203. E. L. Way, F. H. Shen
The Effects of Narcotic Analgesics on Specific Systems. Pp. 229-253 in: "Narcotic Drugs," Biochemical Pharmacological. Edited by D. H. Clouet.
Plenum Press, New York (1971).

204. C. B. Smith
Neurotransmitters and the Narcotic Analgesics. Pp. 495-504 in: Chemical and Biological Aspects of Drug Dependence. Edited by S. Y. Mule, H. Brill,
C. R. C. Press, Cleveland, Ohio (1974).

205. D. H. Clouet, K. Iwatsubo
Methods of Producing Tolerance and Dependence in Laboratory Animals: In Mechanisms of Tolerance to and Dependence on Narcotic Analgesic Drugs.
Annu. Rev. Pharmacol. 15, 49-71 (1975).

206. J. C. Johnson, M. Ratner, G. J. Gold, D. H. Clouet
Morphine Effects on the Levels and Turnover of Catecholamines in Rat Brain.
Res. Commun. Chem. Pathol. Pharmacol. 9(1), 41-53 (1974).

207. M. Ko Fian, T. Reigle, P. Orsulak, L. J. Schidr
The Effects of Acute and Chronic Morphine Administration on the Levels of 3-Methoxy-4-Hydroxyphenylglycol Sulphate in Rat Brain.
Res. Commun. Chem. Pathol. Pharmacol. 10(3), 403-417.

208. C. B. Smith
Neurotransmitters and the Narcotic Analgesics. P. 495 in: Chemical and Biological Aspects of Drug Dependence. Edited by S. J. Mule, H. Brill.
CRC Press, Cleveland, Ohio (1972).

209. H. Weil-Malherbe, E. R. B. Smith, A. J. Eisenman, H. F. Fraser
Plasma Catecholamine Levels and Urinary Excretion of Catecholamines and Metabolites in Two Human Subjects During a Cycle of Morphine Addiction and Withdrawal.
Biochem. Pharmacol. 14, 1621-1633 (1965).

210. C. R. Rethy, C. B. Smith, J. E. Villareal
Effects of Narcotic Analgesics Upon the Locomotor Activity and Brain Catecholamine Content of the Mouse.
J. Pharmacol. Exp. Ther. 17, 472-479 (1971).

211. A. Bertler, E. Rosengren
Occurrence and Distribution of Dopamine in Brain and Other Tissues.
Experientia 15, 10-11 (1959).

212. A. Carlsson, M. Lindqvist
Effects of Chlorpromazine or Haloperidol on Formation of 3-Methoxytyramine and Norepinephrine in Mouse Brain.
Acta Pharmacol. Toxicol. 20, 140-144 (1963).

213. N. E. Anden
Dopamine Turnover in the Corpus Striatum and the Limbic System after Treatment with Neuroleptic and Anticholinergic Drugs.
J. Pharm. Pharmacol. 24, 905-906 (1972).

214. U. Ungerstedt
Striatal Dopamine Release after Amphetamine or Nerve Degeneration Revealed by Rotational Behavior.
Acta Physiol. Scand. Suppl. 367, 49-68 (1971).

215. U. Ungerstedt
Postsynaptic Supersensitivity after 6-Hydroxydopamine-Induced Degeneration of the Nigro-Striatal System in the Rat Brain.
Acta Physiol. Scand. Suppl. 267, 69-93 (1971).

216. M. Vogt
Functional Aspects of the Role of Catecholamines in the Central Nervous System.
Br. Med. Bull. 29, 168-171 (1973).

217. H. Ehringer, O. Hornykiewicz
Distribution of Noradrenaline and Dopamine (3-Hydroxytyramine) in the Human Brain and Their Behavior in Diseases of the Extrapyramidal System.
Wein Klin. Wochenschr. 38, 1236-1239 (1960).

218. O. Hornykiewicz
Dopamine in the Basal Ganglia. Its Role and Therapeutic Implications (Including the Clinical Use of L-DOPA).
Br. Med. Bull. 29, 172-178 (1973).

219. S. H. Snyder, S. P. Banerjee, H. I. Yamamura, D. Greenberg
Drugs, Neurotransmitters and Schizophrenia.
Science 184, 1243-1253 (1974).

220. E. Eidelberg, R. Erspamer
Dopaminergic Mechanisms of Opiate Actions in Brain.
J. Pharmacol. Exp. Ther. 192, 50-57 (1975).

221. J. R. Lee, M. R. Fennessy
Effects of Morphine on Brain Histamine Antinociception and Activity in Mice.
Clin. Exp. Pharmacol. Physiol. 3, 179-189 (1976).

222. R. Laverty, D. F. Sharman
Modification by Drugs on the Metabolism of 3,4-dihydroxyphenethylamine, Noradrenaline, and 5-hydroxytryptamine in the Brain.
Br. J. Pharmacol. Chemother. 24, 759-772 (1965).

223. H. Takagi, M. Nakama
Effects of Morphine and Nalorphine on the Content of Dopamine in Mouse Brain.
Jap. J. Pharmacol. 16, 483-484 (1966).

224. L. M. Gunne, J. Jonsson, K. Fuxe
Effects of Morphine Intoxication on Brain Catecholamine Neurons.
Eur. J. Pharmacol. 5, 338-342 (1969).

225. H. A. Sasame, J. Perez-Cuet, G. DiChiara, A. Tagliamonte, P. Tagliamonte, G. L. Gessa
Evidence that Methadone Blocks Dopamine Receptors in the Brain.
J. Neurochem. 19, 1953-1957 (1972).

226. K. Kuschinski, O. Hornykiewicz
Morphine Catalepsy in the Rat: Relation to Striatal Dopamine Metabolism.
Eur. J. Pharmacol. 19, 119-122 (1972).

227. L. Ahtee, J. Kaariaienen
The Effects of Narcotic Analgesics on the Homovanillic Acid Content of Rat Nucleus Caudatus.
Eur. J. Pharmacol. 22, 206-208 (1973).

228. E. Contreras, L. Tamayo
Effects of Drugs Acting in Relation to Sympathetic Functions on the Analgesic Action of Morphine.
Arch. Int. Pharmacodyn. 166, 321-330 (1966).

229. C. R. Calcutt, N. S. Doggett, P. S. Spencer
Modification of the Antinociceptive Activity of Morphine by Centrally Administered Ouabain and Dopamine.
Psychopharmacologia 21, 111-117 (1971).

230. K. Kuschinski, O. Hornykiewicz
Effects of Morphine on Striatal Dopamine Metabolism: Possible Mechanism of Its Opposite Effect on Locomotor Activity in Rats and Mice.
Eur. J. Pharmacol. 26, 41-50 (1974).

231. M. J. Besson, A. Cheramy, P. Feltz, J. Glowinski
Release of Newly Synthesized Dopamine from Dopamine-Containing Terminals in the Striatum of the Rat.
Proc. Nat. Acad. Sci. (Wash.) 62, 741 (1969).

232. C. Vander Wende, M. T. Spoerlein, N. C. Luc
Studies on the Role of Dopaminergic Systems in Morphine-Induced Motor Activity. Comparison with Noradrenergic and Cholinergic Systems.
Res. Commun. Chem. Pathol. Pharmacol. 11, 79-88 (1975).

233. D. Slaughter, D. W. Nunsell
Some New Aspects of Morphine Action: Effects on Pain.
J. Pharmacol. Exp. Ther. 68, 104-112 (1940).

234. J. A. Schneider, M. McArthur
Potentiation Action of Ibogaine (bogadin TM) on Morphine Analgesia.
Experientia 12, 323-324 (1956).

235. S. C. Lin, V. C. Sutherland, E. L. Way
Brain Amino Acids in Morphine Tolerant and Non-Tolerant Rats.
Proc. West. Pharmacol. Soc. 16, 8-13 (1973).

236. C. B. Pert, A. M. Snowman, S. J. Snyder
Localization of Opiate Receptor Binding in Synaptic Membranes of Rat Brain.
Brain Res. 70, 184-188 (1974).

237. I. K. Ho, H. H. Loh, E. L. Way
Influence of GABA on Morphine Analgesia, Tolerance, and Physical Dependence.
Proc. West. Pharmacol. Soc. 16, 4-7 (1973).

238. A. D. Sherman, C. L. Mitchell
Effect of Morphine on Regional Levels of Gamma-Hydroxybutyrate on Mouse Brain.
Neuropharmacol. 13, 239-243 (1974).

239. R. Roth, N. Giarman
Natural Occurrence of Gamma-Hydroxybutyrate in Mammalian Brain.
Biochem. Pharmacol. 19, 1087-1094 (1970).

240. A. D. Sherman, G. F. Gebhart
An Evaluation of the Analgesia Induced by Morphine and Gamma-Hydroxybutyrate.
Arch. Int. Pharmacodyn. 213, 195-199 (1975).

241. H. L. Cardenas, D. H. Ross
Morphine Induced Calcium Depletion in Discrete Regions of Rat Brain.
J. Neurochem. 24, 487-493 (1975).

242. D. R. Jasinski, W. R. Martin, R. D. Hoeldtke
Effects of Short and Long Term Administration of Pentazocine in Man.
Clin. Pharmacol. Ther. 11, 385-403 (1970).

243. W. T. Beaver
The Pharmacological Basis for the Choice of an Analgesic I. Potent Analgesies.
Pharmacol. Physicians 4, 1-7 (1970).

244. C. G. Moertel, D. L. Ashmann, W. F. Taylor
A Comparative Evaluation of Marketed Analgesic Drugs.
N. Engl. J. Med. 286, 813-815 (1972).

245. A. J. J. Wood, D. C. Moir, C. Campbell, J. F. Davidson, S. C. Gallon, E. Henney, S. McAllion
Medicines Evaluation and Monitoring Group: Control on Effects of PTZ.
Br. Med. J. 1, 305-307 (1974).

246. S. G. Holtzman, R. E. Jewett
Some Actions of Pentazocine on Behavior and Brain Monoamines in the Rat.
J. Pharmacol. Exp. Ther. 181, 346-356 (1972).

247. S. G. Holtzman
Interactions of Pentazocine and Naloxone on the Monoamine Content of Discrete Regions of the Rat Brain.
Biochem. Pharmacol. 23, 3029-3035 (1974).

248. K. J. Rogers, J. A. Thornton
The Interaction Between MAOI and Narcotic Analgesics in Mice.
Br. J. Pharmacol. 36, 470-480 (1969).

249. H. Blumberg, H. B. Dayton, M. George, D. N. Rappaport.
N-Allylnoroxymorphone: A Potent Narcotic Antagonist.
Fed. Proc. 20, 311 (1961).

250. D. R. Jasinski, W. R. Martin, C. A. Haertzen
The Human Pharmacology and Abuse Potential of N-Allyluoroxymorphone (Naloxone).
J. Pharmacol. Exp. Ther. 157, 420-426 (1967).

251. H. Blumberg, H. B. Dayton, P. S. Wolf
Counteraction of Narcotic Antagonist Analgesies by the Narcotic Antagonist Naloxone.
Proc. Soc. Exp. Biol. Med. 123, 755-758 (1966).

252. T. Kallos, T. G. Smith
Naloxone Reversal of Pentazocine-Induced Respiratory Depression.
J. Amer. Med. Assoc. 204, 932-933 (1968).

253. M. F. Sugrue
Effects of Morphine and Pentazocine on the Turnover of Naradrenaline and Dopamine in Various Regions of Rat Brain.
Br. J. Pharmacol. 47, 644 (1973).

254. B. A. Birkowitz, E. L. Way
Analgesic Activity and Central Nervous System Distribution of the Optical Isomers of Pentazocine in the Rat.
J. Pharmacol. Exp. Ther. 177, 500-508 (1971).

255. J. W. Bellville, W. H. Forrest
Respiratory and Subjective Effects of d- and l-pentazocine.
Clin. Pharmacol. Ther. 9, 142-151 (1968).

256. S. G. Holtzman, R. E. Jewett
Some Actions of Pentazocine on Behavior.
J. Pharmacol. Exp. Ther. 187, 380-390 (1973).

257. B. A. Berkowitz
Effects of the Optical Isomers of the Narcotic Antagonist Analgesic Pentazocine on Brain and Heart Biogenic Amines.
Eur. J. Pharmacol. 26, 359-365 (1974).

258. G. E. Kochansky, T. S. Hemenway, C. Salzman, R. I. Saher
Methaqualone Abusers: A Preliminary Survey of College Students.
Dis. Nerv. Syst. 36, 348-351 (1974).

259. F. S. Tennant Jr.
Drug Abuse in the US Army, Europe.
J. Amer. Med. Assoc. 221, 1146-1149 (1972).

260. I. Linné
Attempted Drug Suicide in a Swedish City.
Acta Med. Scand. 195, 521-525 (1974).

261. M. Kato
An Epidemiological Analysis of the Fluctuation of Drug Dependence in Japan.
Int. J. Addict. 4, 591-621 (1969).

262. C. Distasio, M. Nawrot
Methaqualone.
Am. J. Nursing 73, 1922-1925 (1973).

263. K. Etzler, R. Hoenle, E. H. Joswig, C. Kohler, H. J. Mallach
Study on the Statistical Interactions Between Ethylalcohol and Carbamazepine, Methaqualone, and Nitrazepam.
Arzneim. Forsch. 19, 988-995 (1969).

264. R. de Alarçon
Methaqualone.
Br. Med. J. 1, 122-123 (1969).

265. M. Swartzburg. J. Lieb, A. H. Schwartz
Methaqualone Withdrawal.
Arch. Gen. Psychiatry 29, 46-47 (1973).

266. J. S. Madden
Dependency on Methaqualone Hydrocholoride (Melsedin).
Br. Med. J. 1, 676 (1966).

267. D. R. Benady
Mandrax.
Br. Med. J. 1, 577-578 (1969).

268. S. S. Brown, S. Goenechea
Methaqualone: Metabolic, Kinetic, and Clinical Pharmacologic Observations.
Clin. Pharmacol. Ther. 14, 314-324 (1972).

269. R. K. Nayak, R. D. Snyth, J. H. Chamberlain, A. Polk, A. F. DeLong, T. Herczeg, P. B. Chemburkar, R. S. Joslin, N. H. Reavy-Cantwell
Methaqualone Pharmacokinetics after Single- and Multiple-Dose Administration in Man.
J. Pharmacokin. Biopharm. 2, 107-121 (1974).

270. M. C. Gerald, P. M. Schwirian
Nonmedial Use of Methaqualone.
Arch. Gen. Psychiatry 28, 627-631 (1973).

271. F. S. Tennant Jr.
Complications of Methaqualone-Diphenhydramine (Mandrax) Abuse.
Br. J. Addict. 68, 327-330 (1973).

272. H. Matthew, A. T. Proudfood, S. S. Brown, A. C. A. Smith
Mandrax Poisoning: Conservative Management of 116 Patients.
Br. Med. J. 2, 101-102 (1968).

273. S. Gitelson
Methaqualone-Meprobamate Poisoning.
J.A.M.A. 201, 977-979 (1967).

274. G. R. Burston
Self-Poisoning with Mandrax.
Practioner 199, 340-344 (1967).

275. I. H. Breslow, S. Z. Haidri
Methylquinazolone and Hepatic Function.
Clin. Pharmacol. & Therap. 7, 359-361 (1966).

276. D. T. Caridis, G. M. McAndrew, N. A. Matheson
Haemodialysis in Poisoning with Methaqualone and Diphenhydramine.
Lancet 1, 51-52 (1967).

277. R. E. Johnstone, G. T. Manitsas, E. J. Smith
Apnea Following Methaqualone Ingestion.
Ohio State Med. J. 67, 1018-1920 (1971).

278. R. W. Piepho, M. F. O'Connor
Hypnotic and Hypothermic Responses Following Chronic Administration of Methaqualone in the Rat.
Res. Commun. Chem. Path. Pharmacol. 6, 101-108 (1973).

279. R. P. Kohli, N. Singh, V. K. Kulshrestha
An Experimental Investigation of Dependence Liability of Methaqualone in Rats.
Psychopharmacologia 35, 327-334 (1974).

280. R. B. L. Ewart, R. G. Priest
Methaqualone Addiction and Delirium Tremens.
Br. Med. J. 3, 92-93 (1967).

281. J. R. Lewis, E. M. Steindler
Methaqualone.
J. Amer. Med. Assoc. 224, 1521-1522 (1973).

282. D. S. Inaba, G. R. Gay, J. A. Newmeyer, C. Whiteheat
Methaqualone Abuse "Luding Out."
J. Amer. Med. Assoc. 224, 1505-1509 (1973).

283. E. F. Pascarelli
Methaqualone Abuse, the Quiet Epidemic.
J. Amer. Med. Assoc. 224, 1512-1514 (1973).

284. J. A. Ostrenga
Methaqualone—A Dr. Jekyll and Mr. Hyde?
Clin. Toxicol. 6, 607-609 (1973).

285. M. L. Gujral, P. N. Sazena, R. S. Tiwari
Comparative Evaluation of Quinazolones: A New Class of Hypnotics.
Ind. J. Med. Res. 43, 637-641 (1955).

286. M. M. Glatt
The Changing British Drug Scene.
Lancet 2, 429-430 (1969).

287. H. Matthew
A Clinical Comparison of Two Nonbarbiturate Hypnotics, Mogadon and Mandrax.
Med. J. Aust. 1, 1271 (1972).

288. J. Schlict, M. Mitcheson, M. Henry
Medical Aspects of Large Outdoor Festivals.
Lancet 1, 948-952 (1972).

289. L. M. Gunne, E. Anggard, L. E. Jonsson
Blockade of Amphetamine Effects in Human Subjects. Pp. 249-255 in: International Institute on the Prevention and Treatment of Drug Dependence. Tongue and Tongue, Lausanne (1970).

290. E. Gutman, W. Sargenth
Observations on Benzedrine.
Br. Med. J. 1, 1013 (1937).

291. J. Axelrod
Studies on Sympathomimetic Amines II. The Biotransformation and Physiological Disposition of D-Amphetamine, D-p-Hydroxyamphetamine and D-Methyl-Amphetamine.
J. Pharmacol. Exp. Ther. 110, 315-326 (1954).

292. T. Lewander
Displacement of Brain and Heart Noradrenaline by p-Hydroxynorephedrine after Administration of p-Hydroxyamphetamine.
Acta Pharmac. Tox. 29, 20-32 (1971).

293. A. Tori, S. Caccia
Distribution of Amphetamine and Its Hydroxylated Metabolites in Various Areas of the Rat Brain.
J. Pharm. Pharmacol. 26, 746-848 (1974).

294. J. M. Davis, J. J. Kopin, L. Lemberger, J. Axelrod
Effects of Urinary pH in Amphetamine Metabolism.
Ann. N.Y. Acad. Sci. 179, 493-501 (1971).

295. L. M. Gunne, E. Anggard
Pharmacokinetic Studies with Amphetamines-Relationship to Neuropsychiatric Disorders.
J. Pharmacokinet. and Biopharm. 1, 481-494 (1973).

296. A. Weissman, B. K. Kae, S. S. Tenen
Antiamphetamine Effects Following Inhibition of Tyrosine Hydroxylase.
J. Pharmacol. Exp. Ther. 151, 339-352 (1966).

297. L. C. F. Hanson
Evidence that the Central Action of (+)-Amphetamine is Mediated Via Catecholamines.
Psychopharmacologia (Berl.) 10, 289-296 (1967).

298. R. J. Boakes, P. B. Bradley, J. M. Candy
A Neuronal Basis for the Alerting Action of (+)-Amphetamine.
Br. J. Pharmacol. 45, 391-403 (1972).

299. M. J. Besson, A. Cheramy, P. Feltz, J. Glowinski
Release of Newly Synthesized Dopamine from Dopamine-Containing Terminals in the Striatum of the Rat.
Proc. Nat. Acad. Sci. (U.S.A.) 62, 741 (1969).

300. A. Carlsson
Amphetamine and Brain Catecholamines. P. 389 in: Amphetamines and Related Compounds. Edited by E. Costa and S. Garattini. Raven Press, New York (1970).

301. L. A. Carr, K. E. Moore
Norepinephrine: Release from Brain by d-Amphetamine In Vivo. Science 164, 322-323 (1969).

302. D. A. Hutchins, K. J. Rogers
Effect of Depletion of Cerebral Monoamines on the Concentration of Clycogen and Amphetamine-Induced Glycogenolysis in the Brain. Br. J. Pharmacol. 48, 19-29 (1973).

303. G. M. McKenzie, J. C. Szerb
The Effects of Dihydroxyphenylalanine, Pheniprazine and Dextroamphetamine in the In Vivo Release of Dopamine from the Caudate Nucleus.
J. Pharmacol. Exp. Ther. 162, 302-311 (1968).

304. S. R. Nahorski, K. J. Rogers
In Vivo Effects of Amphetamine on Metabolites and Metabolic Rate in Brain.
J. Neurochem. 21, 679-686 (1973).

305. K. Y. Ng, T. N. Chase, I. J. Kopin
Drug-Induced Release of ^{3}H-Norepinephrine and ^{3}H-Serotonin from Brain Slices.
Nature (London) 228, 468-469 (1970).

306. R. J. Ziance, A. J. Azzaro, C. O. Rutledge
Characteristics of Amphetamine-Induced Release of Norepinephrine from Rat Cerebral Cortex In Vitro.
J. Pharmacol. Exp. Ther. 182, 284-294 (1972).

307. A. J. Azzaro, R. J. Ziance, C. P. Rutledge
The Importance of Neuronal Uptake of Amines for Amphetamine-Induced Release of ^{3}H-Norepinephrine from Isolated Brain Tissue.
J. Pharmacol. Exp. Ther. 189, 110-114 (1974).

308. M. R. Ferris, F. L. M. Tang, R. A. Maxweel
A Comparison of the Capacities of Isomers of Amphetamine, Deoxypipradol, and Methylphenidate to Inhibit the Uptake of Tritiated Catecholamines into Rat Cerebral Cortex Slices, Synaptosomal Preparations of Rat Cerebral Cortex, Hypothalamus and Striatum and Into Adrenergic Nerves of Rabbit Aorta.
J. Pharmacol. Exp. Ther. 181, 407-416 (1972).

309. J. Glowinski, J. Axelrod
Effect of Drugs on the Uptake, Release and Metabolism of ^{3}H-Norepinephrine in the Rat Brain.
J. Pharmacol. Exp. Ther. 149, 43-49 (1965).

310. M. J. Besson, A. Cheramy, P. Feltz, J. Glowinski
Dopamine: Spontaneous and Drug-Induced Release from the Caudate Nucleus in the Cat.
Brain Res. 32, 407-424 (1971).

311. S. H. Snyder, M. J. Kuhar, A. I. Green, J. T. Coyle, E. G. Shaskan
Uptake and Subcellular Localization of Neurotransmitters in the Brain.
Int. Rev. Neurobiol. 13, 127-158 (1970).

312. P. F. Von Voigtlander, K. E. Moore
Involvement of Nigro-Striatal Neurons in the In Vivo Release of Dopamine by Amphetamine, Amantadine and Tyramine.
J. Pharmacol. Exp. Ther. 184, 542-552 (1973).

313. J. J. M. Stolk, R. H. Rech
Effect of Reserpine on Accumulation and Removal of d-Amphetamine-^{3}H.
Biochem. Pharmacol. 18, 2786-2788 (1969).

314. A. Randrup, I. Mundvad
Role of Catecholamines in Amphetamine Excitatory Response.
Nature 211, 540 (1966).

315. P. Janssen, C. Niemegeers, K. Schellekens
Is it Possible to Predict the Clinical Effects of Neuroleptic Drugs (Major Tranquilizers) from Animal Data?
Drug Res. (Arnheim-Forsch) 15, 104-107 (1965).

316. A. Randrup, I. Munkvad
Stereotyped Activities Produced by Amphetamine in Several Animal Species and Man.
Psychopharmacologia 11, 300-310 (1967).

317. A. Randrup, J. Scheel-Kruger
Diethyldithiocarbamate and Amphetamine Stereotype Behavior.
J. Pharm. Pharmacol. 18, 752 (1966).

318. K. Fuxe, U. Ungerstedt
Pp. 257-288 in: Amphetamine and Related Compounds. Edited by E. Costa, S. Garattini.
Raven Press, New York (1970).

319. R. J. Carey, A. P. Salim
Changes in D-Amphetamine Reactivity Resulting from Septal Forebrain Injury.
Physiol. Behav. 5, 133-136 (1970).

320. H. J. Carlisle
Differential Effects of Amphetamine on Food and Water Intake in Rats with Lateral Hypothalamic Lesions.
J. Comp. Physiol. Phychol. 58, 47-54 (1964).

321. S. O. Cole
Increased Suppression of Food Intake by Amphetamine in Rats with Anterior Hypothalamic Lesions.
J. Comp. Physiol. Psychol. 61, 302-305 (1966).

322. A. Epstein
Suppression of Eating and Drinking by Amphetamine and other Drugs in Normal and Hyperphagis Rats.
J. Comp. Physiol. Psychol. 52, 37-45 (1959).

323. J. B. Appel, D. X. Freedman
Tolerance and Cross-Tolerance among Psychotomimetic Drugs.
Psychopharmacologia 13, 267-274 (1968).

324. B. B. Brodi, A. K. Cho, G. L. Gessa
P. 217 in: Amphetamines and Related Compounds. Edited by E. Costa and S. Garattini.
Raven Press, New York (1970).

325. H. Brown
Drug-Behavior Interaction Affecting Development of Tolerance to D-Amphetamine as Observed in Fixed Ratio Behavior of Rats.
Psychol. Rep. 16, 917 (1975).

326. J. C. Campbell, C. S. Seiden
Performance Influence on the Development of Tolerance to Amphetamine.
Pharmacol. Biochem. Behav. 1, 703-708 (1973).

327. S. B. Sparber, H. A. Tilson
Tolerance and Cross-Tolerance to Mescaline and Amphetamine as a Function of Central and Peripheral Administration.
Psychopharmacologia 23, 220-230 (1972).

328. H. A. Tilson, R. H. Rech
Prior Drug Experience and Effects of Amphetamine on Schedule Controlled Behavior.
Pharmacol. Biochem. Behav. 1, 129-132 (1973).

329. H. A. Tilson, R. H. Rech
Conditioned Drug Effects and Absence of Tolerance to d-Amphetamine Induced Motor Activity.
Pharmacol. Biochem. Behav. 1, 149 (1973).

330. D. S. Segal, A. J. Mandell
Long-Term Administration of d-Amphetamine Progressive Augmentation of Motor Activity and Stereotypy.
Pharmacol. Biochem. Behav. 2, 249-255 (1974).

331. T. Lewander
A Mechanism for the Development of Tolerance to Amphetamine in Rats.
Psychopharmacologia 21, 17-31 (1971).

332. R. L. Smith, L. G. Dring
P. 121 in: Amphetamines and Related Compounds. Edited by E. Costa, S. Garattini.
Raven Press, New York (1970).

333. W. M. Davis, S. G. Smith
Central Cholinergic Influence on Self-Administration of Morphine and Amphetamine.
Life Sci. 16, 237-246 (1975).

334. W. G. Drew, L. C. Miller, L. L. Baugh
Effects of Delta 9-THC, LSD-25 and Scopolamine on Continuous, Spontaneous Alternation in the Y-Maze.
Psychopharmacologia 32, 171-182 (1973).

335. L. Kokkinidis, M. D. Walsh, R. Lahue, H. Anisman
Tolerance to d-Amphetamine: Behavioral Specificity.
Life Sci. 18, 913-918 (1976).

336. A. K. Swonger, R. H. Rech
Serotonergic and Cholinergic Involvement in Habituation of Activity and Spontaneous Alternation of Rats in a Y-Maze.
J. Comp. Physiol. Psyhcol. 81, 509-522 (1972).

337. A. Nistri, A. Bartolini, G. Deffenu, G. Pepeu
Investigations into the Release of Acetylcholine from the Cerebral Cortex of the Cat: Effects of Amphetamine, of Scopolamine and of Septal Lesions.
Neuropharmacology 11, 665-674 (1972).

338. L. E. Hollister
Marihuana in Man: Three Years Later.
Science 172, 21-29 (1971).

339. R. Mechoulam, Y. Gaoni,
A Total Synthesis of dl-Delta-1-Tetra-hydrocannabinol, the Active Constituent of Hashish.
J. Amer. Chem. Soc. 87, 3273-3275 (1965).

340. S. Burstein, J. Rosenfeld, T. Wittstruck
Isolation and Characterization of Two Major Urinary Metabolites of Delta-1-Tetrahydrocannabinol.
Science 176, 422-423 (1971).

341. M. E. Wall
The In Vitro and In Vivo Metabolism of Tetrahydrocannabinol (THC).
Ann. N.Y. Acad. Sci. 191, 23-39 (1971).

342. L. Lemberger, R. E. Crabtree, H. M. Rowe
11-Hydroxy-Delta-9-Tetrahydrocannabinol: Pharmacological Disposition, and Metabolism of a Major Metabolite of Marihuana in Man.
Science 177, 62-63 (1972).

343. P. Bracs, D. M. Jackson, G. B. Chesher
The Effect of Δ^9-tetrahydrocannabinol on Brain Amine Concentration and Turnover in Whole Rat Brain and in Various Regions of the Brain.
J. Pharm. Pharmac. 27, 713-715 (1975).

344. L. Maitre, M. Staehelin, H. J. Bein
Effects of Benzoctamine (30803-Ba, Tacitin), A New Psychoactive Drug, on Catecholamine Metabolism.
Agents Actions 1, 136-143 (1970).

345. B. E. Leonard
The Effect of $\Delta^{1,6}$-tetrahydrocannabinol on Biogenic Amines and Their Amino Acid Precursors in the Rat Brain.
Pharmacol. Res. Comm. 3, 139-145 (1971).

346. L. Maitre, P. A. Baumann, A. Delini-Strila
Pp. 101-119 in: Cannabis and Its Derivatives. Edited by W. D. M. Paton and J. Crown.
Oxford University Press, London (1972).

347. J. J. Schildkraut, D. H. Efron
The Effects of Delta-9-tetrahydrocannabinol on the Metabolism of Norepinephrine in Rat Brain.
Psychopharmacologia 20, 191-196 (1971).

348. R. D. Sofia, B. N. Dixit, H. Barry
The Effect of Delta-1-tetrahydrocannabinol on Serotonin Metabolism in the Rat Brain.
Life Sci. 1(10), 425-436 (1971).

349. D. W. Gallagher, E. Sanders-Bush, F. Sulser
Dissociation between Behavioral Effects and Changes in Metabolism of Cerebral Serotonin Following Delta-9-tetrahydrocannabinol.
Psychopharmacologia 26, 337-345 (1972).

350. D. Holtzman, R. A. Lovell, J. H. Jaffe, D. X. Freedman
1-delta-9-tetrahydrocannabinol: Neurochemical and Behavioral Effects in the Mouse.
Science 163, 1464-1467 (1969).

351. L. E. Hollister
Tetrahydrocannabinol Isomers and Homologs: Contrasted Effects of Smoking.
Nature (London) 227, 968-969 (1970).

352. C. B. Tart
Marihuana Intoxication: Common Experiences.
Nature (London) 226, 701-704 (1970).

353. E. Abel
Marihuana and Memory.
Nature (London) 227, 1151-1152 (1970).

354. W. G. Drew, L. L. MIller
Cannabis: Neural Mechanisms and Behavior—A Theoretical Review.
Pharmacology (Basel) 11, 12-32 (1974).

355. E. Friedman, I. Hanin, S. Gershon
Effect of Tetrahydrocannabinols on ^{3}H-acetylcholine Biosynthesis in Various Rat Brain Slices.
J. Pharm. Exp. Ther. 196, 339-345 (1976).

356. J. Glowinski and L. L. Iversen
Regional Studies on Catecholamines in the Rat Brain.
J. Neurochem. 13, 655-669 (1966).

357. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall
Protein Measurement with the Folin Phenol Reagent.
J. Biol. Chem. 193, 265-275 (1951).

358. A. E. Takemori
Enzymic Studies on Morphine Gucuronide Synthesis in Acutely and Chronically Morphinized Rats.
J. Pharm. Exp. Ther. 130, 370-374 (1960).

359. C. O. Enwonwu, B. S. Worthington
Elevation of Brain Histamine Content in Protein-Deficient Rats.
J. Neurochem. 24, 941-945 (1975).

360. J. Blasig, A. Herz, K. Reinhold, S. Zieglansberger
Development of Physical Dependence on Morphine in Respect to Time and Dosage and Quantification of the Precipitated Withdrawal Syndrone in Rats.
Psychopharmacologia (Berl.) 33, 19-38 (1973).

361. I. M. Mazurkiewicz-Kwilecki, R. W. Henwood
Alterations in Brain Endogenous Histamine Levels in Rats after Chronic Morphine Treatment and Morphine Withdrawal.
Agents Actions 6, 402-409 (1976).

362. J. C. Foreman, J. L. Mongar
Effect of Calcium on Dextran-Induced Histamine Release from Isolated Mast Cells.
Br. J. Pharmacol. 46, 767-769 (1973).

363. L. Tuomisto
Ontogenesis and Regional Distribution of Histamine and Histamine-N-methyltransferase in the Guinea Pig Brain.
J. Neurochem. 28, 271-276 (1977).

364. P. G. Kruger
Demonstration of Mast Cells in the Albino Rat Brain.
Experientia 30, 810-811 (1974).

365. H. V. Ellis III, A. R. Johnson, N. C. Moran
Selective Release of Histamine from Rat Mast Cells by Several Drugs.
J. Pharm. Exp. Ther. 175, 627-631 (1970).

366. I. H. Jaffe
Narcotic Analgesics. Pp. 237-275 in: Pharmacological Basis of Therapeutics. Edited by L. S. Goodman and A. Gilman.
MacMillan Publishing Co., Toronto (1970).

367. R. Henwood
Final Studentship Report to the Ontario Mental Health Foundation.

368. E. L. Way, F. H. Shen
The Effects of Narcotic Analgesics on Specific Systems. Pp. 229-253 in: Narcotic Drugs, Biochemical Pharmacology.
Edited by D. H. Clouet.
Plenum Press, New York (1971).

369. K. Dismukes, S. H. Snyder
Dynamics of Brain Histamine.
Adv. Neurol. 5, 101-109 (1974).

370. J. R. Lee, M. R. Fennessy
Effects of Morphine on Brain Histamine, Antinociception and Activity in Mice.
Clin. Exp. Pharmacol. Physiol. 3. 179-189 (1976).

371. I. M. Mazurkiewicz-Kwilecki, B. Bielkiewicz
The Effect of Chronic Morphine Treatment on Histamine Concentration and Histidine Decarboxylase Activity in Rat Brain.
Progress in Neuropsychopharmacol (1977). (In press)

372. J. C. Schwartz, M. Baudry, S. Bischoff, M. P. Martres, H. Pollard, C. Rose, M. Verdiere
Pharmacological Studies of Histamine as a Central Neurotransmitter. Pp. 371-382 in: Drugs and Central Synaptic Transmittion. Edited by P. B. Bradley and B. N. Dhawan.
University Park Press, Baltimore (1976).

373. W. Feldberg, J. Talesnik
Reduction of Tissue Histamine by Compound 48/80.
J. Physiol. 120, 550-568 (1953).

374. C, A, Baile
Putative Neurotransmitters in the Hypothalamus and Feeding.
Fed. Proc. 33, 1166-1175 (1974).

375. R. Henwood
Ontario Mental Health Foundation Research Studentship Report (1974).

376. C. Maslinski, U. Lambrecht, J. Z. Nowak, A. Pilc, Z. Wieczorek-Fila
Catalepsia-like Symptoms Produced by Histidine in Rats.
Agents Actions 3, 185-186 (1973).

377. M. D. Green, M. L. Simon, P. Lomax
Histamine as a Neurotransmitter in the Central Thermoregulatory Pathways of the Rat.
Proc. West. Pharmacol. Soc. 18, 110-113 (1975).

378. H. A. Campos, H. Jurupe
Evidence for a Cholinergic Mechanism Inducing Histamine Release in Rat Brain In Vivo.
Experientia 27, 746-747 (1970).

379. R. D. Smyth, J. K. Lee, A. Polk, P. B. Chemburkar, A. M. Savacool
Bioavailability of Methaqualone.
J. Clin. Pharmacol. 13, 391-400 (1973).

380. R. N. Morris, G. A. Gunderson, S. W. Babcock, J. F. Zaroslinski
Plasma Levels and Absorption of Methaqualone after Oral Administration to Man.
Clin. Pharmacol. Ther. 13(1), 719-723 (1972).

381. Y. Cohen, J. Wepierre, Y. Font du Picard, J. R. Boissier
Elimination of Radioactive Methaqualone in the Mouse.
A Quantitative Study and Identification Assay of the Metabolites.
Therapie. 20, 101-110 (1965).

382. H. Pollard, S. Bischoff, J. C. Schwartz
Turnover of Histamine in Rat Brain and Its Decrease under Barbiturate Anesthesia.
J. Pharmacol. Exp. Ther. 100, 88-99 (1974).

383. J. Beaubein, F. E. Kristoff, H. E. Lehman, T. A. Ban
A Comparison of the Hypnotic Properties of Mandrax and its Two Constituents.
Curr. Ther. Res. 10, 231-232 (1968).

384. W. Hoffmeister, S. Koller
Clinical Arial of a Combination of Methaqualone and Diphenhydramine in a Double-Blind Test.
Arzneim Frosch. 20, 261-264 (1970).

385. M. E. Williams, M. J. Kendall. M. Mitchard, S. S. Davis, R. Poxon
Availability of Methaqualone from Commercial Preparations:
In Vitro and In Vivo Studies in Man.
Br. J. Clin. Pharmacol. 1, 99-105 (1974).

386. J. B. Harman
Preparations Containing Methaqualone.
Prescribers J. 10, 129-131 (1970).

387. M. E. Williams. S. S. Davis, R. Poxon, M. J. Kendall, M. Mitchard
The Influence of Diphenhydramine on the Absorption of Methaqualone in Man.
Br. J. Clin. Pharmacol. 1, 259-260 (1974).

388. G. Douglas
Antihistamines. P. 635 in: The Pharmacological Basis of Therapeutics. Edited by L. S. Goodman and A. Gilman.
MacMillan Publishing Co., New York (1970).

389. T. J. Danielson, A. A. Boulton
Distribution and Occurrence of Amphetamine and P-Hydroxyamphetamine in Tissues of the Rat after Injection of d-Amphetamine Sulfate.
Eur. J. Pharmacol. 37, 257-264 (1976).

390. P. Bracs, D. M. Jackson, G. B. Chesher
The Effect of Δ^9-tetrahydrocannabinol on Brain Amine Concentration and Turnover in Whole Rat Brain and in Various Regions of the Brain.
J. Pharm. Pharmac. 27, 713-715 (1975).

391. D. Holtzman, R. A. Lovell, J. H. Jaffe, D. X. Freedman
1-Δ^9-Tetrahydrocannabinol: Neurochemical and Behavioral Effects in the Mouse.
Science 103, 1464-1467 (1969).

392. B. L. Welch, A. S. Welch, F. S. Messiha, H. J. Berger
Rapid Depletion of Adrenal Epinephrine and Elevation of Telencephalic Serotonin by (-)-trans-9-tetrahydrocannabinol in Mice.
Res. Comm. Chem. Path. Pharmac. 2, 382-391 (1971).

393. B. T. Ho, D. Taylor, G. E. Fritchie, L. F. Englert, W. M. McIsaac
Neuropharmacological Study of 9 and 8 -L- tetrahydrocannabinols in Monkeys and Mice.
Brain Res. 38, 163-170 (1972).

394. B. T. Ho, D. Taylor, L. F. Englert
The Effect of Repeated Administration of (-)-9-tetrahydrocannabinol on the Biosynthesis of Brain Amines.
Res. Comm. Chem. Pathol. Pharmac. 5, 851-854 (1973).

395. J. A. Yagiela, K. D. McCarthy, J. W. Gill
The Effect of Hypothermic Doses of 1-Δ^9-tetrahydrocannabinol on Biogenic Amine Metabolism in Selected Parts of the Rat Brain.
Life Sci. 14, 2367-2378 (1974).

396. R. W. Henwood, I. M. Mazurkiewicz-Kwilecki
Possible Role of Histamine in Morphine Addiction.
Life Sci. 17, 55-56 (1975).

397. R. Henwood, I. M. Mazurkiewicz-Kwilecki
Effect of Histidine on Morphine-Induced Changes in Brain Histamine.
(Submitted for publication)