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ANGIOTENSIN II MODULATES GLUTAMATE-INDUCED DEPOLARIZATIONS AND SYNAPTIC TRANSMISSION IN RAT LOCUS COERULEUS NEURONS IN VITRO

BY

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ABBREVIATIONS

ACE	angiotensin converting enzyme
ACh	acetylcholine
ACPD	trans-1-amino-cyclopentyl-1,3-dicarboxylate
ACSF	artificial cerebrospinal fluid
ACTH	adrenocorticotropic hormone
AHP	afterhyperpolarization
AI	angiotensin I
AII	angiotensin II
AIII	angiotensin III
Ama	amastatin
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANF	atrial natriuretic factor
AP4	2-amino-4-phosphonobutyrate
APV	2-amino-5-phosphonovalerate
Asp	aspartate
Bes	bestatin
CCK	cholecystokinin
cGMP	cyclic guanosine 3',5'-monophosphate
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNS	central nervous system
CPP	DL-3-(2-carboxypiperazin-4-yl)propyl-1-phosphate
CRF	corticotrophin releasing factor

DßH	dopamine-ß-hydroxylase
DGG	gamma-D-glutamylglycine
DNQX	6,7-dinitro-quinoxaline-2,3-dione
Dom	domoic acid
DTT	DL-dithiothreitol
EAA	excitatory amino acid
EPSP	excitatory postsynaptic potential
GABA	gamma-aminobutyrate
GDEE	glutamate diethyl ester
Glu	glutamate
5-HT	5-hydroxytryptamine
HPLC	high pressure liquid chromatography
HRP	horseradish peroxidase
Ibo	ibotenic acid
IP_3	inositol trisphosphate
I-V	current-voltage
KA	kainate
KYN	kynurenic acid
LC	locus coeruleus
LTP	long term potentiation
MeV	mesencephalic trigeminal nucleus
MK-801	(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine
mRNA	messenger ribonucleic acid

NA	noradrenaline
NH_4^+	ammonium ion
NH_4Cl	ammonium chloride
NMDA	N-methyl-D-aspartic acid
NTS	nucleus tractus solitarius
OVLT	organum vasculosum laminae terminalis
2,4-PDC	L-trans-pyrolidine-2,4-dicarboxylic acid
PGi	paragigantocellularis
Plu	2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (Plummer's inhibitor)
PNMT	phenylethanolamine N-methyltransferase
PrH	prepositus hypoglossi
Psi	pounds per square inch
PVH	paraventricular nucleus of hypothalamus
QA	quisqualic acid
RAS	renin angiotensin system
Sar^1-AII	Sar^1-angiotensin II
SFO	subfornical organ
SLX	spinal lamina X
TRH	thyrotropin releasing hormone
TTX	tetrodotoxin
VIP	vasoactive intestinal polypeptide
WGA	wheat germ agglutinin
Yoh	yohimbine

ABSTRACT

The existence of a brain renin-angiotensin system (RAS) has been well established. All biochemical components of the renin-angiotensin system have been found to be present in the brain. Considerable evidence now supports a neurotransmitter or neuromodulatory role for angiotensin II (AII) in the central nervous system (CNS). It has been demonstrated that nucleus locus coeruleus (LC) is innervated by angiotensinergic fibers and terminals, and that all components of an RAS are present in the LC, including renin, angiotensinogen, angiotensin converting enzyme, immunoreactive AII as well as high density receptor binding sites for AII. The LC also receives glutamatergic fibers, and physiological studies have revealed that there are three major subtypes of glutamate (Glu) receptors in rat LC. The objectives of this research are to study the actions of AII on spontaneous activity and on Glu-induced responses in rat LC neurons.

Experiments were done on transverse or horizontal brain slices (350 μm in thickness) taken from male Sprague-Dawley rats weighing 50 - 135 gms in body weight. The LC was visually located and the LC neurons were identified by their electrical characteristics. LC neurons from which we obtained intracellular recordings had steady spontaneous discharges of 0.5 - 5 Hz. Membrane potentials were -50 to - 70 mV. Measured at the level of threshold, the durations of action potentials were relatively long (1.3 - 2.5 ms) in comparison to those of the neurons adjacent to LC. The amplitudes of action potentials

were 60 to 80 mV (also measured at threshold), and had a characteristic "shoulder" on the falling phase.

The effects of AII on spontaneous activity of the LC neurons and on Glu-induced depolarization and resultant excitation were studied on intracellularly recorded LC neurons. Iontophoretic and pressure applied AII strongly depressed the depolarizing effect of Glu in most cells tested, in the absence of other effects on membrane properties. The action of AII appears to be selective since it had no effect on the depolarizations evoked by acetylcholine (ACh), or by depolarizing current injections. Selectivity is also indicated by the observation that AII had little or no effect on Glu responses in brain stem neurons outside of the LC where AII receptors have not been reported to be present. The effects of AII on Glu-induced responses appear to be independent of other neuronal elements since the action of AII was unaltered when yohimbine or bicuculline was added to the perfusate. The AII action does not result from AII stimulating Glu uptake systems, since the depressant action of AII on Glu-induced responses was not significantly affected by Glu uptake blocker 2,4-PDC. The active site for AII appears to be on the postsynaptic membrane, since the action is sustained in a low Ca^{++}/high Mg^{++} solution which blocks synaptic transmission.

Application of H^{+} ions from a pH control barrel had various actions on Glu responses, with depressant action on a number of cells. In some cells, H^{+} and AII had almost equal effect on Glu-induced responses. In most cells, however, the degree of

depression of Glu-induced responses by H^+ is generally mild in comparison to that caused by AII. The correlation between AII action and H^+ effect in the same cell was poor. AII receptor antagonists failed in antagonizing the actions of H^+, in contrast, they reversibly antagonized the actions of AII.

NH_4^+ was also found to depress Glu-induced responses in LC neurons. The action of NH_4^+ appears to be non-specific since it also had depressant actions on Glu-induced responses in non-LC brain stem neurons as well as ACh-induced excitatory responses in the LC. It seems that the mechanisms underlying the action of AII are different from that underlying the action of NH_4^+. The action of AII on Glu responses can be reversibly blocked by both peptide and non-peptide AII receptor antagonists. The peptide AII receptor blocker saralasin partially blocked the depressant action of AII on Glu-induced responses in about half neurons tested, with almost complete blockade in some. Using the selective non-peptide AII antagonists losartan (AT_1-selective) and PD123177 (AT_2-selective), it was found that losartan had very mild or no effects in antagonizing the action of AII. In contrast, the depressant action of AII was consistently reduced in a concentration dependent manner by bath application of the AT_2-selective antagonist PD123177, with almost complete blockade at a concentration of 4.5 μM to 5.0 μM. This indicates that the AT_2 receptor subtype is predominantly involved in the mediation of this action of AII.

AII was found to be subject to rapid peptidase degradation. When AII was applied by bath perfusion, rather than by

iontophoretic or pressure application, it had little or no effect on Glu responses. However, when Sar^1-AII, an analogue which is resistant to aminopeptidase degradation was bath-applied, or when AII was applied together with peptidase inhibitors, the depressant action on Glu responses was observed.

Like AII, angiotensin III (AIII) also had depressant actions on Glu responses in LC neurons, but the action of AIII appeared to be less potent than that of AII.

In addition to its strong depressant actions on applied Glu-induced responses, AII was also found to depress responses to synaptically released excitatory amino acids (EAAs). In fact we were able to show that excitatory postsynaptic potentials (EPSPs) evoked by electrical stimulation were blocked by Glu receptor antagonists, indicating involvement of EAAs in the mediation of the EPSPs. Sar^1-AII or AII applied together with peptidase inhibitors depressed the amplitudes of the EPSPs in a concentration dependent manner. The depressant actions of AII on EPSPs were also antagonized by the AT_2-selective antagonist PD123177, indicating again the involvement of AT_2 receptors in the mediation of AII action on EAA-induced responses.

The functional significance of the depressant action of AII on Glu responses in the LC neurons is at present unclear. It may represent a role for a brain angiotensinergic system in control of cardiovascular functions through modulation of the actions of excitatory amino acid neurotransmitters on LC neuronal activity.

1. INTRODUCTION

It is well known that the connection pattern of the nucleus locus coeruleus (LC) is quite different from other central nervous system (CNS) cell groups. The LC has widespread efferent projections from the spinal cord to the cerebral cortex. In contrast, the afferent projections are from quite limited brain areas (Aston-Jones et al., 1990). It appears that such a widespread noradrenergic output is regulated by a limited input. The regulation of LC neuron activity is therefore of considerable importance, since it may influence target cell activity in very extensive regions of the CNS.

Recent studies have shown that many chemical substances may function as potential neurotransmitters in the LC. One of them is angiotensin II (AII), a naturally occurring octapeptide hormone. It has been demonstrated that all components of an angiotensin system are present in the LC, including angiotensinogen, angiotensin converting enzyme, AII and high density receptor binding sites for AII. It has also been demonstrated that the LC receives innervation by angiotensinergic fibers and terminals. These findings suggest that AII may function as a neurotransmitter or neuromodulator in the LC, and may play an important role in regulating the activity of the LC neurons. The LC is also innervated by glutamatergic fibers. It is possible that AII may affect LC neuronal activity through either direct actions, or by modulating the actions of other neurotransmitters, for example

L-glutamate (Glu).

1. 1. Nucleus Locus Coeruleus

The LC is the largest nucleus of central noradrenergic neurons in the mammalian CNS, and is the origin of most noradrenergic fibers in the brain, including the entire supply to the hippocampus (Loy et al., 1980) and neocortex (Moore and Card, 1984). It is situated in the dorsal part of the pons on the lateral border of the fourth ventricle. This nucleus is limited at its widest extent by the fourth ventricle and pontine central grey medially, mesencephalic V (MeV) laterally and the superior cerebellar peduncle dorsolaterally. It occupies this location in all vertebrate species in which it is found (Russell, 1955; Dahlstrom and Fuxe, 1964; Foote et al., 1983). The findings reviewed here are largely based on the most studied species, the rat.

The LC of rat extends for approximately 900μm rostro-caudally and occupies a volume of approximately 0.8 mm^3. The maximum width of this nucleus is about 340μm, but generally is between 250 - 300μm. Dorsoventrally, it attains a maximum development of 600μm. There are about 1400 - 1600 neurons in the LC (Descarries and Saucier, 1972; Swanson 1976), approximately 200 of which are located in the ventral portion of the nucleus (Swanson, 1976).

The nucleus can be divided into two portions: a dorsal main region containing tightly packed fusiform or bipolar cells

having a major and minor axis, and a contiguous smaller ventral area, containing primarily larger multipolar neurons which have no major axis (Swanson, 1976). The cell bodies of neurons in the dorsal portion are smaller than those in the ventral portion (Dahlstrom and Fuxe, 1964; Olson and Fuxe, 1972; Swanson and Hartman, 1975). The somata size of the LC neurons in the dorsal portion of the nucleus is approximately 20 - 25 μm, while those LC neurons in the ventral portion are 30 - 40 μm (Shimizu and Imamoto, 1970; Grzanna and Molliver, 1980a,b). Both neuron types have long dendrites that branch once or twice and extend outside the nucleus (Swanson, 1976; Cintra et al., 1982). The dendrites of these cells have a moderate number of regularly spaced spines. The soma of the larger LC neurons have prominent protoplasmic appendages which are spine-like but larger than typical spines (Swanson, 1976).

Based on the experimental results of formaldehyde-induced fluorescence in rat LC, Dahlstrom and Fuxe (1964) reported that all or at least practically all of its closely packed neurons belong to the catecholamine type (They described many groups of catecholamine-containing neurons and systematized them as groups A1 to A12. The LC corresponds to group A6). This catecholamine was lately demonstrated to be almost exclusively noradrenaline (NA) at least in the rat brain (Kuhar et al., 1972). In rat, NA was reported to make up 90% of the catecholamine synthesized from ^{3}H-tyrosine (Kuhar et al., 1972).

1. 1. 1. Locus Coeruleus Efferents

The efferent projections of the LC have been thoroughly studied using various methods. It has been demonstrated that the LC provides widespread projections to the entire neuraxis (Fig. 1), including neocortex (Levitt and Moore, 1978; Loughlin et al., 1982; Waterhouse et al., 1983; Moore and Card, 1984; Jones and Yang, 1985), hippocampus (Segal and Landis, 1974; Loy et al., 1980; Harding and Davis, 1985), hypothalamus (Sawchenko and Swanson, 1981; Swanson et al., 1981), amygdala (Ottersen, 1981), thalamus (Lindvall et al., 1974; Westlund et al., 1990), olfactory bulb (Shipley et al., 1985), cerebellum (Bloom et al., 1971; Olson and Fuxe, 1971; Pasquier et al., 1980), nuclei in the pons and medulla (Loizou, 1969) and spinal cord (Nygren and Olson, 1977; Commissiong et al., 1978; Nakazato, 1987). These projections arise from both ipsilateral (predominant) and contralateral LC (For more details, see following reviews: Amaral and Sinnamon, 1977; Moore and Bloom, 1979; Foote et al., 1983).

The projections of subpopulations of LC neurons have also been determined in rat. The majority of spinally-projecting LC neurons in rat are located in the caudal and ventral parts of LC, as determined from injections into the spinal cord of various retrograde tracers, such as Fast Blue (Holets et al.,

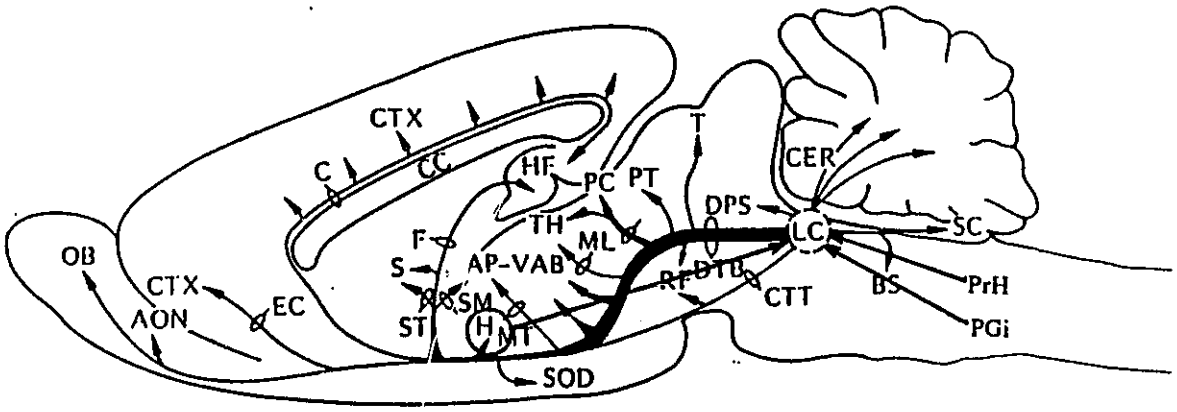


Fig. 1. Diagram of the efferent and afferent projections of the locus coeruleus viewed in the sagittal section. Abbreviations: AON, anterior olfactory nucleus; AP-VAB, ansa peduncularis-ventral amygdaloid bundle system; BS, brainstem nuclei; C, cingulum; CC, corpus callosum, CER, cerebellum; CTT, central tegmental tract; CTX, cerebral neocortex; DPS, dorsal periventricular system; DTB, dorsal catecholamine bundle; EC, external capsule; F, fornix; H, hypothalamus; HF, hippocampal formation; LC, locus coeruleus; ML, medial lemniscus; MT, mammillothalamic tract; OB, olfactory bulb; PC, posterior commissure; PGi, paragigantocellularis; PrH, prepositus hypoglossi; PT, pretectal area; RF, reticular formation; S, septal area; SC, spinal cord; SLX, spinal lamina X, SM, stria medullaris; SOD, superior decussations; ST,stria terminalis; T, tectum; TH, thalamus (modified from Moore and Bloom, 1979).

1988), True Blue (Lyons and Grzanna, 1988; Lyons et al, 1989), dopamine-ß-hydroxylase (DßH) antisera (Westlund et al., 1983), Horseradish peroxidase (HRP)(Mason and Fibiger, 1979) or [^{3}H]choline (Jones et al., 1986). Projections to the hippocampus primarily arise from the dorsal portion of the LC (Mason and Fibiger, 1979). There is also differentiation along the anterior-posterior axis of LC. Neurons in the anterior portion of the LC project to the thalamus, whereas hypothalamus receives axonal innervation from the posterior part of the LC (Mason and Fibiger, 1979).

The topography of projections from single LC neurons is at present not clear. Double labelling of LC neurons by two different retrogradely transported dyes injected into different target areas has revealed that a single LC neuron could project to two different target areas, for example, cerebral cortex and cerebellum (Nagai et al., 1981; Steindler, 1981). It would be very interesting to determine the relationship between an individual LC neuron and the geometry and distribution of its terminal fields in the target areas.

1. 1. 2. Afferents to the LC

The afferent inputs to the LC have been studied using anatomical and physiological techniques. Earlier retrograde labelling of cell bodies following administration of HRP into the LC has indicated that the LC receives wide and divergent sources of afferent inputs from the cerebral cortex, brain stem and spinal cord (Cedarbaum and Aghajanian, 1978a; Clavier , 1979; Morgane and Jacobs, 1979). Using tritiated amino acids for anterograde transport studies, LC was also found to receive projections from some brain areas (Conrad et al., 1974; Beckstead et al., 1979; Saper et al., 1979; Loewy et al., 1981; Price and Amaral, 1981). Since amino acids may transported transsynaptically, some of labellings may not reflect a direct input to LC (Aston-Jones et al., 1990). Receptor binding, immunocytochemical and neuropharmacological studies indicate that many neurotransmitter systems impinge on this noradrenergic

nucleus, suggesting that the LC receive afferents from wide brain regions (Foote et al., 1983). Also, physiological studies have shown that electrical stimulation of peripheral nerves and a variety of brain structures have robust effects on the activity of LC neurons (Aghajanian et al., 1977; Takigawa and Mogenson, 1977). The activity of the LC neurons is even affected by electrical stimulation of tooth pulp and optic nerves (Igarashi et al., 1979). Also, hypercapnia or hypoxia modulate LC neuronal discharges (Elam et al., 1981), indicating central chemoreceptors in circuits afferent to LC. The results of anatomical and physiological experiments indicate that the LC receives afferents from multiple diverse sources, but the fiber origins of these afferents to LC are not quite clear. It should be pointed out that these studies have generally not investigated whether these physiological responses result from direct inputs to the LC.

A more restricted pattern of direct afferents to LC has been proposed recently by Aston-Jones and his colleagues (Aston-Jones, et al. 1986) when they re-examined the afferents to LC following injections of a more sensitive tract-tracer, wheat germ agglutinin (WGA)-conjugated HRP into the compact caudal part of the LC. In contrast to the results of earlier anatomical and physiological studies, they found that only two cell groups were consistently and strongly retrogradely labelled, the paragigantocellularis lateralis (PGi) in the ventrolateral rostral medulla and the prepositus hypoglossi

(PrH) in the dorsomedial rostral medulla (Fig. 1). Retrogradely labelled PGi neurons were predominantly ipsilateral to the injection site, whereas retrograde labelling in PrH was bilateral. Two additional regions consistently exhibiting retrograde labelling (though only a few cells were sparsely labelled) were the paraventricular nucleus of hypothalamus (PVH) and spinal lamina X (SLX). Based on these experimental data, the LC appears to receive afferent inputs from two major sources (PGi and PrH) and two minor sources (PVH and SLX). These anatomical findings appear to have been confirmed by parallel electrophysiological studies showing that a high percentage of neurons in the PGi and PrH were antidromically activated by LC stimulation (Ennis and Aston-Jones, 1987). These results indicate that this globally projecting nucleus, especially those neurons located at the caudal part of the nucleus where the WGA-HRP injections were placed, is actually innervated by a limited number of afferents. However, little is at present known about how these LC-projecting neurons influence LC neurons.

The discrepancy between Aston-Jones' work and earlier reports of widespread afferents to the LC apparently reflected retrograde labelling spreading from the sites of injections into pericoerulear regions which are innervated by fibers from other brain areas (but not projecting directly to the LC), since when injections encroached on pericoerulear areas, neurons labelled retrogradely were found in widespread brain regions as reported previously (Aston-Jones et al., 1991).

1. 1. 3. Intrinsic Organization and Ultrastructure of Rat LC

Both types of LC neurons have 2 - 5 dendrites that are extensively ramified, extending well beyond the boundaries of the nucleus. It has been demonstrated that the dendrites of somata within the rat LC extend a few hundred micrometers outside the boundaries of the nucleus (Swanson, 1976). The LC dendrites appear to be most extensive in the medioventral direction, extending into the pontine central grey adjacent to LC (Shimizu et al., 1978). This site was considered to be the site of convergence for LC afferents (Foote et al., 1983).

Ultrastructural studies in rat have shown that most of the afferents to LC neurons terminate on distal dendrites 0.5 - 2.5 μM in diameter or onto spine-like appendages derived from somata and dendrites. In contrast, relatively few synaptic endings were found to terminate on large proximal dendrites and somata. In addition to afferent synapses, there are dendrodendritic and dendrosomatic contacts between LC neurons (Shimizu et al., 1979).

The somata of LC neurons are covered by several layers of protoplasmic astrocytes. Glia-free surfaces on LC neurons are covered by axo-somatic synapses with boutons terminating either directly on the soma or on a small appendage. The large number of these appendages are characteristic of LC neurons. They vary greatly in width, length and shape , and have cytoplasmic constituents including vesicles and endoplasmic reticulum.

1. 1. 4. Neurotransmitter substances in the LC

Many neurotransmitter systems have been found to impinge on the LC (Foote et al., 1983; Marshall and Finlayson, 1988). The presence of neurotransmitter substances in afferent nerve terminals or in axonal collaterals of the LC neurons indicates that these neurotransmitter substances may play important roles in regulating LC neuronal activity. More than 20 chemical substances have so far been reported to function as neurotransmitters in the LC (for review, see Marshall and Finlayson, 1988). Most of these substances which include catecholamines, excitatory amino acids (EAA), neuropeptides and other transmitter substances (e.g. acetylcholine) are putative neurotransmitters in the CNS. It has been demonstrated that LC neurons are excited by substance P (Guyenet and Aghajanian, 1977, 1979), ACh (Bird and Kuhar, 1977; Engberg and Svensson, 1980; Guyenet, 1980), Glu (Henderson et al., 1982; Olpe and Jones, 1982; Olpe et al., 1989; Xiong and Marshall, 1990), adrenocorticotropic hormone (ACTH)(Olpe and Baltzer, 1981; Valentino et al., 1983), corticotrophin releasing factor (CRF)(Valentino et al., 1983; Valentino and Foote, 1988; Valentino, 1989), adenosine 5'-triphosphate (Harms et al., 1992) and thyrotropin releasing hormone (TRH)(Osmanovic, 1991). They are inhibited by α_2-adrenergic agonists (Aghajanian et al., 1977; Cedarbaum and Aghajanian, 1976, 1977; Williams et al., 1985), γ-aminobutyric acid (GABA) (Cedarbaum and Aghajanian, 1978b; Osmanovic and Shefner, 1990), and mu opiate agonists

(Korf et al., 1974; Bird and Kuhar, 1977; Pepper and Henderson, 1980; Williams et al., 1982, 1988; North and Williams, 1983; Valentino and Wehby, 1989; Seutin et al., 1990). 5-hydroxytryptamine (5-HT) and neurotensin were found to have complex effects on LC neurons (Guyenet and Aghajanian, 1977; Young et al., 1978; Aston-Jones et al., 1991). The only test of AII on the LC neurons was done by Anissimov et al. (1980) and they found the responses of extracellularly recorded rabbit LC neurons to iontophoretically applied AII were complex, with excitation in some cells, inhibition in some other cells or biphasic responses.

In addition to the finding that many putative neurotransmitters have physiological effects on the LC neurons, a variety of neurotransmitter-containing nerve terminals, (Aghajanian et al., 1977; Cuello and Kanzawa, 1978; Fuxe et al., 1976; Hartman et al., 1986; Johansson et al., 1984; Shimizu et al.,, 1979; Steinbusch, 1984; Sutin and Jacobowitz, 1988,1991; Swanson, 1976; Vanderhaeghen et al., 1980; Watson et al., 1978) and transmitter-containing cell bodies (Chronwall et al., 1985; Grzanna and Molliver, 1980a; Melander et al., 1986; Milner et al., 1984; Sakanaka et al., 1987; Sofroniew, 1985; Sutin and Jacobowitz, 1988,1991; Uhl et al., 1979) have been found in the LC. Moreover, the corresponding synthetic enzymes, for example, DßH (Cimarusti et al., 1979), phenyethanolamine N-methyltransferase (PNMT)(Hokfelt et al., 1974), choline acetyltransferase (Kobayashi et al.,1975), tryptophan

hydroxylase (Pickel et al., 1977) and glutamic acid decarboxylase (Ottersen and Storm-Mathisen, 1984), the synthetic enzymes for NA, adrenaline, ACh, 5-HT and glutamate respectively have also been found in nerve terminals in LC. All these data strongly suggest the innervation of the LC by these transmitter-containing fibers mentioned above, though the origins of some remain to be determined.

In correlation to the finding of many neurotransmitters in the LC, the corresponding neurotransmitter receptor binding sites have also been localized in the LC by autoradiographic studies. The LC has been found to have moderate to high binding for several transmitter substances. There is a high concentration for α_2-adrenergic receptor binding site in LC, while α_1-adrenergic binding site is relatively low (Cedarbaum and Aghajanian, 1977; Young and Kuhar, 1980, 1981). Experiments, both in vivo and in vitro, have demonstrated that NA potently inhibits the spontaneous activity of LC neurons via α_2- receptors (Cedarbaum and Aghajanian, 1976; Williams et al., 1985). These data clearly indicate the existence of autoreceptors for NA on the noradrenergic LC neurons. A high density of receptor binding has also been observed for opiates (Pert et al., 1975; Atweh and Kuhar, 1977), with the mu subtype reported to be predominant in LC neurons (Quirion et al., 1983). Many investigations have demonstrated that LC spontaneous activity is strongly suppressed by directly applied opiates and enkephalins in a naloxone reversible manner (Bird and Kuhar,

1977; Guyenet and Aghajanian, 1979; Williams et al., 1982, 1984). These opiate effects are mediated primarily through a mu subtype receptor on LC neurons (Williams and North, 1984). Also present, in moderate to high levels, are the binding sites for AII (Gehlert et al., 1986b; Mendelsohn et al., 1984), substance P (Shults et al., 1984), histamine H_1 receptors (Palacios et al., 1981), muscarinic receptors (Rotter et al., 1979) and somatostatin receptors (Leroux et al., 1985). Low densities of receptor binding sites for 5-HT (Meibach, 1984), Vasoactive intestinal peptide (VIP) (Shaffer and Moody, 1986), atrial natriuretic factor (ANF) (Quirion et al., 1984) and CRF (Wynn et al., 1984) have also been reported. Physiological studies indicate that there is at least one subtype of purinoceptor (P_2-purinoceptor) in the rat LC (Tschopl et al., 1992; Harms et al., 1992). The parallelism in the existence of neurotransmitter-containing fibers, neurotransmitters and their receptor binding sites have been demonstrated for AII, ANF, cholecystokinin (CCK), CRF, EAA, enkephalins, GABA, 5-HT, [illegible], substance P and somatostatin (Marshall and Finlayson, 1988; Aston-Jones et al., 1990).

1. 1. 5. Physiological role of the Locus Coeruleus

With cell bodies in the upper pons and their axon terminals innervating virtually all areas of the CNS with highly branched axons, the LC neurons occupy a strategic position for exerting a broad influence on CNS functions. A large number of

experiments have attempted to elucidate the function of this nucleus since the discovery of its widespread projections (Amaral and Sinnamon, 1977; Foote et al., 1983). Also, many investigators have studied the effects of NA, the neurotransmitter of almost all LC neurons (some also contain certain peptides), on target neurons in many brain regions. It has been found that NA decreases background activity of target neurons and increases signal-to-noise ratio which presumably results from noradrenergic transmission, suggesting LC neurons act to enhance the signal processing capabilities of target neurons. Several postsynaptic actions of NA have now been determined. In addition, the discharge characteristics of the LC neurons have been described in both anaesthetized and in behaving animals, in studies of animal behavior during which NA was supposed to be released from nerve terminals. Based on neuroanatomical studies of LC connections, and the studies of single unit recording of LC neurons and their effects on target neurons, it has been suggested that these LC neurons are associated with autonomic control, such as the control of cardiovascular function (Scriabine et al., 1976; Kawamura et al., 1978; Chida et al., 1983; Sved and Felsten, 1987); control of motor and sensory systems, for examples the control of the spinal motor output (Marshall, 1983; Barnes CD, et al., 1989; Lai et al., 1989; Fung et al., 1991) and the influences on pain (Proudfit, 1988; Jones SL, 1991); sleep-waking activities (Hobson et al., 1975; Jones BE, 1990, 1991); brain development

and plasticity (Kasamatsu, 1991; Nakamura and Sakaguchi, 1990) and the higher functions such as attention (Robbins, 1984; Foote and Morrison, 1987), vigilance (Aston-Jones et al., 1984), orientation (Foote et al., 1991), learning and memory (Sara and Devauges,1988; Velley et al., 1991).

Based on the earlier HRP retrograde studies, the LC was considered to receive afferents from regions as diverse as its efferent projections. This would imply that neurons in the LC may be driven by a great variety of different afferent sources. This makes it difficult to determine how these LC neurons are regulated by afferent neurons and to interpret the physiological functions of this noradrenergic nucleus. However, as outlined above, recent observations by Aston-Jones and his colleagues (1986, 1990) reveal that the afferents to LC originate from more restricted brain structures, mainly from medulla oblongata. Electrophysiological studies have shown that these medullary afferents have powerful influence on the activity of LC neurons, especially those projecting to the LC core (Aston-Jones et al., 1986). It has been found that electrical stimulation of the ventrolateral medulla, specifically the PGi, yields two kinds of effects on LC neuronal discharges: a marked excitatory influence (73% of LC neurons) and a strong inhibitory influence (16% of LC neurons) (Ennis and Aston-Jones, 1986). The excitatory responses can be blocked by EAA antagonists, e.g. kynurenic acid, suggesting that EAAs are the transmitters released at the medullary afferent terminals (Ennis and Aston-Jones, 1986).

Inhibitory responses were blocked by the α_2 adrenoceptor antagonist idazoxan, indicating the PGi inhibitory input to the LC is mediated by an inhibitory adrenergic pathway (Aston-Jones et al., 1992). In contrast, electrical stimulation of another medullary region, the PrH, potently inhibited almost 85% of LC neurons tested (Ennis and Aston-Jones, 1989a,b). This inhibitory response can be antagonized by picrotoxin or bicuculline, indicating that this inhibitory response is mediated by GABA acting primarily at the $GABA_A$ receptor subtype. The role(s) that the medullary afferents may play in the well established behavioral responsiveness of LC neurons remains to be determined.

It has been known for many years that the pattern of the LC efferent connection is quite different from other cell groups in the CNS. Unlike other CNS neurons, individual LC neurons innervate huge territories with highly branched axons. For example, individual LC neurons have been found to project divergently to cerebellum and cerebral cortex. Such widespread connections indicate that LC neurons may be involved in diverse aspects of central processing, rather than in a limited range of sensory, motor or integrative functions.

1. 2. The Brain Renin-Angiotensin System

More than 20 different peptides in the CNS have been described in recent years (Gardiner and Bennett, 1989). Some of these peptides are well defined with respect to their

biosynthetic pathways, their distribution in peripheral and CNS, and their biological functions. AII is one such well-defined neuropeptide. Its biosynthetic pathway and functions in the periphery as a plasma hormone are well recognized and characterized (Fig. 2), but its central biosynthetic pathway and functions are not clear.

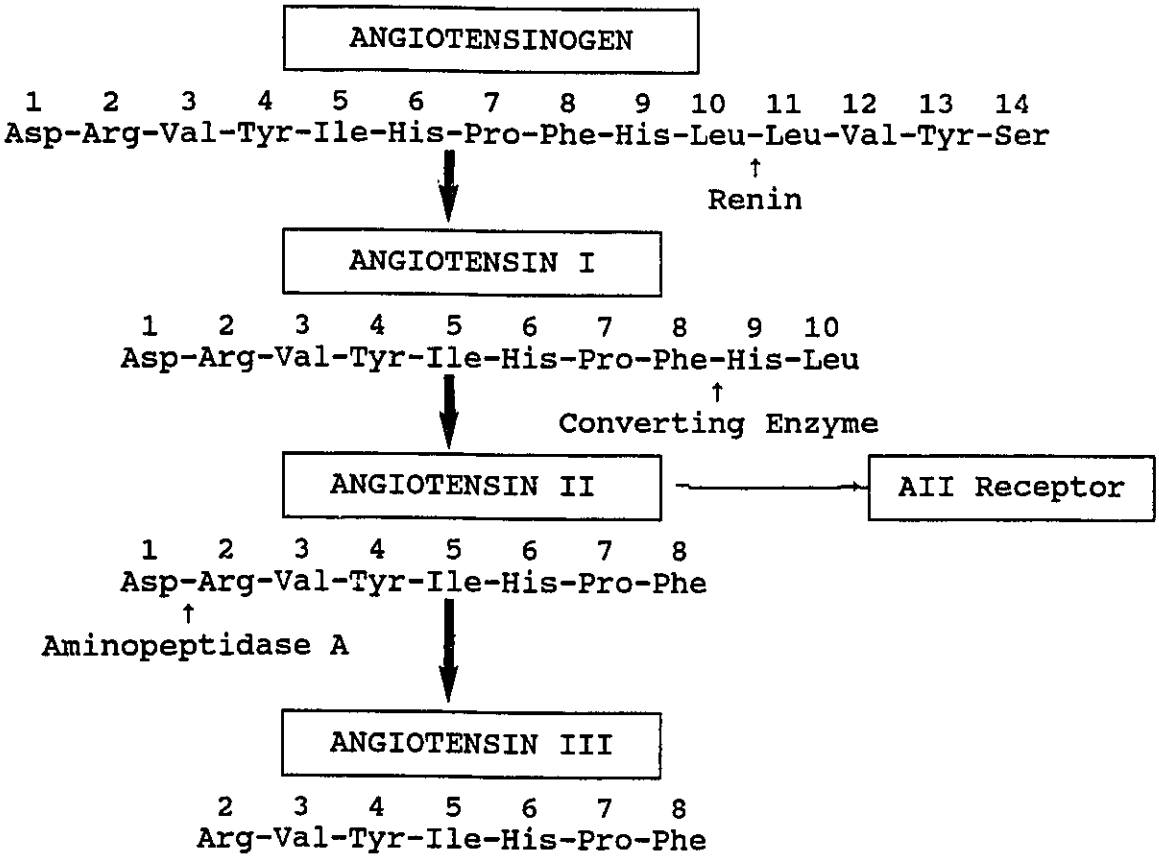


Fig. 2. The renin-angiotensin system showing the amino acid sequences of the components and the peptide bond at which they are hydrolysed by the enzymes.

AII, the major active peptide of brain renin-angiotensin system (RAS), was identified in the 1950's as the agent

responsible for the pressor responses. The pathway by which renin, an aspartate protease released from the kidney, cleaves a decapeptide angiotensin I (AI) residue from angiotensinogen and subsequent formation of the active octapeptide AII by the action of the decarboxypeptidase-converting enzyme is well established. AII is found to stimulate aldosterone release, to influence renal salt and water balance and blood pressure in addition to its direct vascular effect. The possibility that circulating AII could influence the CNS was not recognized until the early 1970's (Ferrario et al., 1970). Although the octapeptide would not be expected to penetrate the blood-brain barrier, sites such as the area postrema, the subfornical organ (SFO), and organum vaculosum of the lamina terminalis (OVLT) offer the potential for central influences by a circulatory peptide (Joy and Lowe, 1970, Fink et al., 1980).

The existence of an endogenous brain RAS was proposed by Ganten et al (1971b) and Fischer-Ferrario et al.(1971) when they found that the components required for the formation of AII were present in the brain. This proposal has now gained widespread acceptance (Ganten et al., 1984; 1987). The evidence in support of a role of AII released from brain neurons as a transmitter is now strong (Phillips, 1987). The details of brain processing and release of AII remain to be determined, and the relationship of central AII mechanisms to peripheral AII remains to be clarified.

1. 2. 1. Components of Brain Renin-Angiotensin System

1. 2. 1. 1. Brain Renin

There is considerable evidence to support the existence of intrinsic brain renin. It has been reported that renin is present in the brains of rat (Fischer-Ferraro et al., 1971; Fuxe et al., 1980; Schelling et al., 1982), mouse (Speck et al., 1981), dogs (Fischer-Ferraro et al., 1971; Ganten et al., 1971b; Osman et al., 1979), hog (Hirose et al., 1980), and human (Ganten and Speck, 1978; Slater et al., 1980). Its presence is measured by its action on angiotensinogen to form angiotensin I.

The regional distribution of the renin in brain has been studied biochemically and immunohistochemically. Biochemical distribution is extensive, with large amounts in hypothalamus and cerebellum, and small amounts in brain stem, spinal cord and amygdala (Inagami et al., 1982a; Paul et al., 1985). Renin is also found in the choroid plexus, adenohypophysis and pineal. Immunohistochemical studies have shown wide distribution of renin positive cells in various regions of rat brain (Fuxe et al., 1980; Calza et al., 1982; Inagami et al., 1982b). Among the brain regions staining for renin positive cells were deep medial cerebellar nuclei, cerebellar Purkinje cells, the nuclei in the brain stem, including NTS, raphe nucleus and the LC (Schelling et al., 1982), and the nuclei in septal region, thalamus and hypothalamus, such as supraoptic nucleus, suprachiasmic nucleus and paraventricular nucleus (Calza et al., 1982; Healy and Printz, 1984). Pyramidal cells of the

hippocampus and some cells in the cerebral cortex and olfactory bulb were stained. The pineal gland and adenohypophysis were also positively stained. The area postrema and SFO are well recognized for their role in mediating pressor effects of blood-borne AII were, however, not stained by antibody to renin. Most of the staining was found in neuronal cells. Some renin positive cells showed granular staining in the perinuclear regions and this was suggested to be an indication of intraneuronal synthesis of renin (Inagami et al., 1982b). The ultimate proof of local synthesis of renin in the central nervous system will stem from recombinant DNA techniques. Using a renin cDNA probe for hybridization studies with mRNA isolated from various mouse tissues, Ganten et al. (1984) have found that the renin gene is expressed and the protein is synthesized locally in brain.

Renin has been confirmed to have some of the physiological effects associated with AII. It has been demonstrated in dog (Reid and Ramsay, 1975) and in rat (Epstein et al., 1973) that the injection of renin centrally induces drinking behaviour. This drinking could be blocked by the angiotensin receptor antagonist saralasin.

1. 2. 1. 2. Angiotensinogen

One component of the evidence in favour of the existence of an endogenous brain RAS is that angiotensinogen on which renin acts to produce AI is present in the central nervous system and

that angiotensinogen can be synthesized in the brain. The first evidence for the presence of angiotensinogen in the brain was obtained by Ganten et al (1971a, 1975, 1976) when brain extracts were incubated with excess renin and tested for AI generating capacity. This was later confirmed in the brains of rats (Lewicki et al., 1978; Imboden et al., 1987), rabbit (Printz and Lewicki, 1977), sheep and dogs (Morris and Reid, 1978) and humans (Allen et al., 1988a), in concentrations of up to 205 ng per ml/CSF (Reid and Ramsay, 1975). The molecular weight of plasma angiotensinogen is 40,000-60,000 which is presumably too large to cross the brain blood barrier. Therefore it is most likely endogenous to the brain and not derived from substrate in the plasma.

The synthesis of angiotensinogen within the brain is supported by the experimental results that cerebrospinal fluid angiotensinogen concentrations do not change when plasma angiotensinogen concentration changes (Reid, 1979) and that radioiodinated angiotensinogen injected intravenously does not appear in cerebrospinal fluid (Morris and Reid, 1978).

Using the technique of cell free translation of mRNA, Campbell et al (1984) provided definite evidence for brain synthesis of angiotensinogen and demonstrated that the angiotensinogen precursors synthesized in liver and in brain are immunoreactively identical, produce similar renin cleavage products, and show equivalent electrophoretic mobility. Recent studies have shown the presence of angiotensinogen mRNA in the

brain by Northern blot analyses using angiotensinogen cDNA as a hybridization probe (Campbell and Habener, 1986; Dzau et al, 1986; Lynch et al., 1986; Ohkubo et al., 1986; Kalinyak and Perlman, 1987). Angiotensinogen mRNA sequences have been reported to be present in the RNA isolated from both neuronal and glial cultures, with three times higher concentration in neuronal cultures than in glial cultures (Kumar et al., 1988). The differences in isoelectric focusing of the angiotensinogens in plasma and brain, together with the findings of angiotensinogen precursors (Campbell et al., 1984) and angiotensinogen mRNA (Campbell and Habener, 1986; Dzau et al, 1986; Lynch et al., 1986; Ohkubo et al., 1986; Kalinyak and Perlman, 1987; Kalinyak et al., 1991) in brain strongly indicate that angiotensinogen is synthesized within brain.

Angiotensinogen is distributed widely throughout the CNS and has been measured in all areas of the brain (Lewicki et al., 1978; Wallis and Printz, 1980; Gregory et al., 1982; Sernia and Reid, 1980; Sernia and Mowchanuk, 1983). A moderate concentration of angiotensinogen has been detected in the LC of rat (Lewicki et al, 1978).

It has been reported that angiotensinogen is synthesized by astrocytes in the rat brain (Deschepper et al., 1986; Stornetta et al., 1988). Cell-associated immunoreactive angiotensinogen is associated predominantly with glia (Deschepper et al., 1986; Imboden et al., 1987; Thomas and Sernia, 1988), although some angiotensinogen immunoreactive

neurons have also been described (Thomas and Sernia, 1988). Intebi et al. (1990) have recently confirmed that angiotensinogen is produced by primary cultures (containing 80% astroglia) of rat brain. They found that astroglia-enriched cultures that contained no identifiable neurons produced angiotensinogen and its mRNA. In another study, however, angiotensinogen mRNA was found in much higher concentrations in neuronal (containing 75 -85% neurons) cultures, as compared with glial cultures (Kumar et al., 1988).

1. 2. 1. 3. Angiotensin Converting Enzyme

Angiotensin converting enzyme (ACE), a dipeptidyl peptidase, required to cleave the histidine-leucine amino acids from the carboxyl end of the decapeptide (AI) to yield the active octapeptide (AII) is widely distributed in the mammalian CNS, including human. In animal and human brain, the highest levels of ACE activity were found in the choroid plexus, hypothalamus, SFO, OVLT, the area postrema, the basal ganglia and the posterior lobe of the pituitary. In other brain areas, different levels of ACE were reported (Yang and Neff, 1972,1973; Arregui et al., 1978,1980; Chai et al, 1987; Tani, 1991). The LC and the NTS exhibit a substantial ACE activity (Arregui et al., 1978; Chevillard and Saavedra, 1982). Using molecular genetic approaches, two forms of ACE have so far been identified, a pulmonary form and a testicular form. cDNAs encoding human pulmonary ACE and rabbit testicular ACE have been

cloned and sequenced (Soubrier et al., 1988; Kumar et al., 1989). The two enzymes sequences share domains which have very high homology (~90%), especially around the putative active centre of the enzyme. The expression of the ACE mRNA in the rat brain has been reported following localization of this enzyme by quantitative *in vitro* autoradiography (Chai et al., 1987; Whiting et al., 1991). Both expression of specific mRNA and enzyme activity were found in the choroid plexus, the SFO, the caudate putamen and the cerebellum. The distribution correlated well with the expression of ACE mapped by radioligand binding and autoradiography. The expression of ACE mRNA in the rat brain has been reported to be pulmonary ACE mRNA rather than the testicular form of ACE mRNA (Whiting et al., 1991)

1. 2. 1. 4. Angiotensins

It has been confirmed that both AI and AII are found in the brains of rats (Changaris et al., 1978; Ganten et al., 1978; Fischer-Ferrao et al., 1971), dogs (Fischer-Ferraro et al., 1971), rabbits and man (Phillips et al., 1979). They have also been extracted from the brains of nephrectomized rats (Ganten et al., 1983). Brain angiotensin appears to have the same amino acid sequence as plasma angiotensin since identical peptides were cleaved from brain and plasma angiotensinogen upon incubation with renin *in vitro* and *in vivo* (Ganten et al., 1983). Also, the increased accumulation of AI has been observed in

nephrectomized hypertensive rats when the conversion to AII was blocked *in vivo* by central application of ACE inhibitor captopril.

AI and AII in brain have been quantified and characterized by high pressure liquid chromatography (HPLC). The actual quantities of AI and AII in the brain are vary between species, but the levels are in the picogram range (Raizada et al., 1981; Hermann et al., 1984, 1986; Mikami et al., 1985; Phillips and Stenstrom, 1985). The AII-like immunoreactivity has been reported to be less than 80pg/g tissue in extracts of whole brain (Phillips, 1987).

Biochemical and immunohistological studies have clarified the distribution of AII in the brain (Ganten et al., 1983; Healy and Printz, 1984; Lind et al., 1985). The main locations are the hypothalamus, the limbic system, the medulla oblongata and the spinal cord. Of particular relevance to circulatory regulation would appear to be the AII found in the paraventricular and supraoptic nuclei of the hypothalamus, the SFO, and the OVLT.

High densities of AII-positive nerve terminals exist within median eminence, the paraventricul nucleus, the supraoptic nucleus and the SFO. Other brain areas containing AII-positive never terminals are the nucleus dorsomedial hypothalami, ventrolateral part of the caudal medulla oblongata, the substantia gelatinosa of the spinal cord, the nucleus tractus spinalis nerve trigemini and the nucleus amygdala centralis (Fuxe et al., 1976; Healy and Printz, 1984). The LC

was found to be innervated by fibres containing AII-like immunoreactivity (Fuxe et al., 1976; Foder et al., 1992).

Most angiotensin immunoreactive cell bodies are found in the nucleus supraopticus and in the nucleus paraventricularis of hypothalamus. According to Swanson and Sawchenko (1980), these angiotensin positive cell bodies in the paraventricular part belong to magnocellular type of nerve cell bodies. These neurons send their angiotensin immunoreactive fibers to the median eminence, posterior pituitary gland and the LC (Fuxe et al., 1976; Ganten et al, 1978; Hirose et al., 1980). Scattered angiotensin immunoreactive cell bodies have also been found in the perifornical area and in the dorsal and ventral part of the lateral hypothalamic area and in amygdala. In the medulla oblongata, cell bodies are found only in the NTS and area postrema. These distributions would strongly support an important functional role for AII in the brain in cardiovascular control. In addition, high concentrations of angiotensin are found in the cerebrospinal fluid of hypertensive patients (Finkielman et al, 1972), and in the cerebrospinal fluid of hypertensive rats (Hutchinson et al., 1975), indicating possible involvement of angiotensin in the pathogenesis of hypertension.

Since the neurons and glia cells in the CNS are equipped with all components of the brain RAS, the likelihood that angiotensins can be synthesized in the brain rather than being taken up across the blood-brain barrier is supported by the findings that AI, AII and renin are found to exist in both

neuronal and glia cells in culture which exclude the peripheral RAS (Hermann et al., 1988b), and that brain cells are capable of synthesizing immunoprecipitable radioactive AII in incorporation experiments using radioactive-labelled amino acids (Raizada et al., 1983; Hermann et al., 1988a). Also, AII-like peptide has been reported to be released from cultured rat brain cells in response to chemical stimulation (Meyer and Weyhenmeyer, 1986). The release of AII by cultured neurons supports the proposed role for AII as a neurotransmitter/neuromodulator in the CNS.

1. 2. 2. Angiotensin II Receptors

To identify discrete neuronal entities in the CNS bearing AII specific binding sites, autoradiographic studies enable the exact histological localization and spatial distribution of AII receptors in the brain of rat (Mendelsohn et al., 1984; Gehlert et al., 1986b; Saavedra et al., 1986b; Tsutsumi and Saavedra, 1991d), dog (Speth et al., 1985), rabbit (Mendelsohn et al., 1988), calf (Bennett and Synder, 1976), sheep (McKinley et al., 1986) and human (McKinley et al., 1987; Allen et al., 1988b, 1991; Barnes JM, et al., 1991). These results clearly indicate the presence of specific receptors for AII in the CNS. The earlier studies utilized ^{125}I-AII, and examined the binding of this radioligand to membranes prepared from brain tissue. In the calf brain the highest area of ^{125}I-AII specific binding was found in the cerebellum (Bennett and Snyder, 1976). In rat brain, cerebellum contains only low levels of ^{125}I-AII specific

binding, with the majority of the binding being located in hypothalamus, thalamus, midbrain and septum (Sirett et al., 1977). Subsequent ligand and in vitro autoradiographic studies have demonstrated a discrete localization of specific receptors for AII in the rat CNS (Gehlert et al., 1986b; Healy et al., 1986; Mendelsohn et al., 1984; Sirett et al., 1979; Walters and Speth, 1988). There is a very high density of AII receptors in areas outside of the blood-brain barrier, such as the SFO, OVLT and the area postrema (Mendelsohn, 1985; Van Houten et al., 1980). These findings are not surprising because AII has been shown to act at these sites to elicit dramatic changes in blood pressure and drinking behaviour (Phillips, 1987). These areas can be affected by peripherally generated AII. High densities of AII receptors are found in the paraventricular and periventricular nuclei of the hypothalamus, and in the NTS in the brain stem. Other brain areas which contain high density of AII receptors are the LC, the lateral olfactory tract, subthalamic nucleus and inferior olive. Low densities of specific AII receptors are found in areas such as the striatum and interomediolateral column of the spinal cord. These AII receptors are presumably acted on by AII generated from the brain endogenous renin-angiotensin system (Ganong, 1984; Lynch et al., 1986; Phillips, 1987). The presence of AII receptors on neuronal and astrocytic glial cells has been confirmed using fluorescence and immunocytochemical methods. Stamler et al., (1980) have demonstrated that AII receptors specifically

localize to the cell bodies and neurites of the neurons in mixed cultures. This finding was later confirmed by other researchers (Raizada et al., 1984) by using light microscopic autoradiography with ^{125}I-AII. AII receptors were also found in the cell bodies and neurites of neuronal cells in mouse spinal cord cultures (Simmonet et al., 1988). The localization of AII receptors to different parts of the neuronal and astrocytes may indicate a functionally distinct population of receptors.

With the development of non-peptide selective AII receptor antagonists, AII receptors have further been classified into two subtypes (Chiu et al., 1989b; Whitebread et al., 1989). Receptors with highest affinity for losartan (DuP753, K_i = 10-50 nM) and the lowest affinity for PD123177 (K_i > 10 μM), are refered to as AT_1 receptors (proposed nomenclature by Bumpus et al., 1991). In contrast, binding sites displaying highest affinity for PD123177 (K_i = 10-100 nM) and lowest affinity for losartan (K_i > 1 μM), are refered to as AT_2 receptors (Bumpus et al., 1991). The prototypical antagonist of the AT_1 receptor is losartan. The prototypical antagonists of the AT_2 receptor are PD123177, PD123319 and CGP42112A (Catt and Abbott, 1991).

As in the peripheral tissues, two subtypes of AII receptors have also been identified in the CNS of mammals, including human (Barnes JM, et al., 1991), and characterized by their sensitivity to the sulfhydryl reducing agent DL-dithiothreitol (DTT) (Gehlert et al., 1991a; Speth et al., 1991; Tsutsumi and Saavedra, 1991c) and by displacement with specific AII receptor

antagonists (Gehlert et al., 1990; Rowe et al., 1990b; Wamsley et al., 1990; Leung et al., 1991; Song et al., 1992). Binding to AT_1 receptors is sensitive to relatively low concentrations of DTT and losartan (DuP753) and insensitive to PD123177 and CGP42112A (Gehlert et al., 1986b). Binding to AT_2 subtype receptors is inhibited by the AII antagonist PD123177 or CGP42112A but not with relatively low concentration of DTT or the AT_1 antagonist losartan (Speth and Kim, 1990; Tsutsumi and Saavedra, 1991c).

A high density of receptor binding sites has been found in the LC of rats (Mendelsohn et al., 1984; Song et al., 1992). Further localization studies indicate that AII receptors in the rat LC are located on noradrenergic nerve cell bodies or their dendritic and axonal ramifications within the LC (Rowe et al., 1990a). The majority of AII receptors in the rat LC are of the AT_2 subtype (Rowe et al., 1991; Song et al., 1992).

The primary evidence supporting the idea that AT_1 and AT_2 receptors probably play different roles in the brain are the findings of differences in their distribution and developmental expression. AT_1 receptors are mainly located in the brain areas outside the brain blood barrier related to body fluid regulation, such as SFO, and in brain areas related to blood pressure control, for example, the NTS. The actions of AII in these areas are well-known, for instance cardiovascular and fluid control. AT_2 receptors are expressed in brain regions involved in sensory and motor control, such as the ventral

thalamic nuclei, the LC and the inferior olive. The actions of AII in these regions are unclear. The expression of AT_1 receptors in young and adult animals is similar. In contrast, AT_2 receptors are more highly expressed in earlier developmental periods.

1. 2. 3. Functions of Angiotensin II in the CNS

The physiological role of AII in the CNS is at present uncertain. There is accumulating evidence that AII may play an important role in the neural control of body fluid homeostasis and cardiovascular regulation. Evidence for this comes largely from the studies in which AII was directly injected into the cerebral ventricles. Direct injection into the cerebral ventricles allows AII to reach many receptor sites. In contrast, peripherally administrated AII can only reach CNS sites outside blood-brain barrier.

One of the most compelling biological effects produced by intraventricular administration of AII is drinking. The OVLT and its adjacent median preoptic nucleus were suggested to be the sites on which intraventricularly injected AII acts to mediate the drinking response. In contrast, the SFO was postulated as an important site of action for peripherally administrated AII in mediating this drinking response, since experimental lesion of the SFO abolishes the drinking response to intravenous AII, but injections directly into the brain still cause drinking even following such lesions (Phillips, 1978).

Receptor binding studies have confirmed that these brain areas have high densities of receptor binding sites for AII. In addition, AII was found to have neurotrophic effects on spinal cord in cultures (Iwasaki et al., 1991), to participate in learning and memory (Yonkov and Georgiev, 1990), to interact with other neurotransmitters in the CNS, such as catecholamine, 5-HT.

In summary, considerable evidence indicates that there is an endogenous brain RAS. Biochemical, immunohistochemical and immunocytochemical studies have revealed the localizations and distributions of all components of the brain RAS. The major brain structures involved in the RAS are hypothalamus, brain stem and spinal cord. Two types of AII receptors have so far been identified at least in rat CNS. The LC is associated with high a density of binding sites for AII and is innervated by fibres containing AII-like immunoreactivity, in addition to renin, angiotensinogen and ACE being found in this nucleus. Although the functions of brain RAS remain to be elucidated, many experimental results indicate the involvement of the brain RAS at least in the control of body fluid homostatsis and cardiovascular functions.

1. 3. Excitatory Amino Acids

The EAAs for example Glu and L-aspartate (Asp) exert strong excitatory actions on most neurons in the mammalian CNS. Earlier biochemical studies revealed a central role in neural

function for Glu (Weil-Malherbe, 1936). Subsequent electrophysiological studies showed that Glu and other naturally occurring EAAs excited single neurons in the mammalian brain (Curtis et al., 1959). Considerable evidence has later accumulated that Glu and some other EAAs may function as excitatory neurotransmitters in the mammalian CNS. Today Glu satisfies, to a large extent, the main criteria for classification as a neurotransmitter in the mammalian CNS (Fonnum, 1984).

1. 3. 1. EAA receptors in the CNS

It is generally believed that the majority of synapses in the mammalian CNS use EAAs as their neurotransmitters. The excitation of mammalian central neurons by the EAAs has been demonstrated to be the consequence of activating EAA receptors (Watkins and Evans, 1981; Mayer and Westbrook, 1987; Watkins and Olverman, 1987). As with other neurotransmitters, multiple receptor subtypes for EAAs have been found in the mammalian CNS. The characterization and classification of EAA receptors were originally made on the basis of electrophysiological studies (Watkins and Evans, 1981). This classification has since been supported by neurochemical, biochemical and anatomical studies using brain membrane preparations and autoradiography (Foster and Fagg, 1984; Greenamyre et al., 1985; Monaghan and Cotman, 1986). Based on the use of exogenous compounds and on the relative susceptibility to a number of agonists and antagonists,

it is currently thought that there are five subtypes of receptors for EAAs in the CNS.

1). NMDA receptors, where N-methyl-D-aspartate acid (NMDA) and Asp are potent and specific agonists, and D-2-amimo-5-phosphonovalerate (APV) and DL-3-(2-carboxypiperazin-4-yl)propyl-1-phosphate (CPP), act competitively as potent and selective antagonists.

2). AMPA receptors (formerly called QA receptors), where L-quisqualate (QA) and DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) are potent agonists, differing in their specificity. 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) and 6,7-dinitro-quinoxaline-2,3-dione (DNQX) are potent antagonists.

3). KA receptors, where kainate (KA), domoate (Dom) are potent and specific agonists. CNQX and DNQX are potent antagonists.

4). AP4 receptors, where 2-amino-4-phosphonobutyrate (AP4) is a selective agonist. No selective antagonist is available.

5). ACPD or metabotropic receptors, where trans-1-amino-cyclopentyl-1,3-dicarboxylate (ACPD) is a potent and selective agonist. No selective antagonist is available at present.

The presence of selective and potent NMDA receptor antagonists allows a clear identification of NMDA receptors.

However, the distinction between QA and KA receptors is still obscure. These two subtype EAA receptors were originally distinguished by the selective antagonists at QA/AMPA receptor by glutamate diethyl ester (GDEE) (McLennan and Lodge, 1979), and by neuronal sensitivity to KA (rather than to QA) (Agrawal and Evans, 1986). Further distinctions have been made by autoradiographic localization of QA and KA receptors (Greenamyre et al., 1985; Monaghan et al., 1985). These differences are less distinctive than the differences separating NMDA receptors from QA and KA receptors, so the latter two EAA receptors are considered and named collectively as non-NMDA receptors.

1. 3. 2. Membrane Response to EAA Receptor Activation

Among the EAA receptors mentioned above, the three major subtypes are NMDA, AMPA and KA. These three subtypes of EAA receptors are well studied in comparison with the other two. The AMPA receptor is also activated by QA and was formerly called QA receptor (Foster and Fagg, 1984; Mayer and Westbrook, 1987; Watkins et al., 1990). It appears that different EAA receptor subtypes are coupled to separate ion channels (Ascher and Nowak, 1988). Activation of any one of these three subtype receptors causes depolarization of postsynaptic neuronal membrane, allowing an influx of Na^+ and an efflux of K^+ ions through the ion channels coupled to the postsynaptic receptors. The ion channels coupled to NMDA receptors also allow an influx of Ca^{2+} ions in addition to the influx of Na^+ ions (MacDermott et al.,

1986).

NMDA receptors are connected to ion channels which are susceptible to a voltage-dependent blockade by Mg^{2+} ions. The Mg^{2+} ion blockade of NMDA receptor coupled channel can be released when the membrane potential is partially depolarized (Mayer et al., 1984; Nowak et al., 1984). It is generally accepted that at normal membrane potential and physiological concentration of Mg^{2+}, the postsynaptic neuronal membrane is in such a condition that it is insufficiently depolarized to release the Mg^{2+} blockade of NMDA receptor-coupled ion channel, therefore the NMDA component in membrane depolarization is generally small. However, when the membrane potential is depolarized to a certain level by strong stimuli, such as excitatory transmitter release or even after the depolarization mediated by activation of non-NMDA receptors, the Mg^{2+} blockade is then released, causing further depolarization. Patch clamp analyses have revealed that, in the absence of Mg^{2+}, NMDA channels are voltage independent. However, when experiments are performed in the presence of Mg^{2+}, currents are blocked in a voltage-dependent manner (Mayer et al., 1984; Nowak et al., 1984). The effect of Mg^{2+} is maximal at hyperpolarizing conditions and virtually absent at -20 mV and above (Mayer et al., 1984; Nowak et al., 1984).

The NMDA receptor-ion channel complex contains an integral ion channel that gates Na^{+}, K^{+} and Ca^{2+} ions and is blocked in a voltage dependent manner by Mg^{2+}. It also contains

several binding sites in addition to the recognition site for the agonists and competitive antagonists and the binding site for Mg^{2+}. It has been demonstrated that a number of compounds, including the dissociative anaesthetic ketamine and anticonvulsant (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), selectively antagonize NMDA receptor-mediated excitations. Glycine, an inhibitory neurotransmitter, is found to potentiate NMDA-induced responses in cultured neurons by increasing the frequency of channel openings (Johnson and Ascher, 1987). This effect of glycine was demonstrated to be strychnine insensitive, and was considered to depend on allosteric interactions with the NMDA receptor instead of acting on traditional strychnine sensitive receptors (Ascher and Nowak, 1987; Bonhaus et al., 1987).

Ion channels coupled to non-NMDA receptors show characteristics different from ion channels operated by NMDA. Activation of non-NMDA receptors causes a rapid depolarization of postsynaptic neuronal membrane accompanied by increases in membrane conductance (Engberg et al., 1978; MacDonald and Wojtowicz, 1980; MacDonald et al., 1982; Yamamoto and Sato, 1985). The conductances due to activation of non-NMDA receptors are voltage independent and have a reversal potential close to 0mV (Yamamoto and Sato, 1985). These conductances involve Na^+ and K^+ currents (no Ca^{2+} currents).

1. 3. 3. Physiological Roles of EAA in the CNS

EAAs are generally considered to be the major excitatory neurotransmitters in the CNS. There are so many EAA-utilizing pathways in the CNS that the physiological functions of EAAs remain to be elucidated. EAAs have been demonstrated to be crucial for fast excitatory neurotransmission in the CNS and their receptor activation has been proposed as the molecular basis of synaptic plasticity in developing and mature brain. One of the best characterized system for synaptic plasticity is the phenomenon of long-term potentiation (LTP).

EAAs have also been found to reduce the growth rates and length of dendrites without altering axonal growth in cultured hippocampal pyramidal cells (Mattson et al., 1988). The effect of EAAs on growth can be attenuated by either gamma-D-glutamylglycine (DGG) or calcium channel blocker, but not by APV, indicating the effect on growth occurred as a result of the opening of voltage-gated calcium channels by quisqualate and kainate receptor activation. Brewer and Cotman (1988) have reported that NMDA and noncompetitive NMDA antagonist MK-801 can modify the length and branching pattern of neural growth in cultured dentate granule cells. These findings suggest that EAAs not only function as neurotransmitters in the CNS but also play a role in neural growth. EAAs have been shown to influence other brain functions, such as sleep (Fox and Armstrong-James, 1986; Armstrong-James and Fox, 1988) and locomotion (Grillner et al., 1981; Donzanti and Uretsky, 1984).

In addition to their normal functions in the CNS, EAAs will produce neurotoxicity when administered into the CNS. It has been suggested that cell death is essentially due to excessive excitation caused by EAAs (Olney, 1984). EAA receptor antagonists have been found to have the effect of protecting against neuronal cell death in models of ischaemia (Simon et al., 1984), hypoglycaemia(Wieloch, 1985) and trauma (Faden et al., 1989). Besides, EAAs are found to be involved in epilepsy since EAA receptor antagonists were found to have the ability to prevent seizures (Croucher et al., 1982; Hayes and Balster, 1985).

1. 4. Modulation of Glutamate Actions by Other Neurotransmitters.

Modulation, as defined by Kaczmarek and Levitan (1987) is the process whereby a neuroactive substance, say a locally released biogenic amine, peptide or a systemic hormone acts on synaptic transmission. Several different mechanisms have been postulated to transduce these extracellular signals into changes in excitability. These mechanisms usually involve specific membrane receptors and a chain of biochemical reactions such as protein phosphorylation which often requires calcium as well as second messengers, or through direct action on membrane ion channels. The net result of these reactions may be an altered state of a channel or receptor which in turn may cause a change

in the strength of synaptic transmission.

It has been reported that the actions of some neurotransmitters in the CNS are modulated by some other neurotransmitters (for a recent review, see Marshall and Xiong (1991). Glu is an important and widespread transmitter substance in the mammalian CNS. Recent studies have shown that the actions of Glu in the CNS are subject to modulation by some other neurotransmitters, such as NA, 5-HT, and so on (For review, see Marshall and Xiong, 1991). It has been shown in rat hippocampal slices that NA enhances the responsiveness of CA1 pyramidal neurons to iontophoretically applied Glu by increasing spike frequency and duration of the spike train (Madison and Nicoll, 1986). This action of NA is mediated via β_1-noradrenergic receptors by reducing the slow calcium-activated potassium conductance. Also in rat hippocampal slices, Segal (1982) reported that NA or isoproterenol potentiated the depolarizing responses to Glu in intracellularly recorded CA1 pyramidal neurons. This effect was suggested to be the action of NA on the postsynaptic dendritic site to increase the reactivity to application of Glu (Segal, 1982).

NA has also been found to potentiate the actions of Glu on Purkinje cells. In earlier studies, NA was found to facilitate responses of cerebellar neurons to iontophoretically applied Glu (Moises et al., 1979; Woodward et al., 1979). Subsequent studies revealed that NA could enhance Glu-induced depolarizations on cultured chick Purkinje neurons as well

(Mori-Okamoto and Tatsuno, 1988, 1989). Using explant cultures of mouse cerebellum, Marshall and Tsai (1988) demonstrated that NA produced potent increases in Glu activations of Purkinje cells. They found that these potentiations had short or long durations, and demonstrated that the short duration potentiation was mediated by α_1 adrenoceptors, whereas the long duration potentiation was induced through activation of ß-adrenoceptors. The modulation of Glu actions by NA has been found in other brain areas, such as spinal cord (White and Neuman, 1983).

5-HT has also been found to have modulatory actions on Glu effects in several brain areas, including the LC (Aston-Jones et al., 1991). In the neocortex, 5-HT potentiates Glu-induced depolarizations without changing membrane input resistance (Nedergaard et al., 1987). Similar potentiating effects on Glu-induced excitations have been demonstrated on spinal cord motoneurons (White and Neuman, 1983). These potentiating actions of 5-HT are blocked by 5-HT receptor antagonists. In addition to its potentiating actions in aforementioned brain areas, 5-HT has been reported to exert inhibitory actions on Glu-induced responses in rat cerebellar Purkinje cells (Lee et al., 1986). The inhibition of Glu-induced excitations by 5-HT was observable in the absence of other changes in the membrane properties of intracellularly recorded Purkinje neurons in brain slices (Hicks et al., 1989). Potent inhibitory actions of iontophoretically applied 5-HT on Glu-induced responses have also been observed in rat LC neurons in vivo (Aston-Jones et

al., 1991). 5-HT was found to have no consistent effect on LC spontaneous firing, but reliably depressed the responses of LC neurons to iontophoretically applied Glu. This inhibitory action of 5-HT is antagonized by 5-HT receptor antagonists methysergide and methiothepin, indicating 5-HT receptors are involved in the mediation of this inhibitory action.

In a whole-cell patch-clamp study of rat spinal dorsal horn neuorns (layer I-III), substance P was found to potentiate Glu-induced current in 65% of the cells tested, mainly by potentiating NMDA receptor-induced current (Randic et al., 1990). The site of substance P modulation of Glu-induced current was on the postsynaptic membrane, and the mechanism underlying this modulation has been demonstrated that substance P modulates the basal efflux of endogenous Glu (Kangrga et al., 1990).

Modulation of Glu effects by GABA was also observed in neocortical and archicortical structures (Walden et al., 1990). In brain slices containing neostriatum, cortex and corpus callosum, Glu-mediated synaptic potentials recorded in neostriatal neurons were depressed by a $GABA_B$ receptor agonist, baclofen in a dose-dependent manner. This depression was not coupled with changes of membrane potential and apparent membrane input resistance (Calabresi et al., 1992). The depression action of baclofen (applied by iontophoresis or pressure ejection) on EPSPs (mediated via Glu) was also found in intracellularly recorded rat frontal neocortical neurons in a slice preparation (Howe et al., 1987). These experimental

results suggest that at least some neurotransmitters in the CNS may express their physiological functions by modulating the actions of Glu, instead of acting directly on CNS neurons.

In summary, Glu is an important excitatory neurotransmitter in the CNS. Five subtype Glu receptors have been identified so far and the major three of them (NMDA, AMPA and KA) have been well characterized. Glu receptors are widely distributed in the CNS, including the LC. Depolarization is the membrane response to the activation of these major subtype Glu receptors. The actions mediated by Glu can be blocked by a variety of antagonists. Glu plays important roles in the neurotransmission and synaptic plasticity in the CNS. The actions of Glu in the CNS have tendency of being modulated by other neurotransmitters.

1. 5. Objectives

LC receives angiotensinergic fibers from hypothalamus and all components of RAS have been found to exist in the LC, in addition to high density binding sites for AII receptor. The LC is also innervated by glutamatergic fibers originating from medulla oblongata. The overall objective of this study is to find out what kind of action and function AII may play in this central noradrenergic nucleus and its possible interaction with Glu. The specific objectives are as follows:

1). To study the actions of AII on spontaneous activity of LC neurons.

2) To study the actions of AII on applied Glu evoked

activity. We hypothesize that AII might have modulatory actions on Glu evoked activity, since the action of Glu in the CNS is subject to be modulated by some other neurotransmitters (Marshall and Xiong, 1991).

3). If AII has effects on applied Glu evoked activity, the effects of AII on endogenously released Glu evoked activity will be tested since it has been reported that electrically evoked EPSPs in LC are mediated by endogenously released Glu (Cherubini et al, 1988).

4). To determine which subtype(s) of Glu receptor is (are) affected by AII.

5). To find out which subtype(s) of AII receptor is (are) involved in the mediation of the action of AII on Glu.

6). To investigate possible mechanisms which are responsible for the action of AII.

2. MATERIALS AND METHODS

2. 1. Surgery

Subjects for this study were 213 male Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) Weighing 50 - 135 grams. The animals were given intramuscular ketamine (35mg/kg) injection followed ten minutes later by exposure to an oxygen-rich anaesthetic chamber for five minutes at a flow rate of 2L/min., followed by 2-3.5% halothane in oxygen for another three minutes at the same flow rate. Immediately after removal from the anaesthetic, the animals were surgically decapitated at the atlanto-occipital joint following a rostro-caudal cut over the scalp. The skull of the animal was removed using a surgical forceps. Before the brain was lifted out of cranium, the dura mater was carefully cut and lifted away. A transverse section was made at the level of approximate 1.0 - 1.5mm rostral to cerebellum and the cranial nerves attached to pons were sectioned. The brain was then carefully lifted out of the cranium cavity and placed into a cold and oxygenated artificial cerebrospinal fluid (ACSF) to cool it and to wash away blood and debris. The pia mater was, with caution, picked off from the ventral side of the brain stem when the brain was in cold ACSF solution. The brain is then ready for blocking.

2. 2. Brain Slice Preparation

The brain was transferred from the petri dish containing

cool and oxygenated ACSF to a flat surface on which there was a piece of filter paper moistened with ACSF. The pons with part of cerebellum was separated from the rest of the brain tissue by two sections made at the caudal and rostral limits of the pons using razor blades. The pons was then trimmed. The cranial nerve roots and some pial tissue were removed with cuts from both dorsal and ventral sides as well as the lateral sides, making a final tissue block of approximate 6 x 5 x 5 mm. The tissue block was glued to the plexiglass stage cover using cyanoacrylate glue (Japan) with rostral (in transverse slice) or dorsal (in horizontal slice) surface uppermost. Its ventral side (in transverse slice) or caudal side (in horizontal slice) rested against a plexiglass block cushioned with an agar slab. Either transverse or horizontal brain stem slices of 350μM in thickness containing the LC were taken from the tissue block using a vibratome (Vibroslice - Campden Instruments LTD, England). Serial slicing took place in a vessel (~100ml in volume) containing cold (4°C) ACSF which was oxygenated while slicing. Blade speed was set on the highest vibration frequency and advance of the blade was set on the lowest speed. The determination of the level of slicing was in most cases made by naked eye, and in some cases, a dissecting microscope (Carl Zeiss, Jena Instrument LTD, Germany) was employed in order to identify the level of the LC. One or two slices were usually taken in one rat at the LC level. The slice cut in more caudal (in transverse section) or a little dorsal (in horizontal

section) was, in most cases, used for this study if two slices were cut at the LC level in one rat. The selected slice was transferred from the slicing vessel to a petri dish containing chilled oxygenated ACSF with a wide bore plastic tube and suction bulb, and then transferred to the slice chamber. The total time from surgical decapitation to transfer of the selected slice to the slice chamber was in most cases less than 10 minutes.

2. 3. Slice Chamber and Perfusate

Two kinds of slice chambers made in plexiglass were used in this study. The slice chamber used in our earlier experiments was perfused from below the slice through a side hole at the bottom level of the chamber and sucked away from top through a 22G1 1/2 needle (Becton-Dickinson, U.S.A.) which was connected to the laboratory vacuum system (Fig. 3A). The volume of this chamber is about 0.5ml. Since the fluid level in this kind of slice chamber is not easily adjustable, it was not possible to reduce the fluid level in this slice chamber and to minimize the capacitance of recording electrode. This chamber was later replaced by another kind of slice chamber which was perfused from above the slice also through a side hole and sucked away from a small separated chamber which connected to the slice chamber through a tunnel beneath (Fig. 3B), so that the fluid level in the slice chamber was adjustable. The total volume of this chamber is 0.8ml, but the working volume is approximate the

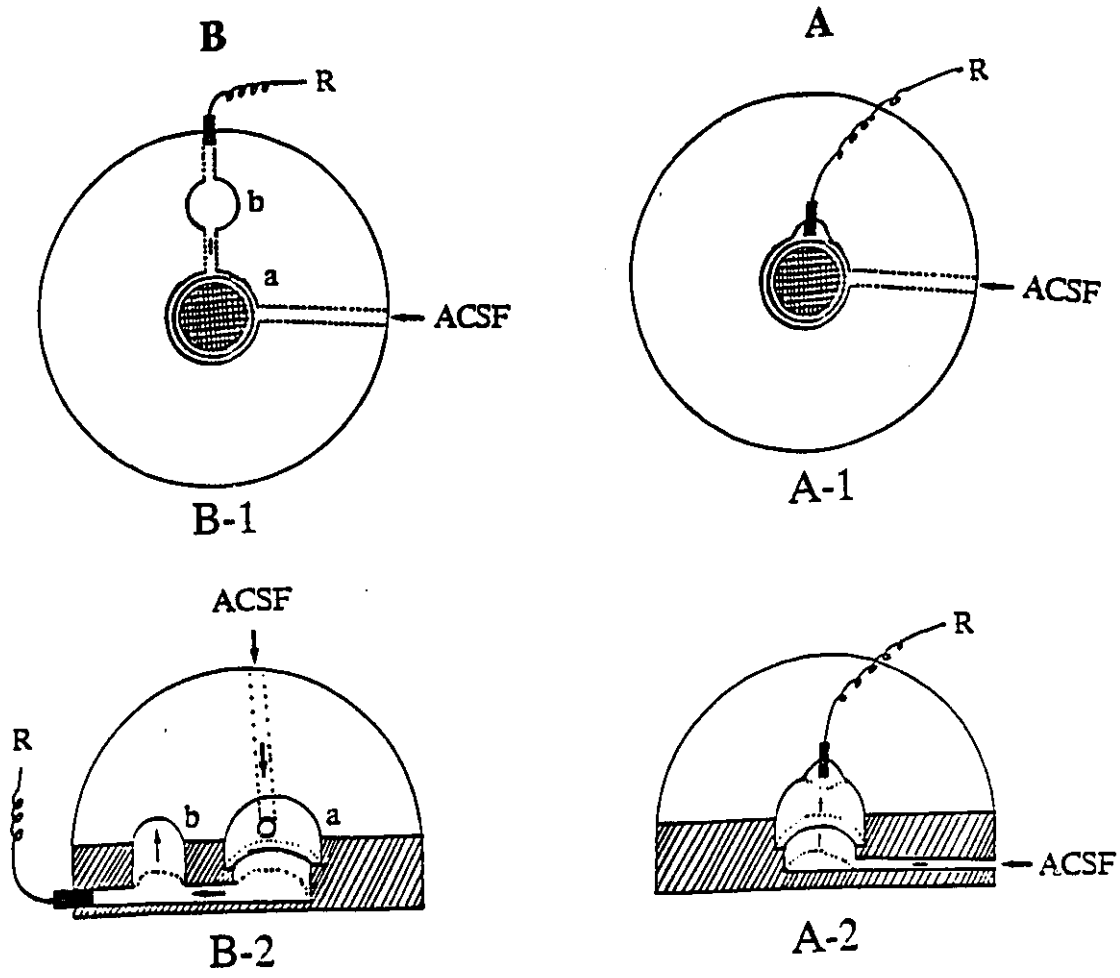


Fig. 3. Diagrams of plastic chambers for in vitro studies of brain slices. **A.** A simple chamber. **A-1** is a top view of the chamber. **A-2** is a side view of the chamber. The perfusate flows into the chamber from the bottom and is sucked away from the top. The fluid level can not be adjusted in this chamber. **B.** A modified chamber. **B-1** is a top view, showing that there is a side hole (b) beside the chamber (a). **B-2** is a side view of the chamber. Both the chamber and the side hole are connected through a tunnel beneath. Perfusate flows over the slice and down to the bottom of the chamber, then through the tunnel to the side hole where the perfusate is sucked away. The fluid level in this chamber is easily adjustable. R: reference electrodes. ACSF: artificial cerebrospinal fluid.

same as previous one since the fluid level was adjusted in each experiment. Inside the chamber near the bottom, there is a circular step on which rests an O-shaped plastic ring made from a plastic syringe. This O-shaped ring supports a piece of nylon mesh made of ladies' stocking.

The selected slice rested on a nylon mesh on which there was a small piece of triangular lens paper which supports the slice, and superfused with ACSF at a flow rate of 1.5ml/min., which was measured and adjusted before the slice was transferred into the slice chamber. The ACSF was made on the experimental day by dilution of a 5 times stock solution with deionized, bi-distilled water and contained (in mM): NaCl 118.0, KCl 3.0, $CaCl_2$ 2.5, $MgSO_4$ 0.8, NaH_2PO_4 1.0, $NaHCO_3$ 20.0, and D-glucose 10.0. The stock solution contained all above ingredients except $NaHCO_3$ and D-glucose. $NaHCO_3$ and D-glucose were added to the ACSF on the experimental day. Osmolarity was in the range of 290 - 300 mOsm. The ACSF contained in reservoirs was continuously heated and bubbled with 95% O_2/ 5% CO_2. The ACSF in the slice chamber had a pH of 7.4.

2. 4. Heating

The ACSF was contained in a double layer jar and heated through a water circulation heating system (Heto, Denmark). The temperature of the circulating water was set at 35°C. In order to prevent the ACSF from losing heat on the way into the slice chamber, the flow line of the ACSF was run through a plastic

jacket (near the slice chamber) which was also heated by the same water circulation heating system. The ACSF in the slice chamber was maintained at 32 ± 0.5°C.

2. 5. Electrodes

2. 5. 1. Intracellular Recording Electrodes

Microelectrodes for intracellular recording were pulled from glass-fibre filled, borosilicate glass pipettes (1.2mm O.D., 0.7 I.D., Dagan Co., Minneapolis, Minnesota, U.S.A.) using a Brown-Flaming puller (Sutter Instruments Co., Model P-87, California, USA). Those electrodes which had a desired tip shape (Fig. 4A) and were 8 - 10μ in length in the fine parts of their tips were used. The microelectrodes were filled with 2M KAc just before use since they contained glass filaments for quick filling (Tasaki, et al., 1968). The d.c. tip resistances of these electrodes were in the range of 60 - 120 MΩ.

2. 5. 2. Iontophoresis Electrodes

Seven glass-fibre filled, borosilicate glass pipettes (1.2mm O.D., 0.9mm I.D., Dagan, U.S.A.) were held together by heating a shrinking rubber tubing over an open flame and pulled on a Stoelting vertical puller in one step while twisting them 90 - 180° (to help the capillaries adhere together), producing a fine single tip containing seven barrels. The ends of the pipettes were staggered in order to avoid cross-contamination

when filling and electrical circuit shortage while passing current due to the formation of "salt bridge". The tips of iontophoretic electrodes were broken back to a total diameter of 4 - 8μm by bumping the tip of the electrode against a glass rod under microscope observation (Fig. 4B). Each barrel was filled at least 30 minutes before experiments with different drug solution or control solution by using different hypodermic syringes and needles with fine flexible plastic tubing attached (approximate 5cm in length).

The iontophoresis electrodes were carefully filled in order to avoid solutions spreading from each pipette and their forming a "salt bridge" when current were applied on them. The wires we used to connect the probe of the iontophoresis current generator with the iontophoresis electrodes were teflon coated (0.11" in diameter, Medwire Corp. Mt. Vernon, N.Y.), to prevent possible circuit shortage while passing current. We also used a parafilm coating method (Shi and Bunney, 1990) to avoid "salt bridge" in these iontophoresis experiments.

In some experiments, the capillaries used for making iontophoresis electrodes were coated with sigmacote in order to avoid possible binding of peptide to the inner walls of the glass. A small amount of sigmacote solution was injected into one end of each capillary using a hypodermic syringe, then they were tilted at 30 - 45°, allowing sigmacote solution to slowly flow from one end to the other. The capillaries were then dried in an oven for 30 minutes at 60°C before being used.

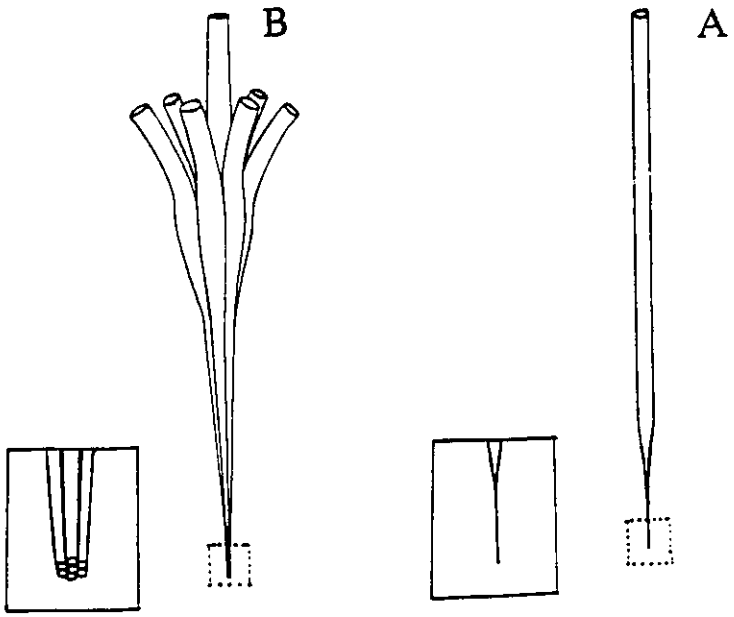


Fig. 4. Examples of intracellular recording electrode (A) and multi-barrel iontophoresis electrode (B).

2. 5. 3. Electrodes for Pressure Ejection

The electrodes for pressure ejection were made from the same pipettes as those used for making iontophoretic electrodes. The pipettes were pulled in four steps which were pre-programmed on the Brown-Flaming puller (Sutter Instruments Co., Model P-87). Each pull produced two usable pipettes. The tip diameter of each pipette was measured under microscope, and pipettes having tip diameters of 2 - 6μm were usually used in our experiments. The difference among the tip diameters of the pipettes was no more than 1.0 μm if more than two pipettes were used in an experiment.

2. 5. 4. Electrical Stimulating Electrode

A bipolar electrical stimulating electrode was made from two pieces of teflon coated wire twisted together. The electrodes were insulated except at the tip.

2. 6. Drug Applications

2. 6. 1. Iontophoresis

The central barrel of the seven-barrel electrode was always filled with 1M NaCl and used for automatic current balancing. One of the side barrels was filled with saline or pH control solutions. The other side barrels were filled with the following agents: Glu (200mM, pH 8.0, Sigma, St. Louis, USA), NMDA (20mM, pH 8.0, Sigma), AMPA (20mM, pH 8.0, Tocris,

Bristol, England), Dom (20mM, pH 8.0, Tocris), QA (20mM, pH 8.0, Sigma), KA (20mM, pH 8.0, Sigma), ibotenic acid (Ibo, 20 mM, pH 8.0, Sigma), Asp (20 mM., pH 8.0, Sigma). These substances were ejected by passing negative current.

Positive current was applied to the side barrels to eject substances as follows: AII (human sequence, 2mM, pH 4.0-5.0, Sigma; Bachem, Torrance, California, USA; Peninsula, Belmont, California, USA), AI (2mM, pH 5.0, Sigma), AIII (human sequence, 2mM, pH 5.0, Sigma), Sar^1-angiotensin II (Sar^1-AII, 2mM, pH 5.0, Bachem), [Sar^1-Val^5-Ala^8]-angiotensin II (saralasin, 1.5-2mM, pH 4.0-5.0, Sigma), ACh (200mM, pH 4.5, Sigma), NA (20mM, pH 4.5, Sigma), 5-HT (100mM, Sigma), ammonium chloride (NH_4Cl, 2-10mM, pH5.0). All agents were dissolved in deionized water and their pHs were adjusted with 1N HCl or 1N NaOH. Solutions of peptides for iontophoresis were made on each experimental day, so were the solutions of the peptides for pressure ejection and bath application as mentioned in the following paragraphs. Other solutions were made in advance and stored in refrigerator.

In earlier iontophoresis experiments, we adjusted the pH of AII solutions to pH 4.0. In order to minimize effects of hydrogen ion iontophoresis, during later experiments we adjusted AII solution and other iontophoresis solutions to pH 5.0 (some of them were adjusted to pH 5.5) when the desired ions being ejected were cations, since hydrogen ions (H^+) have been found to have direct effects on spinal motoneurons (Marshall and Engberg, 1980). The pH of the control solution were adjusted by

adding 1N HCl to deionized water or 2mM NaCl solution to achieve a pH of 3.5 - 5.0. Positive current was applied in order to eject H^+ ions. This solution was sometimes found to be difficult to pass iontophoretic current. In this circumstance, 2 mM NaCl solution of pH 3.5 - 5.0 was used as pH control.

Iontophoresis was controlled by using a Dagan 6400 microiontophoresis current generator. The current used to eject drugs was automatically balanced through the central barrel containing sodium chloride (1M, natural pH). The iontophoresis electrodes were positioned into the tissue adjacent to the intracellularly recorded neurons by an independent micromanipulator.

2. 6. 2. Pressure Ejection

Drugs used for pressure ejection were: Glu (5 - 20mM, Sigma), AII (0.01 -0.1mM, Sigma, Bachem), Sar^1-AII (0.01 - 0.1mM, Bachem), ACh (20mM, Sigma), NMDA (5 mM, Sigma), AMPA (5mM, Tocris, Bristol), Dom (5mM, Tocris, Bristol), NH_4Cl (5mM, Sigma). All drugs were dissolved in ACSF with pH adjusted to 7.4 with 1N HCl or 1N NaOH if necessary. The pressure electrodes were placed about 50 - 100μM above the surfaces of the slices and 200 - 400μM away from the intracellular recording electrodes. Compressed nitrogen gas was used as a pressure source. The pressure applied to the pressure electrode was adjustable through a pneumatic valve installed inside the

Picospritzer II (1 - 150 pounds per square inch [psi]). Pressure ejection was performed and controlled by using a multi-channel Picospritzer II (General Valve Corporation, New Jersey, U.S.A.). For those experiments in which serial pulse pressure ejections were employed, the ejections were triggered by a Grass Stimulator (S88, GRASS Instruments). The control was done by ejecting ACSF from a separate electrode. For hydrogen ion control experiments, the pressure electrode was filled with the ACSF which was pre-adjusted to pH 5.0 with 1N HCl.

2. 6. 3. Bath Application

For bath application of drugs, the drugs were added to ACSF and the pH was adjusted if necessary. The drugs were applied to the slice chamber by switching from the perfusion solution to one that contained the drug by means of a three-way tap. There was about 1.5 minute lag between turning on the three-way tap and entry of the drug solution into the slice chamber. Drugs used were AII (2-10μM, Sigma, Bachem), Sar^1-AII(10μM, Bachem), kynurenic acid (KYN, 0.2-0.5mM, Sigma), APV (50μM, Sigma), CNQX (5-20μM, Tocris), L-trans-pyrolidine-2,4-dicarboxylic acid (2,4-PDC, 0.02mM, Tocris), Amastatin hydrochloride (Ama., 5-10μM, Sigma), Bestatin hydrochloride (Bes., 5-10μM, Sigma), 2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid, also called Plummer's inhibitor (Plu., 50μM, Calbiochem), losartan (DuP753., 5μM, DuPont Co.), PD123177 (5μM, DuPont Co.), (-)-bicuculline methochloride (20μM, Sigma), yohimbine hydrochloride (1μM,

Sigma), DTT (1-5mM, Sigma), Tetrodotoxin (TTX, 0.5μM, Japan).

2. 7. Electrophysiological Recordings

2. 7. 1. Intracellular Recording

The intracellular recording electrode was connected to an HS-2 headstage of an Axoclamp 2A amplifier (gain: 0.1L, Axon Instruments Inc., Burlingame, California, USA) by an electrode holder(EH-2MSW, 1.2mm, E.W.Wright, Guilford, Connecticut, USA) through a chloride coated silver wire (0.25mm in diameter). The electrodes were manually manipulated into the region of the LC under microscopic control. Current pulses, which are generated by a Grass stimulator as voltage pulses and converted to constant current pulses by the Axoclamp-2A amplifier were routinely applied to the recording electrode to detect an apparent increase in resistance when the electrode contacted the cell membrane.

Intracellular potentials were amplified, filtered and displayed by conventional methods (Fig. 5), and recorded on a chart recorder (Gould 2200, USA). Some results were also recorded on a magnetic tape recorder (Racal Recorders LTD, England) for further analysis. Spike frequency was integrated by a frequency counter (Model RD-1, Vancouver, B.C., Canada) and recorded on the chart recorder too. Single action potentials were captured by using a Gould Digital Storage oscilloscope (Model 1421, USA) and subsequently replayed onto the chart

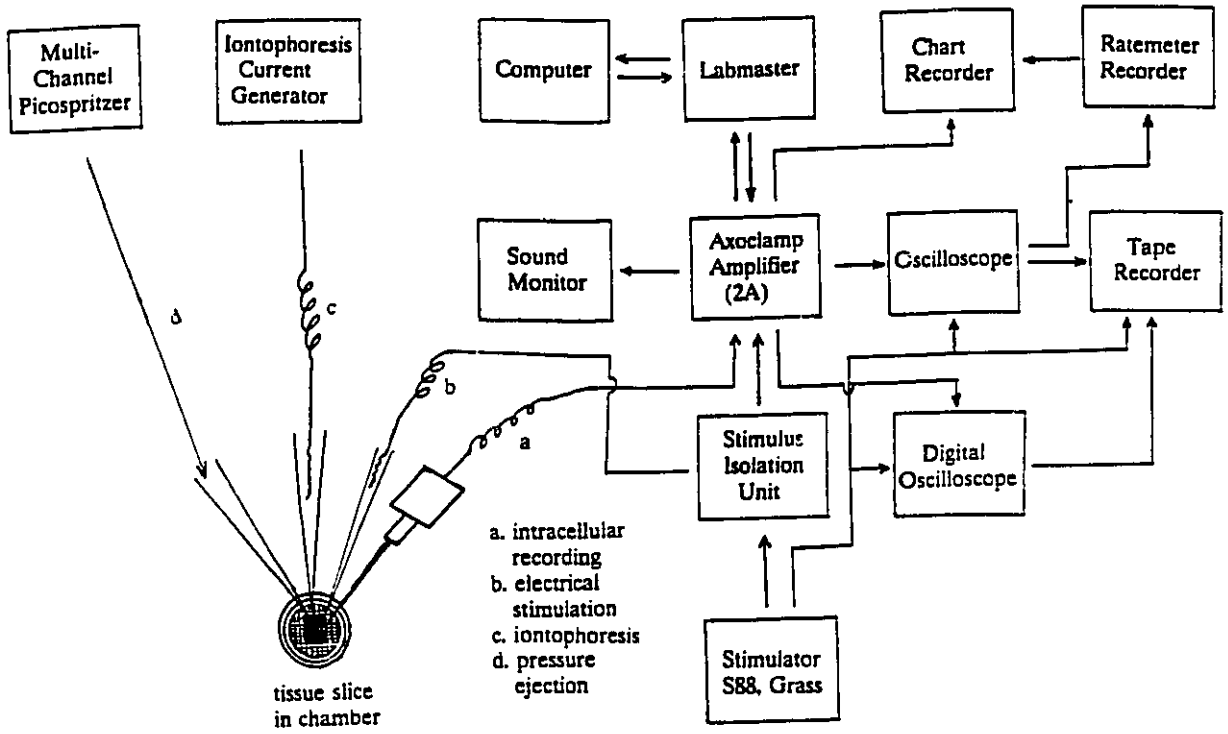


Fig. 5. Schematic drawing of experimental setup.

recorder. Hyperpolarizing currents were passed across the cell membrane by an active bridge circuit (Axoclamp-2A) in order to stop spontaneous discharges. The bridge was balanced prior to impalement of the cell. In some cells, when membrane input resistance was measured, the bridge was carefully re-adjusted after impalement of the cells.

The measurements of membrane input resistances were conducted in some experiments by passing hyperpolarizing current pulses through intracellular recording electrode and measuring the resultant voltage changes. In some other experiments, the measurements of membrane input resistance were done by passing step current pulses generated by computer (pCLAMP 5.5) and measuring membrane responses to these current pulses. Current-Voltage (I-V) curves were constructed according to the current applied and voltage responses measured. The duration of current pulses (200ms or 300ms) was sufficient to charge the membrane capacitance and to reach a steady-state voltage response.

2. 7. 2. Recording of Synaptic Potential

Synaptic potentials were evoked by applying a single rectangular voltage pulse (5 - 60 V, 0.25Hz, 0.3ms) to a bipolar electrical stimulating electrode. The voltage pulses generated by a Grass Stimulator (S88, Grass Instruments, U.S.A.) were delivered to the slices through an isolation unit. The tip of the stimulatory electrode was inserted about 50 μm into the slice tissue at a distance of 300 - 800 μm rostral to the

recording site in horizontal slices. Synaptic potentials were amplified, filtered and displayed as shown in Fig. 3. The synaptic potentials were also recorded on magnetic tape using a tape recorder (Racal Recorders LTD, England) for after-experiment analyses.

Effects of drugs on EPSPs were tested by pressure ejection or bath application. Ten to sixteen consecutive episodes of the EPSP were recorded on the tape during the periods of control, drug application and wash. The EPSPs were recorded at least 10 minutes after the drug containing solution reach the slice chamber during the period of drug application, and 15 - 30 minutes later during washing period. For pressure application of drugs, the EPSPs were recorded while this drug was applied by pressure ejection at 0.5 Hz throughout the sampling period.

2. 8. Identification of LC Neurons

The LC neurons were identified by their location and their electrical characteristics. The area of the LC was easily discernible in the living slice preparation when viewed under a microscope (Stereo Star, Reichart) at a magnification of 10 - 40× with transillumination. In the transverse brain slice, it is located at the lateral edge of, and ventral to, the 4th ventricle (Fig. 6A). In the horizontal brain slice, it is situated at the lateral edge of, and slightly caudal to, the lateral recess of the 4th ventricle (Fig. 6B). Electrophysiological identification was made from a regular

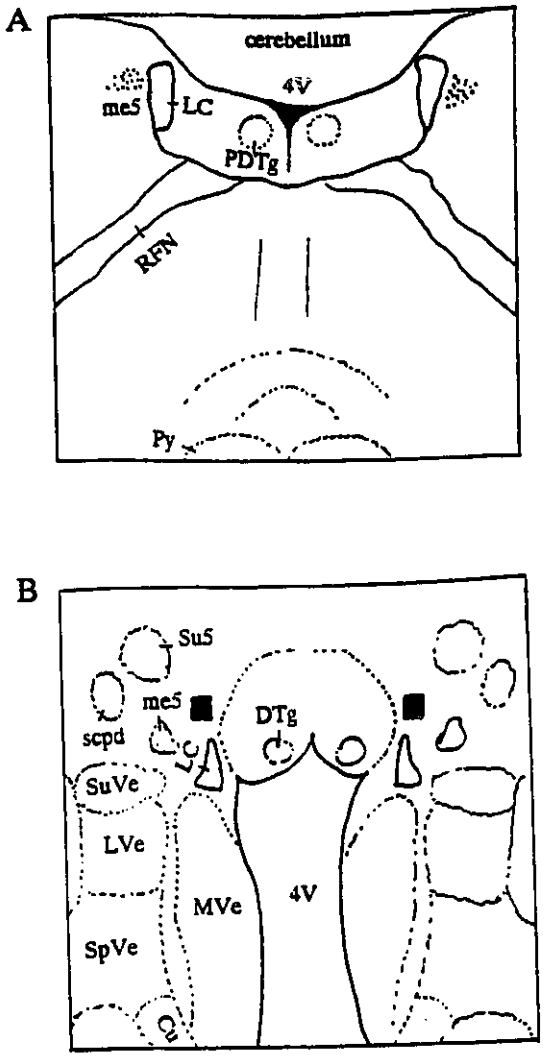


Fig. 6. Schematic drawings of both transverse (A) and horizontal (B) brain slices used in this study.

DTg: dorsal tegmental nucleus; 4V: 4th ventricle;
LVe: lateral vestibular nucleus; LC: locus coeruleus;
me5: mesencephalic trigeminal tract; Py: pyramidal tract
MVe: medial vestibular nucleus;
PDTg: posterodorsal tegmental nucleus;
RFN: root of facial nerve;
scpd: superior cerebellar peduncle
Su5: supratrigeminal nucleus;
SpVe: spinal vestibular nucleus;
■ in horizontal slice represents the place where electrical stimulating electrode was placed.

pattern of spontaneous discharge between 0.5 - 5 Hz (following stabilization), a relatively long duration action potential (in a range of 1.3 - 2.5 ms) and a characteristic "shoulder" on the falling phase of the action potential.

2. 9. Voltage Clamp Experiments

In voltage clamp experiments, a single electrode voltage clamp circuit (Axoclamp-2A, Axon Instruments Co., U.S.A.) was used. The capacitance of intracellular recording electrodes was minimized by using carefully selected electrodes (40 - 60 MΩ in resistance) and by lowering the bathing fluid level in the recording chamber. The switching frequency (3 - 9 KHz) and capacity compensation were adjusted in discontinuous current clamp mode while monitoring the head stage voltage responses to current pulses. During each experiment the headstage voltage was continuously monitored on a separate oscilloscope to ensure that the voltage transient across the microelectrode had decayed completely before voltage sampling. Neurons were usually clamped at the holding potentials close to their resting membrane potentials. Ligand-induced current was recorded and the effect of AII on ligand-induced current was studied.

2. 10. Experimental Procedures

10 - 15 minutes after the selected brain slice was transferred to the slice chamber, two titanium electron

microscope grids were placed over the areas of the LC on each side of the slice to stabilize the tissue in the areas where the microelectrode would penetrate, and these were held in place by two small platinum logs (approximate 0.3 mm in diameter and 1.0 - 1.5 mm in length). This was necessary to decrease tissue instability which occurred even at the relatively slow flow rate of 1.5 ml/min.. No intracellular recordings were conducted until the slice was perfused with the ACSF saturated with 95% O_2/5% CO_2 for at least one hour. When a single LC neuron was penetrated, a hyperpolarizing current of 0.05 - 0.1 nA was routinely applied to the intracellular recording electrode in order to help the cell stabilize, and to reduce or stop spontaneous firing. Once the cell condition was stable, this current was reduced or removed. Stable impalements were used in our experiments when membrane potential was greater than -50 mV and the spike amplitude of spontaneous firing was greater than 60 mV (measured from resting potential).

Iontophoresis electrodes were, in most cases carefully placed adjacent to the intracellular recording electrodes after recordings were stable, and pulses of Glu were then applied to assess the placement of iontophoresis electrodes and the responsiveness of the recorded LC neurons to Glu. Glu was regularly pulsed (typically 15 or 20 second pulses) and the ejecting current was adjusted to, in most cases, produce a response of 10 - 15mV membrane depolarization or produce spike activation at rates of 15 - 30 Hz. When a stable Glu response

was established, AII was applied simultaneously (usually 2 to 3 minutes) with pulsed Glu over two Glu pulses in most cases. Glu pulses were continued after cessation of AII until full recovery was obtained. In some experiments, ACh was regularly pulsed and AII was similarly tested in the same or in a different cell. In some other experiments, Glu and ACh were pulsed alternatively, then AII was continuously applied over Glu and Ach pulses. In addition, Glu was continuously applied, while AII was pulsed (5 - 15 seconds in duration), overlapping Glu application. Saralasin was applied over two pulses of Glu ahead of Application of AII in most cases, and in some cases, it was applied at the same time of application of AII.

The tests of the effects of AII on Glu responses by pressure ejection were conducted in a similar way to that of iontophoresis. Single pulses were employed to eject AII, Glu and Glu agonists. As to test for an effect of AII on EPSPs, AII was ejected (at 0.5 Hz) throughout the sampling period.

In order to prevent possible drug leakage from the pressure pipette tips, the pressure electrodes were not manipulated into solution until the tests began. When tests were finished, the pressure electrodes were withdrawn from the solution. The pressure strength and the duration of the pressure pulses for Glu and its agonists were adjusted to produce an excitation of the LC neurons at 10 - 30 spikes/s. Once the response of the LC neuron to Glu and its agonists were stable and consistent, the same pressure strength and the duration of a single pulse was

applied to AII and its agonist, Sar^1-AII.

For pressure ejection of NH_4Cl, 10 pulses (1Hz) were applied just before pressure application of Glu and its agonists.

2. 11. Data Analysis

The LC neurons yielding stable, high-quality recording throughout experimental procedures were subjected to qualitative or quantitative analyses. Spike frequency was calculated by comparing the integrated recordings of spike frequency with the integrated recording of standard pulses frequency. The influence of locally applied transmitter substance on the LC depolarization or discharge frequency was quantified using the mean depolarization or mean firing rate averaged of 2 - 3 applications. The changes of membrane depolarization amplitude or spike frequency were measured and compared with the average of pre- and after controls, taking the average of pre- and after controls as 100%. In comparing with the average of the controls, a 30 - 45%, 50 - 70% or >75% decrease in membrane potential amplitude or spike frequency were considered as mild, moderate or strong depression respectively. A 30% or greater increase in membrane potential amplitude or spike frequency was treated as a potentiation. Since the effects of both AII and its analog Sar^1-AII on Glu were similar, they were referred to collectively as AII.

A criterion of at least 30% change in spike frequency or in amplitude of depolarization was used for individual cells to

estimate the number of neurons responsive to a particular transmitter substance. In some cells, the effects of AII on spontaneous firing was tested, in some other cells, this was determined by comparing the spontaneous discharges between Glu pulses without application of AII with the spontaneous discharges between Glu pulses with AII application.

EPSPs evoked by electrical stimulation were averaged on a computer using pCLAMP 5.5 software. Each EPSP shown in this thesis was the average of 10 - 16 consecutive episodes. The peak amplitudes, the time to reach peak amplitudes and the time of 50% decay in amplitudes of the EPSPs were measured. Comparisons between controls and tests were statistically conducted among these parameters. Again, at least a 30% change in the amplitude and the duration of the EPSPs was used as a criterion to determine the number of neurons which were responsive to applied substances.

The quantitative data was statistically analyzed using student's *t* test. The qualitative data was analyzed by Chi Squared (X^2) test.

3. RESULTS

3. 1. Properties of Intracellular Recorded LC Neurons

A total of 324 LC neurons were recorded intracellularly from 213 brain slices (213 rats). Once impaled, the LC neurons had an "injury" discharge at rates of 10 - 30 Hz, lasting for about 30 seconds to several minutes in most neurons recorded, or even longer in some other neurons, and then gradually returned to a resting condition if no hyperpolarizing current was applied through the intracellular recording electrode to these neurons. When a hyperpolarizing current of 0.05 - 0.1 nA was applied to most LC neurons after the penetrations of these LC neurons, the durations of the "injury" discharges were greatly shortened. This was an easy and fast way to stop the "injury" and spontaneous firings and to stablize the cells.

When the hyperpolarizing current was removed or in a stabilizing condition following the "injury" discharge, the LC neurons showed spontaneous discharges with firing rates of 0.5 - 5 Hz in the absence of any stimulus. The threshold for firing an action potential was about -50 mV in the majority of cells. Following the action potential, there was a large afterhyperpolarization, with an amplitude of 30.0 ± 1.6mV (measured from the level of threshold point to the lowest point of the afterhyperpolarization), lasting 50 - 150ms. This afterhyperpolarization merged into a slow depolarization that

brought the cell to the threshold again (Fig. 7C). Since the mergence of this afterhyperpolarization into membrane spontaneous depolarization, it is difficult to separate the afterhyperpolarization from the membrane spontaneous depolarization (i.e. pacemaker potential) in younger animals. Therefore the amplitude of the afterhyperpolarization includes the component of membrane spontaneous depolarization. Apparent membrane potentials were in the range of -50 - -70 mV, with an average of 57.7 ± 5.6mV (Mean ± S.D.). The spontaneous firing of the LC neurons were depressed when TTX was added to the perfusate (n=3).

The amplitudes of the spontaneous action potentials were, in most cells 60 - 80 mV measured from threshold, and the durations of the action potentials were within a range of 1.3 - 2.5 ms, most of them were 1.6 - 2.0 ms measured also from threshold. The falling phase of these spontaneous firing action potentials had three components, an initial rapid repolarization followed by a relative slow repolarization, and then again a rapid repolarization, giving the appearance of a small "shoulder" on the falling phase when viewed at a fast sweep speed (Fig. 7A). This shoulder, which reflected a decrease in rate of repolarization was one of the standards to identify the LC neurons. The repolarization of the action potential carried the membrane potential 26 - 32 mV negative to the threshold level (Fig. 7B. 7C).

Passage of hyperpolarizing current pulses of 200ms in

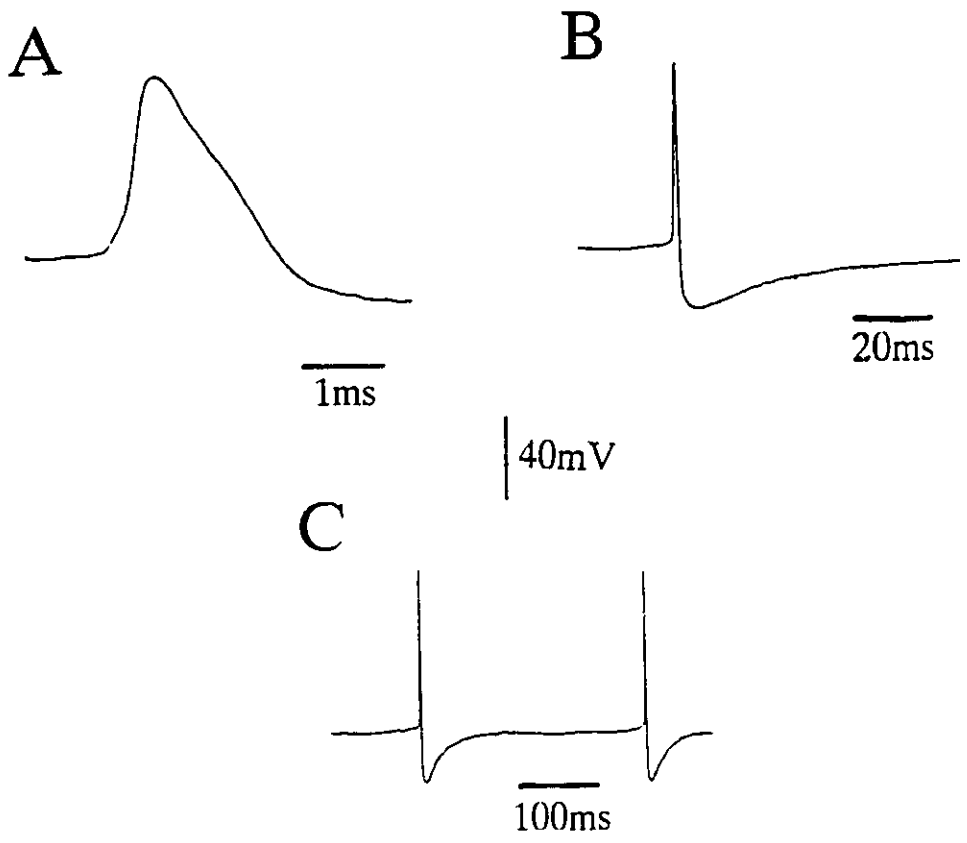


Fig. 7. Spontaneous action potentials recorded during normal ACSF perfusion. A. is fast sweep speed recording, note a "shoulder" on the falling phase. B. is a relatively slow sweep speed recording. The action potential was followed by a large afterhyperpolarization. C. This large afterhyperpolarization merged into a slow depolarization, brought the cell to the threshold and fired action potential again.

duration, which was sufficient to charge the cell membrane capacitance through the intracellular recording electrode caused voltage deflections which followed an ohmic relationship to the current passed (Fig. 8). Rectification occurred at more hyperpolarized levels. According to Ohm's law, the current-voltage (I-V) relationship measured during the linear part of the curve gave apparent input resistance in a range of 117 - 236MΩ.

The time course of the potential change resulting from hyperpolarizing rectangular current pulses was measured. The membrane time constant of single exponential was in the range of 8.8 to 23.2 ms, with an average of 15.8 ± 4.6 ms (n = 12).

In both transverse and horizontal slices of young rats whose body weights were less than 100 gms, especially those with body weight around 50 gms, LC neurons displayed slow rhythmic depolarizations with an amplitude of 5 - 10 mV. These depolarizations had slow rise and fall times, and were insensitive to TTX, but could be blocked by perfusing the slice with a modified ACSF containing low Ca^{2+} and high Mg^{2+}.

During passage of depolarizing current pulses (0.2nA, 3-10s) through the intracellular recording electrode, LC neurons were activated to firing repetitively, with acommodation during the pulse (Fig. 9). In addition to a large after-hyperpolarization following individual spikes, there was also a large, long-lasting hyperpolarization following the spike cluster (Fig. 10).

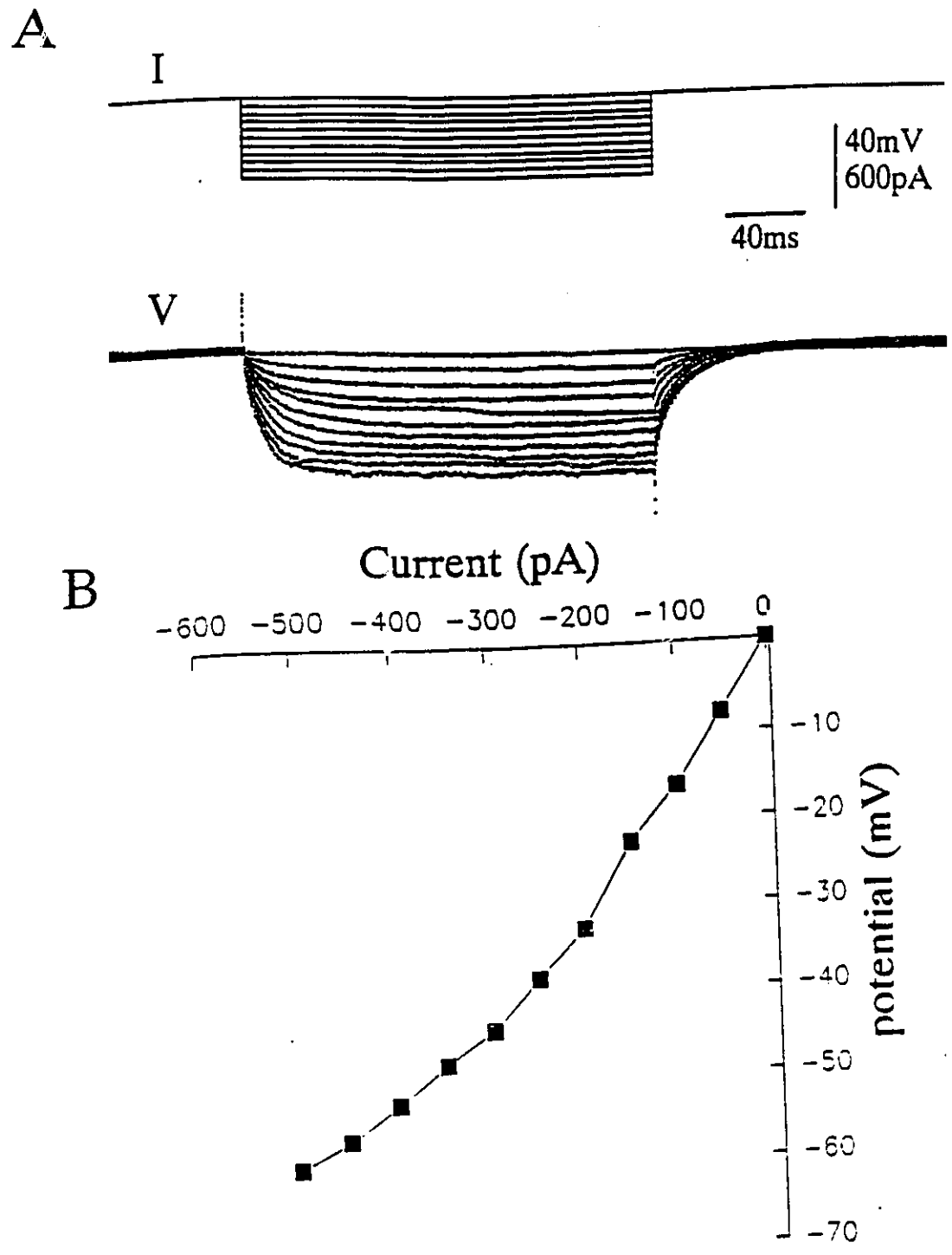


Fig. 8. Apparent membrane input resistance in an LC neuron. A. Membrane responses (V) induced by 200 ms current injections (I). B. I-V plot of the results in A (measured at 150 ms). Membrane input resistance of this cell was 160 MΩ. Resting membrane potential of this cell was -65mV.

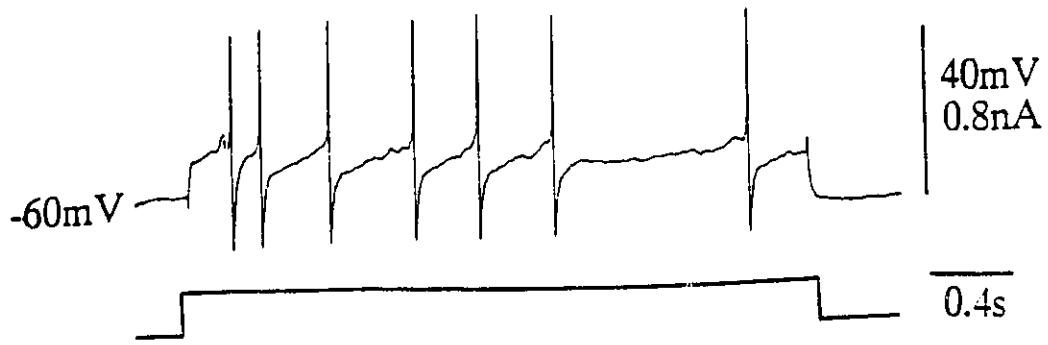


Fig. 9. Discharges induced by current injection in an LC neuron. Passing a depolarizing current pulse (0.2nA, 3s) through intracellular recording electrode, the LC neuron was activated to firing with accommodation.

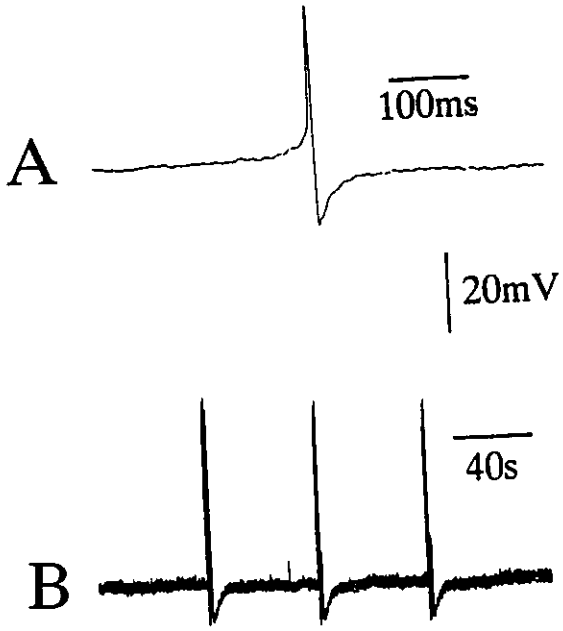


Fig. 10. There is a large after-hyperpolarization following an individual spike (A) and a large long-lasting hyperpolarization following the spike cluster generated by passing depolarizing current (B). 0.1 nA hyperpolarizing current was applied to the recording electrode during the period of recording spike clusters in order to stop spontaneous firing.

LC neurons could also be activated by electrical stimulation of rostral regions in horizontal slices. Normally, a short pulse (0.3ms, 0.5-2.0 mA) cause a single spike, which also had a large after-hyperpolarization.

3. 2. Effects of AII on Spontaneous Firing of LC Neurons

AII molecules are positively charged in aqueous solution when the pH of this solution is adjusted to 5.0 or 4.0. The AII molecules can therefore be ejected out of the pipette containing AII solution by iontophoresis, when positive current is applied to this pipette.

Applied iontophoretically (20-60nA in most cases), AII had little or no apparent actions on spontaneous firing of LC neurons tested (Fig. 11) except in some cells in which AII caused a small hyperpolarization (see below). Pressure ejection of AII (9 - 35psi, n=6) or bath applied AII in the presence of peptidase inhibitors (n=4) also showed no apparent effect on spontaneous firing of the LC neurons. In contrast, other putative neurotransmitter substances yielded profound, consistent effects on spontaneous firing of LC neurons when applied iontophoretically to these same LC neurons. Potent inhibitory responses were elicited by iontophoretic application of GABA (n=4). NA was found to have a depressant action on LC neurons (n=4). Iontophoretically applied Glu exerted a potent excitatory action on 187 cut of 197 LC neurons tested. The other 10 cells showed no responses to iontophoretically applied Glu.

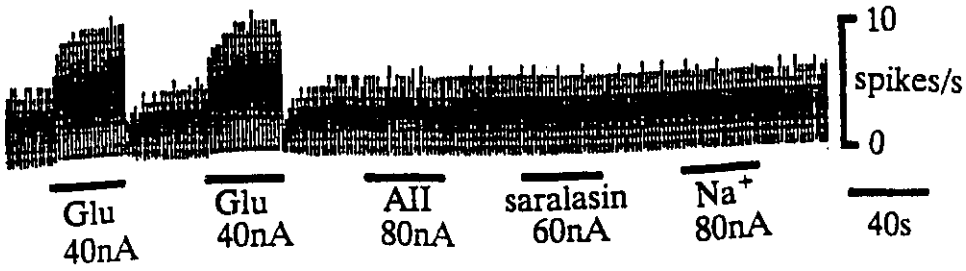


Fig. 11. Effects of AII and Glu on spontaneous firing of an intracellularly recorded LC neuron. This figure is a ratemeter recording of spontaneous firing frequency. The LC neuron discharged spontaneously in the absence of any stimulus. Time of iontophoretic applications is indicated by short bars underneath the recording trace with negative current (in Ampere $\times 10^{-9}$) for Glu, and positive current for AII, saralasin and Na^+. Application of Glu (0.2M, pH 8.0) increased the spontaneous firing. Application of AII had no apparent effect on the spontaneous firing. No effect was found with iontophoretic application of saralasin, or when positive current was applied to a saline-containing barrel, ejecting Na^+ ions.

The activation of the LC neurons by Glu occurred a few seconds after turning on the iontophoretic current and stopped immediately after current was turned off. The current intensities required to activate the LC neurons in most cases were of 15 - 40 nA applied for 3-5 seconds. LC neurons were also activated by Glu receptor agonists, e.g. NMDA, AMPA or domic acid. Sensitivity of the LC neurons to these agonists appeared to be higher than to Glu (data not shown here), since almost the same degree of excitatory responses was observed when the smaller amount of iontophoretic current was applied to these agonist-containing barrels. In addition, LC neurons were reliably activated by ACh (n=11) though larger currents were needed to generate almost equal responses to those generated by Glu.

In addition, iontophoretic application of AII caused a 2 - 10 mV membrane hyperpolarization in 18 out of 187 LC neurons. Spontaneous discharges of these LC neurons were reduced or depressed due to the membrane hyperpolarization.

3. 3. Effects of AII on Glu-induced Depolarizations and Resultant Excitations

3. 3. 1. Effects of iontophoretically applied AII

Iontophoretic application of AII was found in most LC neurons to strongly depress the depolarizations (Fig. 12) and resultant excitations (Fig. 13) produced by iontophoretic

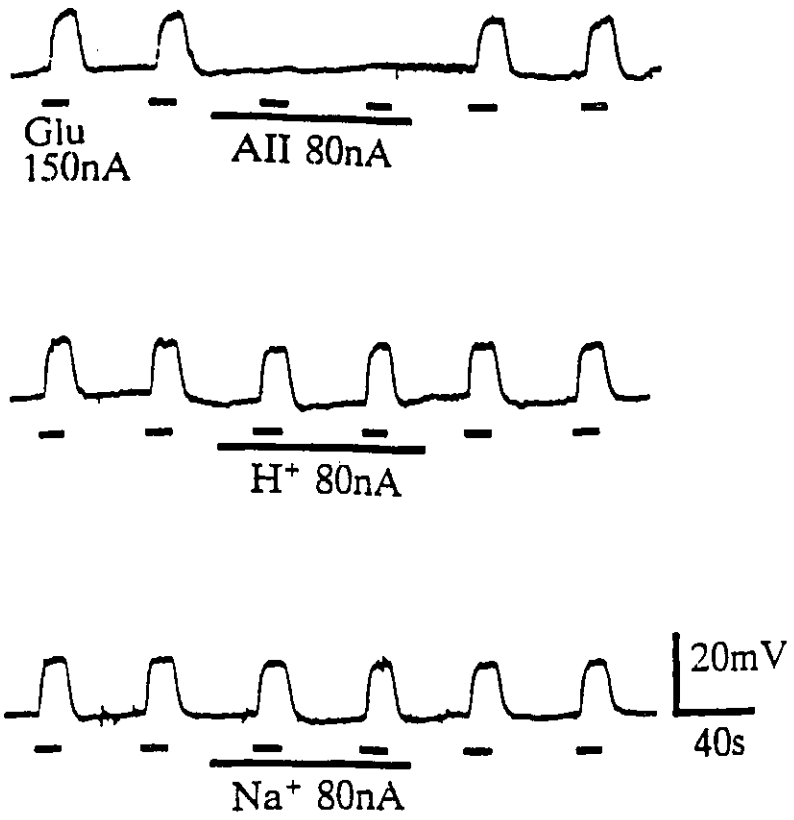


Fig. 12. An example of depression of Glu-induced depolarizations by AII in an intracellularly recorded LC neuron. A steady hyperpolarizing current was applied to avoid spontaneous firing. Time of iontophoretic application of Glu is indicated by short bars underneath the recording trace with negative current used. Application of AII (pH 5.0, passing positive current) is indicated by a long bar. AII completely blocked Glu-induced depolarizations (upper trace). Iontophoretic application of H^+ from a pH control barrel appears to have no effect on Glu-induced depolarizations in this cell (middle trace). Passing the same amount of current with the same polarity through a NaCl containing barrel (ejecting Na^+) had no apparent effect on Glu responses (lower trace). Membrane potential was hyperpolarized to - 70 mV by passing negative current through the recording electrode.

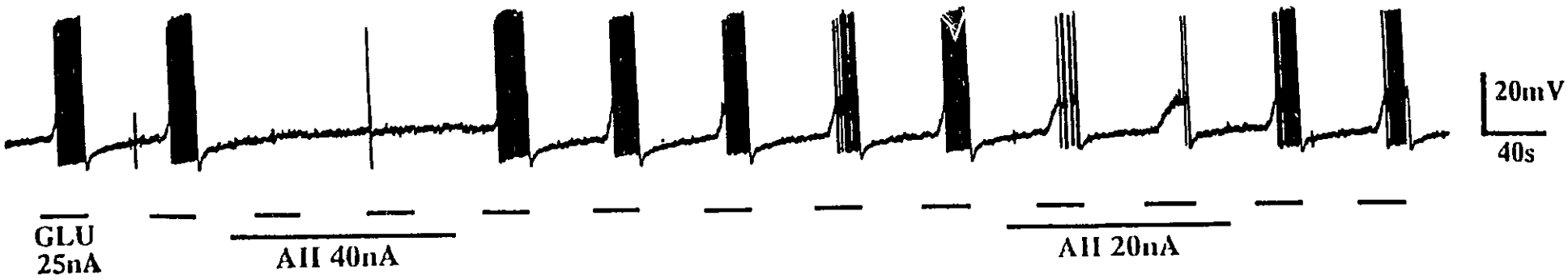


Fig. 13. Blockade of Glu depolarizations by AII in an intracellularly recorded LC neuron. Time of iontophoretic applications are indicated by bars as in Fig. 11. Glu-induced depolarization is reduced by passing positive current of 20nA through AII-containing barrel and completely blocked by passing 40 nA through the same barrel. Spike amplitudes in this and other figures are truncated due to frequency response limitations of the pen recorder.

application of Glu, with little or no effects on spontaneous firing of the LC neurons when applied alone (see Fig. 11). The depression mediated by AII on Glu-induced responses occurred quickly after turning on ejecting current and disappeared quickly when the ejecting current was turned off (Fig. 14). The degree of depression of Glu responses was related to the current used to eject AII (Fig. 15, also see Fig. 13). Typically, AII (20 - 60 nA) gave a moderate to strong depression of the Glu response. Fig. 16A shows the membrane hyperpolarization caused by AII in an LC neuron. AII depressed Glu responses and caused about 8 mV membrane hyperpolarization. When the membrane potential was manually hyperpolarized (by applying hyperpolarizing current to the intracellular recording electrode) about 10 mV as a control, the action of Glu was still present though the spike frequency was somewhat reduced (Fig. 16D), indicating that most of blockade of Glu depolarization by AII was not attributable to the membrane hyperpolarization.

3. 3. 2. Current Control

Application of D.C. current near a cell membrane might affect the excitability of a cell membrane. AII is positively charged in a pH 4.0-5.0 solution. Positive current is used in order to eject AII molecule out of the AII-containing barrel. In order to rule out any possible contribution by positive current to the AII actions on Glu-induced depolarization, current control was carried out by passing current through a NaCl-

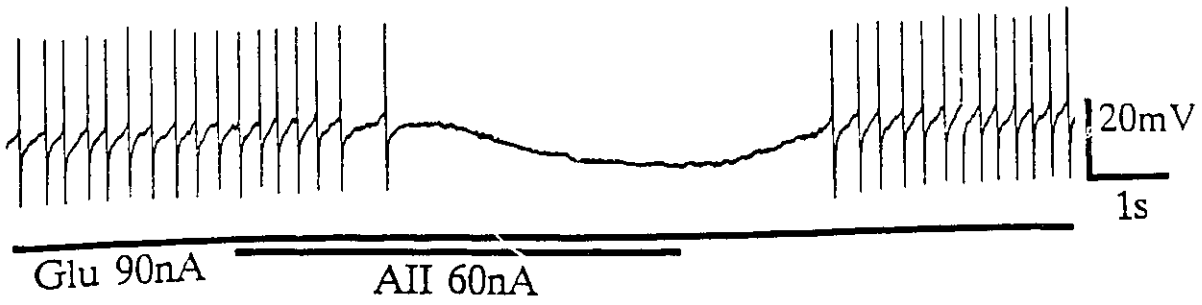


Fig. 14. The time course of AII action on Glu-induced responses in another LC neuron recorded intracellularly. This cell has been depolarized by continuous iontophoretic application of Glu as indicated by a long bar underneath the recording. Iontophoretic application of AII (indicated by a short bar) completely depressed Glu-induced responses and restored the membrane potential to control level before application of Glu, even during the continued application of Glu. The depression occurred rapidly after the current for ejecting AII was turned on and rapidly disappeared when the current was turned off. Application of AII alone had no effect on membrane potential on this same cell. Membrane potential was hyperpolarized to - 62 mV.

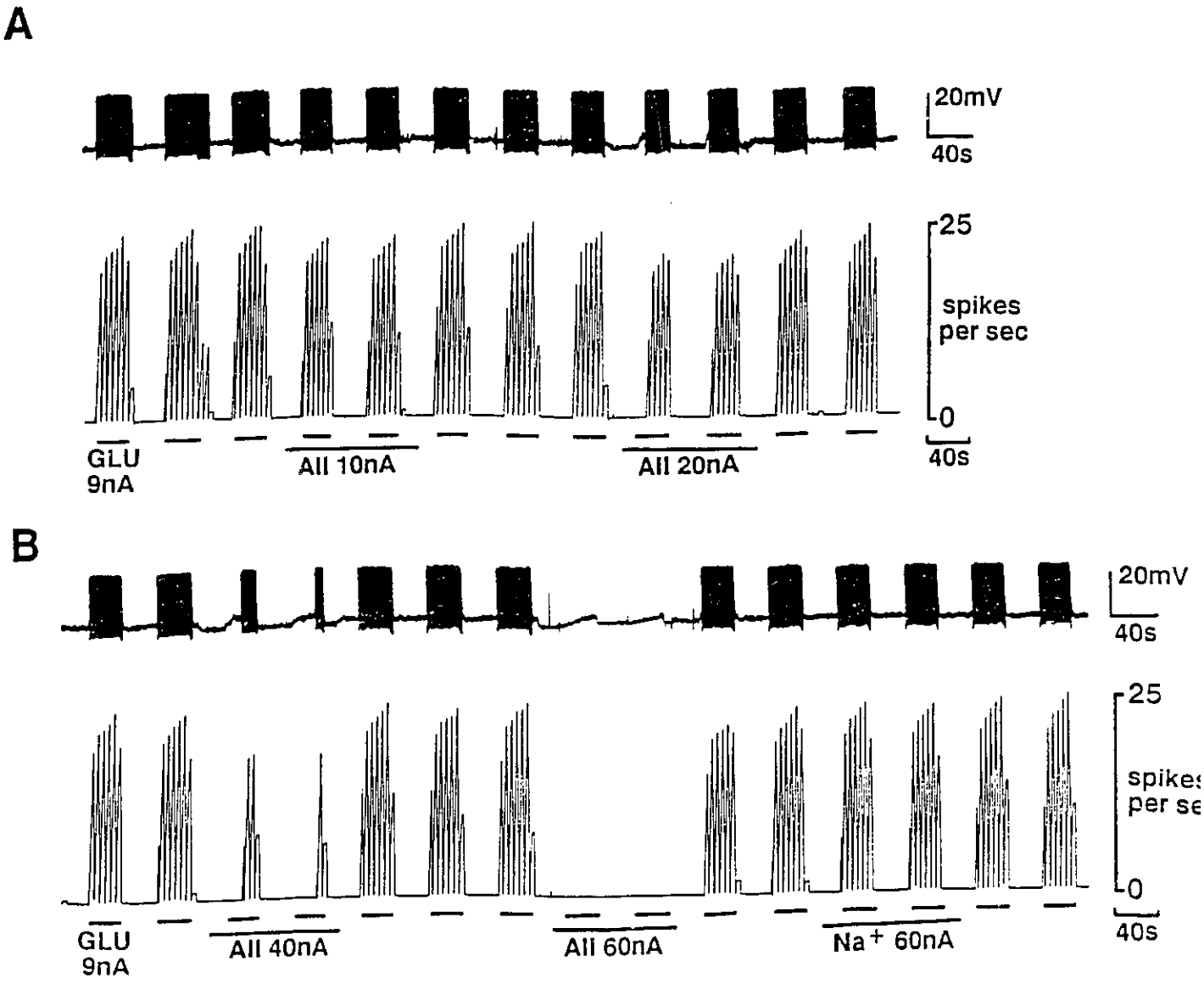
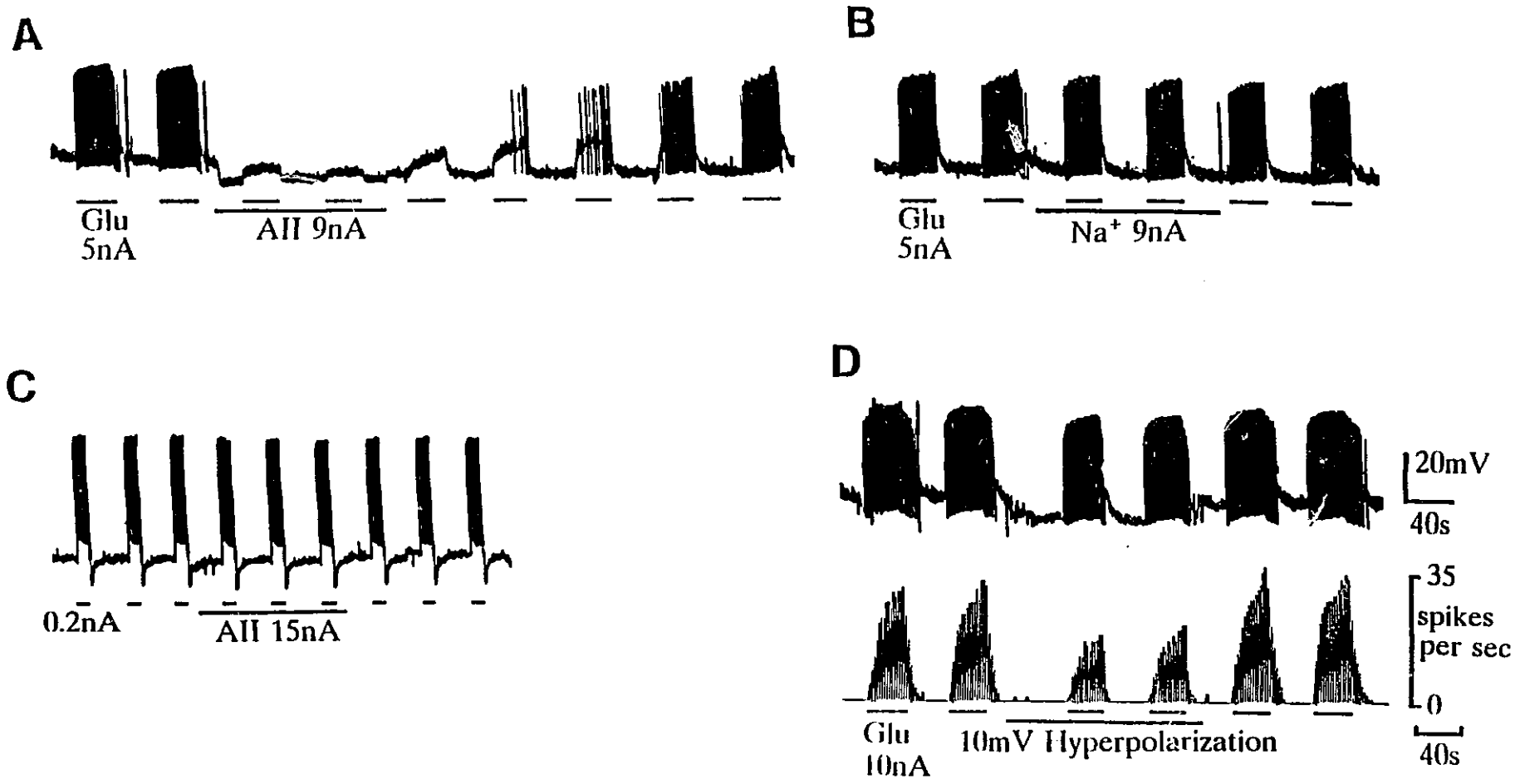


Fig. 15. The inhibitory effect of AII on Glu-induced excitation was dose dependent. In A and B, upper traces are intracellular recordings; lower traces are corresponding ratemeter recordings of spike frequency. Glu excitation is almost unchanged by passing 10 nA current through AII containing barrel, slightly reduced by passing 20nA, greatly reduced by 40 nA, and completely blocked by 60 nA.

Fig. 16. AII slightly hyperpolarized some LC neurons in addition to its depressant action on Glu-induced response. **A.** The depressant action occurred quickly, though in this case, the time for recovery was longer. **B.** Glu responses were unaffected by passing the same current through another barrel containing saline. **C.** AII had no effect on the excitatory responses induced by depolarizing current pulses. **D.** 10mV hyperpolarization reduced (not abolished) Glu response. Upper trace is the intracellular recording and lower trace is the corresponding ratemeter recording of spike frequency.


A
Glu
5nA
AII 9nA
B
Glu
5nA
Na+ 9nA
C
0.2nA
AII 15nA
D
20mV
40s
35
spikes
per sec
0
Glu
10nA
10mV Hyperpolarization
40s

containing barrel even though the Dagan 6400 current generator has an automatic current balance system.

Application of a positive current through the NaCl-containing barrel (ejecting Na^+) equal to or greater than that used to eject AII caused little or no change in the Glu response or other neuronal properties (e.g. Fig. 16B). In 28 current controls of this type, no depressant effects similar to those of AII or other effects were observed (Table 1). It could also be shown that the AII effect was not due to interaction with a voltage dependent ionic current, since depolarization and spike activation caused by depolarizing current injection were unaffected by AII (Fig. 16C).

TABLE 1

Effects of Iontophoretically Applied AII on Glu-induced Responses

Tests	N	Depression				—	P
		mild	moder.	strong	total		
AII → Glu	187	28	63	74	165*	17	5
Na^+ → Glu	28	0	0	0	0*	28	0
H^+ → Glu	120	35	18	7	60*	57	3

N: number of cell; moder.: moderate; --: no effect;
P: potentiation
± S.D. (the same in the following tables)
AII → Glu: the test of AII actions on Glu-induced responses;
Na^+ → Glu: current control test (ejecting Na^+);
H^+ → Glu; pH control test (ejecting H^+)
mild, moder. and strong depression represent 30-45%, 50-70% and >75% reduction of spike frequencies of amplitude of

depolarizations in comparsion with control
*: AII is statistically compared with Na^+ and H^+ for total number of cells depressed, significance is found in all the comparisons ($p<0.01$).

3. 3. 3. Effects of AII on Action Potentials

AII appears to have no significant effect ($p>0.05$) on the action potentials generated by depolarizing current pulses in LC neurons (Fig. 17). The amplitudes, durations and afterhyperpolarization of the action potentials during control, application of AII and after control were summarized in Table 2. Fig. 18 is an example of the effect of iontophoretically applied AII on the membrane input resistance. AII was found to have no significant effect on membrane input resistance (n=11) though the Glu responses were strongly depressed by AII on this same cell. We also measured membrane input resistance during the period when AII and Glu were co-applied and the Glu-induced responses were completely depressed by AII. No significant change was found (n=4).

TABLE 2

Effects of AII on Action Potentials*

	Amplitude (mV)	N	Duration (ms)	N	AHP (mV)	N
Control	67.7 ± 8.9	9	1.81 ± 0.25	9	27.8 ± 4.4	9
AII	68.1 ± 9.9	9	1.81 ± 0.29	9	28.2 ± 4.6	9
After control	68.2 ± 8.1	9	1.81 ± 0.27	9	28.2 ± 4.2	9

*: AII had moderate or strong depressent actions on Glu-induced

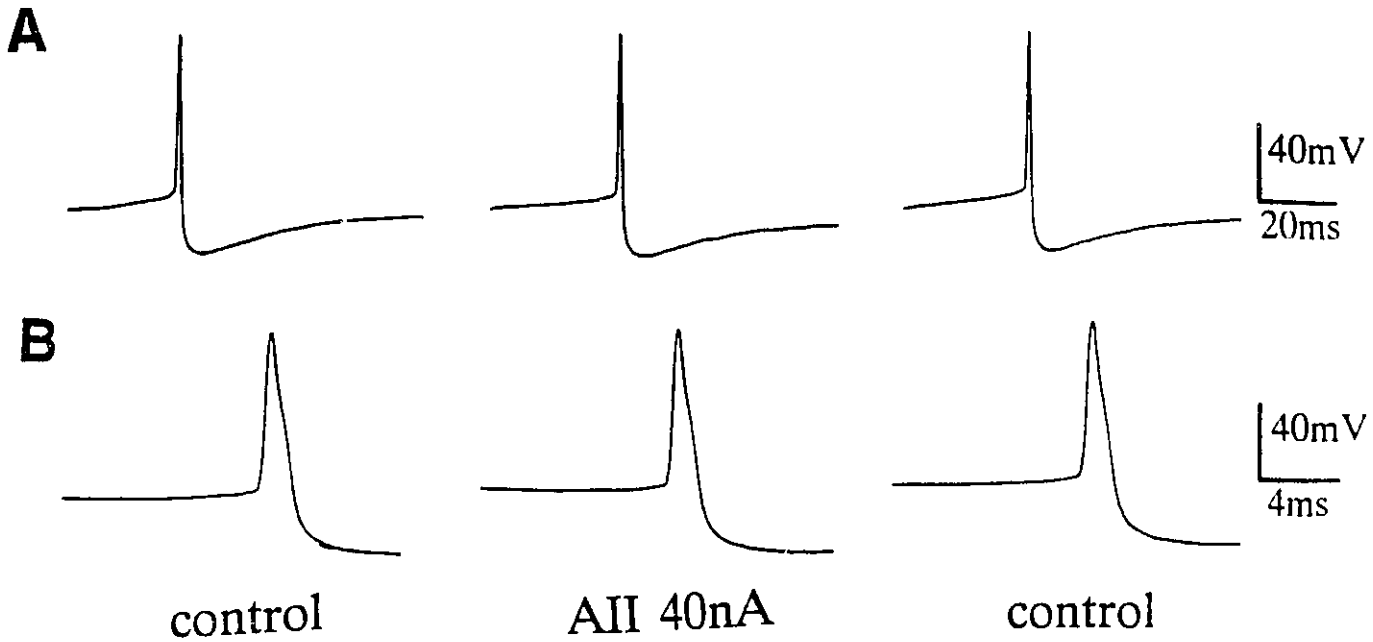


Fig. 17. Intracellular recording of action potentials from a neuron during control periods (left and right) and during the period of iontophoretically applied AII which completely suppressed glutamate responses. No apparent change was found in the amplitude, duration or afterhyperpolarization of the action potentials generated by current injection during AII suppression. Note calcium "shoulder" on action potential (lower trace).

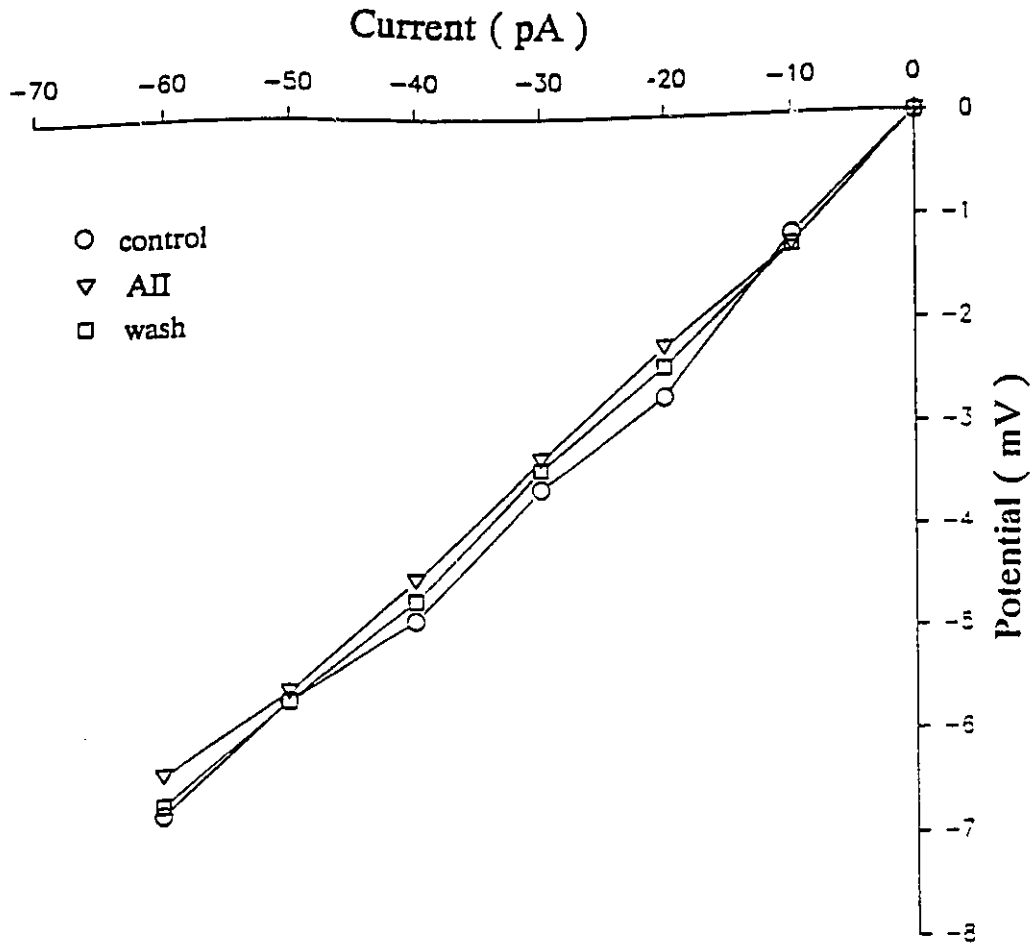


Fig. 18. Effect of AII on membrane input resistance. Abscissa indicates the current (pA) injected to an LC neuron through intracellular recording electrode, ordinate is the membrane voltage response to intracellular current injection. Membrane potential was at -60mV. AII applied iontophoretically had no apparent action on membrane input resistance, while Glu responses were completely blocked by AII in this same LC neuron.

responses in these cells. AHP: afterhyperpolarization; measured from threshold. N: number of cells

3. 3. 4. Effects of H^+ on Glu Responses

Because AII is passed by a positive current from a low pH solution (pH 4.0-5.0), some protons will be ejected with the AII molecules. The effect of hydrogen ions (H^+) on Glu responses was therefore tested in many LC neurons by passing positive current through a pH control barrel which had the same or lower pH in comparison with the solution in the AII-containing barrel (Fig. 19.). In these tests, H^+ was found to have depressant actions on Glu responses in 60 out of 120 cells. The depression caused by H^+ was varied, but was mild in most cells tested (Table 1). Among all the cells on which H^+ had effects (n=60), the effects of AII were found to be greater than that of H^+ in most cells (Fig.19, n=42); in 7 cells, both H^+ and AII had approximately equal effect on Glu responses; in 5 cells, H^+ had a depressant effect while AII had no effect on these same cells; in addition, AII increased Glu responses in some cells while H^+ depressed Glu responses in these same cells (n=3); in some other cells, AII depressed Glu responses while H^+ increased Glu responses (Fig.20, n=3). The correlation between the effects of AII and H^+ was poor.

3. 3. 5. Effects of Ammonium ions (NH_4^+) on Glu Responses

Initial tests of AII were carried out with AII from Sigma, but we obtained information that these lots contained

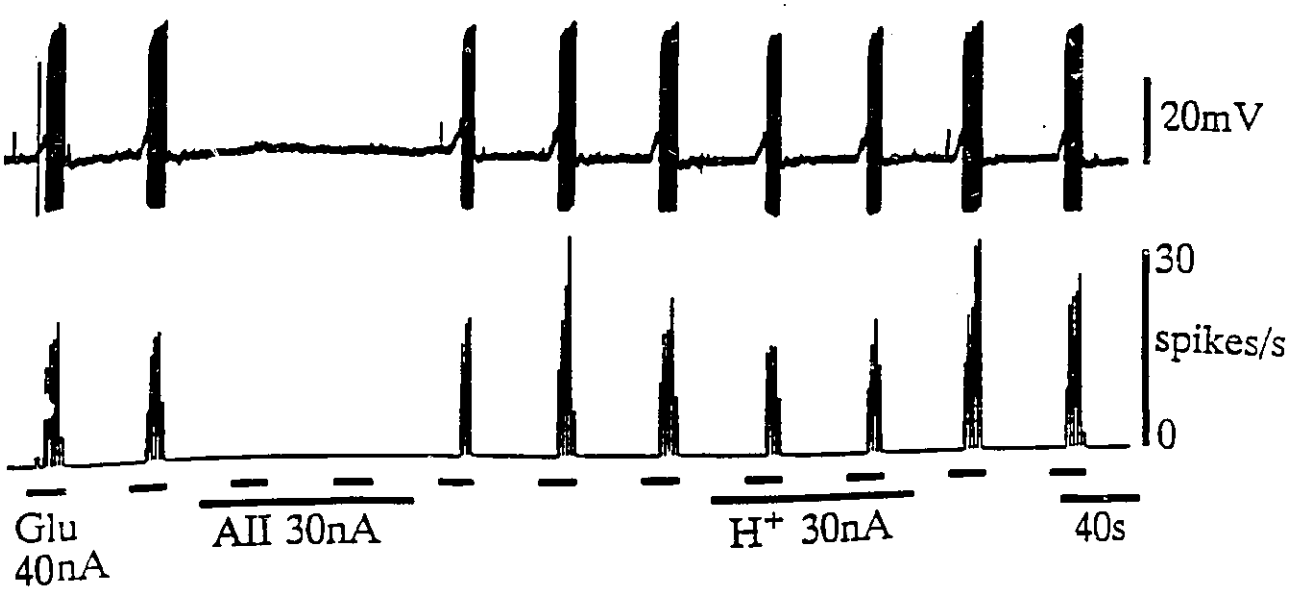


Fig. 19. The effects of AII and H^+ on Glu responses. Upper trace is intracellular recording lower trace is the corresponding ratemeter recordings of spike frequency. Iontophoretic application of AII (pH 5.0) completely eliminates Glu-induced depolarization and resultant excitation. Iontophoretic application of H^+ (pH 5.0) attenuates Glu-induced response.

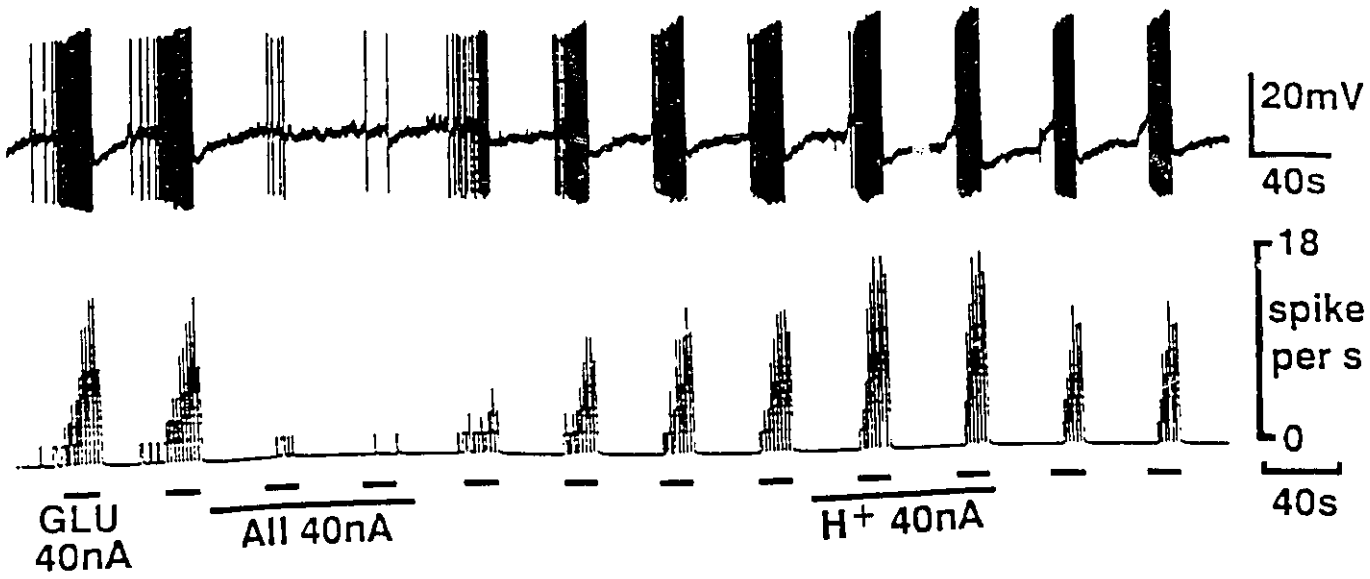


Fig. 20. Another example of H^+ effect on Glu-induced responses in an LC neuron. Upper trace is a chart recording of intracellular recorded potential. Lower trace is a ratemeter recording corresponding in time to upper trace. Iontophoretic application of glutamate (Short black bars under lower trace) causes depolarization and spike activation. Application of AII by cationic current almost completely blocks depolarization and activation by glutamate. Later application of hydrogen ion from a pH 3.0 solution causes enhancement of the glutamate responses.

ammonium in sufficient concentration to give approximate 50 μM NH_4^+ in a 1 mM solution. This raises a possibility that the action of AII on Glu we have observed could be due to the action of NH_4^+ carried out by the positive current used to eject AII since NH_4^+ has been reported to inhibit Glu responses in the hippocampus (Fan et al., 1990). We tested the effect of NH_4^+ on Glu responses in LC neurons and found it did depress Glu responses when applied by iontophoresis or by pressure ejection, with almost the same potency as that of AII.

When applied by iontophoresis, NH_4^+ was found to depress Glu responses (at least 30% decrease either in firing frequency or the amplitude of depolarization) in 41 out of 51 LC neurons tested (Fig. 21). NH_4^+ had little or no apparent action on Glu responses in 9 of the remaining 10 LC neurons. An increase of the Glu response by NH_4^+ was found only in one cell. Like the action of AII on Glu responses, the action of NH_4^+ on Glu responses in most cases also occurred rapidly when iontophoretic currents were turned on and disappeared rapidly when iontophoretic currents were turned off. Membrane potentials were unchanged in response to iontophoretically applied NH_4^+ in the majority of LC neurons tested though a 4 - 10 mV hyperpolarization was found in 6 LC neurons during iontophoretic application of NH_4^+. NH_4^+ was also found to have no aparent action on the amplitudes, durations and afterhyperpolarizations of the action potentials.

Pressure ejection of NH_4Cl was found to have similar

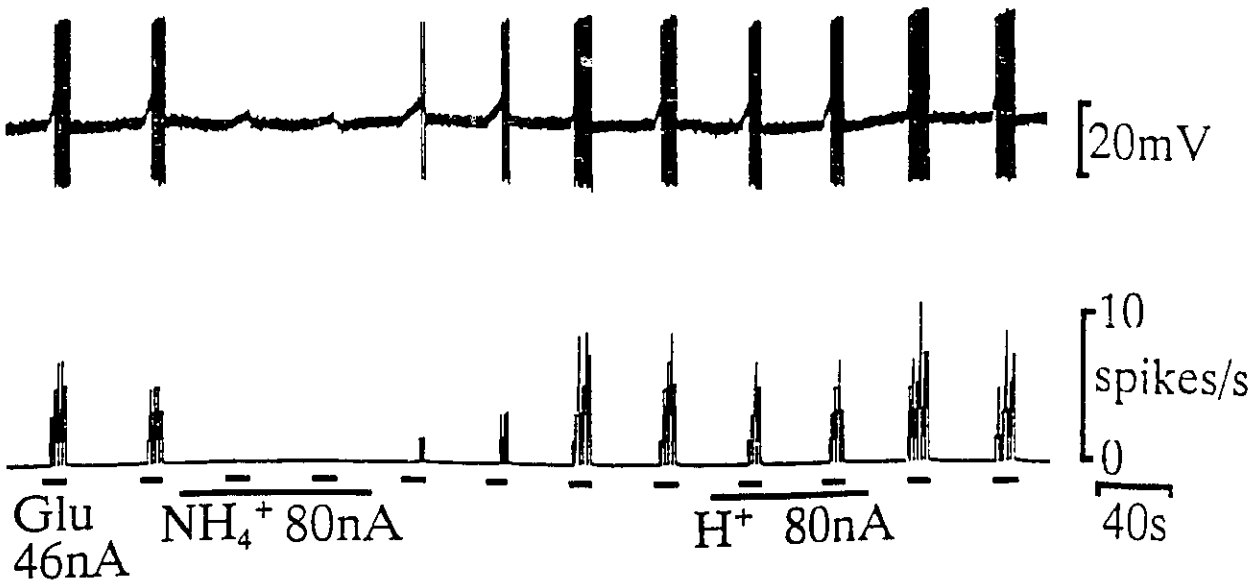


Fig. 21. Depression of Glu response by ammonium ions (NH_4^+) in LC neurons. Upper trace is intracellular recording; lower trace is the corresponding ratemeter recording of spike frequency. Time of iontophoretic application of Glu is indicated by the short bars underneath the recording trace with negative current used. Application of NH_4^+ with positive current was indicated by a longer bar. Iontophoretically applied NH_4^+ almost totally depressed the excitatory responses caused by iontophoretically applied Glu. Application of H^+ attenuated Glu response in this same neuron.

action on Glu response to iontophoretically applied NH_4^+. Pressure application of NH_4Cl depressed Glu responses in 18/20 LC neurons (Fig. 22). In the other 2 cells, Glu responses were increased during pressure ejection of NH_4Cl.

Bath application of NH_4Cl at a relative high dosage (2 - 5mM) had exclusively excitatory action on all the LC neurons and increased Glu responses in all cells tested (n=5). When the dosage was lowered to 0.25 - 1.0mM, NH_4Cl was found to have varied actions on LC neurons. Among 15 LC neurons tested, NH_4Cl attenuated Glu responses in 6 cells (Fig. 23A), excited the LC neuron and slightly increased Glu responses in 4 cells (Fig, 23B), and had no effect in 3 cells. In another 2 cells, NH_4Cl was found to increase Glu responses followed by a decrease of Glu response in one cell, and to decrease Glu responses followed by an increase of Glu response in the other cell. Effective concentration for NH_4Cl applied by bath appears to be at least 250μM. Low concentration (10 -100μM) had no apparent effects on Glu-induced responses or on cell excitablity.

The acting site of NH_4^+ on Glu appears on the postsynaptic membrane by the fact that NH_4^+ still had depressant action on Glu responses during perfusion of the slice with a modified ACSF containing low Ca^{2+}/high Mg^{2+} (n=3) which has been demonstrated to block synaptic transmission (see below).

In spite of the depressant action on Glu-induced responses, NH_4^+ was also found to depress ACh-induced responses in LC neurons. As mentioned earlier, the LC neurons can be

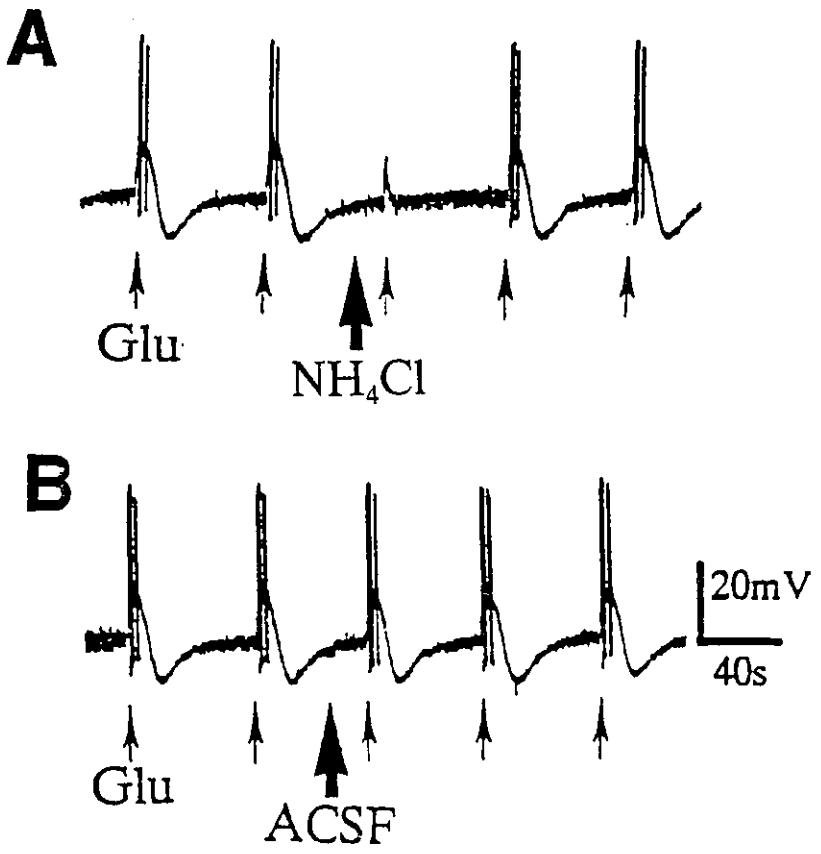
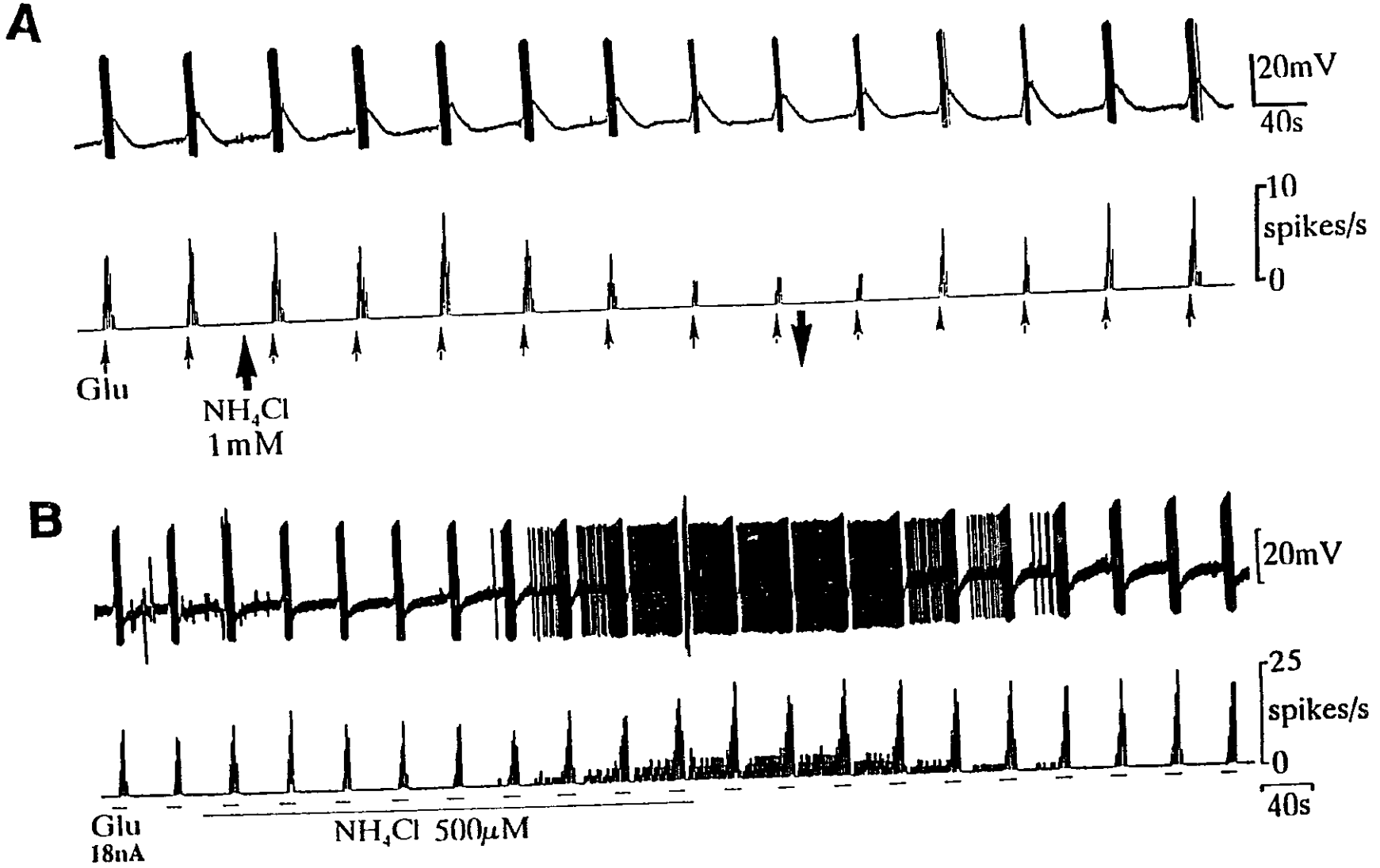


Fig. 22. Pressure ejection of NH_4Cl had the same kind effect on Glu responses as that applied iontophoretically. In both **A** and **B**, Small arrows indicate the time of pressure ejection of Glu (5mM, 9psi, 2ms, 1 pulse). Big arrows indicate the time of pressure ejection of NH_4Cl (5mM, 9psi, 2ms, 10 pulses at 1Hz)(**A**) or ACSF(9psi, 2ms, 10 pulses at 1 Hz)(**B**). Glu-induced depolarizations were almost completely blocked by pressure applied NH_4Cl. Pressure application of ACSF as a control had no apparent effect.

Fig. 23. Effects of bath applied NH_4Cl on Glu -induced responses in LC neurons. In both **A** and **B**, upper traces are intracellular recordings , lower traces are corresponding ratemeter recordings of spike frequency. **A.** an example of inhibitory action of NH_4Cl on Glu responses. Small arrows indicate pressure ejections of Glu, Big upward arrow marks the time of switching on the bath media containing NH_4Cl. Big downward arrow indicates the time of switching off the bath media containing NH_4Cl. There is 90 seconds lag between switch on the bath media containing NH_4Cl and the entry of the bath media to the recording chamber. Glu responses were attenuated by bath applied NH_4Cl. **B.** this result was obtained from another LC neuron. short bars indicate iontophoretic application of Glu, longer bar indicates bath application (from switch on to switch off) of NH_4Cl. NH_4Cl produced an excitatory response on this LC neuron and slightly increased Glu responses.

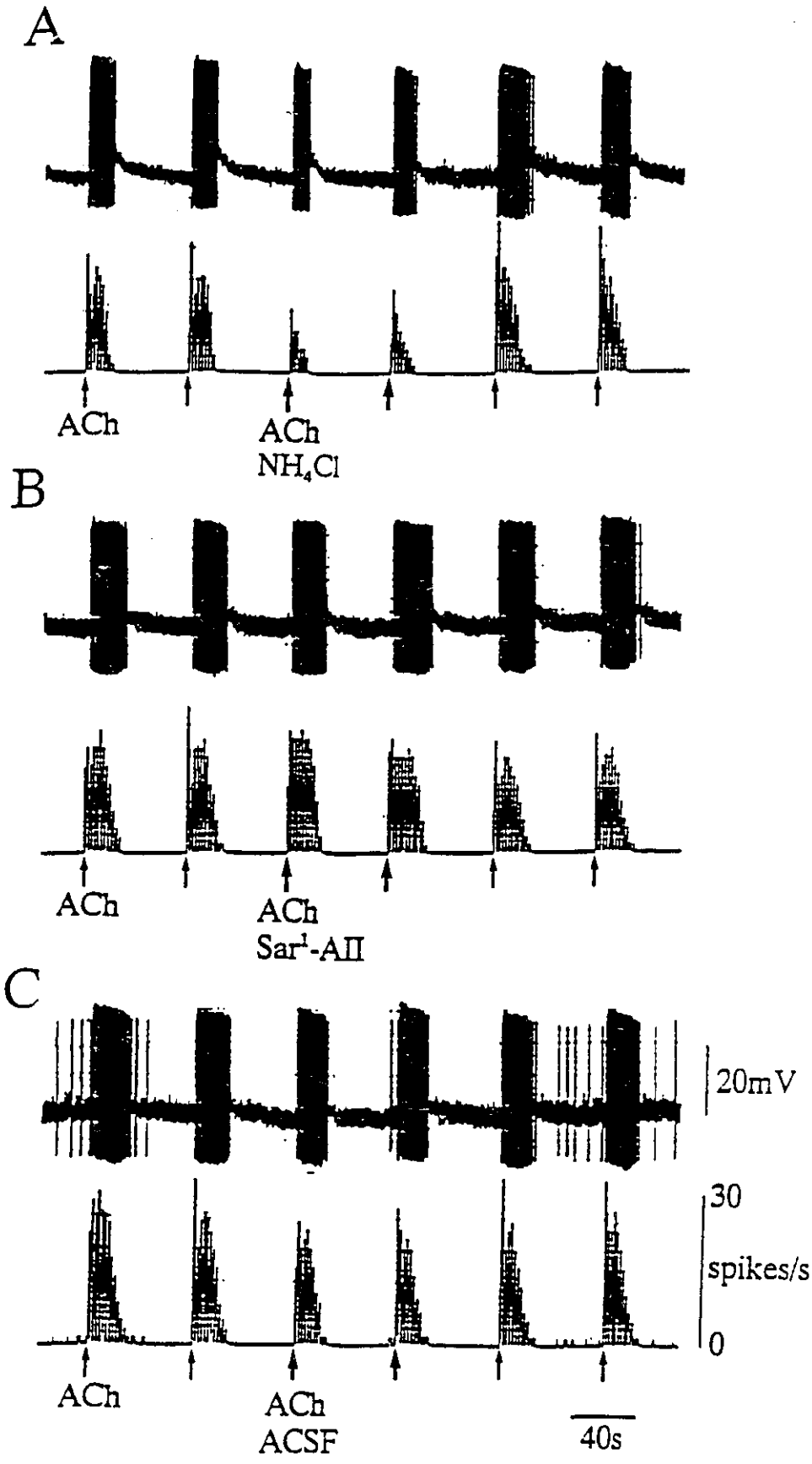

A
20mV
40s
10
spikes/s
0
Glu
NH4Cl
1mM
B
20mV
25
spikes/s
0
40s
Glu
18nA
NH4Cl 500μM

activated by ACh. These ACh-induced responses in the LC neurons were depressed by either iontophoretically applied NH_4^+ (4 out of 6 cells. data not shown) or presssure applied NH_4Cl (Fig. 24, n=2).

To further clarify the action of AII on Glu response, tests were made with AII from two other sources (Bachem and Peninsula), which were NH_4^+-free. No significant differences were found among the actions of AIIs from different companies (Fig. 25). We also found in a number of cells that there was no parallelism between the effects of AII and the effects of NH_4^+, since AII depressed Glu responses in some cells whereas NH_4^+ had no effects on the same cells (Fig. 26.).

In addition, the effects of both AII and NH_4^+ on Glu responses were tested in adjacent non-LC brain stem neurons (e.g. neurons recorded from the regions of Barrington's nucleus and lateral vestibular nucleus) as summarized in Fig. 27. The selectivity of AII on Glu responses in LC neurons was demonstrated by the fact that AII had no effects on Glu responses on most non-LC brain stem neurons, such as neurons recorded from regions of Barrington's nucleus and lateral vestibular nucleus. It was found that AII had depressant actions on Glu responses in 3/18 non-LC neurons. In the other 15 of these neurons, AII showed no apparent action on Glu responses (Fig. 28). In contrast, NH_4^+ depressed Glu responses in 8 out of 10 non-LC neurons tested. The difference between the actions of AII on LC and non-LC brain stem neurons was significant (Fig.

Fig. 24. ACh-induced responses were depressed by NH_4Cl, but not Sar^1-AII. Upper traces in **A**, **B** and **C** were intracellular recordings from an LC neuron, lower traces were corresponding ratemeter recordings. Small arrows indicate pressure applications of ACh (12 psi, 4ms, 1 pulse). Big arrows indicate simultaneous application of ACh with NH_4Cl, or Sar^1-AII or ACSF. NH_4Cl depressed ACh-induced responses (**A**). Sar^1-AII had no apparent effect on ACh-induced responses (**B**). Application of ACSF as a control had no apparent action on ACh responses (**C**).


A
ACh
ACh
NH_4Cl
B
ACh
ACh
Sar^1-AII
C
20mV
30
spikes/s
0
ACh
ACh
ACSF
40s

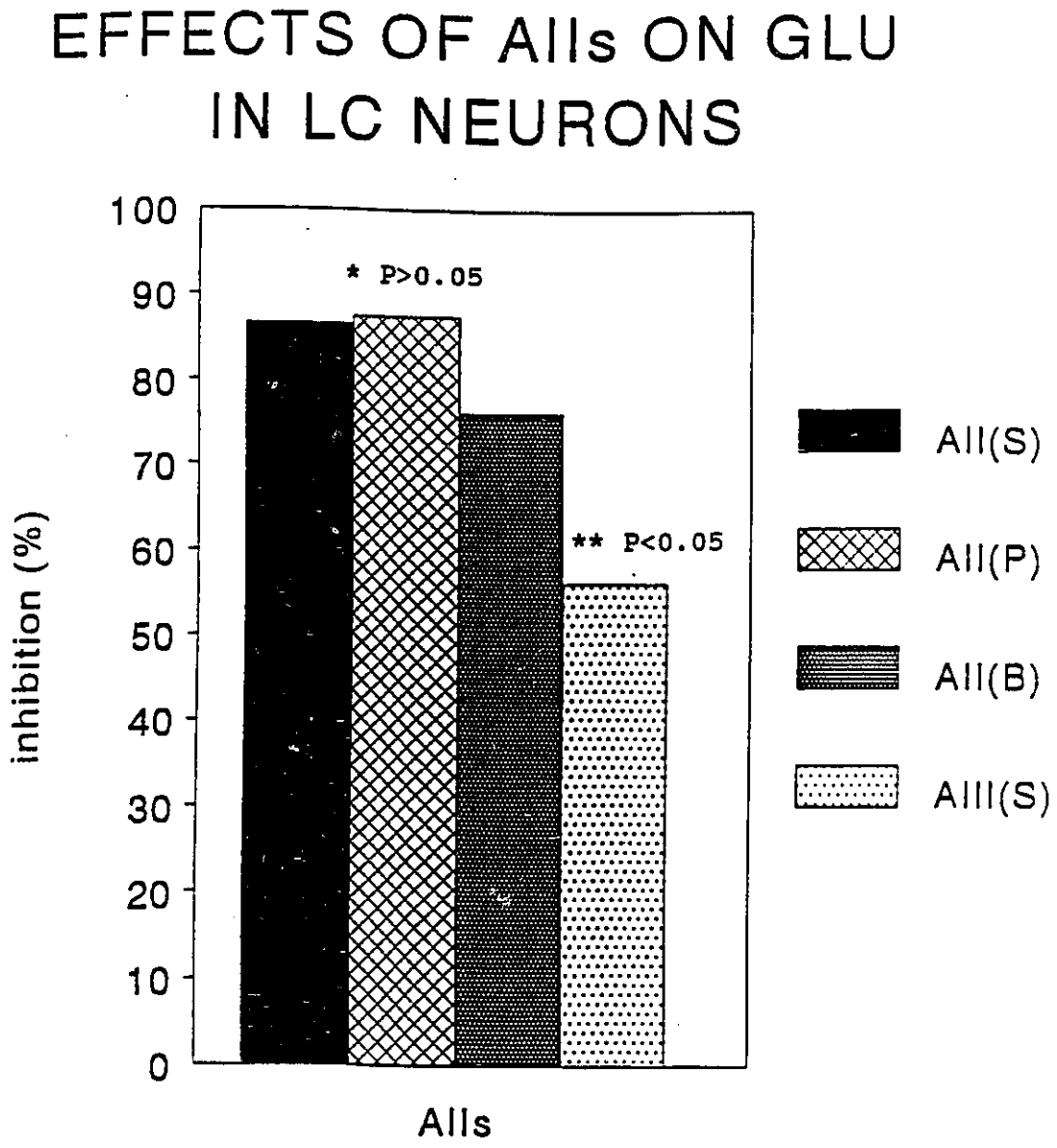


Fig. 25. Comparison of the depressant actions of iontophoretically applied AIIs purchased form different companies on Glu responses in the LC neurons. Ordinate shows percentage of neurons in which Glu response was depressed by at least 30%. No significant differences were found between different samples of AII. Comparison of AIII with AII actions showed that AIII was less effective than AII. *: represent comparisons among AIIs, **: represent comparisons between AIIs and AIII.

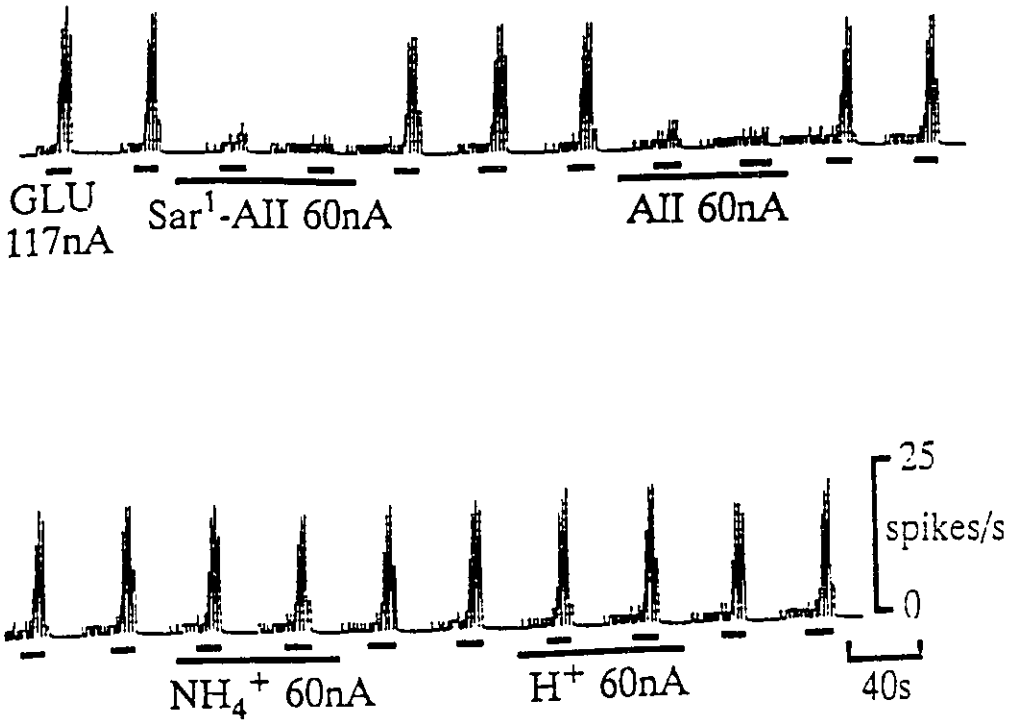


Fig. 26. Effects of AII and NH_4^+ on an LC neuron. Both traces are the ratemeter recordings of the number of spikes activated by iontophoretically applied Glu (indicated by short bars). Application of Sar^1-AII or AII strongly depressed Glu-induced spikes (upper trace), Application of NH_4^+ or H^+ had little or no effect on Glu responses.

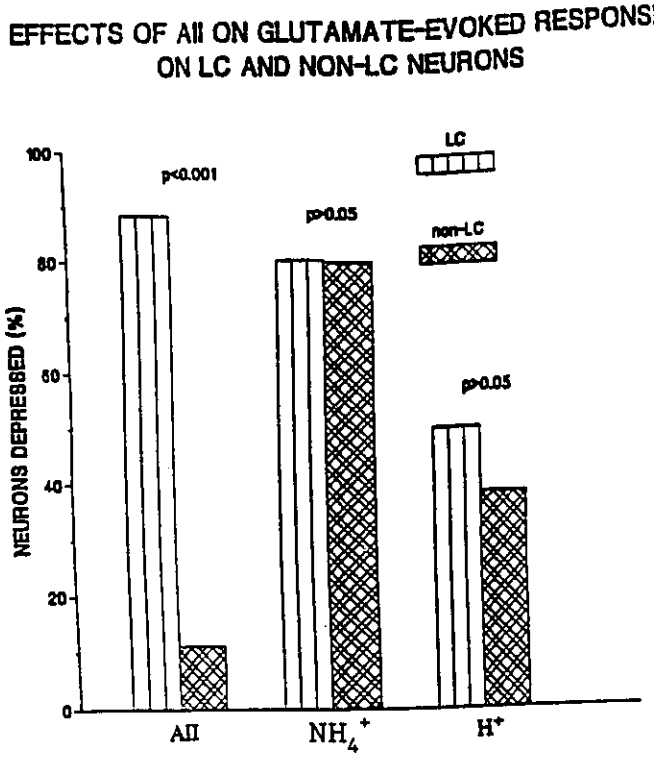


Fig. 27. The effects of AII, NH_4^+ and H^+ on Glu responses in LC and non-LC brain stem neurons. The ordinate indicates percentage of neurons depressed at least 30% by AII. The actions of AII on Glu responses in the LC and non-LC brain stem neurons are significantly different ($p<0.001$). In contrast, no significant differences were found between the actions of H^+ and NH_4^+ in the LC and non-LC brain stem neurons ($p>0.05$).

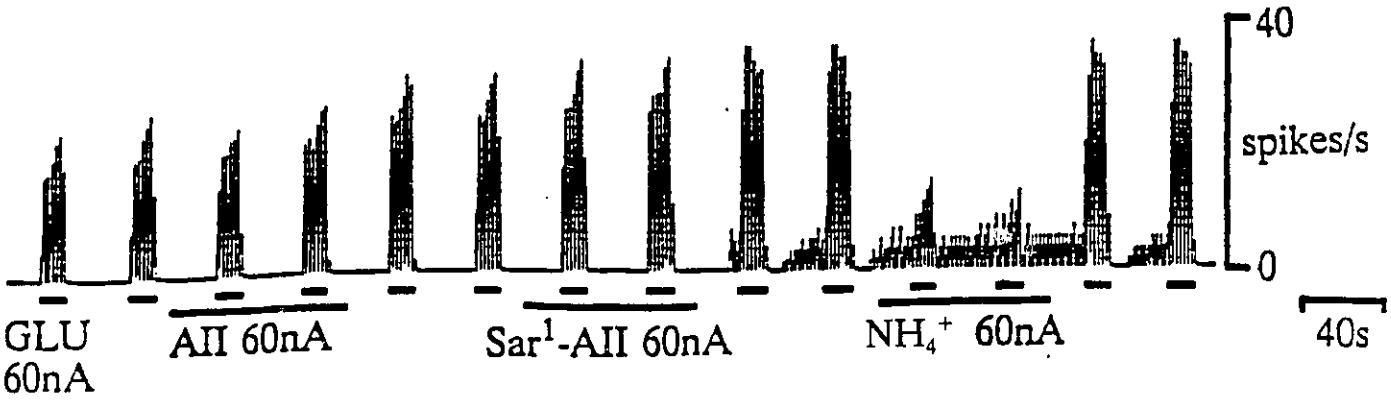


Fig. 28. An example of effects of AII and NH_4^+ on a non-LC brain stem neuron (recorded in the region of Barrington's nucleus). This is a ratemeter recording of spike frequency. Application of Glu (indicated by short bars) activated this neuron. Application of AII or Sar^1-AII appears to have no apparent actions on Glu-induced responses. In contrast, application of NH_4^+ strongly depressed Glu activated spikes, though this cell fired more rapidly due to spontaneous depolarization during recording.

27). These data indicate AII effects are not due to contaminating NH_4^+ though there are similarities in the actions of these two substances on Glu responses in LC neurons. The difference was confirmed in tests of AII receptor antagonists (see below).

3. 3. 6. Effects of Sar^1-AII Applied by Pressure

The effects of Sar^1-AII (including AII), applied by pressure ejection (0.1 mM), on Glu responses have also been tested, and the results are very similar to those observed with iontophoretic application. Pressure application of Sar^1-AII had strong depressant actions on Glu, applied either by iontophoresis (n=11) or by pressure (n=29). No hyperpolarizing responses were seen when AII or Sar^1-AII was applied by pressure ejection. These results are summarized in Table 3 (iontophoretically applied Glu) and Table 4 (pressure applied Glu). The effects of pressure applied Sar^1-AII (including AII seem to be consistent, and to be more potent and than those applied by iontophoresis. Fig. 29A. illustrates an example of this action using the AII analogue Sar^1-AII. Control applications of ACSF by pressure had no effect on Glu responses (n=4).

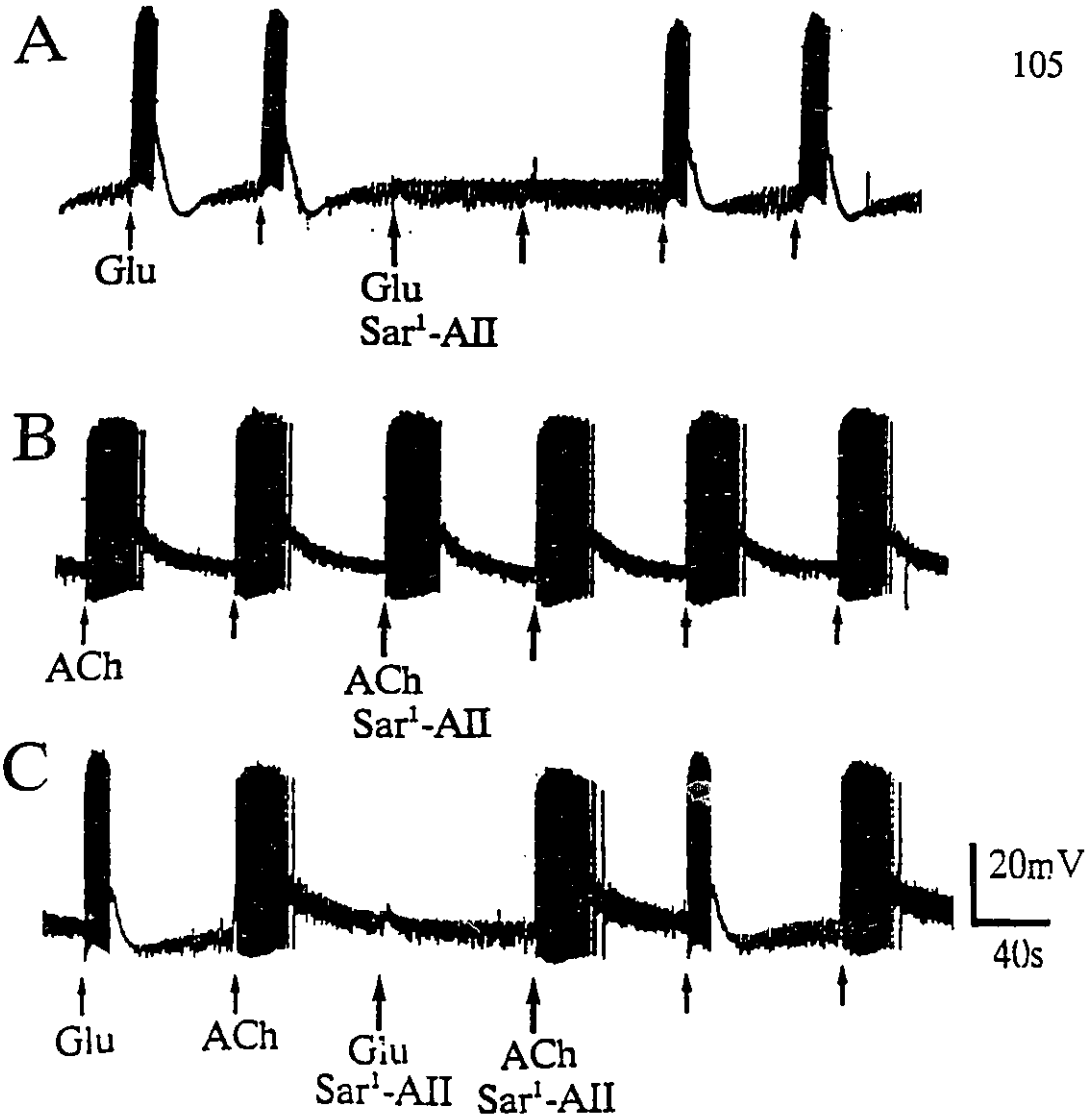


Fig. 29. Pressure applied Sar¹-AII depressed Glu-induced responses without effects on ACh-induced responses. All traces are intracellular recordings from the same LC neuron. In the upper trace (A), Small arrows indicate pressure application of Glu (9psi, 2ms, 1pulse), big arrows indicate pressure ejections of Sar¹-AII (9psi, 2ms, 1 pulse) and Glu at the same time. Pressure ejection of Sar¹-AII completely depressed depolarizations and resultant excitations induced by pressure ejection of Glu. In the middle trace (B), small arrows indicate pressure application of ACh (9psi, 2ms 1 pulse), big arrows indicate simultaneous application of ACh and Sar¹-AII by pressure. Sar¹-AII had no effect on ACh-induced responses. In the bottom trace (C), small arrows indicate alternative application of Glu and ACh by pressure, big arrows indicate simultaneous pressure application of Sar¹-AII and Glu or ACh. Sar¹-AII selectively depressed Glu-induced responses without effect on ACh-induced responses.

TABLE 3

Effects of Pressure Applied AII on the Responses induced by Glu applied iontophoretically

Tests	N	depression of Glu response				--
		mild	moder.	strong	total	
AII(S)→Glu	3	1	1	1	3	0
AII(B)→Glu	5	1	3	1	5	0
Sar^1-AII→Glu	3	0	2	1	3	0
ACSF→Glu	2	0	0	0	0	2

S: Sigma; B: Bachem
N: number of cells; moder.: moderate; --: no effect;
*: When the total number of cells which Glu responses were depressed by AIIs was compared with the number of cells of ACSF control, the difference is significant ($p<0.01$).

TABLE 4

Effects of Pressure Applied AII on the Responses induced by Glu applied by pressure

Tests	N	depression of Glu response				--
		mild	moder.	strong	total	
AII(S)→Glu	4	0	1	3	4	0
AII(B)→Glu	7	0	1	6	7	0
Sar^1-AII→Glu	18	0	0	18	18	0
ACSF→Glu	3	0	0	0	0	3

S: Sigma; B: Bachem
N: number of cells; moder.: moderate; --: no effect;
*: When the total number of cells which Glu responses were depressed by AIIs was compared with the number of cells of ACSF control, the difference is significant ($p<0.01$).

3. 3. 7. Effect of AII on ACh-induced Responses

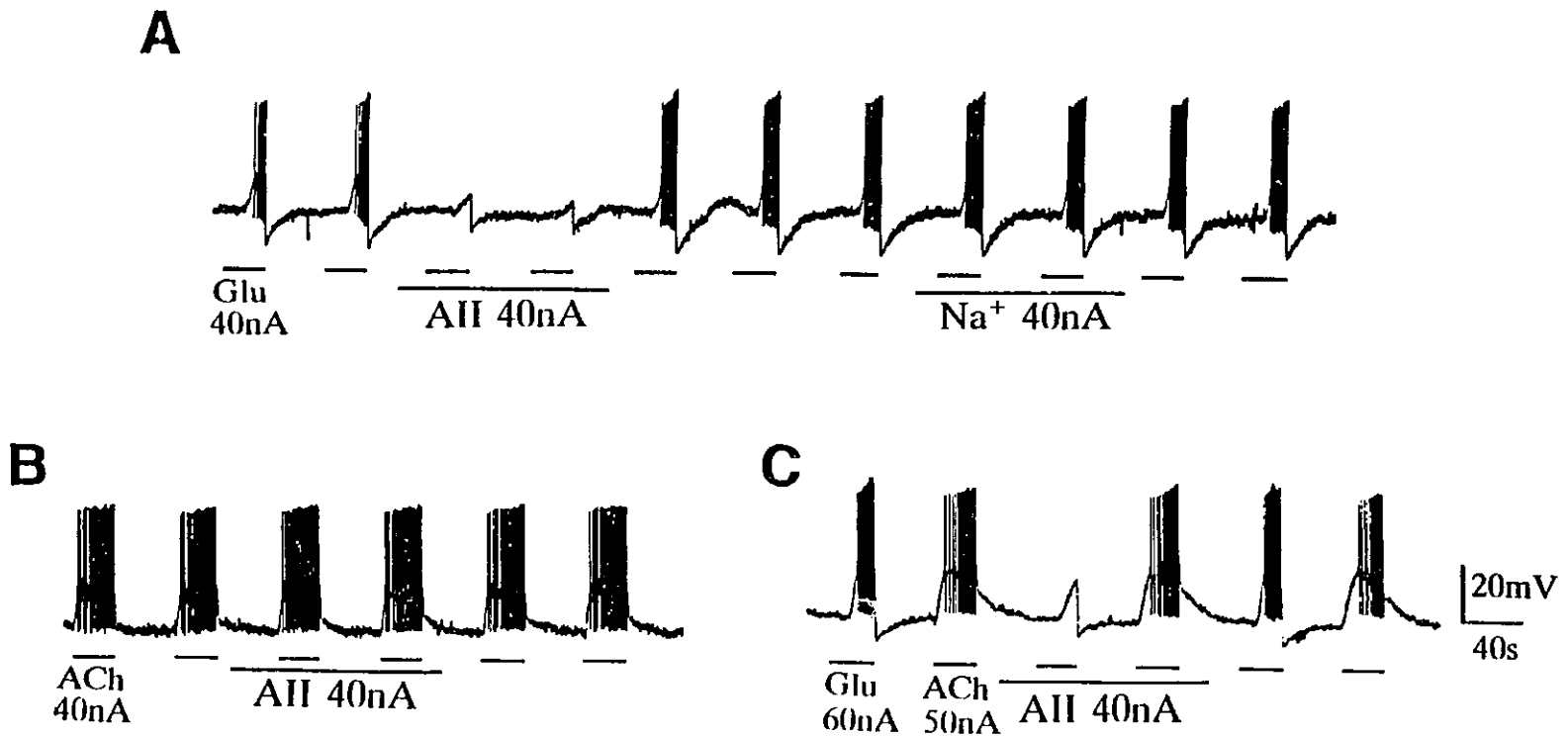
As Glu, ACh also exerts excitatory action on LC neurons. The selectivity of the AII effects was tested by comparing its influence on depolarizations and resultant excitations evoked by both Glu and ACh. AII was found to have no effect on ACh-induced depolarizations and resultant excitations when applied by iontophoresis (Fig. 30B, n=9) or by pressure ejection (Fig. 29B, n=4). When Glu and ACh were alternatively applied by iontophoresis (Fig. 30C, n=6), AII strongly and selectively depressed the Glu responses without affecting the ACh responses. Similar results were obtained with pressure ejection (Fig. 29C, n=4).

3. 4. Effects of AII Receptor Antagonists

To test whether the observed actions of AII were mediated by AII receptors, both the peptide antagonist, saralasin and non-peptide antagonists, losartan (AT_1-selective) and PD123177 (AT_2-selective), were tested.

When co-applied with AII iontophoretically, saralasin was found to block AII action in 20 out 35 LC neurons tested, with mild blockade in 7 cells, moderate blockade in 10 cells and almost total blockade in 3 cells (Fig. 31). Saralasin was applied 2 minutes ahead of application of AII in some cells. In some other cells , saralasin was applied at the same time as AII. The currents used to eject saralasin from pipettes in each

Fig. 30. Glu, but not ACh depolarizations are depressed by AII. A. Glu depolarizations are depressed during AII application, application of same of current through another electrode barrel containing saline has no effect on Glu response. B. A similar application of AII has no effect on ACh-induced excitations in the same neuron. C. Alternating application of Glu and ACh demonstrates the selectivity of AII depression on Glu actions.


A
Glu
40nA
AII 40nA
Na+ 40nA
B
ACh
40nA
AII 40nA
C
Glu
60nA
ACh
50nA
AII 40nA
20mV
40s

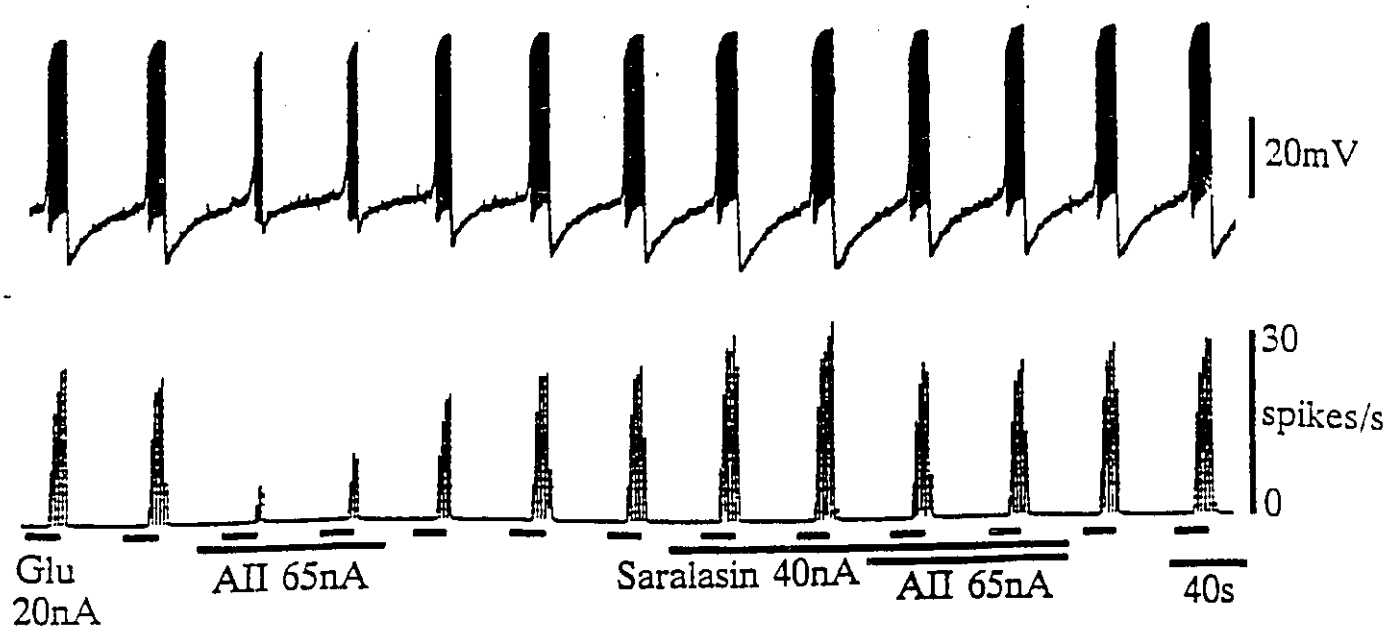


Fig. 31. Effects of saralasin, a peptide AII receptor antagonist on AII-induced depression of Glu responses. Upper trace is intracellular recording, lower trace is the corresponding ratemeter recordings of spike frequency. Iontophoretic application (indicated by a longer bar on left) of AII depressed the excitatory responses caused by iontophoretically applied Glu indicted by short bars. Application of AII receptor antagonist saralasin (indicated by the longest bar) blocked the depressant action of AII on Glu responses.

experiment were close to maximal, since further increase of ejecting current resulted in the blockade of that channel due to the failure of limited ions to conduct a large current. Application of saralasin alone was also found to cause mild to moderate depressions of Glu responses in 10/39 LC neurons (data not shown).

Combined application of AT_1-slective antagonist losartan(5μM) and AT_2 selective antagonist PD123177(5μM) strongly and reversibly blocked AII actions on Glu responses in all cells tested (n=7). Application of losartan alone had a mild blockade of AII actions in 3 out of 9 cells and no effect on the other six. In contrast, during application of PD123177, moderate to strong blockade of AII effects was observed in all cells tested (n=10), indicating that the AT_2 subtype receptors were primarily responsible for the AII actions on Glu responses (Fig. 32). The action of PD123177 in antagonizing AII depression of Glu-induced depolarizations was found to occur in a concentration dependent manner with an IC_{50} of approximate 1.5 μM (Fig. 33, n=6). Also, we tested the influence of DTT, a AT_1 receptor inactivator on the action of AII on Glu response, and found that DTT (1 - 5 mM) had no influence on the depressant action of AII on Glu responses.

3. 5. Effects of AII Receptor Antagonists on the NH_4^+ and H^+-induced Depression of Glu Responses in LC Neurons

Using these potent and selective AII receptor antagonists,

Fig. 32. Effects of non-peptide AII receptor antagonists on the action of AII. Upper traces are the intracellular recordings, lower traces are corresponding ratemeter recordings of spike frequency. Small arrows indicate pressure ejection of Glu (20mM, 12psi, 2ms, 1 pulse), big arrows indicate pressure ejection of AII(0.1mM, 12psi, 2ms, 1 pulse) and Glu (20mM, 12psi, 2ms, 1 pulse) at the same time through separate barrels. **A.** Under normal perfusing media (ACSF), AII completely eliminated Glu response. **B.** When losartan (5μM) and PD123177(5μM) were added to perfusing media, the action of AII was completely blocked. **C.** Bath applied PD123177(5μM) alone had almost the same blocking effect on Glu response as that of co-applied with losartan. **D.** Application of losartan (5μM) alone had little effect on AII action. All recordings are obtained from the same LC neuron.

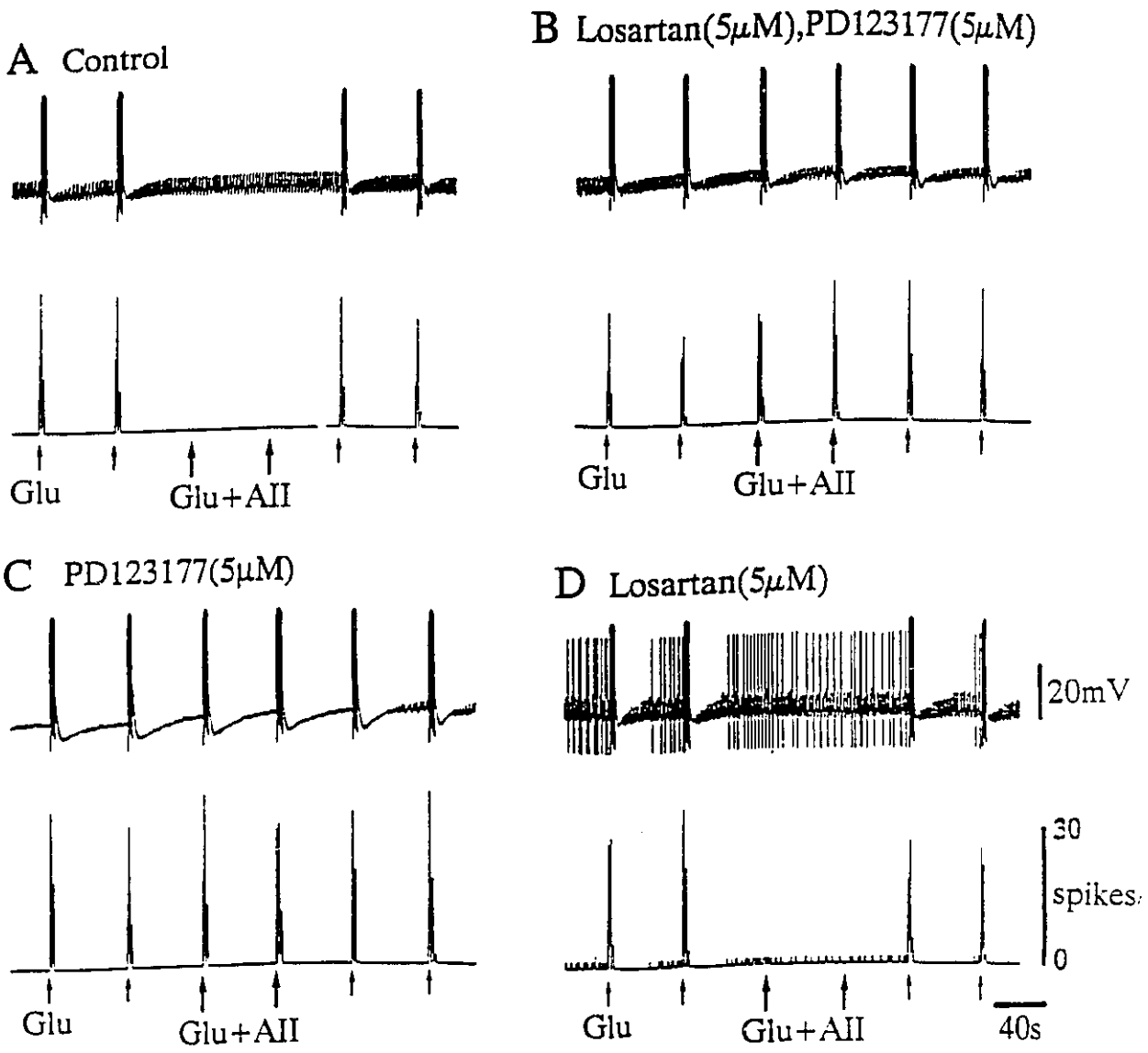
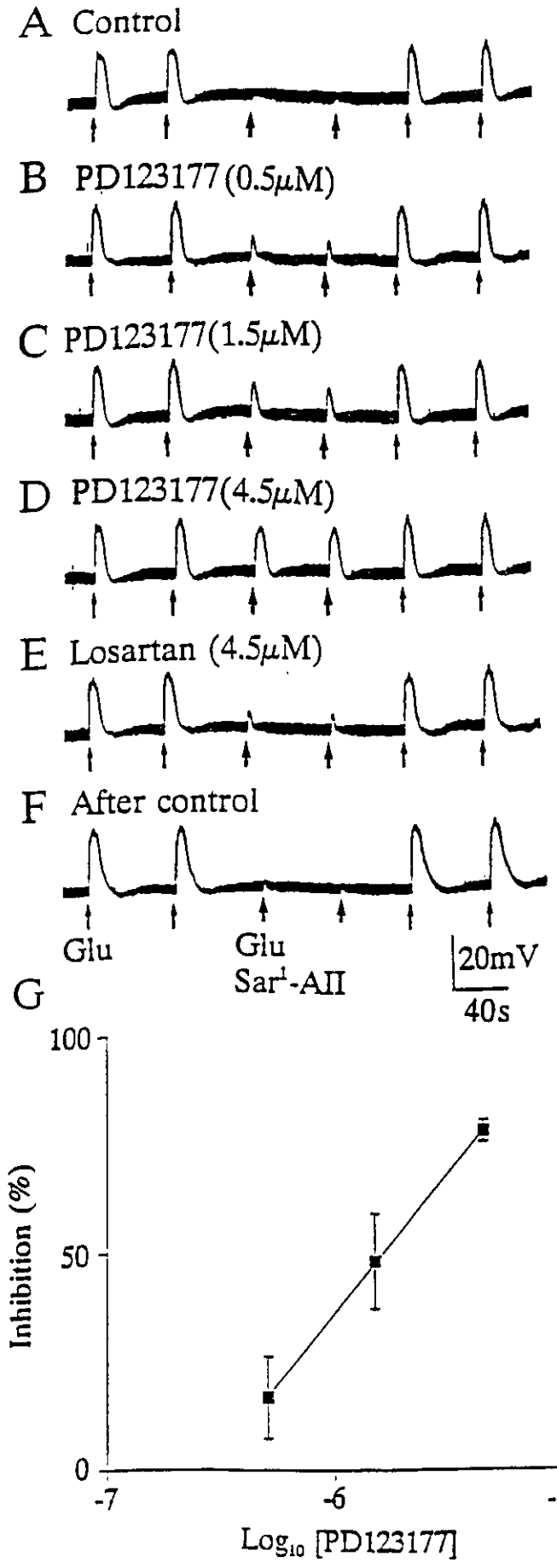

A Control
Glu
Glu+AII
B Losartan(5μM),PD123177(5μM)
Glu
Glu+AII
C PD123177(5μM)
Glu
Glu+AII
D Losartan(5μM)
20mV
30
spikes
0
Glu
Glu+AII
40s

Fig. 33. Effects of non-peptide AII receptor antagonists on the action of AII. In all traces, small arrows indicate pressure ejection of Glu (5mM, 20psi, 7ms, 1 pulse), big arrows indicate pressure ejection of AII(0.1mM, 20psi, 7ms, 1 pulse) and Glu (5mM, 20psi, 7ms, 1 pulse) at the same time through separate barrels. In normal perfusion media (ACSF), AII completely eliminated Glu-induced depolarizations (A). Bath applied PD123177, a selective AT_2 receptor blocker, antagonized the action of AII in a concentration dependent manner (B, C and D; n=6). Bath application of losartan (DuP753, 4.5μM), an AT_1 receptor antagonist, had a mild blockade of the action of AII (E). F. is an after control showing Sar^1-AII had the same potency as control in depressing Glu-induced depolarization.
G. Concentration-response curve of the of PD123177 on the inhibition of AII action. The estimated IC_{50} for PD 123177 is about 1.5μM. Membrane potential was hyperpolarized to -80mV throughout the recording.

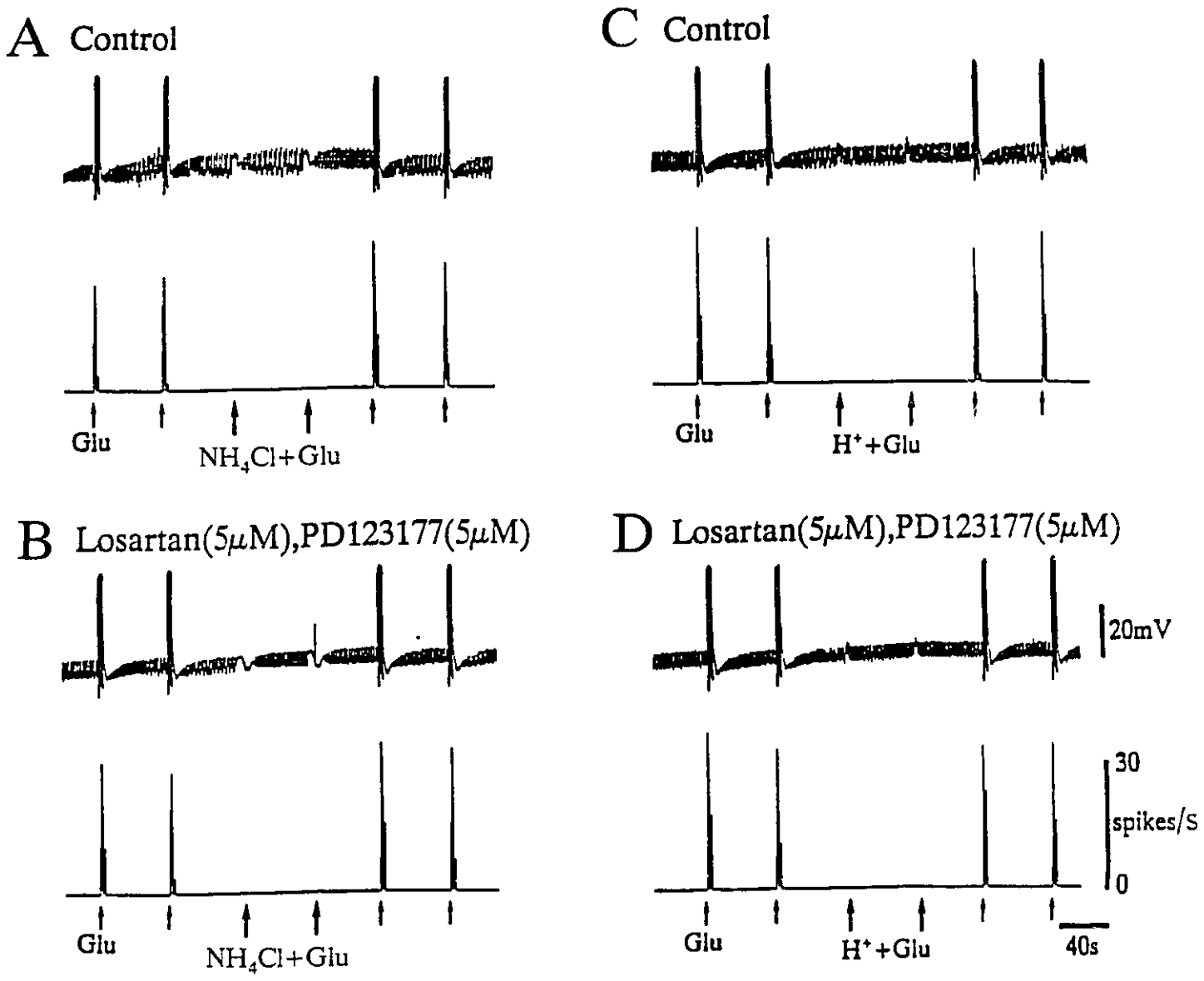

A Control
B PD123177 (0.5μM)
C PD123177(1.5μM)
D PD123177(4.5μM)
E Losartan (4.5μM)
F After control
Glu
Glu
Sar¹-AII
20mV
40s
G
100
50
0
Inhibition (%)
-7
-6
-5
Log10 [PD123177]

we were able to test their antagonizing actions on the depressant effects induced by H^+ and NH_4^+. The results show that these blockers were ineffective in antagonizing either H^+ or NH_4^+-induced depressions of Glu responses (Fig. 34, n=3) while the AII action was antagonized in these same cells (see Fig.31).

3. 6. Effects of AIII on Glu Responses

We also tested the effects of iontophoretically applied AIII, a normal metabolic degradation product of AII, on Glu responses in LC neurons. Of 16 LC neurons tested, there were 4 cells on which neither AII nor AIII had effects on Glu responses. Of the remaining 12 cells, AIII was found to depress Glu responses in 9 cells (Fig. 35), with mild depression in 4, moderate in 3 and strong in 2. In contrast, AII had depressant action on all of them, with mild depression in 2, moderate in 2 and strong in 8. There were 9 cells in which both AII and AIII depressed Glu-induced responses. Among these 9 cells, AIII had a mild depression in 4, a moderate depression in 3, and a strong depression in 2, whereas AII had a mild depression in 1, a moderate depression in 1, and a strong depression in 7. It seems that the actions of AIII on Glu responses were less potent than that of AII in terms of the strong depression. The difference between the strong depressant actions of AII and AIII is statistically significant ($p<0.05$). In addition, the effects of AI, a precursor of AII, on Glu responses has been tested in four LC neurons, and a small reduction of spike frequency caused by

Fig 34. These results were obtained from the same neurons as in Fig. 32. Non-peptide antagonists had no effects on the depressant actions of H^+ (ACSF adjusted to pH 5.0, 12psi, 2ms, 1 pulse) and NH_4Cl (5mM, 12psi, 2ms, 1 pulse) on Glu (20mM, 12psi, 2ms, 1 pulse). **A.** and **C.** are the controls which show both NH_4Cl and H^+ had depressant actions on Glu responses. **B.** and **D.** show that the depressant actions of NH_4Cl and H^+ were unaffected by bath co-applied losartan (5μM) and PD123177(5μM) which antagonized AII action on the same neuron (see Fig. 32B and 32C.).


A Control
Glu
NH4Cl+Glu
C Control
Glu
H+ +Glu
B Losartan(5μM),PD123177(5μM)
Glu
NH4Cl+Glu
D Losartan(5μM),PD123177(5μM)
20mV
30
spikes/S
0
Glu
H+ +Glu
40s

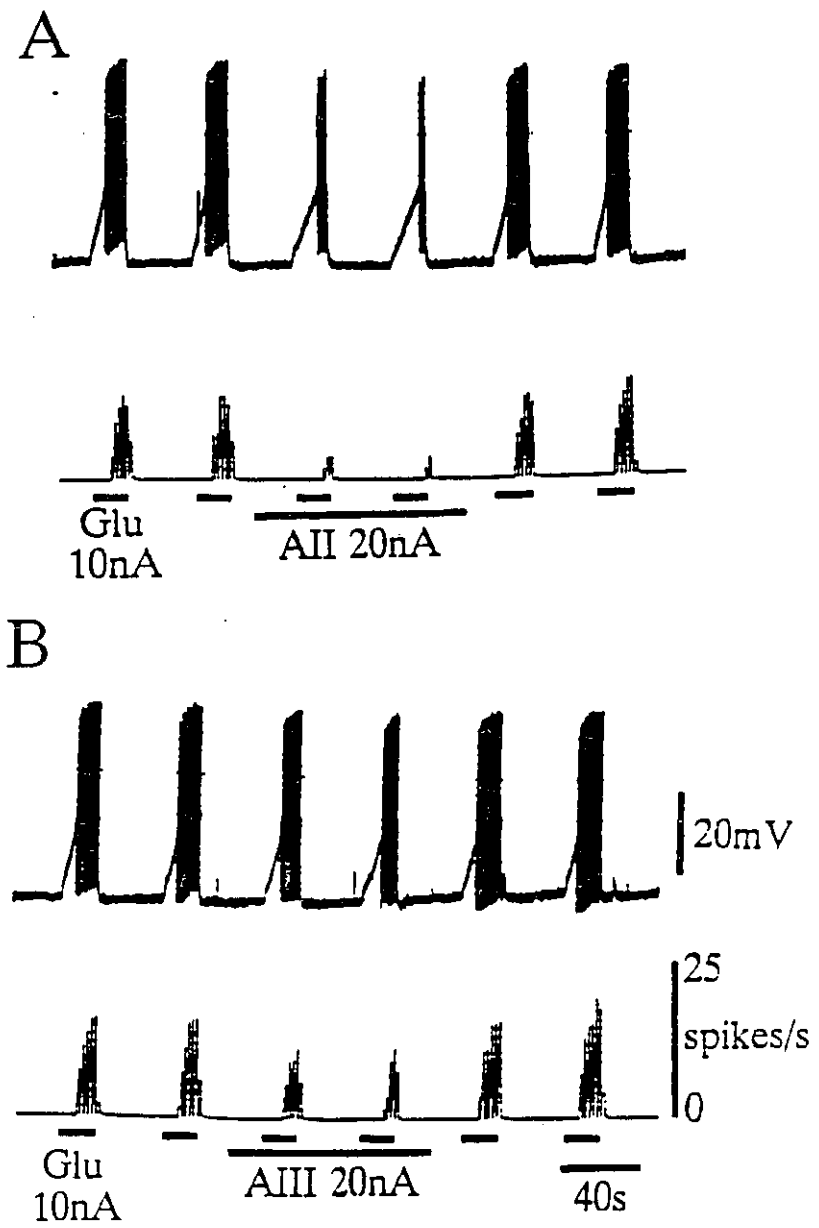


Fig. 35. Effect of AIII on Glu-induced responses in an LC neuron. In both A and B, upper traces are intracellular recordings, lower traces are the corresponding ratemeter recordings of spike frequency. Iontophoretic applications are indicated as in Fig. 11. AII strongly reduced the number of spikes induced by Glu (A), in contrast to the action of AII, AIII had only a mild depression on this parameter (B). Membrane potential was at - 62mV.

AI was found in only one of them (Fig. 36.)

3. 7. Effects of Bath Applied AII on Glu Responses

While the effects of AII applied by pressure were very similar to those with iontophoresis, bath application of AII was found to have only a mild depressant action on Glu responses in 2 out of 13 cells tested, even at a dosage as high as 10μM. Several possible explanations for this result were considered. One possibility is due to the peptide degradation in vehicle solution; another possibility is due to the peptide binding to the inner walls of the reservoir and the tubing leading to bath chamber; the third possible explanation is that the activity of AII is diminished by peptidases.

In order to check characteristics of AII and eliminate the possibility that AII degrades in vehicle solution owing to the adjustment of the pH of the solution, the potency of AII purchased from both Sigma and Bachem was tested on rat arterial blood pressure. AII from the both companies was, at the same concentration, found to have almost the same potency in raising arterial blood pressure of rats when administered intravenously. We also measured the concentration of AII purchased from different companies in solutions prepared as normal experimental procedure using high performance liquid chromatography. No evidence was found to support the degradation of AII in vehicle solution.

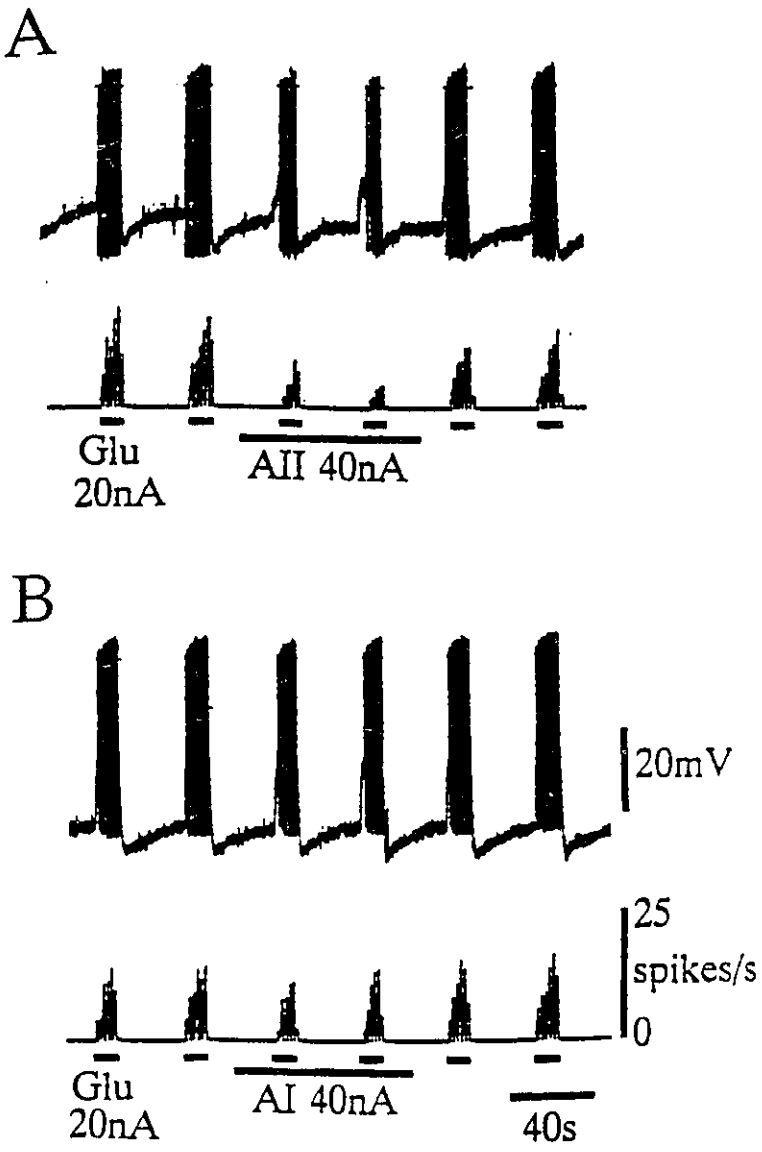


Fig. 36. Effect of angiotensin I (AI) on Glu-induced responses. In both A and B, upper traces are intracellular recordings, lower traces are corresponding spike frequency. Iontophoretic application of AII (indicated by a long bar in A) had a moderate reduction of spikes activated by iontophoretic applied Glu (indicated by short bars). Application of AI, a precursor of AII, had little effect on Glu responses (B). Membrane potential was at - 60 mV.

Using Sigmacote, to prevent peptides from binding to the glass walls of containers, the depressant action of AII on Glu appeared to be somewhat greater in some cells though no large differences were observed.

Bath application of Sar^1-AII, which is considered to be resistant to the peptidase degradation (Hall et al., 1974), was found to depress Glu responses (Fig. 37), suggesting that peptidase activity may be responsible for the ineffectiveness of AII when applied by bath. Moreover, when AII was bath-applied together with peptidase inhibitors, the depressant actions of AII on Glu responses were observed on both iontophoretically applied (5/7) or pressure ejected Glu (17/20). Fig. 38 shows that bath application of AII alone had little effect (Fig.38A), but when co-application of AII with peptidase inhibitor Ama. (inhibiting peptidase A and M) and Plu. (inhibiting carboxypeptidase N), the Glu responses were clearly depressed (Fig.38B). The peptidase inhibitors themselves had no apparent action on Glu responses when tested as a control (Fig. 38D). The combination of peptidase inhibitors we used in most of our experiments ought to prevent AII from degradation at both the N-terminal (Ama.) and C-terminal (Plu.). When co-applied with both Ama and Plu, AII had moderate or strong depressant actions on Glu responses in all the cells tested (n=11). At a dosage as low as 2μM, the depressant action was still seen (Fig. 38C). When co-applied with Ama and Bes (n=3), with Ama alone (n=2) or with Plu (n=5), AII had mild depressions of Glu responses. Bes

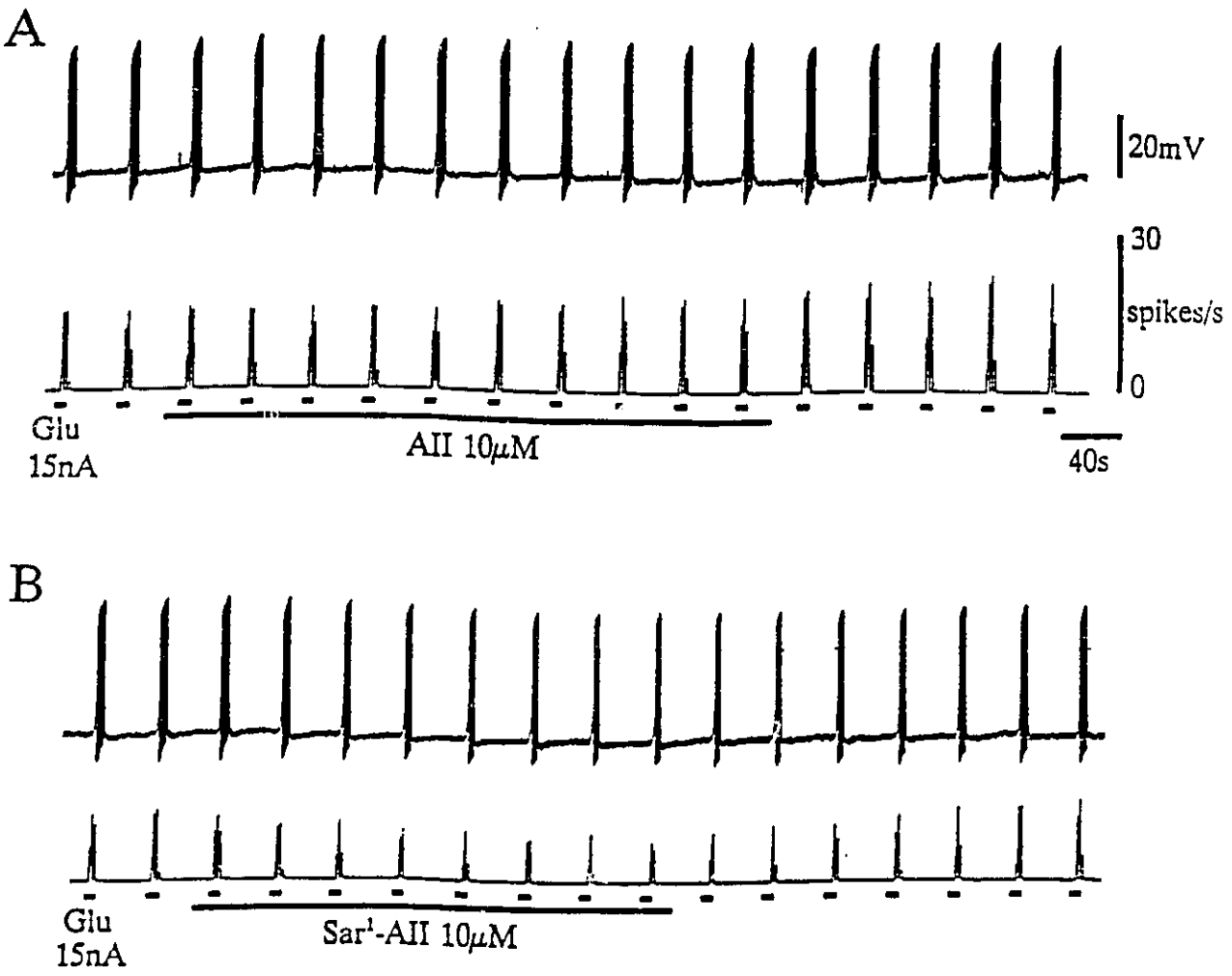
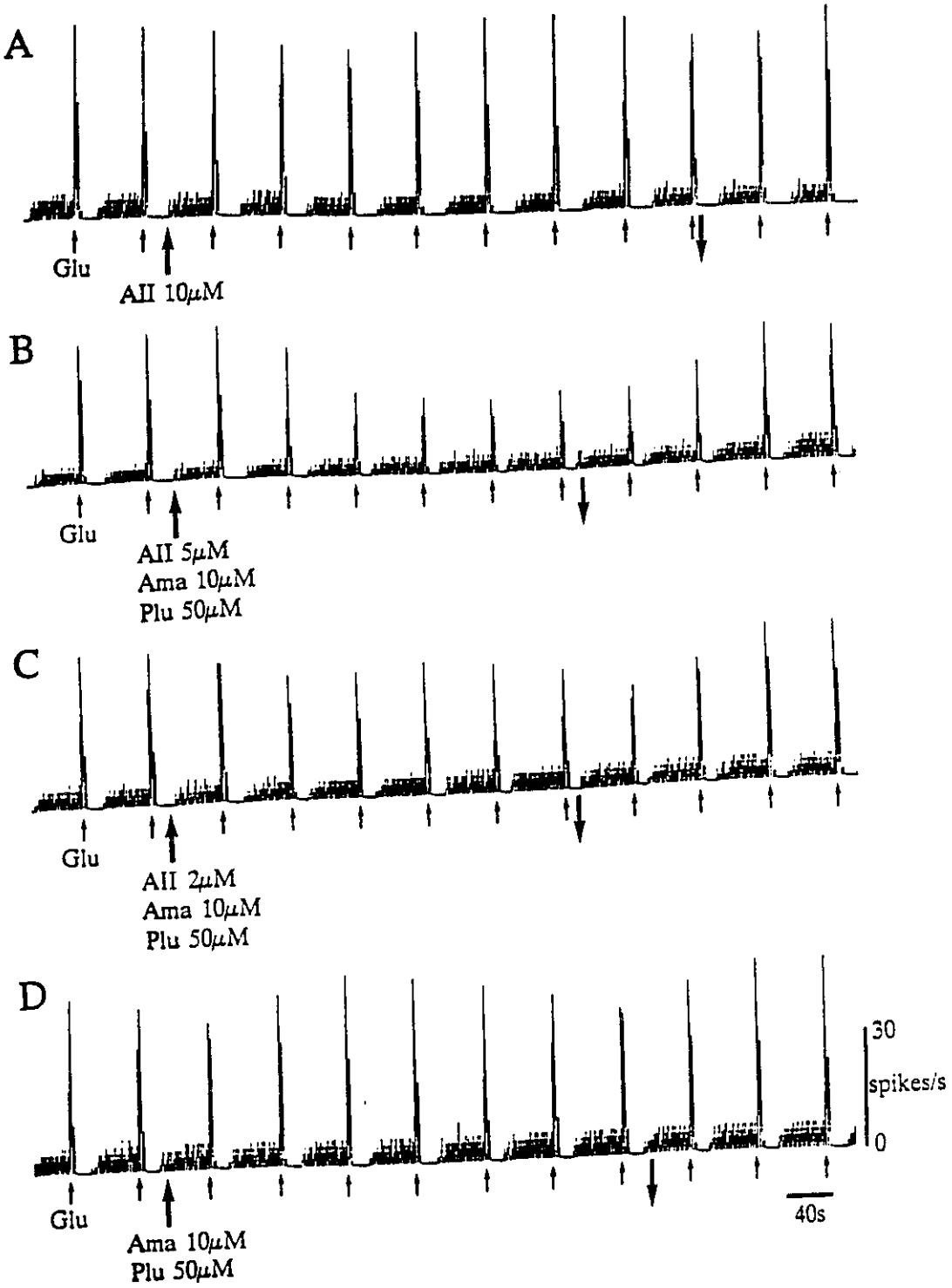


Fig. 37. Bath applied AII had little effect on Glu-induced response (A), while bath applied Sar^1-AII (resistant to peptidase degradation) depressed the Glu-induced response (B). Short bars indicate iontophoretically applied Glu. Longer bars indicate the time between the AII or Sar^1-AII containing solutions and the control ACSF entering the bath.

Fig. 38. Peptidase inhibitors applied together with AII by bath increased the depressant action of AII on Glu-induced excitation. Small arrows indicate pressure ejection of Glu (20mM, 9psi, 2ms, 1 pulse), Upward big arrows indicate the start of bath application of AII or AII with peptidase inhibitors, downward big arrows indicate the end of bath applications. **A.** Without peptidase inhibitor, AII (10μM) had little effect on Glu response. **B.** By addition of amastatin (Ama, 10μM) and Plummer's reagent (Plu, 50μM), AII depressed the Glu response by about 50% at a concentration of 5μM. **C.** Further lowering of the concentration of AII to 2μM, resulted in a small reduction (about 15%) of Glu response when applied together with peptidase inhibitors. **D.** Application of peptidase inhibitors alone had no effect on Glu response. All traces are the ratemeter recordings of spike frequency from the same neuron.

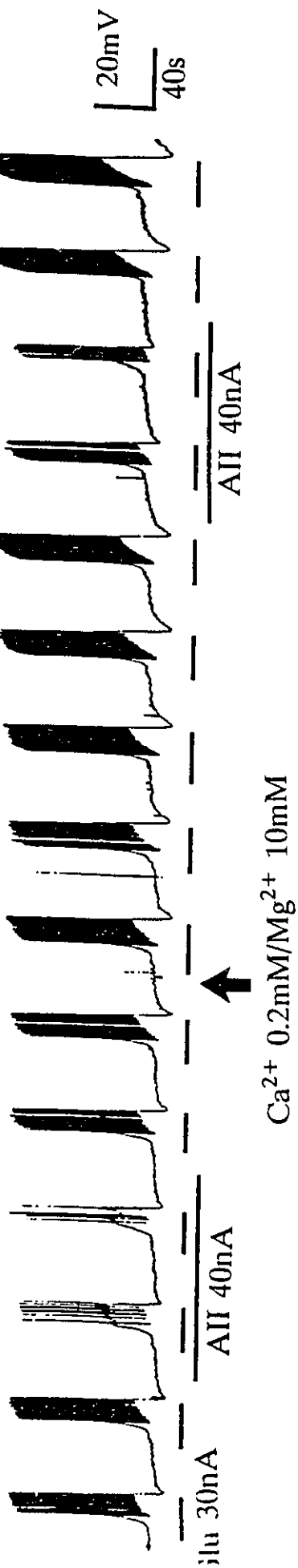

A
Glu
AII 10μM
B
Glu
AII 5μM
Ama 10μM
Plu 50μM
C
Glu
AII 2μM
Ama 10μM
Plu 50μM
D
Glu
Ama 10μM
Plu 50μM
30
spikes/s
0
40s

was not tested alone.

3. 8. Effects of AII on Glu Are Independent of Other Neuronal Elements

Both GABA (via $GABA_A$ receptors, Ennis and Aston-Jones, 1988) and NA (via α_2 receptors, Marshall and finlayson, 1988) were found to inhibit LC neuronal activity. In order to eliminate the possibility that the action of AII was dependent on involvement of other neuronal elements, e.g. GABA inhibitory interneurons or release of NA (we observed that iontophoretically applied NA inhibited Glu-induced responses in several tests [n=3]) from LC neurons, the actions of AII on Glu-induced responses were tested in the presence of antagonists for NA or GABA. It was found that the depressant action of AII on Glu responses were unaffected when the α_2-adrenoceptor antagonist yohimbine (1μM; N=4) or the $GABA_A$ receptor antagonist, bicuculline (20μM; N=4) wrer added to bath media. The likelihood of an indirect or presynaptic action of AII was further reduced by the observation that the depressant effect persisted during perfusion with a low calcium-high magnesium solution which strongly suppressed excitatory synaptic potentials evoked in LC neurons (Fig. 39). As shown in section 3.3.7., this depressant action of AII on Glu-induced responses was selective since AII had no apparent actions on ACh-induced responses.

Fig. 39. An intracellular recording from an LC neuron, showing the action of AII on Glu-induced responses during the perfusion with a modified ACSF containing low Ca^{2+}/high Mg^{2+}. At up arrow, bathing solution containing low Ca^{2+}/high Mg^{2+} reaches bath. Although the cell depolarizes and becomes more strongly activated during the low calcium perfusion, AII still causes a marked reduction in the Glu-induced responses. Short bars indicate iontophoretic application of Glu. Longer bars indicate iontophoretic application of AII.


20mV
40s
AII 40nA
AII 40nA
ilu 30nA
Ca^{2+} 0.2mM/Mg^{2+} 10mM

3. 9. Effects of AII on Glu Responses in the Presence of a Glu Uptake Blocker

There is also a possibility that the depressant action of AII on Glu response is due to the activation of the Glu uptake system of glia cells. Our experimental results did not support this since the depressant action of AII was unaffected when a Glu transport blocker (2,4-PDC) was added to the perfusion solution (Fig. 40, n=4). In addition, AII was also found, in 5 out of 7 cells, to depress the excitation induced by iontophoretically applied ibotenate which does not appear to be a substrate for the Glu transport system (Fig. 41)(Roberts and Watkins,1975; Drejer et al., 1982).

3. 10. Effects of AII on Glu Agonists

In order to determine whether a specific subtype of Glu receptor was affected by AII, the effects of AII on Glu agonists were tested by iontophoresis. AII was found to have mild to strong depressant actions on these Glu agonists. (Table 5). From the results of those experiments in which Glu (activating both NMDA and non-NMDA receptors), NMDA (acting on NMDA receptors) and AMPA (acting on AMPA (non-NMDA) receptors) were tested on the same cells, it was found that the depressant actions of AII on Glu agonist-induced responses appeared to be less potent than the actions of AII on Glu-induced responses. These results are summarized in Table 6.

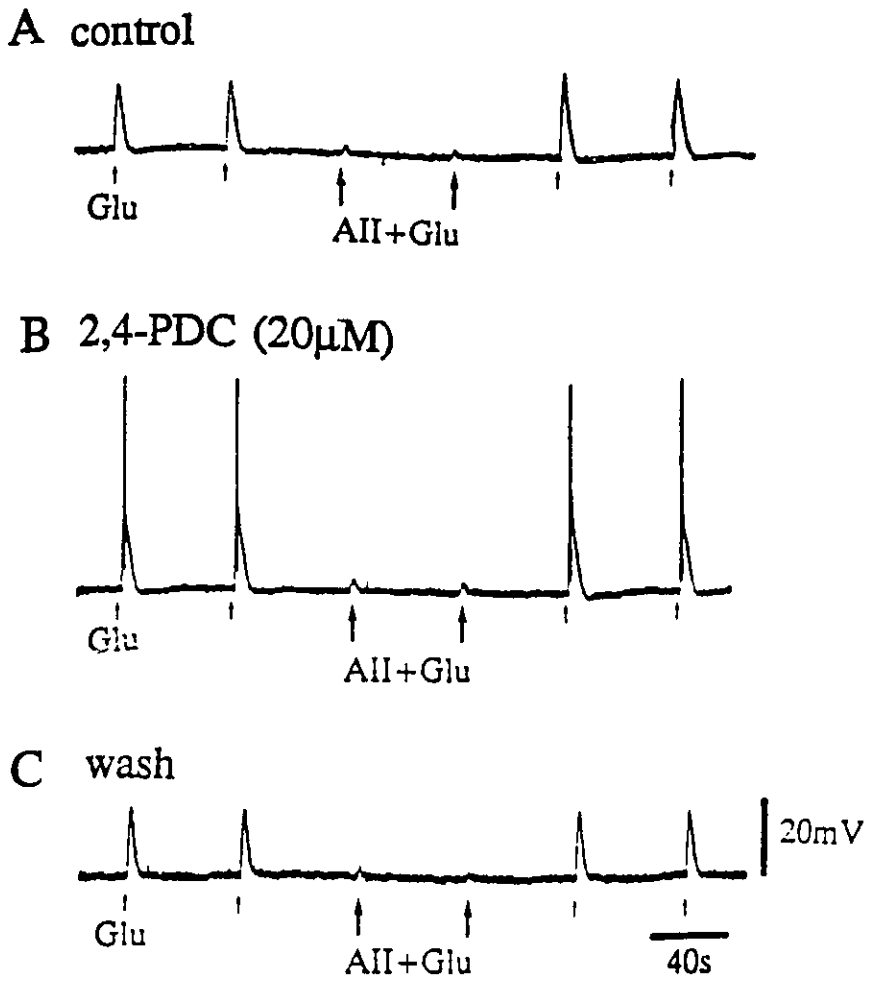


Fig. 40. One possible mechanism of AII action could be the stimulation of a glutamate uptake system. This was tested using the glutamate uptake blocker 2,4-PDC. **A.** Glu-induced depolarizations were almost completely blocked by AII. **B.** Bath application of 2,4-PDC (a Glu uptake blocker) enhanced Glu responses, but the depressant action of AII on Glu response was almost unaffected. **C.** when 2,4-PDC was washed out, Glu response returned to control and the action of AII on Glu was about the same as control. Small arrows indicate pressure ejection of Glu (20mM, 12psi, 2ms, 1 pulse), big arrows indicate pressure ejection of Glu and AII (0.1mM, 12psi, 2ms, 1 pulse) through separate micropipettes at the same time.

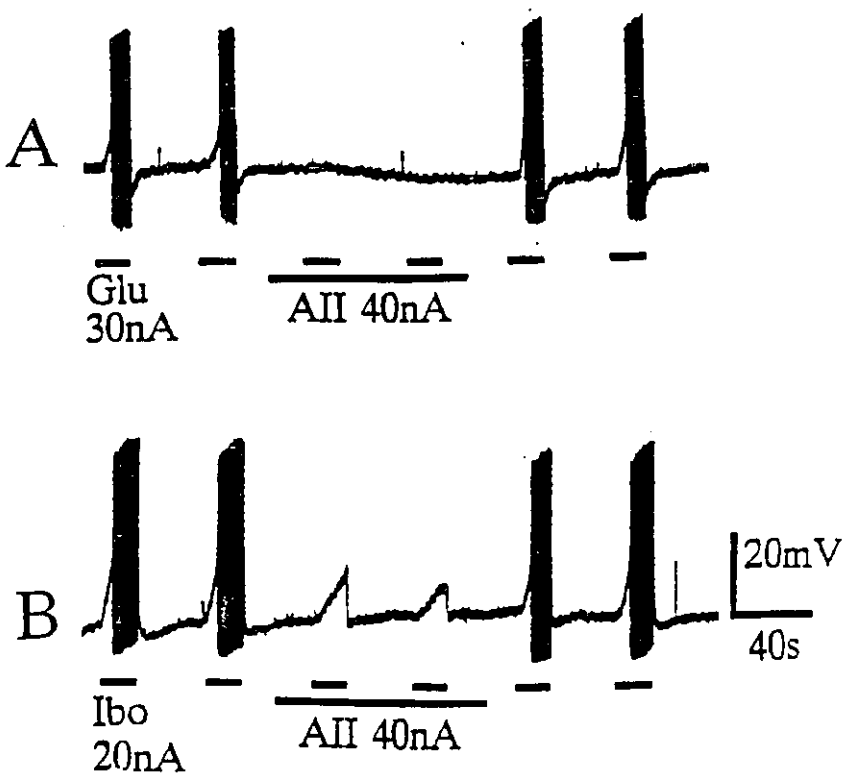


Fig. 41. Effect of AII on ibotenate (Ibo)-induced depolarizations and resultant excitations. Both traces (A and B) are intracellular recordings from an LC neuron. Iontophoretic applications are as indicated in Fig.11. AII (40nA) completely depressed Glu-induced depolarizations and spike activation (A). At the same dosage (40nA) AII strongly depressed Ibo-induced depolarizations and resultant excitations(B).

TABLE 5

Effects of Iontophoretic Applied AII on Glu Agonists

Cells	Glu	NMDA	AMPA	QA	KA	Dom
tested	25	23	18	3	4	5
depressed*	22	12	9	1	1	4

* at least 30% reduction

TABLE 6

Effects of iontophoretically Applied AII on Glu Agonists in the Same Cells

Tests	N	depression				--
		mild	moder.	strong	total	
AII→Glu	15	2	4	8	14	1
AII→NMDA	15	2	4	4	10	5
AII→AMPA	15	4	3	2	9	6

N: number of cells tested; moder.: moderate; --: no effect;

The pattern of depressant actions of AII on these selective agonists varied in different neurons. Thus AII did not strongly depress the responses of all three Glu agonists in the same cell when tested separately, even when Glu responses were almost completely blocked by AII in that neuron. When applied by pressure, Sar^1-AII (0.1 mM) was found to have depressant actions on both NMDA- (n=9) and AMPA-induced (n=5) responses in

all cells tested. In 3 cells in which Glu-induced responses were almost completely blocked by Sar^1-AII applied by pressure, NMDA- and AMPA-induced responses were also almost completely blocked by pressure applied Sar^1-AII in 2 cells. One of them is shown in Fig. 42. Sar^1-AII was found to have stronger depressant action on AMPA-induced responses than on NMDA-induced responses.

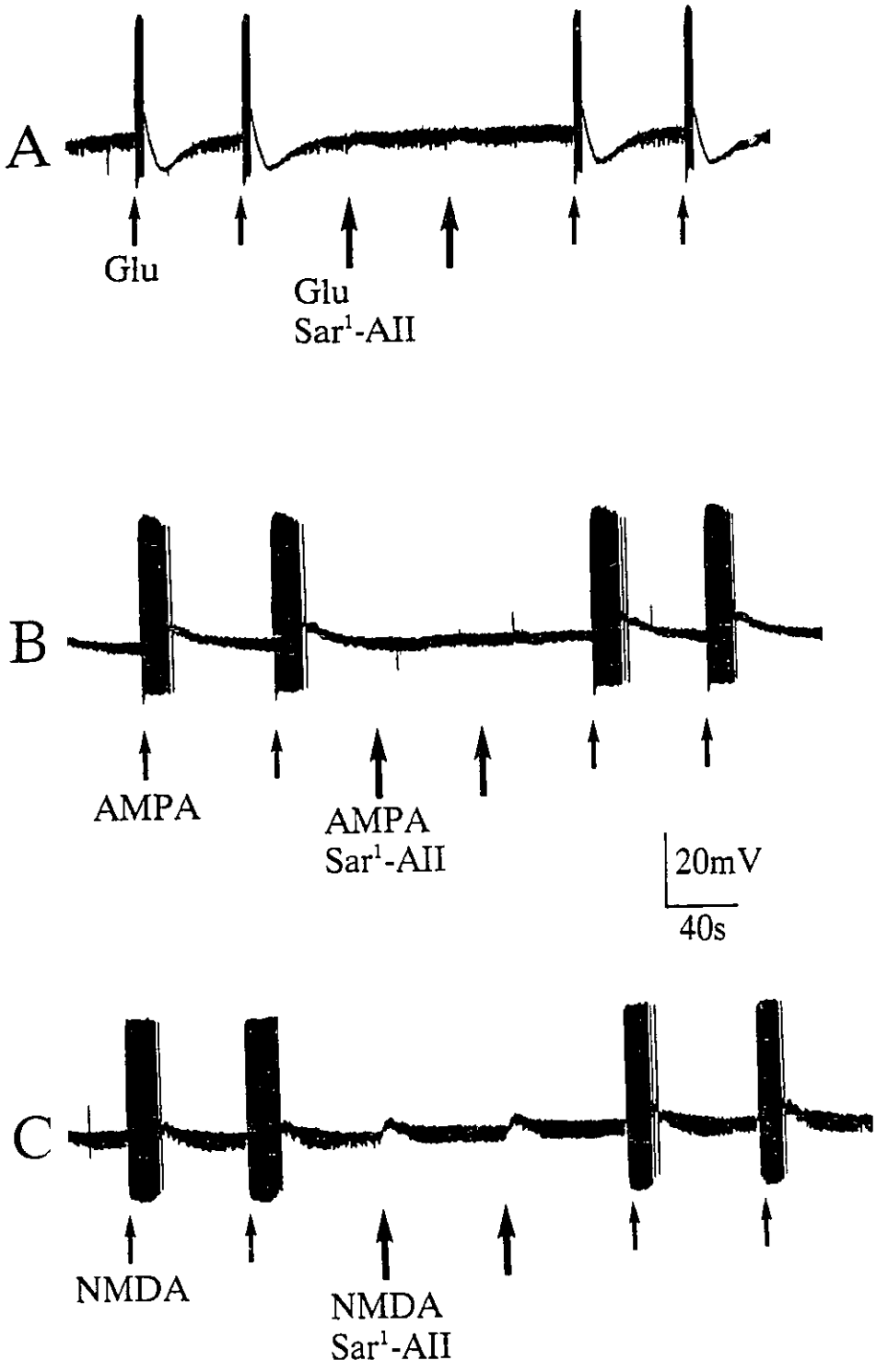
We also tested the effects of Glu receptor antagonists on Glu responses in those LC neurons in which the Glu responses were completely depressed by AII. In 4 cells in which both APV and CNQX were tested, APV (an NMDA receptor blocker) was found to reduce Glu response in one LC neuron; while CNQX (a non-NMDA receptor antagonist) attenuated Glu responses in 3 LC neurons.

3. 11. Effects of AII on Excitatory Postsynaptic Potentials (EPSPs)

3. 11. 1. Properties of Electrically Evoked EPSPs

In horizontal slices, but not transverse slices, single pulse (300μs in duration) applied to a stimulating electrode placed 300 - 800 μm rostral to the recording electrode induced depolarizing synaptic potentials (EPSPs) in all neurons tested. The latency from the beginning of stimulus artifact to the onset of the EPSPs was, depending on the distance of placement of the stimulating electrode from the recording electrode, in a range of 2.0 - 4.2ms, with the average of 2.8 ± 0.7ms (mean ± S.D., n=15). The EPSP was graded in amplitude according to the intensity of stimulation (Fig.43). When the intensity of the

Fig. 42. An example of the effects of Sar^1-AII on Glu-induced and Glu agonist-induced depolarizations and resultant excitations. All results were obtained from the same cell. Small arrows in trace **A**, **B** and **C** indicate pressure applications of Glu, AMPA and NMDA respectively (18 Psi, 2ms, 1 pulse), Bigger arrows represent simultaneous pressure applications of Sar^1-AII with Glu (**A**), AMPA (**B**) and NMDA (**C**). Sar^1-AII completely depressed Glu-induced responses (**A**) and AMPA-induced responses (**B**), and had strong depressant action on NMDA-induced responses (**C**).


A
Glu
Glu
Sar¹-AII
B
AMPA
AMPA
Sar¹-AII
20mV
40s
C
NMDA
NMDA
Sar¹-AII

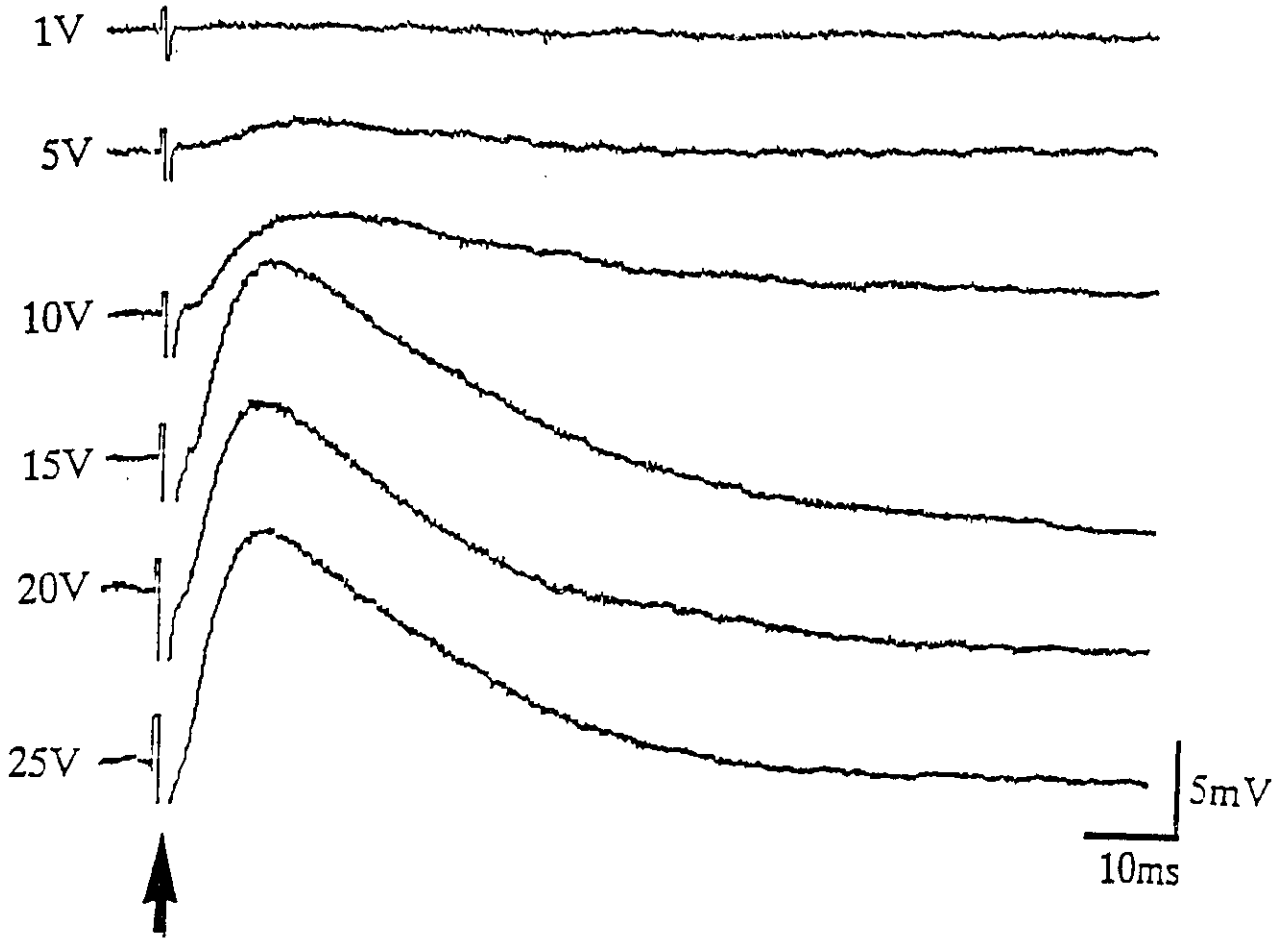


Fig. 43. EPSPs in a LC neuron evoked by a single stimulus. The EPSPs was graded in amplitude according to the strength of stimulation (indicated). The arrow at the bottom indicates the time when a single stimulus pulse was applied.

stimulating pulse was strong enough, this EPSP reached threshold for action potential generation. The time to peak amplitude (measured from stimulating artifact to the peak point of the EPSP) of EPSPs was 10.3 ± 2.4 ms (n=22). The time of 50% decay was 29.2 ± 1.1 ms (n=22). The amplitude of the EPSP increased as the membrane was hyperpolarized (Fig. 44). The slow component was more evident when membrane potential was held at -75 mV than -105 mV. The EPSP was almost completely blocked when TTX was added to the perfusate (n=2), or by switching the perfusate to a modified low Ca^{2+} / high Mg^{2+} (0.2/10mM) ACSF (n=4)(Fig. 45).

3. 11. 2. Effects of Glu Receptor Antagonists on EPSPs

The EPSPs generated by electrical stimulation were reversibly blocked by addition of Glu receptor antagonists to the perfusing media. Superfusion of KYN (500μM), a non-competitive Glu receptor antagonist, blocked the EPSPs in all cells tested (n=8). KYN appears to block the amplitudes of EPSPs, shorten the duration of the EPSP and reduce the time of 50% decay (Table 7). The control amplitude of averaged EPSPs in the experiments in which KYN was tested was 7.2 ± 1.2 mV. This amplitude was reduced to 2.5 ± 1.0 mV when KYN was added to the bath media, producing an inhibition with 65% reduction in the amplitude of EPSPs in comparing with control (Fig. 46A., Table 7). These data indicate that Glu receptor(s) were involved in the mediation of the EPSPs in LC neurons.

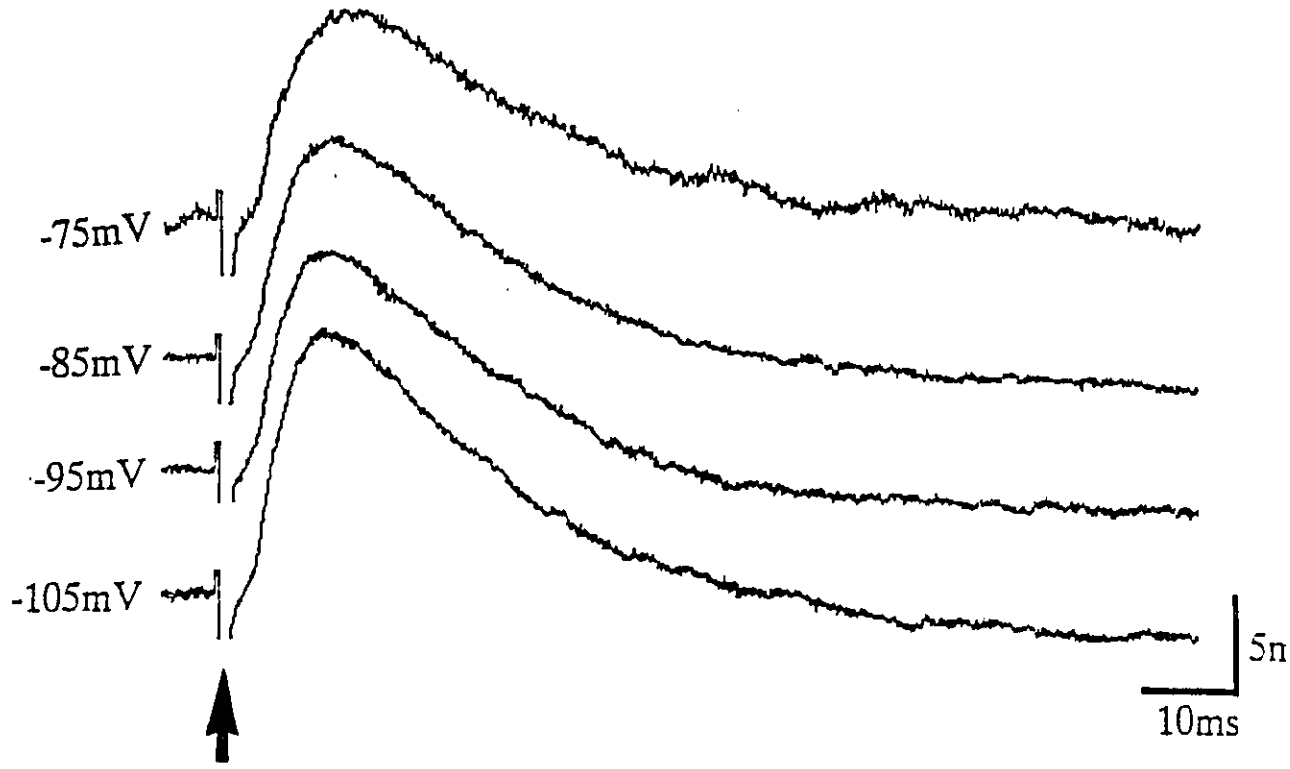


Fig. 44. EPSPs evoked by electrical stimulations. These recordings were obtained from the same cell as that in Fig. 43. The black arrow at bottom left indicates the artifacts of stimuli. The amplitudes of the EPSPs increased and membrane time constants(37ms at -75mv, 30.7ms at -105mv) decreased when membrane potential was hyperpolarized as indicated by the numbers on the left in each trace.

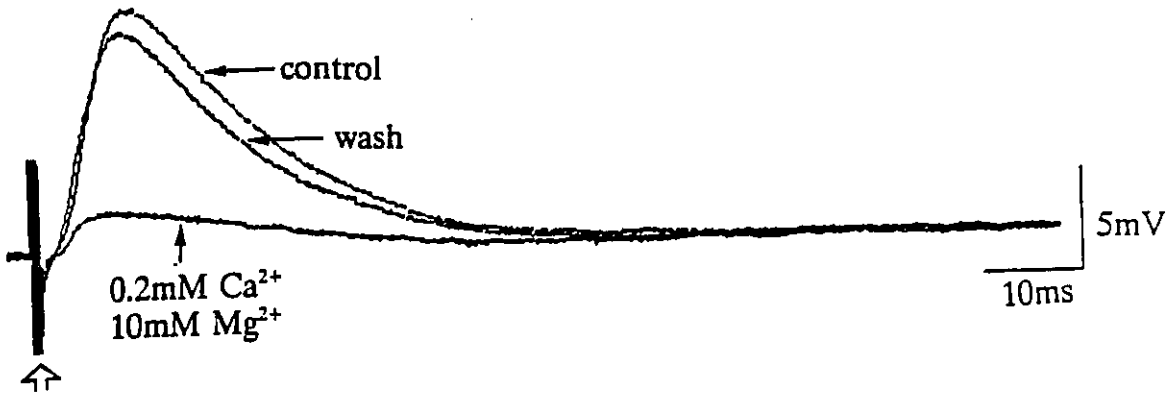


Fig. 45. Excitatory postsynaptic potential (EPSP) recorded from another LC neuron. Each EPSP was the average of 16 consecutive episodes evoked by electrical stimulation. The EPSP was almost completely blocked by perfusing the slice with a low Ca^{2+} and high Mg^{2+} solution.

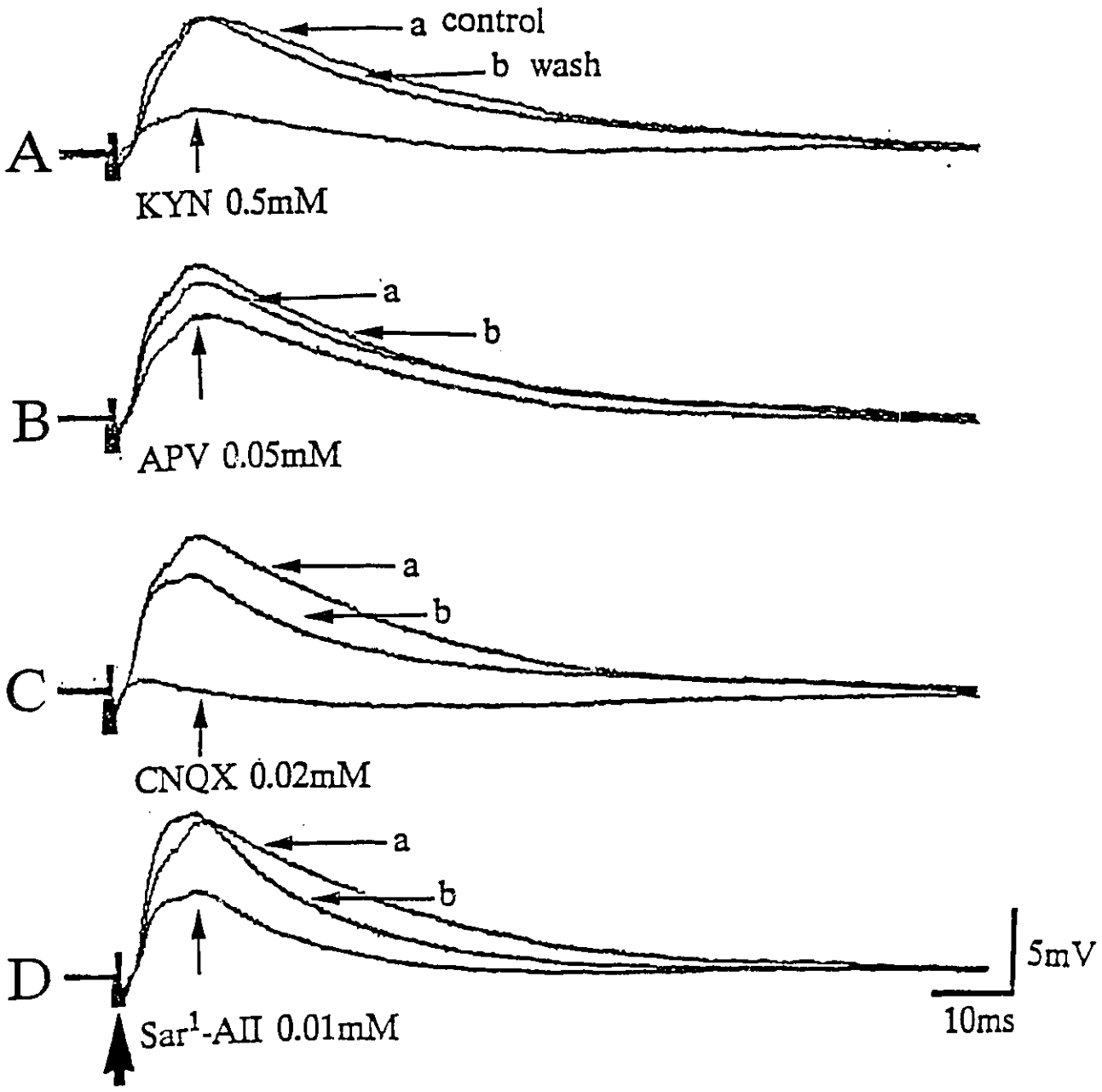


Fig. 46. The depressant action of Sar[1]-AII on EPSPs. A. EPSP evoked by electrical stimulation was antagonized by KYN. B. APV reduced the EPSP amplitude. C. CNQX abolished the EPSP. Note the effect of CNQX was even stronger than that of KYN. D. Sar[1]-AII depressed the amplitude of the EPSP (n=7). All recordings were obtained from the same cell.

TABLE 7

EFFECTS OF KYN ON EPSPs

	Peak amplitude (n) (mV)	Time reach peak amplit. (n) (ms)	Time of 50% decay (n) (ms)
Control	7.2 ± 1.2 (8)	9.3 ± 1.3 (8)	27.0 ± 9.3 (8)
Kyn	2.5 ± 1.0 (8)**	8.2 ± 1.0 (8)	18.8 ± 3.9 (7)*
Wash	6.6 ± 1.5 (8)	9.8 ± 0.9 (8)	26.3 ± 6.1 (8)

*: $p<0.05$, **: $p<0.01$

To further clarify which subtype of Glu receptors was responsible for the generation of the EPSPs, the NMDA receptor blocker, APV, and non-NMDA receptor blocker, CNQX, were tested. We found that both NMDA and non-NMDA receptor blockers had inhibitory actions on the EPSPs. CNQX (2-10μM) was found to have strong antagonizing action on EPSPs, with an average of 91.5% reduction in the amplitude of the EPSPs (Fig. 46C, n=8, Table 8) in addition to its significant actions on the duration, the time to reach peak amplitude and the time constants. In contrast, APV (50μM) had a milder antagonizing effect on the EPSP, in the presence of normal concentration of Mg^{2+}, with an average of 33.3% reduction in amplitude of the EPSPs in comparison to control (Fig. 46B, n=7, Table 9). APV appears to have little effect on other parameters (Table 9).

TABLE 8

EFFECTS OF CNQX ON EPSPs

	Peak amplitude (n) (mV)	Time reach peak amplit. (n) (ms)	Time of 50% decay (n) (ms)
Control	7.8 ± 1.7 (8)	9.4 ± 1.3 (8)	28.6 ± 8.6 (7)
CNQX	0.7 ± 0.8 (8)*	3.1 ± 3.7 (8)*	4.2 ± 8.2 (7)*
Wash	5.6 ± 1.0 (8)	8.7 ± 2.9 (8)	32.9 ± 15.6 (7)

*: $p<0.01$

TABLE 9

EFFECTS OF APV ON EPSPs

	Peak amplitude (n) (mV)	Time reach peak amplit. (n) (ms)	Time of 50% decay (n) (ms)
Control	7.1 ± 1.5 (7)	8.9 ± 1.2 (7)	28.0 ± 8.7 (7)
APV	4.8 ± 1.6 (7)*	8.6 ± 1.3 (7)	25.1 ± 3.7 (7)
Wash	7.5 ± 1.9 (7)	9.6 ± 1.5 (6)	25.1 ± 3.7 (6)

*: $p<0.01$

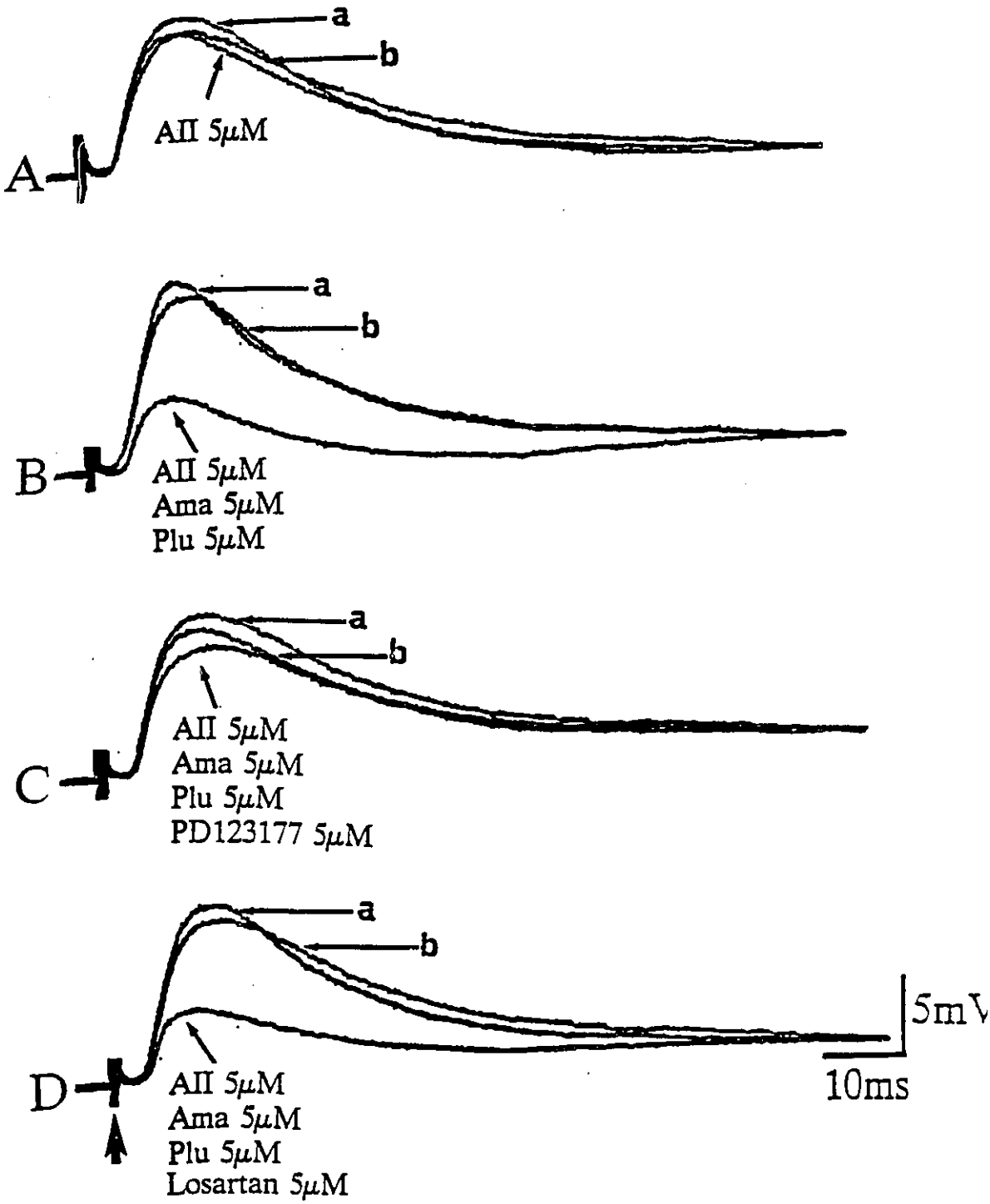
3. 11. 3. Effects of AII on EPSPs

Bath application of AII (10μM, Bachem) without peptidase inhibitor(s) was found to have no apparent action in 3 out of 7 or mild attenuation in 4 out of 7 on the amplitudes of the EPSPs. In contrast, when co-applied with both Ama. (5μM) and

Plu. (5μM), AII (5μM, Bachem) had moderate to strong depressant actions on EPSPs evoked by electrical stimulation in the 4 cells tested (Fig. 47A, 47B, n=4). These results indicate once again that the ineffectiveness of bath applied AII is associated with the peptidase activity. The average depression of the amplitudes of the EPSPs was 63.8 ± 4.8% (± S.D.) in comparison with control. Also, bath application of Sar^1-AII (10μM, Bachem) had similar depressant actions on the EPSPs in all the cell tested (Fig. 46D, n=15). Among these cells, the degree of depression was between 30 - 50 % in 9 cells in comparison with the amplitude of control. In another 6 cells, the degree of depression was found to be greater than 50 per cent, but less than 80 percent. Sar^1-AII appears to depress the amplitude of the EPSPs (Fig. 48A) without apparent action on the time of 50% decay (Fig. 48B) and the rising and the falling time constants (Table 10). Since AII and Sar^1-AII are supplied with trifluoroacetic acid, a control of bath applied trifluoroacetic acid (10μM) was carried out in three cells, and trifluoroacetic acid was found to have no apparent action on EPSPs (data not shown). We also tested the actions of AII on EPSPs in the presence of yohimbine (1μM, n=2) or bicuculline (20μM, n=2) in the perfusate. Neither yohimbine nor bicuculline appears to have influence on the action of AII on the EPSPs. Also, neither of them has effects on the amplitudes of EPSPs.

Pressure ejection of Sar^1-AII (10 μM) into the perfusing solution close to the recording site caused the same kind of

Fig. 47. The action of AII on EPSPs was antagonized by non-peptide AII receptor antagonist. Black arrow at bottom indicates artifacts of electrical stimuli. Application of AII (5μM) alone had no effect on EPSP (**A**). However, when co-applied with peptidase inhibitors, AII strongly depressed EPSP (**B**). Addition of AT_1 AII receptor antagonist losartan to the perfusate, The depressant action of AII on EPSP seems to be unaltered (**D**). When AT_2 AII receptor antagonist PD123177 was added to bath media, the depressant action of AII on EPSP was blocked by PD123177 (**C**), indicating AT_2 subtype of AII receptor was involved in the mediation of the depressant action of AII on EPSP.



a: control **b**: wash

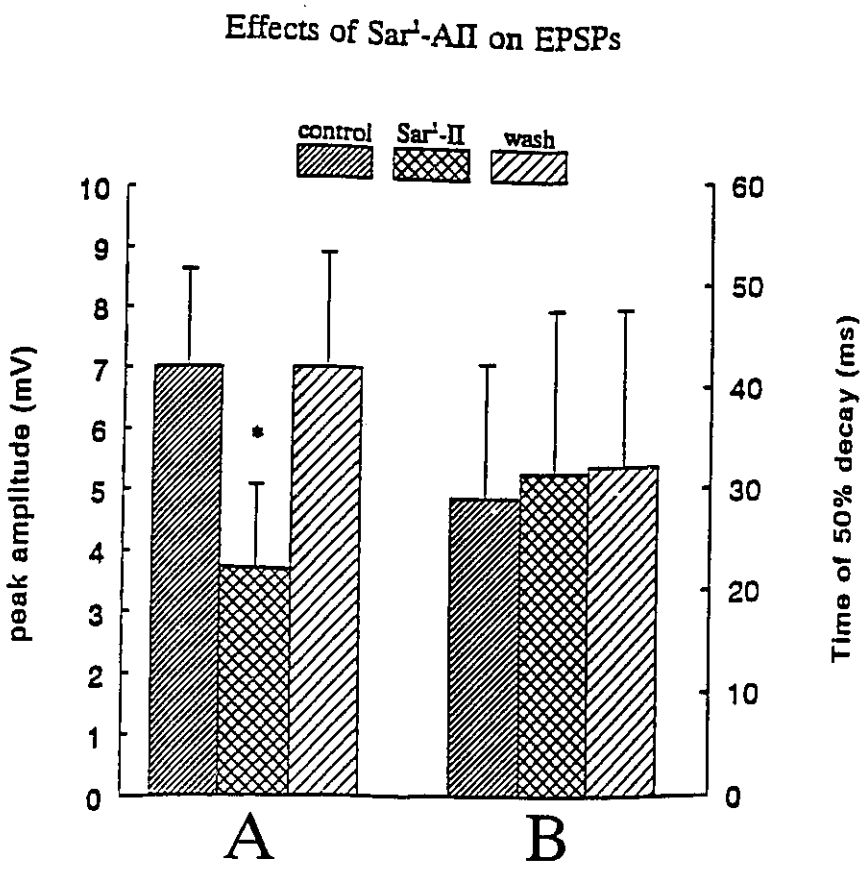


Fig. 48. Effects of bath applied Sar¹-AII (10μM) on peak amplitude of EPSPs and the time of 50% decay in amplitude of EPSPs (n=7). **A**: effect of Sar¹-AII on peak amplitude; **B**: Effect of Sar¹-AII on the time of 50% decay in amplitude. Note that Sar¹-AII significant depressed peak amplitude of EPSPs and had no significant effect on the time of 50% decay in amplitude. Error bars represent standard deviation.

depression of EPSPs as that applied by bath, with an average of about 65% reduction in the amplitudes of the EPSPs. Application of ACSF from a separate barrel as control had no apparent action on EPSPs (Fig. 49, n=5).

TABLE 10 EFFECTS OF AII ON EPSPS

	Peak amplitude (n) (mV)	Time reach peak amplit.(n) (ms)	Time of 50% decay (n) (ms)
Control	7.0 ± 1.6 (22)	10.3 ± 2.4(22)	29.2 ± 13.3(22)
AII	3.8 ± 1.4 (22)*	10.6 ± 2.2(22)	31.7 ± 16.1(22)
Wash	7.0 ± 1.9 (22)	9.9 ± 2.5(22)	32.3 ± 15.5(20)

*: $p<0.05$

3. 11. 4. Actions of AII Receptor Antagonists on AII-mediated Depression of EPSPs

Like the actions of AII on Glu-induced responses, the action of AII on the EPSPs evoked by electrical stimulation can also be antagonized by AII receptor antagonists. Fig. 50 shows the effects of bath applied non-peptide antagonists on pressure applied Sar^1-AII. Pressure applied Sar^1-AII strongly depressed the amplitude of the EPSP. The depressant action of Sar^1-AII was consistently antagonized by the AT_2-selective antagonist PD123177 in a concentration dependent manner (Fig. 50B, 50C, 50D and 50F) in all cells tested (n=6). In contrast, the AT_1-selective antagonist losartan was found to have antagonizing action only in 1 out of 6 cells tested, in the other 5 cells, losartan had small or no effect in blocking AII mediated

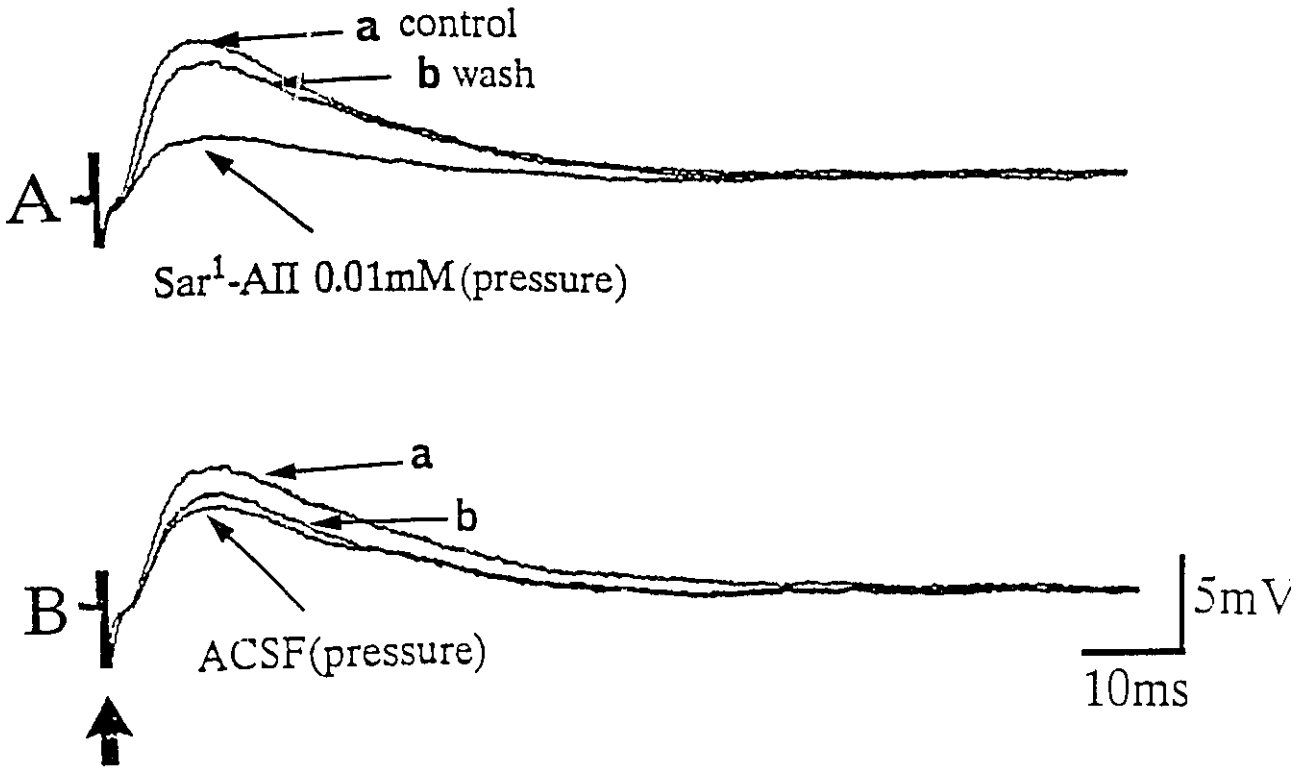
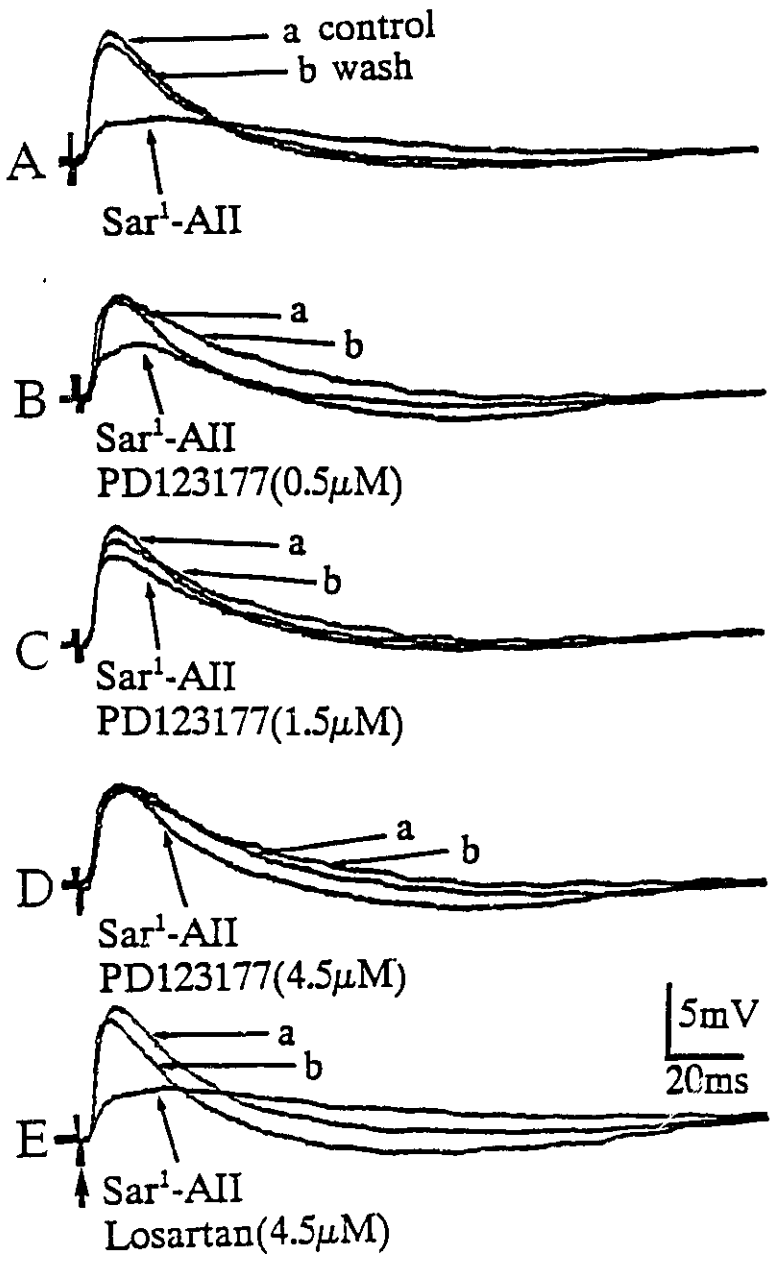
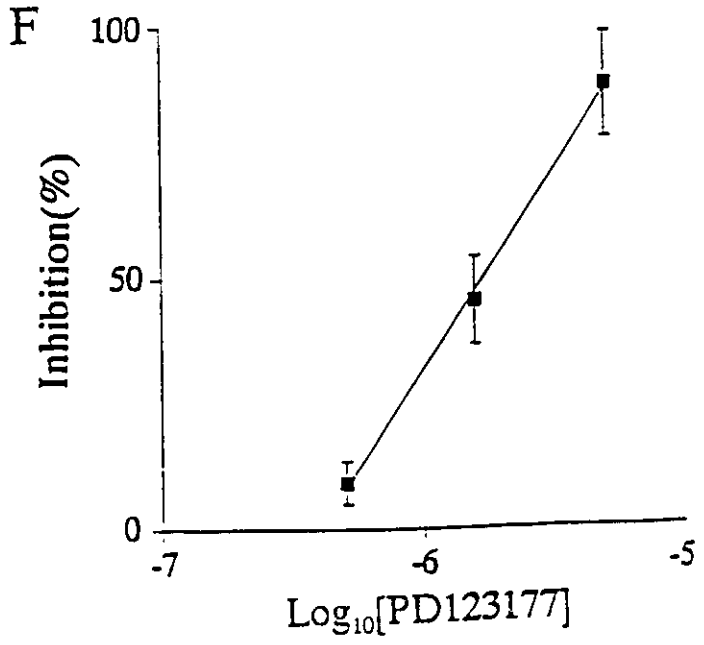


Fig. 49. EPSPs evoked by electrical stimulation can also be attenuated by pressure ejection of Sar^1-AII (**A**). Pressure ejection of ACSF as control had no effect (**B**). Big black arrow at bottom indicates the time when a single stimulus was applied.

Fig. 50. The action of AII on EPSPs was antagonized by non-peptide AII receptor antagonist. Black arrow at left bottom indicates artifacts of electrical stimuli. Pressure ejection of Sar^1-AII (100μM, 12Psi, 2ms, 10 pulses at 1 Hz) strongly depressed the amplitude of EPSP (**A**). Bath application of non-peptide AII receptor antagonist PD123177 (AT_2 selective) at different concentrations antagonized the depressant action of AII on Glu responses at different extent (**B**, **C** and **D**; n=6). Bath application of another non-peptide AII receptor antagonist Losartan (AT_1 selective) had little effect on the action of AII (**E**). All traces of EPSPs are the average of 10 - 15 consecutive episodes and recorded at membrane potential -66 mV.

F. Concentration-response curve of the action of PD123177 on antagonizing AII action. The estimated IC_{50} for PD123177 is about 1.5μM.


a control
b wash
A
Sar¹-AII
a
b
B
Sar¹-AII
PD123177(0.5μM)
a
b
C
Sar¹-AII
PD123177(1.5μM)
a
b
D
Sar¹-AII
PD123177(4.5μM)
5mV
20ms
a
b
E
Sar¹-AII
Losartan(4.5μM)


F
100
50
0
Inhibition(%)
-7
-6
-5
Log10[PD123177]

depression of the EPSPs (Fig. 50E.), even at a high dosage of 10μM. Similar results were obtained in testing bath applied antagonists on bath applied AII with peptidase inhibitors (Fig. 47C, 47D). These data demonstrated that the depressant action of AII on EPSPs in LC neurons was mainly mediated through AT_2 subtype AII receptor.

The results of the effects of AII receptor antagonists in antagonizing the actions of AII on EPSPs are in full agreement with those results that AII-mediated depressions of Glu-induced responses were antagonized by an AT_2-selective antagonist PD123177, but not the AT_1-selective antagonist losartan, indicating that AII depressions of both Glu-induced responses and the EPSPs were mediated by AT_2 subtype AII receptors. In addition, we tested the effect of saralasin in one LC neuron and partial antagonism of AII action on the EPSP was observed.

3. 11. 5. The Effects of NH_4^+ on EPSPs

When applied by bath at a concentration of 0.5mM, NH_4Cl attenuated the the amplitudes of the EPSPs by 30 - 50% without apparent actions on the time reaching peak amplitude and the time of 50% decay in the amplitude of the EPSPs in comparing with the corresponding controls (Table 11). Among 5 LC neurons tested, NH_4Cl was found to attenuate the EPSPs in 4 cells. The EPSP in another one cell showed no change during application of NH_4Cl. To further lower the concentration to 0.25mM, a 30% decrease in amplitudes of the EPSPs was found in 2/5 cells. The

effects of pressure applied NH_4Cl (10 mM, 0.25Hz, 10-16 pulses) were tested on 3 LC neurons. A moderate reduction of amplitude of EPSP was found on one cell. NH_4Cl had no apparent action on the amplitudes of the EPSPs of the other two cells.

TABLE 11

EFFECTS OF NH_4Cl ON EPSPs

	Peak amplitude (mV)	Time reach peak amplit. (ms)	Time of 50% decay (ms)
Control	6.5 ± 1.4	8.9 ± 1.9	23.6 ± 7.5
NH_4Cl	4.5 ± 1.8*	9.8 ± 2.5	23.1 ± 6.5
Wash	6.3 ± 1.8	9.7 ± 2.5	24.1 ± 9.4

* $p<0.05$

3. 12. AII Attenuates Glu-induced Current

Under single electrode voltage clamp, pressure ejection of Glu elicits an inward current under the presence of TTX (0.5μM) in the perfusate. The inward current increased when the cell was hyperpolarized and rectification was observed when membrane potential was held at levels negative to the resting potential (Fig. 51). Bath application of Sar^1-AII attenuated the current induced by pressure applied Glu (n=4; Fig. 52).

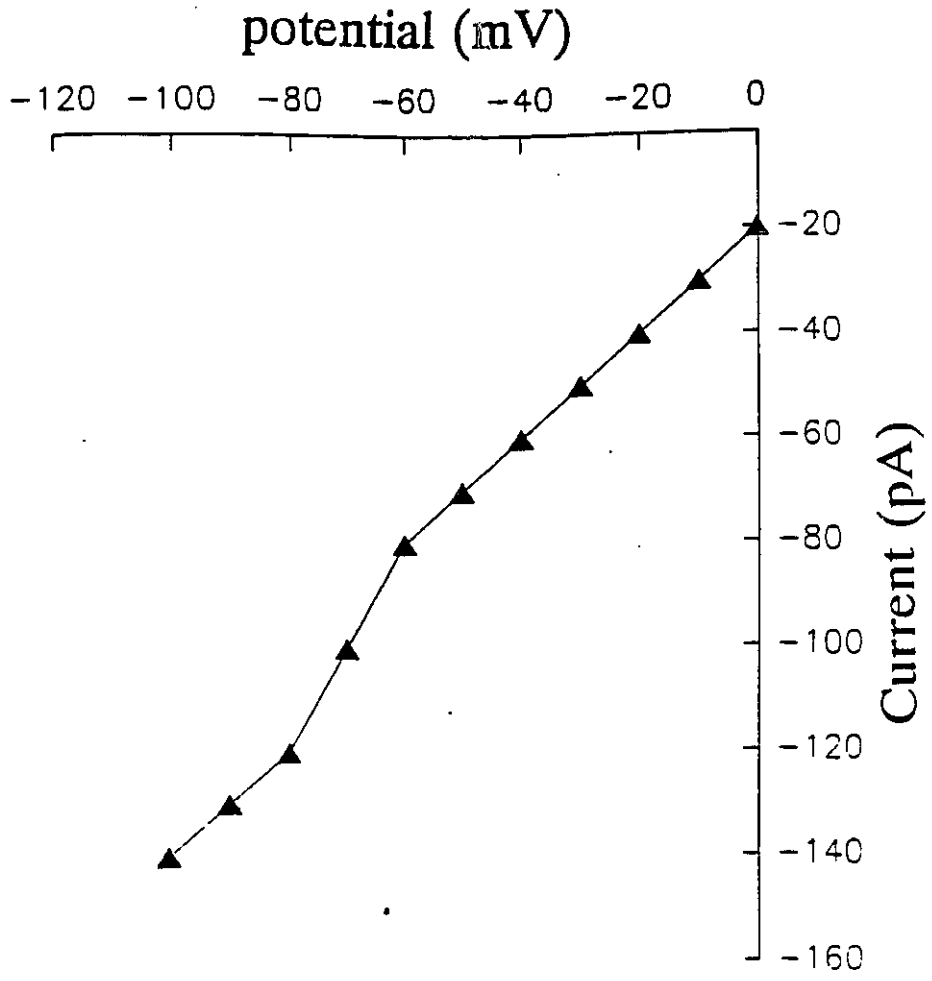


Fig. 51. Glu-induced inward current in an LC neuron. The ordinate represents the current induced by pressure ejection of Glu. The abscissa indicates membrane potential holding at different levels. This result was obtained in the presence of TTX (1μM) in the perfusate.

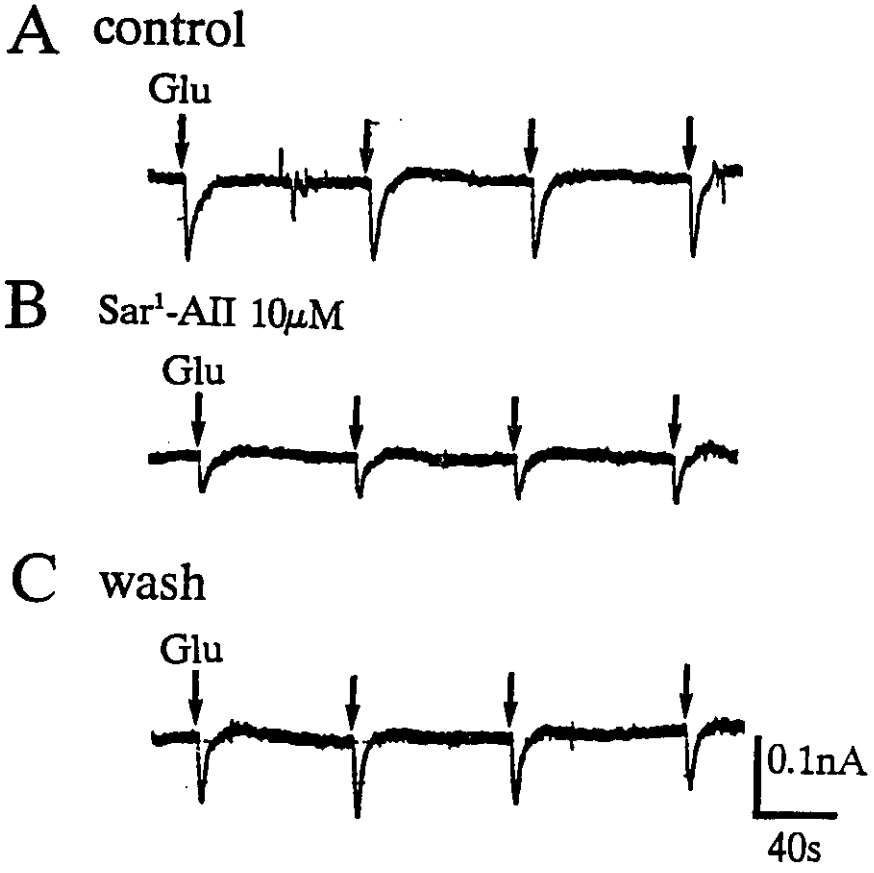


Fig. 52. Sar¹-AII attenuated inward current induced by pressure applied Glu under single electrode voltage clamp. Membrane potential was hold at -60mV. Downward arrows indicate pressure ejection of Glu (15psi, 2ms, 1 pulse). Pressure ejection of Glu induced an inward current (downward deflections following downward arrows in all traces). A. is a control. When Sar¹-AII was added to the perfusate, Glu-induced inward current was attenuated by approximate 60% (B). After 15 minute wash, the amplitude of Glu-induced current restored to its control level (C).

4. DISCUSSION

4. 1. Electrophysiological Properties of LC Neurons

It has been demonstrated that the spontaneous activity of rat LC neurons varies during development (Nakamura and Sakaguchi, 1990). Most LC neurons in fetal and neonatal rats were found to be quiescent. As age increased, the spontaneous discharges of these LC neurons change from a pattern of sporadic firing with long-lasting silent periods to periodic discharge (Nakamura et al., 1987; Sakaguchi and Nakamura, 1987). The animals used in our experiments were at the age of 21 - 35 postnatal days. The reasons for choosing animals of these ages are: (1) these animals relatively young and may be considered more resistant to anoxia, and (2) in rats older than postnatal day 20, LC neurons have a tonic pattern of firing like adult animals (Nakamura and Sakaguchi, 1990). We found that the spontaneous activity of the LC neurons recorded from brain slice preparations taken from these relatively young animals showed properties similar to those reported in adult animals (Henderson et al., 1982; Williams et al., 1984; Williams and Marshall, 1987; Nakamura and Sakaguchi, 1990), with firing rate of 0.5 - 5 Hz in the absence of any stimuli. The LC neurons recorded in our studies exhibited a tonic firing pattern, with a large after-hyperpolarization following each individual spike. This after-hyperpolarization merged into a slow depolarization in

younger animals that brought the membrane potential to threshold. The amplitudes of these slow depolarizations are about 5 - 10 mV. The spontaneous activities of the LC neurons were abolished by TTX, indicating the involvement of voltage dependent Na^+ conductance in the generation of spontaneous discharges.

In addition to changes during development, the firing pattern of LC neuron appears to be also dependent on the preparation. In tissue culture, LC neurons have been found to have synchronous bursting activity (Marshall and Finlayson, 1988; Finlayson and Marshall, 1988). This bursting activity was also found to be present in LC neurons recorded in unanesthetized animals in vivo (Aston-Jones and Bloom, 1981a,b; Foote et al., 1980). Such a synchronous bursting was not seen in our brain slice preparation experiments. This difference is perhaps not surprising since the synchronous bursting of LC neurons has been suggested to be the result of a common excitatory input to the LC from its adjacent regions in explant tissue culture (Finlayson and Marshall, 1988; Marshall and Finlayson, 1988). This excitatory input may be cut away, so that excitatory synaptic inputs to the LC from other brain regions were removed in our slice preparations.

In some brain slices taken from young rats (about 50 gms in body weight), or from some older animals with body weights about 80 - 100 gms, spontaneous depolarizing events were seen. These were slow rhythmic depolarizations and were superimposed by

spontaneous action potentials as reported by Williams and Marshall (1987). These depolarizations were insensitive to TTX, but can be blocked by superfusion of a solution containing a low Ca^{2+}/high Mg^{2+}(0.2/10mM). These depolarizations were suggested to be generated by the inward movement of calcium ions, probably in the dendritic regions of the neuron (Williams and Marshall, 1987). Also, spontaneous depolarizations can be seen in horizontal slices taken from some older animals.

4. 2. Effects of AII on CNS Neurons

Electrophysiological studies have shown that AII exerts excitatory actions on magnocellular hypothalamic neurons (Nicoll and Baker, 1971; Akaishi et al., 1981; Harding and Felix, 1987a; Felix et al, 1991), hippocampal neurons (Haas et al., 1982), supraoptic neurons (Yang et al., 1992), preoptic neurons (Gornan and York, 1978), paraventricular neurons (Ambuhl et al., 1992a), inferior olivary neurons (Ambuhl et al., 1992b) as well as the neurons in the OVLT (Knowles and Phillips, 1980), SFO (Tanaka et al., 1986) and anteroventral region of the third ventricle (Hattori and Koizumi, 1990). Cortical neurons were reported to be mostly inhibited by AII (Nicoll and Baker, 1971, Anissimov et al., 1980). The actions of AII we observed on the LC neurons appear different from effects mentioned above. This may be due to different responsiveness of central neurons to AII in different regions. However, it may also be explained in part by

the absence of testing for interactions of AII with Glu in most other studies.

It is interesting that other researchers (Wayner et al., 1973.) have reported that AII has depressant actions on neuronal activity when Glu is used to induce excitatory responses in lateral hypothalamic neurons recorded extracellularly in adult rats.

Anissimov et al., (1980) tested the effects of AII on extracellularly recorded LC neurons in rabbits in vivo, and found that some LC neurons (~20%) were excited and some (~40%) were depressed by AII when applied iontophoretically. In contrast, we found that AII had no apparent action on resting activity of most LC neurons in brain slice preparation. These differences may be due to differences in species or preparation. It is also possible that the depressant actions of AII reported in some brain regions were caused by depression of tonic Glu-mediated excitatory influences by AII, or by the small hyperpolarizations we observed with AII application to some neurons.

4. 3. Modulatory Actions of AII on Glu-induced responses in LC Neurons

Recent physiological and pharmacological experiments indicate that there are numerous interactions between AII and other neurotransmitters in the CNS, e.g. catecholamines (Huang et al., 1987; Maclean et al., 1990; Qadri et al., 1991), 5-HT (Nahmod et al., 1978), opiates (Summy-Long et al., 1983),

substance P (Barnes KL, et al., 1991), ACh (Barnes JM, et al., 1990) and GABA (Georgiev et al., 1991). An important consideration for establishing a neuromodulatory role for a transmitter substance is the demonstration of selectivity of the action on the second transmitter (Marshall and Xiong, 1991). It has been found that EAAs are subject to modulation by other neurotransmitters, for example NA (Moises et al., 1979; Segal, 1982; Mori-Okamoto and Tatsuno, 1988) and 5-HT (Lee et al., 1986; Aston-Jones et al., 1991). These modulatory actions have been observed in different brain areas, such as cerebellum (Moises et al., 1979; Lee et al., 1986; Mori-Okamoto and Tatsuno, 1988,1989), hippocampus (Segal, 1982) and LC (Aston-Jones et al., 1991). Our results demonstrate that AII strongly depresses Glu-induced depolarization in LC neurons while it has no apparent action on their other properties. The depressant action of AII on Glu-induced responses is potent and concentration dependent, and we have observed complete blockade of Glu depolarization in many cells. This action of AII on Glu-induced responses in LC neurons was selective in the following ways: firstly, AII had no apparent action on spontaneous firing when applied by iontophoresis or by pressure ejection to most neurons. Secondly, AII had no effect on membrane potential, input resistance, or amplitude, duration and after-hyperpolarization of the action potential. Thirdly, AII had no effect on the depolarization and excitation caused by ACh, and fourthly, AII had little or no effect on Glu responses in most

non-LC brain stem neurons in regions where no AII receptors have been reported. Conversely, the effects of AII on LC have been demonstrated to be mediated by one specific sub-type of AII receptor. AII appears to exert its action on Glu-induced responses on the postsynaptic membrane, since the depressant action of AII remains during perfusion with a low calcium - high magnesium solution that has been demonstrated to effectively block post-synaptic potentials evoked by electrical stimulation in rat LC.

In addition to its action on exogenous Glu-induced responses, Sar^1-AII has been found to depress the EPSPs mediated by a Glu-like transmitter released from the pre-synaptic terminal as a consequence of electrical stimulation. These evoked EPSPs can be blocked by low calcium/high magnesium solution and attenuated by the Glu receptor antagonists kynurenic acid, APV and CNQX as observed by Cherubini et al. (1988). Sar^1-AII has been shown to depress the amplitude of the EPSPs by an average of 65% when applied by pressure ejection, without significant effects on duration as assessed by measurement of 50% decay or time constants. These data indicate that Sar^1-AII probably interacts mainly with non-NMDA receptors since at many synapses it has been found that under physiological conditions NMDA receptors contribute only a small and variable amount to the EPSPs (Ganong et al., 1983; Jahr and Jessell, 1985; Jahr and Yoshioka, 1986; Collingridge and Lester, 1989). The depressant action of Sar^1-AII was consistently

reduced in a concentration dependent manner by bath application of the AT_2-selective antagonist PD123177, with almost complete blockade at a concentration of 4.5 μM to 5.0 μM. This further suggests that the action of AII on Glu-induced responses is mediated through actions at AT_2 receptors. The available literature regarding the action of AII on Glu appears to be only the report by Wayner et al. (1973), who found that iontophoretic application of Glu had excitatory action on extracellularly recorded rat lateral hypothalamic neurons in vivo and that the Glu-induced excitatory responses were depressed by iontophoretically applied AII in a dose dependent manner. It is possible that the mechanisms underlying the depressant actions of AII on Glu-induced responses in rat lateral hypothalamus observed by Wayner et al. (1973) and in rat LC observed in our experiments are the same. However, the major AII receptor subtype in the LC is reportedly AT_2 receptors (Rowe et al., 1991; Song et al., 1992), while the major AII receptor subtype in the hypothalamus is AT_1 receptors (Rowe et al., 1991; Song et al., 1992). This suggests that AII can also modulate Glu-induced responses through activating AT_1 receptors, in addition to its interaction with AT_2 receptors. Further experiments are needed to confirm this suggestion.

We have gone to considerable lengths to eliminate other possible explanations for the observed effects. The depression of Glu responses by AII was demonstrated to be independent of the mode of application, since similar results were obtained

when AII was applied by iontophoresis, pressure ejection or by addition to the perfusion solution. It was not an artifact of ionophoretic current since current control experiments showed no effect on Glu responses. The action of AII seems to be also independent of voltage dependent changes due to membrane depolarization, since depolarization and spike activation induced by depolarizing current pulses were unaffected by AII. While a mild hyperpolarization was observed with AII application to some cells, the effect on Glu responses in these cells appears to be unrelated to the coincident membrane hyperpolarization since injection of hyperpolarizing current did not alter the Glu response except for some reduction in the number of spikes generated, presumably due to the greater difference between membrane potential and threshold for spike generation.

4. 4. Effects of AII on Glu-induced Responses Are Independent of Glu Uptake System

Unlike most non-amino-acid transmitters, whose lifetimes are limited by enzymatic degradation, the action of L-Glu is determined by clearing the synaptic cleft via high affinity uptake of the amino acid into the surrounding cells (Schousboe et al., 1983). While for other systems (e.g. GABA) it is known that the transmitter is taken up at high affinity into glia cells as well as in presynaptic nerve terminals, for Glu, the sites of uptake are not as well established. Some studies

suggest that Glu uptake is mainly into glia cells rather than neurons (Currie and Kelly, 1981; Mclennan, 1976). Indeed, the highest density of high affinity Glu transport sites have been found on astrocytes cultured from prefrontal cortex and Neostriatum, i.e., areas which are considered to be rich in glutaminergic terminals (Divac et al., 1977; Kim et al., 1977; Fonnum et al., 1981).

Much of the early work on the brain RAS focused on neuronal elements, but recent evidence suggests that AII receptors are present in glia cells in primary cultures derived from normotensive rat hypothalamus and brain stem (Raizada et al., 1987; Sumners et al., 1990) and in human astrocytoma cell line CRTG1, STTG1 and WITG2 (Tallant et al., 1991). AII has been reported to stimulate a dose-dependent hydrolysis of phosphatidylinositols in glia cells (Raizada et al., 1987, Sumners et al., 1990). It is conceivable that the depressant action of AII on Glu responses we have observed in LC neurons could be the result of AII binding to AII receptors located on glia cell membrane, activating these AII receptors and stimulating the glia cell Glu uptake system, so that the applied Glu was taken up into glia cells before reaching Glu receptors located on the postsynaptic membrane of the LC neurons. This possibility seems unlikely in view of our observation that the AII action remains during bath application of 2,4-PDC, a potent and selective competitive inhibitor of Glu transport, but does not appreciably bind to the NMDA, AMPA and KA receptors (Bridges

et al., 1991). The Glu response was enhanced by bath applied 2,4-PDC, indicating at least partial blockade of Glu uptake. Other evidence against this possibility was that AII also had depressant actions on the excitatory responses elicited by Ibo, KA, NMDA and AMPA, Glu receptor agonists which appear not to be taken up by glia cells (Balcar and Johnston, 1972; Roberts and Watkins, 1975; Stallcup et al., 1979; Drejer et al., 1982). It has been noticed that activation of the subtype(s) of Glu receptor has inhibitory action on glia cell uptake system (Barbour et al., 1989; Flott and Seifert, 1991). When Glu activates neuronal NMDA receptor, the resulting Ca^{2+} influx leads to activation of phospholipase A_2, and release of arachidonic acid into extracellular space (Dumuis et al., 1988). The arachidonic acid has been found to cause a prolonged depression of Glu uptake by glia cells in whole-cell patch clamp experiments (Barbour et al., 1989). Also, activation of QA receptor was found to reduce Glu uptake almost completely (Flott and Seifert, 1991). These modulatory actions of Glu receptor subtype activation on the glial cell uptake of Glu provide further evidence that the effects of AII on Glu-induced responses are probably not the result of activating glial cell uptake.

4. 5. The Action of AII on Glu-induced Responses is Independent of the Action of NA

In the peripheral nervous system, AII potentiates

noradrenergic neuroeffector transmission by facilitating the release of NA (Ziogas et al., 1985; Szabo et al., 1990). Blockade of re-uptake and increased biosynthesis may also contribute to the potentiation of noradrenergic transmission (Khairallah, 1972; Roth and Hughes, 1972). Conflicting results have been reported concerning the action of AII on noradrenergic transmission in the CNS. AII has been found to increase NA release in hypothalamus of rat (Qadri et al., 1991), rabbit (Garcia-Sevilla et al., 1979); in rat cerebral cortex (Huang et al., 1987), in paraventricular nucleus of conscious rats (Stadler et al., 1992) and in the primary cultures of rat brain (Sumners et al., 1983). However in other studies, AII did not facilitate NA release in rat cortex and hypothalamus (Taube et al., 1977). The contrary results have also been found in NA uptake studies. Sumners and Raizada (1986) reported that AII significantly increased NA uptake in neuronal cultures prepared from the hypothalamus and brain stem of normotensive rats, whereas Fernandez et al. (1990) found that AII decreased NA uptake in an in vitro hypothalamus and medulla oblongata slice preparation.

It has been demonstrated that α_2-adrenoceptor is the major subtype of noradrenergic receptors in the rat LC. The receptors mediating the hyperpolarizing responses of LC neurons to NA have been characterized as α_2-adrenoceptors (Finlayson and Marshall, 1988). Iontophoretically applied NA has been found to have hyperpolarizing actions on rat LC. This raises the question

whether or not the depressant action of AII on Glu-induced responses is due to stimulation of NA release from the collaterals of noradrenergic LC neurons or inhibition of NA uptake since we found that iontophoretically applied NA attenuated Glu-induced responses. However, our results show that addition of α_2-adrenoceptor antagonist yohimbine to the bath media did not change the action of AII on Glu-induced responses, indicating NA was not involved in the mediation of depressant action of AII on Glu-induced responses.

Huang et al. (1987) have reported that AII facilitates the potassium-evoked release of [^{3}H]-NA from slices of parietal cortex innervated by the noradrenergic fibers originating from the LC, but does not significantly alter the release of [^{3}H]-NA evoked in a similar manner from the LC slices. Their experimental results provide further evidence against the possibility that the depressant action of AII was dependent on the release of NA. Gordon et al (1985) have shown that selective lesioning of several noradrenergic pathways in the brain did not influence the central actions of AII, although non-selective ablation, by administration of 6-hydroxydopamine into the lateral ventricles, inhibited the central dipsogenic and cardiovascular actions of AII (Gordon et al., 1979).

It has been shown that AT_1 receptors are mainly located in brain regions (such as circumventricular organs and NTS) associated with cardiovascular control (Steckelings et al., 1992). Stadler et al. (1992) have found most recently that

intraventricular injections of pressor doses of AII (1 to 100 ng) led to significant increase of NA release in rat paraventricular nucleus. This AII-induced release of NA in vivo can be abolished by pretreatment with a non-peptide AT_1 receptor antagonist losartan, indicating the stimulation of AT_1 receptor produces NA release in the paraventricular nucleus. However, the major subtype of AII receptors in the LC is AT_2 receptor (Rowe et al., 1991; Tsutsumi and Saavedra, 1991a; Song et al., 1992). Its activation may not be associated with a function to stimulate NA release, since the activation of AT_1 and AT_2 receptors in rat skin slices has been found to have opposite effects on phosphatidylinositol hydrolysis: activation of AT_1 receptors increases inositol phosphate production, while activation of AT_2 receptors decreases inositol phosphate production (Gyurko et al., 1992). Whether or not activation of central AT_2 receptors has influence on NA release remains to be determined.

It has been reported that GABA inhibition of LC neurons in rat are primarily mediated via $GABA_A$ receptors (Ennis and Aston-Jones, 1989b). Electrically evoked Inhibitory postsynaptic potentials recorded in rat LC neurons in vitro were also mediated through $GABA_A$ receptors by increasing chloride conductance (Cherubini et al., 1988). We observed that the effect of AII on Glu-induced responses was unchanged when bicuculline was added to the perfusate, to block $GABA_A$ receptors. Therefore, the action of AII on Glu-induced responses

was not the result of the activation of GABAergic interneurons. Some studies have shown that activation of $GABA_B$ receptors had modulatory actions on Glu-mediated responses in other brain regions (Howe et al., 1987; Calabresi et al., 1992). However, the action of AII on Glu-induced responses should have been tested in the presence of $GABA_B$ receptor antagonist, to rule out the possible activation of $GABA_B$ receptors by AII though GABA mediated inhibition of LC neurons was not attributed to an action of activating $GABA_B$ receptors. However, in view of our experimental results that the depressant action of AII on Glu-induced responses retained when slices were perfused with a modified ACSF, containing low Ca^{2+} (0.2mM) and high Mg^{2+} (10mM) which was demonstrated to block synaptic transmission, and that there was no significant change in membrane input resisitance during AII depression of Glu-induced responses, the action of AII on Glu-induced responses was not an indirect effect.

4. 6. Effects of H^+ Ion on Glu-induced Responses

It has been shown previously that H^+ ions influence neuronal excitability. H^+ ions have been reported to hyperpolarize motoneurons in cat spinal cord (Marshall and Engberg, 1980) and to depress neuronal activity in rat cerebrocortex (Hewes and Frederickson, 1974) when applied iontophoretically. The effects of H^+ ions on neuronal excitability make it an complicating factor for the studies of testing neurotransmitter substances applied iontophoretically

from acidic solutions (Frederickson et al., 1971). Recent studies have shown that H^+ ions inhibit Glu receptors in hippocampal neurons of mouse (Vyklicky et al., 1990) and rat (Tang et al., 1990), in cortical neuronal cultures (Giffard, et al., 1990). These give us a hint that the depressant action of AII on Glu-induced responses may be due to the action of H^+ ions ejected out through AII-containing barrel during iontophoresis since acidic AII solution was used in our experiments. Therefore we conducted pH controls in most tests by passing positive current through H^+-containing barrels.

We found in our pH control experiments that H^+ ions had various effects on Glu-induced responses in the LC neurons. In some neurons, the Glu-induced responses were depressed (also see White et al., 1988), whereas in some others the Glu-induced responses were enhanced, but in most pH control tests, the Glu-induced responses showed little or no change. The correlation between AII action and H^+ effect was poor in the same cell. It is possible that the depressant action of H^+ on Glu-induced responses in some cells may contribute to the depressant action of AII. It is also possible that in those cells on which AII had weak depressant action or no apparent action on Glu-induced responses, the depressant action of AII on Glu-induced responses was weakened or neutralized by an excitatory action of H^+ ions on these cells. Although we do not know to what extent the depressant action of H^+ ions on Glu-induced responses contributes to the depressant action of AII on Glu-induced

responses, the component contributed by H^+ ions must, if there is any, be generally small, since in most cells tested the H^+-mediated depression of Glu-induced responses was much smaller than that of AII-mediated depression.

Many reports have demonstrated that there are changes in extracellular pH in CNS tissue both *in vitro* (Krishtal et al., 1987) and *in vivo* (Kraig et al., 1983; Mutch and Hansen, 1984) as a result of electrical afferent stimulation or agonist application (Endres et al., 1986; Kaila and Voipio, 1987; Jarolemik et al., 1989; Sykova and Svoboda, 1990). These changes are large enough to influence neuronal function, since small extracellular alkaline shifts have been shown to enhance neuronal excitability and acidic changes to depress neuronal excitability (Balestrino and Somjen, 1988; Jarolemik et al., 1989). It has been demonstrated in turtle cerebellar slice that iontophoretically applied Glu produces a rapid extracellular alkaline transient, typically followed by a prolonged acidification (Chesler and Rice, 1991). It is conceivable that this prolonged acidification may also exist in the LC during iontophoretic application of Glu or Glu receptor agonists. Although extracellular space acidic change results in a decrease in neuronal excitability, it is unlikely that this prolonged extracellular space acidification makes its contribution to the depression of Glu-induced responses by AII since we did not observe any apparent decrease in Glu-induced responses during such a short period of (less than 1 minute) iontophoretic

application of Glu. It remains to be determined whether or not AII has any impact on the prolonged extracellular space acidification evoked by application of Glu.

4. 7. Effects of NH_4^+ on Glu Responses and Synaptic Transmission

4. 7. 1. Effects of NH_4^+ on Glu-induced Responses

NH_4^+ has been reported to inhibit the excitatory responses induced by iontophoretically or bath applied Glu in CA1 pyramidal neurons in rat hippocampal slices (Fan et al., 1990; Fan and Szerb, 1991). The AII from Sigma used in our earlier experiments contained NH_4^+ ions. To find out whether or not NH_4^+ per se has effect on Glu-induced responses, NH_4^+ was tested and found to have a moderate to strong depressant action on Glu-induced responses in many LC neurons when applied iontophoretically or by pressure. The profile of NH_4^+ action on Glu-induced responses seems to be similar to the action of AII. This raises the possibility that the depressant action of AII on Glu-induced responses we have observed in our earlier studies may be due to the action of NH_4^+ ions. This possibility was demonstrated to be unlikely by the following experimental results. Firstly, we tested the action of AII, which was obtained from other companies and confirmed to be NH_4^+ free, on Glu-induced responses and found that the NH_4^+-free AII (the latter tests were conducted using exclusive NH_4^+-free AII) had the same kind of depressant actions on Glu-induced responses in

LC neurons. Secondly , we tested the actions of both AII and NH_4^+ on Glu-induced responses in non-LC brain stem neurons recorded from the regions where no receptor binding for AII has been reported. AII was found to have little or no effect on Glu-induced response in these non-LC brain stem neurons. In contrast, NH_4^+ was found to have depressant actions on Glu-induced responses in these non-LC brain stem neurons. This suggests that the depressant action of AII on Glu-induced responses in the LC neurons were mediated through a mechanism which is different from that of NH_4^+. Thirdly, AII was found to selectively depress Glu-induced responses without effect on ACh-induced responses whereas NH_4^+ was found to inhibit the excitatory responses induced by ACh. Perhaps more affirmative evidence to eliminate the possibility that the action of AII on Glu-induced responses was due to the action of NH_4^+ is the demonstration that the newly discovered non-peptide AII receptor antagonists failed to antagonize the inhibitory action of NH_4^+ in LC neurons while the action of AII was consistently blocked by one of these non-peptide AII receptor antagonists in the same LC neurons. It was possible that the depression of Glu-induced responses by AII obtained in our earlier experiments may contain a component contributed by NH_4^+ since NH_4^+-containing AII samples were used. However, the inhibitory action contributed by NH_4^+ was, if any, probably small since no apparent changes were found on Glu-induced responses when NH_4^+ was applied iontophoretically at a concentration of 100μM (dissolved in 2mM NaCl solution,

dissolution in distilled water was found to be difficult to pass iontophoretic current). It should be pointed out that the concentrations used in our control tests, on which the depressant action of NH_4^+ on Glu-induced responses were observed, were 2 - 10 mM for iontophoresis, which was 20 - 100 times higher than that contained in AII solution, and that passing same amount of ejecting currents through solutions containing NH_4^+ ions as the only cation will drive greater amounts of NH_4^+ ions out of the pipette than could have possibly been ejected from AII solutions containing relatively small concentrations of these ions. Since the later experiments were conducted exclusively with NH_4^+-free peptide, and gave very similar results, we conclude that any such contribution was very small.

Co-application of AII with peptidase inhibitors into the bath depressed Glu-induced responses even at a concentration as low as 2 μM (see section 4.8.). However, the action of bath applied NH_4Cl on Glu-induced responses was different from that of iontophoresis. At high concentrations (2-5 mM), NH_4Cl exclusively increased Glu-induced responses. When the concentration was in the range of 0.25-1.0mM, NH_4Cl had various actions on Glu-induced responses. NH_4Cl was found to have no apparent effect on Glu-induced responses when applied at a concentration between 10 to 100μM. Thus, there was no correlation between the actions of AII and NH_4Cl. These results indicate once again that the action of AII on Glu-induced responses is independent of the of NH_4^+. The various effects of

bath applied NH_4Cl may be due to its exerting inhibitory action on other brain stem structures which have axonal projections to LC.

4. 7. 2. Effect of NH_4^+ on Excitatory Synaptic Transmission

Depression of excitatory synaptic transmission by NH_4^+ has been observed both in vivo (Theoret and Bossu, 1985; Raabe 1989) and in vitro (Theoret et al. 1985, Fan et al. 1990). Perfused through lateral ventricle at a concentration of 10mM, NH_4Cl was found to inhibit excitatory synaptic transmission in rat hippocampus (Theoret and Bossu, 1985). NH_4Cl was also found to depress the monosynaptic excitatory reflex in the spinal cord when 20% NH_4Cl solution was intravenously infused (Raabe, 1989). Similar depressant actions of NH_4Cl on synaptic transmission were observed in hippocampal pyramidal cells recorded extracellularly (Theoret et al., 1985; Fan et al., 1990) and intracellularly (Alger and Nicoll, 1983). We observed that NH_4Cl also had a depressant action on EPSPs evoked by electrical stimulation in rat LC neurons. At a concentration of 0.5mM, NH_4Cl was found to attenuate the amplitudes of the EPSPs by 30 - 50%. Similar depressant action on synaptic transmission was, at the same concentration, found on hippocampal pyramidal neurons (Fan et al., 1990). NH_4Cl had apparent depressant action on the amplitude of the EPSPs without effect on the duration reflected by the time of 50% decay.

4. 8. The Action of AII is Affected by Peptidase Activity

Our earlier experimental results showed that bath applied AII in a concentration to 10 μM has little or no effect on iontophoretically applied Glu in the LC. This might in part be explained by the binding of the peptide to the inner walls of the reservoirs and the tubing, but this does not appear to be the main factor responsible for the ineffectiveness of bath applied AII since no significant improvement of AII action on Glu was observed when the reservoirs and the tubing were coated with sigmacote which is believed to prevent peptides from binding to the glass wall. However, when AII was co-applied by bath with peptidase inhibitors, the depressant action of AII was observed on both iontophoretically applied and pressure ejected Glu, even at a dosage as low as 2 μM. The combination of Amastatin and Plummer's inhibitor as peptidase inhibitors used in our experiments was based on the findings: (1) amastatin is specific inhibitor for aminopeptidase A and M. Addition of amastatin to the bath can inhibit Asp^1 liberation from AII, so as to prevent AII degradation at the N-terminal (Abhold et al., 1987; Nomura, et al., 1990). (2) Plummer's inhibitor is a potent carboxypeptidase N inhibitor. Application of Plummer's inhibitor inhibits Phe^8 dissociation from AII and prevents AII from degradation from the C-terminal (Plummer and Ryan, 1981). The concentrations of these two peptidase inhibitors needed to effectively inhibit their corresponding peptidases have been demonstrated to be in the range of micromolar (Plummer and Ryan,

1981; Abhold et al., 1987; Nomura et al., 1990). The concentrations we used in our experiments were based on the preliminary results showing that they exert their inhibitory actions on peptidases (preserving inhibitory action of AII on Glu when AII was applied by bath) with little or no effect on LC neurons by themselves. It has been demonstrated that substitution of aspartic acid in position one in amino acid chain of AII with sarcosine (N-methylglycine) makes the Sar^1-AII resistant to aminopeptidase degradation (Regoli et al., 1974; Hall et al., 1974; Hinson et al., 1985). The further evidence supporting the conclusion that peptidase activity was responsible for the absence of effect of bath applied AII came from our experimental results that Sar^1-AII was found to depress Glu-induced responses when applied by bath. We found in a number of cells that bath-applied Sar^1-AII depressed Glu responses while bath-applied AII alone at the same concentration had no or little effect on these same cells. These results strongly indicate that AII is subject to peptidase degradation in a brain slice preparation and are in full agreement with those findings reported by other investigators that AII has a very short half-life in the CNS, approximately 23 seconds (Harding et al., 1986) and is degraded very rapidly under normal conditions when angiotensinase activity is not inhibited (Simonnet et al., 1984).

4. 9. Effects of AIII on Glu-induced Responses

AIII, a heptapeptide metabolite of AII (des-Asp^1-AII), is believed to be also a biological active angiotensin. It has been previously demonstrated that AIII appears to be much less potent than AII when applied intracerebroventricularly (Fitzsimons, 1971; Tonnaer et al., 1982). This is consistent with competitive displacement studies that AII had higher affinity than AIII for the binding sites in the CNS (Mann et al., 1981; Tonnaer et al., 1983). In addition, more AII than AIII has been found in the extracts of rat brain tissue in an HPLC study (Phillips and Stenstrom, 1985). These results indicate that AII is the major active component of Brain RAS. We found that AII strongly and selectively depresses Glu-induced responses in rat LC neurons. The depression mediated by AII was much stronger than that mediated by AIII. Our results are in full agreement with those experimental results mentioned above.

In recent studies, however, it has been suggested that AIII may be one of the major active components of brain RAS (Harding et al., 1987; Lin et al., 1988; Chan et al., 1991; Jin et al., 1992) since intracerebroventricularly administered AIII was found to have almost equal effects to AII in producing pressor and dipsogenic effects (Wright et al., 1985; Lin et al., 1988), in modulating renal function (Jin et al., 1992) and in stimulating phosphatidylinositol (PI) hydrolysis in differentiated NG108-15 hybrid cells (Carrithers et al., 1990). AIII was also found to be even more potent than AII when iontophoretically applied to cells of the paraventricular

nucleus (Harding and Felix, 1987a; Felix et al., 1991). Unlike the experimental results mentioned above, we found that AIII, like AII, also has depressant actions on Glu-induced responses. This result supports the suggestion that AIII is one of active components of Brain RAS though its depressant action we have observed is less potent than that of AII.

The differences between our results and those reported by other researchers regarding the potencies of AII and AIII may have something to do with the different preparations used. In those reports that AIII was, when applied by iontophoresis, found to have almost the same or even stronger effect in activating rat paraventricular neurons in comparison with the action of AII (Harding and Felix, 1987a; Felix et al., 1991), the experiments were conducted in vivo and extracellular recorded paraventricular neurons were the testing targets. The direct neuronal responses to these two peptides were tested. In contrast, the experiments in this study were conducted in an *in vitro* brain slice preparation and intracellularly recorded LC neurons were the testing targets. The modulatory action of these two peptides on excitatory amino acids were tested. These may be responsible for the differences between the experimental results of ours and those of other researchers (Harding and Felix, 1987a; Felix et al., 1991). It has been reported that H^+ ions have inhibitory action on neuronal activity (Marshall and Engberg, 1980; White et al., 1988). We also found that H^+ ions may attenuate Glu-induced response in the rat LC. However, in

those experiments conducted by Harding and Felix (1987a), the pHs in solutions of AII and AIII were different, with pH 3.5 in AII solution and pH 4.5 in AIII solution. When same amount positive current was applied to the AII-containing barrel and AIII-containing barrel, it would be expected that more H^+ ions were ejected out from the AII- containing barrel than the AIII-containing barrel, though the concentrations of both AII and AIII were the same, since the H^+ ion concentration in AII-containing barrel is ten times greater than that of AIII-containing barrel. It was possible that AII mediated excitatory responses were at least partially inhibited by H^+ ions. This may make AII-mediated responses to be smaller than those of AIII-mediated. Another possibility is that the affinity to AII and AIII in different cells is different, so that the responses to AII and AIII are not the same even if the dose are the same. It appears to be unlikely that the different potencies of the two peptides are due to the activation of different receptors since the actions of both AII and AIII can be blocked by the AII receptor antagonist [Sar^1, Ile^8]-AII (Wright et al., 1985; Harding and Felix, 1987a), even AII receptor subtype AT_2 antagonist CGP 42112A (Felix et al., 1991), indicating both peptides act on the same receptors. We did not test the action of AII receptor antagonists on both AII- and AIII-mediated depression of Glu-induced responses. It will be interesting to test and compare the effects of AII receptor antagonists on the depressant actions of these two peptides.

Based on the results that the actions of AII and AIII were blocked by the same AII receptor antagonist, it has been hypothesized that there is an obligatory conversion of AII to AIII prior to receptor binding and neuronal activation (Harding and Felix, 1987a). Our results have demonstrated that AII has more potent action in depressing Glu-induced responses than AIII. This suggests that the depressions of Glu-induced responses were mediated directly by AII, instead of AIII converted from AII since direct comparisons showed that iontophoretically applied AII was much more potent than AIII. The more convincing evidence in supporting this suggestion was the finding that bath applied AII alone had no apparent depressant actions on Glu-induced responses. In contrast, when co-applied with the aminopeptidase inhibitor amastatin, preventing degradation from AII to AIII, AII showed to have depressant action on Glu-induced responses. Also, bath applied Sar^1-AII, which is believed to be resistant to peptidase degradation (Hall et al., 1974), had strong depressant actions on Glu-induced responses.

It has been reported that the half-life for AIII is very short (~7seconds) in neural tissue (Harding et al., 1986). The short half-life may be responsible for its less effectiveness than AII in depressing Glu-induced responses in rat LC.

4. 10. Effects of des-Phe^8-AII on Glu Responses

It has been reported that another heptapeptide angiotensin, des-Phe^8-AII (also abbreviated as AII-[1-7] in the literature) is also a biological active neuropeptide (Ferrario et al., 1991; Felix et al., 1991). The production of des-Phe^8-AII from a precursor peptide has been identified by studies of the metabolism of both ^{125}I-AI and ^{125}I-AII in the brain and in a neurally derived cell line. In homogenates of canine brain, des-Phe^8-AII was reported to be a major component of the hydrolysis of ^{125}I-AI or ^{125}I-AII (Santos et al., 1988; Welches et al., 1991). des-Phe^8-AII was also found to be the major product of the metabolism of ^{125}I-AI in NG108-15 neuroblastoma × glioma hybrid cells (Chappell et al., 1990). It seems that des-Phe^8-AII is produced by direct hydrolysis of AI, since Santos et al. (1988) observed the production of des-Phe^8-AII in punches obtained from the canine medulla oblongata was unchanged by pretreatment of the homogenate with enalapril.

Physiological studies have shown that the heptapeptide des-Phe^8-AII is as potent as AII in stimulating vasopressin release from hypothalamic explants of the rat (Schiavone et al., 1988), in its excitatory action on the dorsal vagal complex of the medulla oblongata (Campagnole-Santos, et al., 1989), paraventricular nucleus of the hypothalamus and in augmenting neuronal discharges in *in vitro* slice of the dog's brain (Barnes KL, et al., 1990; Felix et al., 1991). Therefore, the possibility exists that the depressant actions of AII on Glu responses in LC neurons are mediated through the action of des-

Phe^8-AII produced from AII degradation. However, this appears unlikely since we found the actions of AII on Glu-induced responses were greater when AII was co-applied with the carboxypeptidase inhibitor Plummer's reagent (inhibits phenylalanine release from AII peptide chain thereby preventing degradation of AII to des-Phe^8-AII) than when AII was applied alone. Interestingly, when both the aminopeptidase inhibitor amastatin (prevent aspartic acid dissociation from position 1) and the carboxypeptidase inhibitor Plummer's reagent were used in our experiments, the depressant action of AII on Glu responses was stronger than that when amastatin was added alone. This result indicates that dissociation of phenylalanine from position 8 of the AII peptide chain decreases the action of AII on glu-induced responses. This finding seems to be in full agreement with the widely accepted theory that carboxyl-terminal phenylalanine in position 8 is crucial for full agonist potency at the angiotensin receptor (Pendleton et al., 1989). These results clearly indicate that AII-mediated depression of Glu-induced responses is greater than that mediated by des-Phe^8-AII. At the same time, these results indicate that both aminopeptidase and carboxypeptidase activities are responsible for the ineffectiveness of AII on Glu-induced responses observed in our earlier experiments (Marshall and Xiong, 1991)

Recent studies show that des-Phe^8-AII and AII have different actions in the regulation of cell function. In isolated rabbit vasa deferentia, AII was found to increase both

adrenergic neurogenic contractions and prostaglandin E_2 synthesis (Trachte et al., 1990). In contrast, des-Phe^8-AII increased prostaglandin synthesis with a potency almost equivalent to that of AII, but there was no neuromodulatory activity mediated by des-Phe^8-AII (Trachte et al., 1990).

4. 11. The Action of AII on Glu-induced Responses Is Mediated by Activating AII Receptors.

The LC has been demonstrated to contain a high concentration of AII receptors (Mendelsohn et al., 1984; Gehlert et al., 1986a,b; Allen et al., 1991; Rowe et al., 1991; Tsutsumi and Saavedra, 1991d; Song et al., 1992). These receptors are reported to be associated with noradrenaline-containing neuronal elements within the LC, on the basis of autoradiographic studies following treatment with neurotoxins (Rowe et al., 1990a). To determine whether the action of AII on Glu-induced responses was mediated via these specific AII receptors, both peptide and non-peptide AII receptor antagonists were tested.

The peptide antagonist saralasin was reported to be a potent AII receptor antagonist (e.g. Hattori and Koizumi, 1990). It has been shown to strongly antagonize AII-induced responses in the neurons of the circumventricular organs of polydipsic inbred mice (Hattori and Koizumi, 1990), in rat hypothalamic neurons (Tanaka et al., 1986; Qadri et al., 1991) and in primary

cultures of rat brain (Sumners et al., 1983). We found that saralasin partially blocked AII action in about half of the LC neurons tested, with almost complete blockade in some, suggesting that the action of AII on Glu-induced responses is related to the activation of AII receptors. In some cells, saralasin was found to mimic the depressant effect of AII on Glu-induced responses, indicating saralasin also has agonist action at AII receptor as observed by other researchers (Fagard et al., 1981; Ziogas et al., 1985). The difference between our results and those mentioned above on the action of saralasin may be associated with different selectivity of saralasin on different AII receptor subtypes since the major subtype of AII receptor in the LC is reported to be different from the circumventricular organs and hypothalamus, or because the agonist profile of saralasin is different in different brain regions.

Since saralasin is less potent in antagonizing the action of AII on Glu-induced responses and has partial agonist effect at AII receptor, it is not a good antagonist in identifying whether or not the action of AII on Glu-induced responses is mediated by AII receptor. Thus, the recently discovered, more potent and selective non-peptide antagonists, losartan and PD123177 were tested. Using these new non-peptide AII receptor antagonists, we were able to distinguish the subtype of AII receptor mediating the depressant action of AII on Glu-induced responses. When co-applied by bath, losartan and PD123177

antagonized the depressant action of AII on Glu-induced responses in all the cells tested. However when tested separately, losartan was found to only mildly antagonize the depressant action of AII, even at a concentration as high as 20 μM. In contrast, PD123177 antagonized the action of AII on Glu-induced responses in all cells tested. The antagonism of AII depression of Glu-induced responses by PD123177 was concentration dependent, with an approximate IC_{50} of 1.5 μM. These data clearly indicate that AT_2 subtype receptors are mainly involved in mediating this AII action. This finding agrees with those studies reporting that the predominant subtype of AII receptors in the LC is the AT_2 subtype (Gehlert et al., 1991b; Rowe, et al., 1991; Tsutsumi and Saavedra, 1991d; Song et al., 1992).

4. 12. The action of AII on EPSPs

AII has been found to have clear depressant actions on the excitatory responses induced by exogenously applied Glu. In order to determine whether such actions may have physiological relevance, the action of AII on synaptically released Glu-like transmitter was tested. This is testable in the horizontal brain slices, since it has been demonstrated in our experiments that EPSPs mediated by a Glu-like transmitter can be evoked by electrical stimulation of a region rostral to the LC. The evoked EPSPs were antagonized by the broad spectrum Glu receptor antagonists kynurenic acid, suggesting the involvement of EAAs

in the mediation of the EPSPs. In an attempt to find out which subtype(s) of Glu receptors was(were) responsible for the generation of EPSPs, both NMDA and non-NMDA receptor antagonists were tested in the presence of normal concentration of Mg^{2+}. The NMDA receptor-selective antagonist APV partially blocked the amplitude of the EPSPs. In contrast, the non-NMDA receptor selective antagonist CNQX almost completely blocked the EPSPs in all the cells tested, indicating the main involvement of non-NMDA receptors in the generation of the EPSPs. These results are consistent with previous findings that NMDA receptors, at resting membrane potentials, contribute only a small and variable amount to the EPSPs at many synapses, including those in the cortex, spinal cord, neostriatum and hippocampus (Nicoll et al., 1990).

Sar^1-AII applied by pressure ejection or by addition to bath media was found to have moderate depressant actions on the amplitude of the EPSPs, suggesting that Sar^1-AII is primarily affecting non-NMDA receptors. The depressant action of Sar^1-AII was consistently reduced in a concentration-dependent manner by the AT_2-selective antagonist PD123177. The AT_1-selective antagonist losartan generally had little effect on the action of Sar^1-AII on the EPSPs, except on one cell which the action of Sar^1-AII was blocked by losartan. These results indicate that AT_2 receptors are mainly involved in the mediation of the depressant actions of Sar^1-AII on the EPSPs.

So far, we have demonstrated that the actions of AII (or

Sar^1-AII) on the excitatory responses induced by either endogenous released or applied Glu are mainly mediated by activation of one subtype of AII receptors, the AT_2 receptors. These results appear to be novel findings at the cellular level in that AII has an inhibitory action on excitatory synaptic transmission and exerts its inhibitory action on excitatory transmission via the AT_2 subtype receptors.

It has been demonstrated that one of the major inputs to the LC originate from ventrolateral medulla (Aston-Jones et al., 1986) and that EAAs are the most prominent input to the LC from this major afferent (Ennis and Aston-Jones, 1988). We found in horizontal brain-stem slices that stimulation of an area rostral to the LC (see fig. 5) consistently evoked EPSPs. These EPSPs can be blocked by Glu receptor antagonists, indicating EAAs are involved in the generation of the EPSPs. The fiber origin of this pathway is unclear. It may belong to the afferents to LC from paraventricularhypothalamic nucleus as reported by Aston-Jones et al. (1990), or may be some of the major excitatory afferent fibers form ventrolateral medulla project to the LC by their way rostral to the LC (bending to LC). Further experiments are needed to identify the origin of these afferents.

4. 13. Effects of AII on Glu Receptor Agonist-induced Responses

It has been shown that rat LC neurons are activated by the Glu receptor agonists NMDA, QA and KA, (Olpe et al., 1989;

Engberg and Hajos, 1992). Intracoerulear injection of kynurenic acid (a broad-spectrum antagonist of EAAs) or CNQX (a non-NMDA receptor antagonist) almost completely blocked the excitation of the LC neurons induced by electrical stimulation of rear footpad (mediated by endogenous EAAs)(Akaoka and Aston-Jones, 1991). These data indicate that Glu receptors are present in the LC. We also observed that the LC neurons were responsive to Glu and Glu receptor agonists, and that the excitatory responses induced by Glu or Glu receptor agonists were blocked or attenuated by AII. It seems possible that AII might act selectively on one or more than one subtype of these Glu receptors activated by its selective agonist. Our preliminary results indicate that no single subtype of Glu receptor-induced response is consistently affected by AII when applied by iontophoresis. However, it was found that Sar^1-AII had depressant actions on both NMDA and non-NMDA receptor agonist-induced responses when Sar^1-AII was applied by pressure ejection. Based on the results of iontophoretically applied AII, it appears, therefore that AII is more effective as a depressant against Glu-induced depolarizations than against those caused by the selective Glu receptor subtype agonists applied separately, since we observed in several cells that Glu-induced responses were completely blocked by AII, whereas the excitatory responses induced by each of Glu receptor agonists were partially blocked in the same cells. One possible explanation for this difference is that AII may be more effective in antagonizing Glu-induced responses when

more than one subtype of Glu receptors are simultaneously activated than with activation of a single subtype alone since there are interactions between Glu receptor agonists. Recent studies on cerebellar and striatal neurons have demonstrated complex interactions between KA and QA, in which co-application of QA and KA attenuated the release of GABA evoked by KA alone. (Gallo et al., 1989; Pin et al., 1989). In a whole-cell patch clamp study of cultured striatal neurons, QA agonists (QA, AMPA and Glu) have also been found to occlude KA-induced current (Tse et al., 1991). In addition, the same kind of occlusion of KA-induced current by QA, AMPA and Glu has been found in piscine retinal neurons (O'Dell and Christensen, 1989a,b) and chick motoneurons (O'Brien and Fishbach, 1986). It is therefore possible that the inhibitory interactions between Glu receptor subtypes may also exist in the LC when they are activated simultaneously and AII potentiates this inhibitory interaction. If this is the case, this may explain the greater depressant actions of AII on Glu-induced responses than that induced on individual Glu receptor agonists since Glu can activate all subtypes of Glu receptors. Moreover, the discovery of a "metabotropic" Glu receptor which is also selectively activated by quisqualate (Sladeczek et al., 1985; Sugiyama et al., 1987) makes the interactions between Glu receptor agonists more complicatedsince it has been reported that activation of this subtype of Glu receptor potentiates AMPA-induced responses (Dumuis et al, 1990). In addition, an inhibitory Glu receptor

subtype, the AP4 receptor, has also recently been discovered. This subtype of Glu receptor was also reported to interact with QA (Butcher et al., 1983). If this subtype Glu receptor exists in the LC, it may be also involved in the interactions between/among Glu receptor subtype agonists.

Another possible explanation for the difference of the actions of AII on Glu agonist responses might be due to regional differences in the number and types of Glu receptors or due to the different affinities for the same kind Glu receptor in different regions. For example, the numbers, types or affinities of Glu receptor subtypes may be different in distal dendrites from those in proximal dendrites or soma. If such differences exist on LC neurons, the actions of AII on Glu and on Glu receptor agonists when iontophoresis electrodes were placed in distal dendritic regions of recorded cells could be different from those when iontophoresis electrodes were placed in proximal dendritic areas or adjacent to the cell bodies.

By testing the effects of selective Glu receptor antagonists on Glu-induced responses in LC neurons, the Glu-induced responses appear to be mainly mediated through non-NMDA receptors under physiological condition (NMDA receptors made less contribution to Glu-induced responses than non-NMDA receptors due to the presence of the normal concentration of Mg^{2+}), since the non-NMDA receptor antagonist CNQX was found to have strong effects in antagonizing the excitatory responses induced by applied Glu and the EPSPs evoked by electrical

stimulation (mediated via endogenously released Glu). Our results are consistent with those observed by other researchers in pharmacological studies in vivo and in brain slices that Glu activity is blocked by broad spectrum antagonists, but is relatively insensitive to selective NMDA antagonists (Collingridge et al., 1983). This may be because NMDA receptors make less contribution to the responses induced by Glu in the presence of normal Mg^{2+} in the perfusate. However, when testing its effects on Glu agonist-induced responses, AII was found to depress both NMDA and non-NMDA -induced responses, suggesting that the depressant actions of AII on exogenously applied or endogenously released Glu induced responses were achieved by affecting both NMDA and non-NMDA receptors.

To prevent LC neurons from neurotoxic action of Glu released excessively with ischemia/anoxia, during preparation of the slice, ketamine, an NMDA channel blocker, was given intramuscularly to the animals. In an attempt to wash out any effect of ketamine, we did not conduct any recording until slice was perfused in the chamber for at least one hour. The other reason for the use of ketamine was to anaesthetize the animals. We prefer surgically decapitate the animals to decapitate the animals with a chopper, since the animals used in our study were relatively young and small, and decapitation with a chopper may shock the brainstem and cause possible damage of brainstem neurons.

4. 14. Possible Mechanisms Underlying the Action of AII on Glu-induced Responses

We found that AII selectively depresses the excitatory responses induced by endogenously released or exogenously applied Glu without affecting membrane properties. The mechanism underlying the action of AII is unclear. So far, there is little known about the transduction mechanisms of AII receptors in the brain, including those in the LC. In peripheral tissues, AII has been found to mediate its action via a second message system, stimulating phosphoinositide hydrolysis (Pfeilschifter, 1990; Stromberg et al., 1991), increasing calcium influx (Ambroz and Catt, 1992), mobilizing intracellular calcium (Foster et al., 1981; Capponi et al., 1984; Johnson and Aguilera, 1991); activating protein kinase C and inhibiting adenylate cyclase (Marie and Jard, 1981; Pobiner et al., 1991), depending on the tissue or cell type. AII was also reported to increase inositol phospholipid hydrolysis in NG108-15 neuroblastoma × glioma hybrids (Carrithers et al., 1990) and to increase cellular cGMP levels in N1E-115 murine neuroblastoma cells (Gilbert et al., 1984). Some of the actions of AII in the peripheral tissues have now been demonstrated in the brain.

Most recent studies indicate that AII decreases phosphoinositide hydrolysis in the diencephalon-midbrain of rat (Tamura and Speth, 1990), and that the dipsogenic and pressor action of AII are mediated by a mechanism that is not

inactivated by pertussis toxin (Speth and Grove, 1991).

AII has also been reported to decrease cGMP levels in the primary neuronal cultures prepared from the hypothalamus and brain stem of 1-day-old rats (Sumners and Myers, 1991; Sumners et al., 1991), but not in the astrocyte glia cultures. In contrast, AII had no effect on cAMP levels in neuronal cultures (Sumners et al., 1990) though it was found to stimulate cAMP production in the peripheral tissue (Rainey et al., 1991). Thus, there is little evidence for a biochemical transduction for neuronal actions of AII that is similar to its effects in the peripheral tissues. Using quantitative autoradiography after incubation of rat brain sections with AII agonist ^{125}I-Sar^{1}-AII, Tsutsumi and Saavedra (1992) have found in recent studies that AT_2 receptors in the LC are coupled to G-proteins. They found that the binding of ^{125}I-Sar^{1}-AII to AT_2 receptors in the LC of 10-day-old rat were significantly reduced when brain sections were incubated with ^{125}I-Sar^{1}-AII in the presence of GTPγS or pertussis toxin. The regulation of ^{125}I-Sar^{1}-AII binding to AT_2 receptor by GTPγS or pertussis toxin in rat LC suggests that AT_2 receptors in rat LC are coupled to G-protein. These results obtained by pharmacological study (Tsutsumi and Saavedra, 1992) need to be confirmed by physiological and biochemical studies. Further experiments are needed to find out whether the actions of AII on Glu-induced responses in the LC are mediated via pertussis sensitive G-protein. It is at least not the result that AII stimulates IP_3 formation since AII was reported to have

no influence on phosphoinositide hydrolysis in the LC of rats (Huang et al., 1987).

Another possible mechanism for mediating the action of AII may be due to the interaction of AII with ligand-gated ion channels. Iontophoretically or pressure applied AII generally had short latencies in the depression of Glu-induced responses in most cases, but in some cases, the latencies were longer. The mechanisms underlying the shorter and longer latencies may be different. Anissimov et al. (1980) also observed both shorter and longer latencies responses to AII administered to the rabbit LC neurons in vivo by iontophoresis. These observations suggested that AII may have complex actions in the LC, possibly acting as a neuromodulator in modulating the actions of various synaptic transmitters released from afferents to the LC.

In peripheral tissue, such as adrenal glomerulosa cells, it is found that AII-mediated responses are calcium dependent, and mimicked by the calcium ionophore A23187 (Fakunding et al., 1979; Fakunding and Catt, 1980). AII activates these cells of the glomerulosa by increasing the entry of calcium into the cell, which serves as an important messenger function in the responses of these cells to AII. However, the calcium-mobilizing AII was reported to inhibit K^+-induced increase of cytoplasmic Ca^{2+} concentration in adrenal glomerulosa cells and cause a rapid decrease of cytoplasmic Ca^{2+} when added to cells already stimulated with K^+ (Foster et al., 1981).

The increase of cytoplasmic Ca^{2+} concentration evoked by K^+

is due to increased Ca^{2+} entry from extracellular fluid through voltage-sensitive Ca^{2+} channels that are already activated by small depolarizations(Barret et al., 1989). AII, on the other hand, stimulates the release of Ca^{2+} from intracellular Ca^{2+} stores, owing to the very rapid formation of the Ca^{2+}-mobilizing messenger $Ins(1,4,5)P_3$ (Balla et al., 1986; Takahara et al., 1990). The stimulated entry of $^{45}Ca^{2+}$, as well as the cytoplasmic Ca^{2+} increase in AII treated glomerulosa cells, can not be prevented by the dihydropyridine antagonist nifedipine.

It has recently been confirmed that AT_1 receptors are coupled to G-proteins, and their stimulation increases phosphoinositide hydrolysis (Pucell, et al., 1991; Tsutsumi and Saavedra, 1991a). The molecular cloning, sequencing analyses and expression have recently been done in bovine adrenal (Sasaki et al., 1991), rat vascular (Murphy et al., 1991) and human leukocyte genomic (Furuta et al., 1992; Mauzy et al., 1992) AT_1 receptors, respectively. The physiological role of AT_1 receptors has been primarily associated with the control of cardiovascular function and fluid regulation (Chiu et al., 1989a; Whitebread et al., 1989). However, the experimental results concerning the connections of AT_2 receptors to the signal transduction pathways were controversial. AT_2 receptors apparently do not to interact with G-protein in some studies (Bottari et al., 1991; Tsutsumi and Saavedra, 1991a) and their stimulation is not associated with phosphoinositide hydrolysis, whereas in some other studies AT_2 receptors were reported to be

also linked to G-protein (Tsutsumi and Saavedra, 1992). The AT_2 receptors have recently been reported to mediate decreases in cGMP levels in non-neuronal (Bottari et al., 1992) and neuronal cultures (Sumners et al., 1991). The AT_2 receptors in the LC were found to be G-protein-linked (Tsutsumi and Saavedra, 1992). It is conceivable that the action of AII on the excitatory responses induced by endogenously released or exogenously applied Glu may be mediated through activating the G-protein coupled to AT_2 receptors. The role of AT_2 receptors in the CNS is presently unknown. Other AT_2 specific action in the CNS which has been reported is an excitatory action on inferior olivary neurons observed by Ambuhl et al. (1992b). This excitatory action of AII in inferior olivary nucleus was antagonized by AT_2 receptor antagonists PD123177 and CGP42112A.

Our results clearly indicate that the action of AII on GLu-induced responses are mediated by AT_2 receptors. These results are consistent with those reports indicating the predominant form of AII receptor in the LC is AT_2 subtype (Gehlert et al., 1991b; Rowe et al., 1991; Song et al., 1992). In a whole-cell patch clamp study of cultured neurons from neonate rat hypothalamus and brain stem, AII has been found to increase net outward current via AT_2 receptors (Kang et al., 1992a,b) or to decrease net outward current through AT_1 receptor. Further study revealed that the enhanced outward current mediated via AT_2 receptors were potassium currents (I_K and I_A). AII had no effects on calcium current and calcium activated potassium current (Kang

et al., 1992b). In our preliminary experiments, we found that AII attenuated Glu-induced inward current. Further experiments are needed to find out what kind(s) of current(s) was (were) attenuated by AII.

4. 15. Possible Physiological Significance of the Action of AII on Glu-induced Responses in the LC.

It is not clear at present whether the action of AII on Glu-induced responses in the LC represents a role of AII as a component of brain RAS' control of cardiovascular functions and body fluid homeostasis, or as a neurotransmitter to regulate the activity of noradrenergic LC neurons through modulating the actions of other neurotransmitters. It is also difficult to estimate the functional significance of the actions of AII we have observed in the LC neurons since the LC has such wide projections to different brain regions that the regulation of the LC activity probably affects extensive functions of the CNS.

Several lines of evidence indicate that brain AII is associated with the elevation of blood pressure seen in the spontaneously hypertensive rats. These include an increased AII receptor affinity and density (Gehlert et al., 1986a), an increased pressor response to intracerebroventricularly administered AII (Hoffman et al., 1977; Felix and Schelling, 1982) and a reduction of blood pressure in response to centrally administered AII receptor antagonists (Schoelkens et al., 1976;

Hoffman et al., 1977; Mann et al., 1978). Recent studies suggest that there is an interaction between AII and the noradrenergic LC in blood pressure regulation. In parallel with the finding that there is greater sensitivity to AII in hypertensive rats, several studies have demonstrated a selective enhancement of AII receptor binding in cardiovascular regulatory centers of the spontaneous hypertensive rat brain (Stamler et al., 1980; Plunkett and Saavedra, 1985; Gehlert et al., 1986a; Saavedra et al, 1986a; Gutkind et al., 1988), including the LC (Gehlert et al., 1986a). Quantitative autoradiographic study has shown that densities of AII receptor binding sites in the LC of spontaneously hypertensive rats are approximate two-fold higher than that in normotensive Wistar-Kyoto rats (Gehlert et al., 1986a). Also, in the spontaneously hypertensive rat, AII antagonists administered intracerebroventricularly decrease blood pressure while minimally affecting normotensive control rats. (Schoelkens et al., 1976; Hoffman et al., 1977). This suggests that increased AII receptor stimulation is a causal factor in the hypertension. In agreement with this suggestion, an increase in the amount of AII-immunoreactive material has been observed in selected brain nuclei including the LC in the spontaneous hypertensive rats (Weyhenmeyer and Phillips, 1982; Meyer et al., 1989). It has been reported that microinjection of AII into the LC of normotensive rats causes a small pressor response (Berecek et al., 1984). However, the same kind of test has not been done on spontaneously hypertensive rats. It could

be hypothesized that spontaneously hypertensive rats would show a larger pressor response to AII microinjected into the LC.

The LC has been demonstrated to receive a potent excitatory input from the PGi in rostral medulla oblongata (Ennis and Aston-Jones, 1986). The neurotransmitter released at these excitatory afferent terminals has later been confirmed to be excitatory amino acids (Ennis and Aston-Jones, 1988). Interestingly, microinjection of Glu into rat LC decreased arterial blood pressure and heart rate. Destruction of the noradrenergic neurons of the LC by local adminstration of 6-hydroxydopamine eliminated the cardiovascular responses elicited by injection of Glu into the LC (Sved and Felsten, 1987). These findings indicate that the excitatory inputs to the LC have a role in regulation of cardiovascular function. We have found that AII strongly depresses Glu-induced responses in LC neurons. Our results may reflect a pressor role of AII mediated at the LC level by inhibiting an excitatory input. This may be one of the factors that is responsible for the formation of primary hypertension. Since the angiotensinergic fibres projecting to the LC originate from hypothalamus (Fuxe et al., 1976), the depressant action of AII on Glu-induced responses may represent a mechanism of hypothalamic control of cardiovascular function by inhibiting the excitatory inputs to the LC by means of brain renin-angiotensin system.

It has also been found that microinjection of Glu and other excitatory amino acid receptor agonists into the rat NTS which

is, like the LC, associated with the presence of a high density of AII receptor binding, results in decreases in blood pressure and heart rate (Talman et al., 1980). Since NTS receives baroceptor afferent information (Miura and Reis, 1969) and plays an important role in the regulation of baroreflexes (Zandberg et al., 1978), the excitatory amino acids such as Glu were therefore hypothesized to be involved in this regulation (Talman et al., 1980). This hypothesis is supported by the experimental results that excitatory amino acid receptor antagonists, injected both unilaterally (Kubo and Kihara, 1991) or bilaterally (Guyenet et al., 1987; Leone and Gordon, 1989; Talman, 1989) into the NTS, produce blockade of baroreflexes in rats. Microinjection of AII into the NTS attenuates baroreflexes in rats (Casto and Phillips, 1986) and dogs (Averill et al., 1987). It seems quite possible that the action of AII on baroreflexes is mediated by depressing the action of Glu which is believed to be the neurotransmitter of baroceptor afferents (Talman et al., 1980; Leone and Gordon, 1989). Whether the depressant action of AII on Glu in rat LC neuron we have observed also have something to do with baroreflexes is at present unknown.

Ward et al.(1977) found that neurons in the lateral part of the NTS were responsive to volume pulsation of the right atrium. These neurons seem to be antidromically driven from the LC, indicating the information from cardiovascular receptors reaches the LC. Moreover, the noradrenergic LC neurons were inhibited by

blood volume load through vagal afferents probably activated by cardiac arterial receptors (Svensson and Thoren, 1979; Elam et al., 1984)), or by blood pressure elevation through activating arterial baroceptors (Elam et al., 1984) . Also, stimulation of the vagal nerve inhibited LC neurons (Takigawa and Mogenson, 1977). These findings indicate that LC neurons participate in processing information about blood volume homeostasis and blood pressure. Together with the finding that the density and affinity of AII binding are increased in the LC as well as in other nuclei associated with cardiovascular control in the brains of spontaneously hypertensive rats, the action of AII on Glu-induced responses in the rat LC may at least partially be related to the brain renin-angiotensin system's control of cardiovascular function by modulating the actions of other neurotransmitters, for example, Glu.

It is interesting, however, that the AII receptors in the NTS of rats appear to be predominantly the AT_1 receptor subtype (Gehlert et al., 1991b; Tsutsumi and Saavedra, 1991d; Song et al., 1992). This type of AII receptors has been cloned (Iwai et al., 1991; Murphy et al., 1991; Sasaki et al., 1991), and the existence, and regional distribution, of AT_1 receptor subtype mRNA expression in the rat brain have been demonstrated (Bunnemann et al., 1992). AT_1 receptors are coupled to membrane G-protein, and are believed to be involved in the control of cardiovascular functions (Chiu et al., 1989b; Whitebread et al., 1989). In contrast, the major subtype of AII receptors in rat LC

is AT_2 AII receptors (Gehlert et al., 1991b; Tsutsumi and Saavedra, 1991d; Song et al., 1992). As yet, no intracellular effects or physiological functions have been ascribed to the activation of AT_2 receptors.

5. BIBLIOGRAPHY

Abhold RH, Sullivan MJ, Wright JW, Harding JW (1987) Binding, degradation and pressor activity of angiotensins II and III after aminopeptidase inhibition with amastatin and bestatin. J Pharmacol Exp Ther 242:957-962.

Aghajanian GK, Cedarbaum JM, Wang RY (1977) Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. Brain Res 136:570-577.

Agrawal SG, Evans RH (1986) The primary afferent depolarizing action of kainate in the rats. Br J Pharmacol 87:345-355.

Akaishi T, Negoro H, Kobayasi S (1981) Electrophysiological evidence for multiple sites of action of angiotensin II stimulating paraventricular neurosecretory cells in the rat. Brain Res 220:385-390.

Akaoka H, Aston-Jones G (1991) Opiates withdrawal-induced hyperactivity of locus coeruleus neurons is substantially mediated by augmented excitatory amino acid input. J Neurosci 11:3830-3839.

Alger BE, Nicoll RA (1983) Ammonium does not selectively block IPSPs in rat hippocampal cells. J Neurophysiol 59:1381-1392.

Allen AM, Chai SY, Clevers J, McKinley MJ, Pixinos G, Mendelsohn FAO (1988a) Localization and characterization of angiotensin II receptor binding and angiotensin converting enzyme in the human medulla oblongata. J Comp Neurol 269: 249-264.

Allen AM, McKinley MJ, Mendelsohn FA (1988b) Comparative neuroanatomy of angiotensin II receptor localization in the ma mammalian hypothalamus. Clin Exp Pharmacol Physiol 15:137-145.

Allen AM, Paxinos G, McKinley MJ, Chai SY, Mendelsohn FA (1991) Localization and characterization of angiotensin II receptor binding sites in the human basal ganglia, thalamus, midbrain pons, and cerebellum. J Comp Neurol 312:291-298.

Amaral DG, Sinnamon HM (1977) The locus coeruleus: Neurobiology of a central noradrenergic nucleus. Prog Neurobiol 9:147-196.

Ambroz C, Catt KJ (1992) Angiotensin receptor-mediated calcium influx in bovine adrenal glomerulosa cells. Endocrinol 131:408-414.

Ambuhl P, Felix D, Imboden H, Khosla MC, Ferrario CM (1992a) Effects of angiotensin analogues and angiotensin receptor antagonists paraventricular neurons. Regulatory Peptides 38:111-120.

Ambuhl P, Felix D, Imboden H, Khosla MC, Ferrario CM (1992b) Effects of angiotensin II and its selective antagonists on inferior olivary neurons. Regulatory Peptides 41:19-26.

Anissimov JZ, Deleva JI, Nicolov NA, Sudakov KV, Sherstnev VV (1980) Neuronal sensitivity of some brain regions to angiotensin II in rabbits. Agressologie 21:171-175.

Armstrong-James M, Fox K (1988) Evidence for synaptic role for cortical NMDA receptors in slow-wave sleep. Brain Res 451:189-196.

Arregui A, Emson PC, Spokes EG (1978) Angiotensin-converting enzyme in substantia nigra: Reduction of activity in huntingon's disease and after instrastrial kainic acid in rats. Eur J Pharmacol 52:121-124.

Arregui A, MacKay AVP, Spokes EG, Iversen LL (1980) Reduced activity of angiotensin-converting enzyme in basal ganglia in early onset schizophrenia. Psychol Med 10:307-313.

Ascher P, Nowak L (1987) Electrophysiological studies of NMDA receptors. TiNS 10:284-288.

Ascher P, Nowak L (1988) Quisqualate and kainate activated channels in mouse central neurons in culture. J Physiol 399:227-245.

Aston-Jones G, Bloom FE (1981a) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1:876-886.

Aston-Jones G, Bloom FE (1981b) Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. J Neurosci 1:887-900.

Aston-Jones G, Foote SL, Bloom FE (1984) Anatomy and Physiology of locus coeruleus neurons: Functional implications. In *Norepinephrine, Frontiers of Clinical Neuroscience, Vol. 2,* M. Ziegler and C. R. Lake (Eds.), Williams and Wilkins, Baltimore, pp92-116.

Aston-Jones G, Ennis M, Pieribone BA, Nickell W, Shipley MT (1986) The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. Science **234**:734-737.

Aston-Jones G, Shipley MT, Ennis M, Williams JT, Pieribone VA (1990) Restricted afferent control of locus coeruleus neurons revealed by anatomic, physiologic and pharmacologic studies. In *The Pharmacology of Noradrenaline in the Central Nervous System,* C.A Marsden and D.J. Heal (Eds.), Oxford University Press, Oxford, pp.187-247.

Aston-Jones G, Akaoka H, Charlety p, Chouvet G (1991) Serotonin selectively attenuates glutamate-evoked activation of noradrenergic locus coeruleus neurons. J Neurosci 11:760-769.

Aston-Jones G, Astier B, Ennis M (1992) Inhibition of noradrenergic locus coeruleus neurons by C1 adrenergic cells in the rostral ventral medulla. Neuroscience 48:371-381.

Atweh SF, Kuhar MJ (1977) Autoradiographic localization of opiate receptors in the brain. II. The brain stem. Brain Res 129:1-12.

Averill DB, Diz DI, Barnes KL, Ferrario CM (1987) Pressor responses of angiotensin II microinjected into the dorsomedial medulla of the dog. Brain Res 414:294-300.

Balcar VJ, Johnston GAR (1972) The structure specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. J Neurochem 19:2657-2666.

Balestrino M, Somjen GG (1988) Concentration of carbon dioxide , interstitial pH and synaptic transmission in hippocampal formation of the rat. J Physiol 396:247-266.

Balla T, Baukal AJ, Guillemette G, Morgan RO, Catt KJ (1986) Angiotensin-stimulated production of inositol triphosphate isomers and rapid metabolism through inositol 4-monophosphate in adrenal glomerulosa cells. Natl Acad Sci USA 83:9323-9327.

Barbour B, Szatkowski M, Ingledew N, Attwell D (1989) Arachidonic acid induces a prolonged inhibition of glutamate uptake into glial cells. Nature 342:918-920.

Barnes CD, Fung SJ, Pompeiano O (1989) Desending catecholaminergic modulation of spinal cord reflexes in the cat and rat. Ann N.Y. Acad Sci 563:45-48.

Barnes KL, Knowles WD, Ferrario CM (1990) Angiotensin II and angiotensin-(1-7) excite neurons in the canine medulla in vitro. Brain Res Bull 24:275-280.

Barnes KL, Diz DI, Ferrario CM (1991) Functional interactions between angiotensin II and substance P in the dorsal medulla. Hypertension 17:1121-1126

Barnes JM, Barnes NM, Costall B, Horovitz ZP, Ironside JW, Naylor RJ, Williams TJ (1990) Angiotensin II inhibits cortical cholinergic function: Implications for cognition. J. Cardiovascul. Pharmacol. 16:234-238

Barnes JM, Barber PC, Barnes NM (1991) Identification of angiotensin II receptor subtypes in human brain. Neuroreport. 2:605-608.

Barrett PQ, Bollag WB, Isales CM, McCarthy RT, Rasmussen H (1989) Role of calcium in angiotensin II-mediated aldosterone secretion. Endocrinol Rev 10:496-518.

Beckstead RM, Domesick VB, and Nauta (1979) Efferent connections of the substantia nigra and ventral tegmental area in rat. Brain Res 175:191-217.

Bennett JP, Snyder SH (1976) Angiotensin II binding to mammalian brain membranes. J Biol Chem 251:7423-7430.

Berecek JP, Olpe HR, Jones RSG, Hofbauer KG (1984) Microinjection of vasopressin into the locus coeruleus of conscious rats. Am J Physiol 247:H675-H681.

Bird SJ, Kuhar MJ (1977) Iontophoretic application of opiates to the locus coeruleus. Brain Res 122:523-533.

Bloom FE, Hoffer BJ, Siggins GR (1971) Studies on the noradrenergic afferents to the Purkinje cells of the rat cerebellum. I. Localization if the fibers and their synapses. Brain Res 25:501-521.

Bonhaus DW, Burge BC, Mcnamara JO (1987) Biochemical evidence that glycine allosterically regulates an NMDA receptor-coupled ion channel. Eur J Pharmacol 142:489-490.

Bottari SP, Taylor V, King IN, Bogdal Y, Whitebread S (1991) Angiotensin II AT_2 receptors do not interact with guanine nucleotide binding proteins. Eur J Pharmacol 207:157-163

Bottari SP, King IN, Reichlin S, Dahlstroem I, Lydon N, de Gasparo M (1992) The angiotensin AT_2 receptor stimulates protein tyrosine phosphatase activity and mediates inhibition of particulate guanylate cyclase. Bioch Biophysiol Res Comm 183:206-211.

Brewer G, Cotman CW (1988) NMDA promotes branching, MK-801 stimulates elongation of dendrite gyrus granule neurons. Soc Neurosci Abstr 14:115.

Bridges RJ, Stanley MS, Anderson MW, Cotman CW, Chamberlin AR

(1991) Conformationally defined neurotransmitter analogues. selective inhibition of glutamate uptake by one pyrrolidine-2,4-dicarboxylate diastereomer. J Med Chem 34:717-725

Bumpus FM, Catt KJ, Chiu AT, de Gasparo M, Goodfriend T, Husain A, Peach MJ, Taylor Jr. DG, Timmermans BMWM (1991) Nomenclature for angiotensin receptors: a report of the nomenclature committee of council for high blood pressure research. Hypertension 17:720-721.

Bunnemann B, Fuxe K, Ganten D (1992) The brain renin-angiotensin system: localization and significance. J Cardiovasc Pharmacol 19(suppl. 6):S51-S62.

Butcher SP, Collins JF, Roberts PJ (1983) Characterization of the binding of DL-[^{3}H]2-amino-4-phosphonobutyrate to L-glutamate-sensitive sites on rat membranes. Br J Pharmacol 80:355-364.

Calabresi P, Mercuri NB, Stefani A, Bernardi G (1992) Physiological role of $GABA_B$ receptors in the mammalian neostriatum. In: *GABAergic Synaptic Transmission* G.Biggio, A.Concas, E Costa (Eds.) Raven Press, New York, PP217-221.

Calza L, Fuxe K, Agnati F, Zini I Gahten D (1982) Presence of renin-like immunoreactivity in oxytocin immunoreactive nerve cells of the paraventricular and supraoptic nuclei in the rat hypothalamus. Acta Physiol Scand 116:313-316.

Campagnole-Santos MJ, Diz DI, Santos RAS, Khosla MC, Brosnihan KB, Ferrario CM (1989) Cardiovascular effects of angiotensin-(1-7) injected into dorsal medulla of rats. Am J Physiol 257:H324- H329.

Campbell DJ, Bouhnik J Menard J, Corvol P (1984) Identify of angiotensinogen precursors of rat brain and liver. Nature 308:206-208.

Campbell DJ, Habener JF (1986) angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. J Clin Invest 78:31-39.

Capponi AM, Lew PD, Jornot L, Valloton MB (1984) Correlation between cytosolic free Ca^{2+} and aldosterone production in bovine adrenal glomerulosa cells. J Biol Chem 259:8863-8869.

Carrithers MD, Raman VK, Masuda S, Weyhenmyer JA (1990) Effect of angiotensin II and III on inositol polyphosphate production in differentiated NG108-15 hybrid cells. Biochem Biophys Res Comm 167:1200-1205.

Casto R, Phillips MI (1986) Angiotensin II attenuates baroreflexes at nucleus tractus solitarius of rats. Am J Physiol 250:R193-R198.

Catt K, Abbott A (1991) Molecular cloning of angiotensin II receptors may presage further receptor subtypes. TiPS 12:270-281

Cedarbaum JM, Aghajanian G (1976) Noradrenergic neurons of the locus coeruleus: Inhibition of epinephrine and activation by the α-agonist piperocane. Brain Res 112:413-419.

Cedarbaum JM, Aghajanian G (1977) Catecholamine receptors on locus coeruleus neurons:pharmacological characterization. Eur J Pharmacol 44:375-385.

Cedarbaum JM, Aghajanian GK (1978a) Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. J Comp Neurol 178:1-16.

Cedarbaum JM, Aghajanian GK (1978b) Activation of locus coeruleus neurons by peripheral stimuli: Modulation by a collateral inhibitory mechanism. Life Sci 23:1383-1392.

Chai SY, Mendelsohn FAO, Paxinos G (1987) Angiotensin converting enzyme in the brain visualized by quantitative *in vitro* autoradiography. Neuroscience 20:615-627.

Chan JYH, Lee HC, Chan SHH (1991) Reduction in postsynaptic α_2-adrenoceptor activity by endogenous angiotensin III in the nucleus reticularis gigantocellularis of the rat. Neurosci Lett 133:81-85.

Changaris DG, Keil LC, Severs WB (1978) Angiotensin II immunohistochemistry of the rat brain. Neuroendocrinol 25:257-274.

Chappell MC, Tallant EA, Brosnihan KB, Ferrario CM (1990) Processing of angiotensin peptides by NG108-15 neuroblastoma × glioma hybrid cell line. Peptides 11:375-380.

Cherubini E, Herrling PL, Lanfumey L, Stanzione P (1988) Excitatory amino acids in synaptic excitation of rat striatal neurons in vitro. J Physiol 400:677-690.

Chesler M, Rice ME (1991) Extracellular alkaline-acid pH shifts evoked by iontophoresis glutamate and aspartate in turyle cerebellum. Neuroscience 41:257-267.

Chevillard C, Saavedra JM (1982) Distribution of angiotensin-converting enzyme activity in specific areas of the rat brain

stem. J Neurochem 38:281-284.

Chida K, Kawamura H, Hatano M (1983) Participation of the nucleus locus coeruleus in DOCA-salt hypertensive rats. Brain Res 273:53-58.

Chiu AT, Duncia JV, McCall DE, Wong PC, Price WA Jr, Thoolen MJ, Carini DJ, Johnson AL, Timmermans PB (1989a) Nonpeptide angiotensin II receptor antagonists. III. Structure-function studies. J Pharmacol Exp Ther 250:867-874.

Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia JV, Pease LJ, Wong PC, Wexler RR, Johnson AL (1989b) Identification of angiotensin II receptor subtypes. Biochem Biophy Res Comm 165:196-203.

Chronwall BM, DiMaggio DA, Massari VJ, Pikel VM, Ruggiero DA, O'Donohue TL (1985) The anatomy of neuropeptide-Y-containing neurons in rat brain. Neuroscience 15:1159-1181.

Cimarusti DL, Saito K, Vaughn JE, Barber R, Roberts E, Thomas PE (1979) Immunocytochemical localization of dopamine-ß-hydroxylase in rat locus coeruleus and hypothalamus. Brain Res 162:55-67.

Cintra L, Diza-Cintra S, Kemper T, Morgane PU (1982) Nucleus locus coeruleus: a morphometric golgi study in rats of three age group. Brain Res 247:17-28.

Clavier EM (1979) Afferent projections to the self-stimulation regions of the dorsal pons, including the locus coeruleus, in the rat as demonstrated by the horseradish peroxidase technique. Brain Res 4:497-504.

Collingridge GL, Lester RA (1989) Excitatory amino acid receptors in the vertebrate central nerve system. Pharmacol Rev. 40:143-208.

Collingridge GL, Kehl SJ, McLennan H (1983) The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurons in vitro. J Physiol (Lond.) 334:19-31.

Commissiong JW, Hellstrom SO, Neff NH (1978) A new projection from locus coeruleus to the spinal ventral columns: histochemical and biochemical evidence. Brain Res 148:207-213.

Conrad LCA, Leonard CM, Pfaff DW (1974) Connections of the median and dorsal raphe nuclei in the rat: An autoradiographic and degeneration study. J Comp Neurol 156:179-206.

Croucher MJ, Collins JF, Meldrum BS (1982) Anticonvulsant action of excitatory amino acid antagonists. Science 216:899-901.

Cuello AC, Kanzawa J (1978) The distribution of substance P Immunoreactive fibers in the rat central nerve system. J Comp Neurol 178:129-156.

Currie DN, Kelly JS (1981) Glia versus neuronal uptake of glutamate. J Exp Biol 95:181-193.

Curtis DR, Phillis JW, Watkins JC (1959) Chemical excitation of spinal cord neurons. Nature 183:611.

DahlstrÖm A, Fuxe K (1964) Evidence for the existence of monoamine-containing neurons in the CNS. I. demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol Scand (Suppl) 232:1-55.

Deschepper CF, Bouhnik J, Ganong WF (1986) Colocalization of angiotensinogen and gial fririllary acidic protein in astrocytes in rat brain. Brain Res 374:195-198.

Desscarries L Saucier G (1972) Disappearance of the locus coeruleus in the rat after intraventricular 6-hydroxydopamine. Brain Res 37:310-316.

Divac I, Fonnum F, Storm-Mathisen J (1977) High affinity uptake of glutamate in terminals of corticostriatal axons. Nature (Lond) 266:377-378.

Donzanti BA, Uretsky NJ (1984) Magnesium selectively inhibits NMDA induced hypermotility after intra-accumbens injection. Pharmacol Biochem Behav 20:243-246.

Drejer J, Larsson OM, Schousboe A (1982) Characterization of l-glutamate uptake into and release from astrocytes and neurons cultured from different brain region. Exp Brain Res 47:259-269.

Dumuis A, Sebben M, Haynes L, Pin JP Bockaert J (1988) NMDA receptors activate the arachidonic acid cascade system in striatal neurons. Nature 336:68-70.

Dumuis A, Pin JP, Oomagar K, Sebben M, Bockaert J (1990) Arachidonic acid released from striatal neurons by joint stimulation of ionotropic and metabotropic quisqualate receptors. Nature 347:182-184.

Dzau VJ, Ignelfinger J, Pratt RE, Ellison KE (1986) Identification of renin and angiotensinogen mRNA sequences in mouse and rat brains. Hypertention 8:544-548.

Elam M, Yao T, Thoren P, Svensson TH (1981) Hypercapnia and hypoxia: chemoreceptor-mediated control of locus coeruleus neurons and splanchnic, sympathetic nerves.

Brain Res 222:373-381.

Elam M, Yao T, Svensson TH, Thoren P (1984) Regulation of locus coeruleus neurons and splanchnic, sympathetic nerves by cardiovascular afferents. Brain Res 290:281-287.

Endres W, Ballanyi K, Serve G, Grafe P (1986) Excitatory amino acids and intracellular pH in motoneurons of isolated frog spinal cord. Neurosci Lett 72:54-58.

Engberg G, Svensson TH (1980) Pharmacological analysis of a cholinergic receptor mediated regulation of brain norepinephrine neurons. J Neural Transm 49:137-150.

Engberg G, Mihaly Hajos (1992) Ethanol attenuates the response of locus coeruleus neurons to excitatory amino acid agonists in vivo. Naunyn-Schmiedeberg's Arch Pharmacol 345:222-226.

Engberg I, Flatman JA, Lambert JDC (1978) The action of N-methyl-D-aspartate and kainic acids on motoneurons with emphasis on conductance changes. Br J Pharmacol 64:384-385p.

Ennis M, Aston-Jones G (1986) A potent excitatory input to the locus ceruleus from the ventrolateral medulla. Neurosci Lett 71:299-305.

Ennis M, Aston-Jones G (1987) Two physiological distinct populations of neurons in the ventrolateral medulla innervate the locus coeruleus. Brain Res 425:275-282.

Ennis M, Aston-Jones G (1988) Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain. J Neurosci 8:3644-3657

Ennis M, Aston-Jones G (1989a) Potent inhibitory input to locus coeruleus from the nucleus prepositus hypoglossi. Brain Res Bull 22:793-803.

Ennis M, Aston-Jones G (1989b) GABA-mediated inhibition of locus coeruleus from the dorsomedial rostral medulla. J Neurosci 9:2973-2981.

Epstein AN, Fitzsimons JT, Johnson AK (1973) Peptide antagonists of the renin-angiotensin system and elucidation of receptors for angiotensin-induced drinking. J Physiol 238:34p-35p.

Faden A, Demediuk P, Panter SS, Vink R (1989) The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science 244:798-799.

Fagard R, Amery A, Lijnen P (1981) Angiotensin II and sodium as

determinants of the agonistic-antagonistic balance of saralasin's actions. Clin Sci 60:377-385.

Fakunding JL, Chow R, Catt KJ (1979) The role of calcium in the stimulation of aldosterone production by adrenocorticotropin, angiotensin II, and potassium in isolated glomerulosa cells. Endocrinol 105:327-333.

Fakunding JL, Catt KJ (1980) Dependence of aldosterone simulation in adrenal glomerulosa cells on calcium uptake:effects of lanthanum and verapamil. Endocrinol 107:1345-1353.

Fan P, Lavoie J. Le NLO, Szerb JC, Butterworth RF (1990) Neurochemical and electrophysiological studies on the inhibitory effect of ammonium ions on synaptic transmission in slices of rat hippocampus: evidence for a postsynaptic action. Neuroscience 37:327-334.

Fan P, Szerb JC (1991) Effect of NH_4Cl on excitatory transmission in CA1 pyramidal neurons recorded intracellularly. Soc Neurosci Abstr 17:1515.

Felix D, Schelling P (1982) Increased sensitivity of neurons to angiotensin II in SHR as compared to WKY rats. Brain Res 252:63-69.

Felix D, Khosla MC, Barnes KL, Imboden H, Montani B, Ferrario CM (1991) Neurophysiological responses to angiotensin-(1-7). Hypertension 17:1111-1114.

Fernandez B, Dominguez AE, Vatta MS, Mendez MA, Bianciotti LG, Seeber M (1990) Atrial natriuretic peptide and angiotensin II interaction on noradrenaline uptake in the central nervous system. Arch int Pharmacodyn 307:11-17.

Ferrario CM, Dickison CJ, McCubbin JW (1970) Central vasomotor stimulation by angiotensins. Clin Sci 39:239-245.

Ferrario CM, Brosnihan KB, Diz DI, Jaiswal N, Khosla MC, Milsted A, Tallant EA (1991) Angiotensin-(1-7): A new hormone of the angiotensin system. hypertension 18(suppl III):III-126-III-133.

Fink GD, Haywood JR, Bryan WJ, Packwood W, Brody MJ (1980) Central site for pressor action of blood-borne angiotensin in rat. Am J Physiol 239:R358-R361.

Finkielman S, Fischer-Ferraro C, Diza A, Goldstein DJ, Nahmod VE (1972) A pressor substance in the cerebrospinal fluid of normotensive and hypertensive patients. Proc Natl Acad Sci USA 69:3341-3344.

Finlayson PG, Marshall KC (1988) characterization of a developmentally transient adrenergic depolarizing response in cultured locus coeruleus neurons. Can J Physiol Pharmacol 66:1547-1554.

Fischer-Ferraro C, Nahmod VE, Goldstein DJ, Finkielman S (1971) Angiotensin and renin in rat and dog brain. J Exp Med 133:353-361.

Fitzsimons JT (1971) The effect on drinking of peptide precursors and short chain peptides fregments of angiotensin II injected into the rat's diencephalon. J Physiol (Lond) 214:295-303.

Flott B, Seifert W (1991) Characterization of glutamate uptake systems in astrocyte primary cultures from rat brain. Glia 4:293-304.

Foder M, Gorcs TJ, Palkovits M (1992) Immunohistochemical study on the distribution of neuropeptides with the pontine tegmentum - particularly the parabrachial nuclei and the locus coeruleus of the human brain. Neuroscience 46:891-908.

Fonnum F, Storm-Mathisen J, Divac I (1981) Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibers in rat brain. Neuroscience 6:863-873.

Fonnum F (1984) Glutamate: a neurotransmitter in mammalian brain. J Neurochem 42:1-11.

Foote SL, Aston-Jones G, Bloom FE (1980) Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. Proc. Natl Acad Sci USA 77:3033-3037.

Foote SL, Bloom FE, Aston-Jones G (1983) Nucleus locus coeruleus: new evidence of anatomical and physiological specificity. Physiol Rev 63:844-914.

Foote SL, Morrison JH (1987) Extrathalamic modulation of neocortical function. Ann Rev Neurosci 10:67-95.

Foote SL, Berridge CW, Adams LM, Pineda JA (1991) Electrophysiological evidence for the involvement of locus coeruleus in alerting, orienting and attending. In: *Progress in Brain Research. vol. 88, Neurobiology of the locus coeruleus.* C.D.Barnes and O.Pompeiano (Eds.), Elsevier Science Publishers BV, Amsterdam, pp521-532.

Foster AC, Fagg GE (1984) Acidic amino acid binding sites in mammalian neuronal membrane:their characteristics and relationship to synaptic receptors. Brain Res Rev 7:103-164.

Foster R, Lobo MV, Rasmussen H, Marusic ET (1981) Calcium: Its role in the mechanism of action of angiotensin II and potassium in aldosterone production. Endocrinol 109:2196-2201.

Fox K, Armstrong-James M (1986) The role of the anterior intralaminar nuclei and N-methyl-D-aspartate receptors in the generation of spontaneous bursts in rat neocortical neurons. Exp Brain Res 63:505-518.

Frederickson RCA, Jordan LM, Phillis JW (1971) The action of noradrenaline on cortical neurons: effects of pH. Brain Res 35:556-560.

Fung SJ, Manzoni D, Chan JYH, Pompeiano O, Barnes CD (1991) Locus coeruleus control of spinal motor output. In: *Progress in Brain Research, Vol. 88, Neurobiology of the Locus Coeruleus,* C.D.Barnes and O.pompeiano (Eds.), Elsevier Science Publisher BV, Amsterdam, pp395-409.

Furuta H, Guo DF, Inagami T (1992) Molecular cloning and sequencing of the gene encoding human angiotensin II type 1 receptor. Biochem Biophys Res Comm 183:8-13.

Fuxe K, Ganten D, Hokfelt T, Bolme P (1976) Immunohistochemical evidence for the existence of angiotensin II-containing nerve terminals in the brain and spinal cord in the rat. Neurosci Lett 2:229-234.

Fuxe K, Ganten D, Hokfelt T, Locatelli V, Poulsen K, Stock G, Rix E, Taugner R (1980) Renin-like immunocytochemical activity in the rat and mouse brain. Neurosci Lett 18:245-250.

Gallo V, Giovannini C, Levi G (1989) Quisqualic acid modulates kainate responses in cultured cerebellar granule cells. J Neurochem 52:10-16.

Ganong AH, Lanthorn TH, Cotman CW (1983) Kynurenic acid inhibits synaptic and acidic amino acid-induced responses in rat hippocampus and spinal cord. Brain Res 273:170-174.

Ganong WF (1984) The renin angiotensin system. Ann Rev Physiol 46:17-31

Ganten D, Speck G (1978) The brain renin-angiotensin system: A model for the synthesis of peptides in the brain. Biochem Pharmacol 27:2379-2389.

Ganten D, Minnich JL, Granger P, Hayduk K, Brecht HM, Boucher R, Genest J (1971a) Angiotensin-forming enzyme in brain tissue. Science 173:64-65.

Ganten D, Marquez-Julio A, Granger P, Hayduk K, Karsunky KP, Boucher R, Genest J (1971b) Renin in dog brain. Am J Physiol 221:1733-1737.

Ganten D, Hutchinson S, Schelling P (1975) The intrinsic brain iso-renin angiotensin system: its possible role in central mechanisms of blood pressure regulation. Clin Sci Mol Med 48:265s-268s.

Ganten D, Schelling P, Vecsei P, Ganten U (1976) Iso-renin of external origin. The tissue angiotensinogenase system. Am J Med 60:760-722.

Ganten D, Fuxe K, Phillips MI, Mann JFE, Ganten U (1978) The brain isorenin-angiotensin system: Biochemistry, localization, and possible role in drinking and blood pressure regulation. In: *Frontiers in Neuroendocrinology. Vol. 5,* W.F.Ganong, L. Martini (Eds.), Raven, New York, pp 61-99.

Ganten D, Hermann K, Bayer C, Unger T Lang RE (1983) Angiotensin synthesis in the brain and increased turnover in hypertensive rats. Science 221:869-871.

Ganten D, Minnich JL, Lehmann E, Unger T (1984) Brain angiotensin: on the way to become a well-studied neuropeptide system. Neurochem Pharmacol 33:3523-3528.

Ganten D, Lind RW, Unger T, Lang RE, (1987) The renin-angiotensin system in the brain. In: *Integrative Neuroendocrinology: Molecular, Cellualr and Clinical Aspects.* S. McCann, R. Weiner (Eds.), Karger, Basel. pp92-101.

Garcia-Sevilla JA, Dubocovich ML, Langer SZ (1979) Angiotensin II facilitates potassium evoked release of ^{3}H-noradrenaline from the rabbit hypothalamus. Eur J Pharmacol 56:173-176.

Gardiner SM, Bennett T (1989) Brain neuropeptides:actions on central cardiovascular control mechanism. Brain Res Rev 14:79-116.

Gehlert DR, Speth RC, Wamsley JK (1986a) Quantitative autoradiography of angiotensin II receptors in SHR brain. Peptides 7:1021-1027.

Gehlert DR, Speth RC, Wamsley JK (1986b) Distribution of [^{125}I)angiotensin II binding sites in the rat brain: a quantitative autoradiographic study. Neuroscience 18:837-856.

Gehlert DR, Gackenheimer SL, Reel JK, Lin HS, Steinberg MI (1990) Non-peptide angiotensin II receptor antagonists discriminate subtypes of 125I-angiotensin II binding sites in the rat brain.

Eur J Pharmacol 187:123-126.

Gehlert DR, Gackenheimer SL, Schober DA (1991a) Angiotensin II receptor subtypes in rat brain: dithiothreitol inhibits ligand binding to AII-1 and enhances binding to AII-2. Brain Res 546:161-165.

Gehlert DR, Gackenheimer SL, Schober DA (1991b) Autoradiographic localization of subtypes of angiotensin II antagonist binding in the rat brain. Neuroscience 44:501-514.

Georgiev V, Getova D, Opitz M (1991) Mechanisms of the angiotensin II effects on exploratory behavior of rats in open field. III. Modulatory role of GABA. Meth Find Exp Clin Pharmacol 13(1):5-9.

Giffard RG, Monyer H, Christine CW, Choi DW (1990) Acidosis reduces NMDA receptor activation, glutamate neurotoxicity, and oxygen-glucose deprivation neuronal injury in cortical cultures. Brain Res 506:339-342.

Gilbert JA, Pfenning MA, Richelson E (1984) Angiotensin I, II and III-stimulated formation of cyclic GMP in murine neuroblastoma clone N1E-115. Biochem Pharmacol 33:2527-2530.

Gordon FJ, Brody MJ, Fink GD, Buggy J, Johnson AK (1979) Role of central catecholamine in the control of blood pressure and drinking behavior. Brain Res 178:161-173.

Gordon FJ, Brody MJ, Johnson AK (1985) Regional depletion of central nervous system catecholamines:effects on blood pressure and drinking behavior. Brain Res 345:285-297.

Gronan RJ and York DH (1978) Effects of angiotensin II and acetylcholine on neurons in the preoptic area. Brain Res 154:172-177

Greenamyre JT, Olson JMM, Penney JB, Young AB (1985) Autoradiographic characterization of *N*-methyl-D-aspartate-, quisqualate- and kainate-sensitive glutamate binding sites. J Pharmacol Exp Ther 233:254-263.

Gregory TJ, Wallis CJ, Printz MP (1982) Regional changes in rat brain angiotensinogen following bilateral nephrectomy. Hypertention 4:827-838.

Grillner S, McLellan A, Sigvardt K, Wallen P, Willan M (1981) Activation of NMDA receptors elicits fictive locomotion in lamprey spinal cord *in vitro*. Acta Physiol Scand 113:549-551.

Grzanna R, Molliver ME (1980a) The locus coeruleus in the rat: an

immunohistochemical delineation. Neuroscience 5:21-40.

Grzanna R, Molliver ME (1980b) Cytoarchitecture and dendritic morphorlogy of central noradrenergic neurons. In "The Reticular Formation Revisited", Eds. J. A. Hobson and M. A. B. Brazier, Raven Press, NY, PP83-97.

Gutkind JS, Kurihara M, Saavedra JM (1988) Increased angiotensin II receptors in the brain nuclei of DOCA-salt hypertensive rats. Am J Physiol 255:H646-H650.

Guyenet PG (1980) The coeruleospinal noradrenergic neurons: anatomical and physiological studies in the rat. Brain Res 189:121-133.

Guyenet PG, Aghajanian GK (1977) Excitation of neurons in the nucleus locus coeruleus by substance P and related peptides. Brain Res 136:178-184.

Guyenet PG, Aghajanian GK (1979) Acetylcholine, substance P, and met/enkephalin in the locus coeruleus: pharmacological evidence for independent sites of action. Eur J Pharmacol 53:319-328.

Guyenet PG, Filtz TM, Donaldson SR (1987) Role of excitatory amino acids in rat vagal and sympathetic baroreflexes. Brain Res 407:272-284.

Gyurko R, Kimura B, Kurian P, Crews FT, Phillips MI (1992) Angiotensin II receptor subtypes play opposite roles in regulating phosphatidylinositol hydrolysis in rat skin slices. Biochem Biophys Res Comm 186:285-292.

Hall MM, Khosla MC, Khairallan PA, Bumpus FM (1974) Angiotensin analogs: the influence of sarcosine substituted in position 1. J. Pharmacol. Exp. Ther. 188:222-228.

Harding JH, Davis JN (1985) Differencial distribution of locus coeruleus projections to the hippocampal formations: anatomical and biochemical evidence. Brain Res 325:366-369.

Harding JW, Felix D (1987a) Angiotensin-sensitive neurons in the rat paraventricular nucleus:relative potencies of angiotensin II and angiotensin III. Brain Res 410:130-134.

Harding JW, Felix D (1987b) Quantification of angiotensin iontophoresis. J Neurosci Meth 19:209-215.

Harding JW, Yoshida MS, Dilts RP, Woods TM, Wright JW (1986) Cerebroventricular and intravascular metabolism of [^{125}I]angiotensins in rat. J Neurochem 46:1292-1297.

Harding JW, Felix D, Sullivan MJ, Camara CA, Erickson JB, Regulja I, Abhold RF, Wright JW (1987) The pivotal role at angiotensin III in the rat brain angiotensin system. Proc West Pharmacol 30:11-15.

Harms L, Finta EP, Tschopl M, Illes P (1992) Depolarization of rat locus coeruleus neurons by adenosine 5'-triphosphate. Neuroscience 48:941-952.

Hartman BK, Cozzari C, Berod A, Kalmbach SJ, Faris PL (1986) Central cholinergic innervation of the locus coeruleus. Soc Neurosci Abstr 12:770.

Haas HL, Felix D, Davis MD (1982) Angiotensin excites hippocampal pyramidal cells by two mechanisms. Cell Mol Neurobiol 2:21-32.

Hattori Y and Koizumi K (1990) Sensitivity to angiotensin II of neurons in the circumventricular organs of polydipsic inbred mice. Brain Res 524:181-186

Hayer BA, Balster RL (1985) Anticonvulsant properties of phencyclidine-like drugs in mice. Eur J Pharmacol 117:121-126.

Healy DP, Printz MP (1984) Distribution of immunoreactive angiotensin II, angiotensin I, angiotensinogen and renin in central nervous system of intact and nephrectomized rats. Hypertension Suupl. I: I130-I136.

Healy DP, Maciejewski AR, Printz MP (1986) Localization of central angiotensin II receptors with [^{125}I]-Sar^{1},Ile^{8}-angiotensin II: periventricular sites of the anterior third ventricle. Neuroendocrinol 44:15-21.

Henderson G, pepper CM, Shefner SA (1982) Electrophysiological properties of neurons contained in the locus coeruleus and mesencephalic nucleus of the trigeminal nerve in vitro. Exp Brain Res 45:29-37.

Hermann K, Lang RE, Unger T, Bayer C, Ganten D (1984) Combined high-performance liquid chromatography radioimmunoassay for the characterization and quantitative measurement of neuropeptides. J Chromatogr 312:273.

Hermann K, Kimura B, Phillips MI (1986) Isolation and purification of angiotensin II using affinity and high-pressure liquid chromatography. Anal Biochem 159:295-302.

Hermann K, Phillps MI, Hilgenfelt, Raizada MK (1988a) Biosynthesis of angiotensinogen and angiotensins by brain cells in primary culture. J Neurochem 51:398-405.

Hermann K, Raizada MK, Sumners C, Phillips MI (1988b) Immunocytochemical and characterization of angiotensin I and II in cultured neuronal and glia cells from rat brain. Neuroendocrinol 47:125-132.

Hewes CR, Frederickson RCA (1974) Responses of cortical neurones in rat to microiontophoretically applied hydrogen ion. Neuropharmacol 13:1077-1081.

Hicks TP, Krupa M, Crepel F (1989) Selective effects of serotonin upon excitatory amino acid-induced depolarizations of Purkinje cells in cerebellar slices from young rats. Brain Res 492:371-376.

Hinson JP, Vinson GP, Whitehouse BJ, Price G (1985) Control of zona glomerulosa function in the isolated perfused rat adrenal gland in situ. J Endocrinol 104:387-395

Hirose S, Yokosawa H, Inagami T (1978) Immmunochemical identification of renin in rat brain and distinction from acid proteases. Nature 274:392-393.

Hirose S, Yokosawa H, Inagami T, Workman RJ (1980) Renin and prorenin in hog brain: Ubiquitous distribution and high concentration in the pituitary and pineal. Brain Res 191:489-499.

Hobson JA, McCarley RW, Wyzinski PW (1975) Sleep cycle oscillation: reciprocal discharge by two brain stem neuronal groups. Science 189:55-58.

Hoffman WE, Phillips MI, Schmid PG (1977) Central angiotensin II-induced responses in spontaneously hypertensive rats. Am J Physiol 232:H426-H433.

Hokfelt T, Fuxe K, Goldstein M, Johansson O (1974) Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. Brain Res 66:255-257.

Holets VR, Hokfelt T, Rokaeus A, Terenius L, Goldstein M (1988) Locus coeruleus neurons in the rat containing neuropeptide Y, tyrosine hydroxylase or galanin and their efferent projections to the spinal cord, cerebral cortex and hypothalamus. Neuroscience 24:893-906.

Howe JR, Sutor B, Zieglgansberger W (1987) Bacolfen reduces postsynaptic potentials of rat cortical neurons by an action other than its hyperpolarizing action. J Physiol 384:539-569.

Huang Y, Rogers J, Henderson G (1987) Effects of angiotensin II on [^{3}H]noradrenaline release and phosphatidylinositol hydrolysis in the parietal cortex and locus coeruleus of the rat.

J Neurochem 49:1541-1549.

Hutchinson JS, Schelling P, Ganten D (1975) Effect of centrally administered angiotensin II and P113 on blood pressure in conscious rats. Pflugers Arch Ges Physiol 335(suppl.):R28.

Igarashi S, Sasa M, Takaori S (1979) Convergence of sensory input from tooth pulp, optic chiasm and sciatic nerve onto locus coeruleus. Neurosci Lett 12:189-193.

Imboden H, Harding JW, Hilgenfeldt U, Celio MR, Felix D (1987) Localization of angiotensinogen in multiple cell types of rat Brain. Brain Res 410:74-77.

Inagami T, Okamura T, Hirose S, Clemens DL, Cello MR, Naruse K, Takii Y, Yokosawa H (1982a) Identification, characterization and evidence for intraneuronal function of renin in the brain and neuroblastoma cells. In: *Experimental Brain Research, Suppl. 4, The Renin-Angiotensin System in the Brain.* D. Ganten, M. printz, M.I. Phillips, B.A. Scholken (Eds.), Springer-Verlag, Berlin. pp.64-75.

Inagami T, Clemens DL, Hirose S, Okamura T, Naruse K, Takii Y, Yokosawa H (1982b) Brain renin. Clin Exp Hyperten A4:607-622.

Intebi AD, Flaxman MS, Ganong WF, Deschepper CF (1990) Angiotensinogen production by rat astroglial cells in vitro and in vivo. Neuroscience 34:545-554.

Iwai N, Yamano Y, Chaki S, Konishi F, Bardhan S, Tibbetts C, Sasaki K, Hasegawa M, Matsuda Y, Inagami T (1991) Rat angiotensin II receptor: cDNA sequence and regulation of the gene expression. Biochem Biophys Res Comm 177:299-304.

Iwasaki Y, Kinoshita, Ikeda K, Shiojima, Kurihara T, Appel SH (1991) Trophic effect of angiotensin II, vasopressin and other peptides on the cultured ventral spinal cord of rat embryo. J Neurol Sci 103:151-155.

Jahr CE, Jessell TM (1985) Synaptic transmission between dorsal root ganglion and dorsal horn neurons in culture:antagonism of monosynaptic excitatory postsynaptic potentials and glutamate excitation by kynurenate. J Neurosci 5:2281-2289.

Jahr CE, Yoshioka K. (1986) I_{α} afferent excitation of motoneurones in the *in vitro* new-born rat spinal cord is selectively antagonized by kynurenate. J Physiol 370:515-530.

Jarolimek W, Misgeld U, Lux HD (1989) Activity dependent alkaline and acid transients in guinea pig hippocampal slices. Brain Res Brain Res 505:225-232.

Jin JS, Hsieh PS, Huang WC (1992) Enhanced renal response to intracerebroventricular angiotensin II and III in spontaneous hypertensive rats. Brain Res 582:268-276.

Johansson O, Hokfelt T, Elde RP (1984) Immunohistochemical distribution of somatostatin-like immunoreactivity in the central nervous system of the adult rat. Neuroscience 13:265-339.

Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529-531.

Johnson MC, Aguilera G (1991) Angiotensin-II receptor subtypes and coupling to signalling systems in cultured fetal fibroblasts. Endocrinology. 129:1266-1274.

Jones BE, Yang TZ (1985) The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. J Comp Neurol 242:56-92.

Jones BE, Pare M, Beaudet A (1986) Retrograde labelling of neurons in the brain stem following injections of [^{3}H] choline into the rat spinal cord. Neuroscience 18:901-916.

Jones BE (1990) Influence of the brain stem reticular formation, including intrinsic monoaminergic and cholinergic neurons, upon forebrain mechanisms of sleep and waking In: *The Diencephalon and Sleep.* M. Mancia and G. Marini (Eds.), Raven Press, New York, pp.31-48.

Jones BE (1991) The role of noradrenergic locus coeruleus neurons and neighbouring cholinergic neurons of the pontomescencephalic tegmentum in sleep-wake states. In: *Progress in Brain Research, Vol. 88, Neurobiology of the Locus Coeruleus.* C.D.Barnes and O.Pompeiano (Eds.), Elsevier Science Publishers BV, Amsterdam, pp533-543.

Jones SL (1991) Descending noradrenergic influences on pain. In: *Progress in Brain Research, Vol. 88, Neurobiology of the Locus Coeruleus.* C.D.Barnes and O.Pompeiano (Eds.), Elsevier Science Publishers BV, Amsterdam, pp.381-394.

Jones KA, Baughman RW (1988) NMDA-and non-NMDA receptor components of excitatory synaptic potentials recorded from cells in layer V of rat visual cortex. J Neurosci 8:3522-3534.

Joy MD, Lowe RD (1970) Evidence that the area postrema mediates the central cardiovascular response to angiotensin II. Nature 228:1303-1304.

Kaczmarek LK, Levitan IB (1987) What is neuromodulation? In:

Neuromodulation:The biochemical control of neuronal excitability. L.K. Kaczmarek and I.B. Levitan (Eds.), Oxford University Press, pp 1-17.

Kaila K, Voipio J (1987) Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance. Nature 330:163-165.

Kalinyak JE, Perlman AJ (1987) Tissue-specific regulation of angiotensinogen mRNA accumulation by dexamethasone. J Biol Chem 262:460-464.

Kalinyak JE, Hoffman AR, Perlman AJ (1991) Ontogency of angiotensinogen mRNA and angiotensin II receptors in rat brain and liver. J Endocrinol Invest 14:647-653.

Kang J, Sumners C, Posner P (1992a) Angiotensin II (AII) type 2 (AT_2) receptor-mediated effects on potassium currents in cultured neurons. Soc Neurosci Abstr 18:1162.

Kang J, Sumners C, Posner P (1992b) Modulation of net outward current in cultured neurons by angiotensin II: involvement of AT_1 and AT_2 receptors. Brain Res 580:317-324.

Kasamatsu T (1991) Adrenaergic regulation of visuocortical plasticity: a role of the locus coeruleus system. In: *Progress in Brain Research, Vol. 88, Neurobiology of the Locus Coeruleus.* C.B. Barnes and Opompeiano (Eds.), Elsvier Scirncr Publishers, pp599-616.

Kangrga I, Larew JS, Randic M (1990) The effects of substance P and calcitonin gene-related peptides on the efflux of endogenous glutamate and aspartate from the rat spinal dorsal horn in vitro. Neurosci lett 108:155-160.

Kawamura H, Gunn CG, Frolich ED (1978) Cardiovascular alterations by nucleus locus coeruleus in the spontaneously hypertensive rat. Brain Res 140:137-148.

Khairallah PA (1972) Action of angiotensin on adrenergic nerve endings: inhibition of norepinepherine uptake. Fed Proc 31:1351-1357.

Kim JS, Hassler R, Haug P, Paik KS (1977) Effect of frontal cortex ablation on the striatal glutamic acid level in rat. Brain Res 132:370-374.

Knowles WD, Phillips MI (1980) Angiotensin II responsive cells in the organum vasculosum lamina terminals (OVLT) recorded in hypothalamic brain slices. Brain Res 195:256-

Kobayashi RM, Brownstein M, Saavedra JM, Palkovits M (1975)

Choline acetyltransferase content in discrete regions of the rat Brain stem. J Neurochem 24:637-640.

Korf J, Bunney BS, Aghajanian GK (1974) Noradrenergic neurons: Morphine inhibition of spontaneous activity. Eur J Pharmacol 25:165-169.

Kraig RP, Ferreira-Filho CR, Nicholson C (1983) Alkaline and acid transmitters in cerebellar microenvironment. J Neurophysiol 49:831-850.

Krishtal OA, Osipchuk YV, Shelest TN, Smirnoff SV (1987) Rapid extracellular pH transients related to synaptic transmission in rat hippocampal slices. Brain Res 436:352-356.

Kubo T, Kihara M (1991) Unilateral blockade of excitatory amino acid receptors in the nucleus tractus solitarii produces an inhibition of baroreflexes in rats. Naunyn-Schmiedberg's Arch Pharmacol 343:317-322.

Kuhar MJ, Roth RH, Aghajaian GK (1972) Synthesis of catecolamine in the locus coeruleus from ^{3}H-tyrosine in vivo. Biochem Pharmacol 21:2280-2282.

Kumar A, Rassoli A, Raizada MK (1988) Angiotensinogen gene expression in neuronal and gial cells in primary cultures of rat brain. J Neurosci Res 19:287-290.

Kumar RS, Kusari J, Roy SN, Soffer RL, Ganes C (1989) Structure of testicular angiotensin converting enzyme. J Biol Chem 264:16754-16758.

Lai YY, Strahlendorf HK, Fung SJ, Barnes CD (1989) The actions of two monoamines on spinal cord motoneurons from stimulation of locus coeruleus in the cat. Brain Res 484:268-272.

Lee M, Strahlendorf JC, Strahlendorf HK (1986) Modulatory action of serotonin on glutamate-induced excitation of cerebellar Purkinje cells. Brain Res 361:107-113.

Leone C, Gordon FJ (1989) Is L-glutamate a neurotransmitter of baroceptor information in the nucleus of the tractus solitarius? J Pharmacol Exp Ther 250:953-962.

Leroux P, Quirion R, Pelletier G (1985) Localization and characterization of brain somastatin receptors as studied with somastatin-14 and somastatin-28 receptor radioautography. Brain Res 347:74-84.

Levitt P, Moore RY (1978) Noradrenaline neuron innervation of neocortex in the rat. Brain Res 139:219-231.

Leung KH, Smith RD, Pieter, Timmermans BM, Chiu AT (1991) Regional distribution of the two subtypes of angiotensin II receptor in rat brain using selective nonpeptide antagonists. Neurosci Lett 123:95-98.

Lewicki JA, Fallon JH, Printz MP (1978) Regional distribution of angiotensinogen in rat brain. Brain Res 158:359-371

Lin KS, Chan JYH, Chan SHH (1988) Reduction in baroreceptor reflex sensitivity by angiotensin III and its modification by Ile^7- angiotensin III and bestatin in the rat. Neurosci Lett 90:172-176.

Lind RW, Swanson LW, Ganten D (1985) Organization of angiotensin II immunoreactive cells and fiblers in the rat central nerve system. An immunohistochemical study. Neuroendocrinol 40:2-24.

Lindvall O, Bjorklund A, Nobin A, Stenevi U (1974) The adrenergic innervation of the rat thalamus as revealed by the glyoxylic acid fluorescence method. J Comp Neurol 154:317-348.

Loewy AD, Wallach JH, McKeller S (1981) Efferent projections of the ventral medulla oblongata in the rat. Brain Res Rev 3:653-680.

Loizou LA (1969) Projections of the nucleus locus coeruleus in the albino rat. Brain Res 15:563-566.

Loughlin SE, Foote SL, Fallon JH (1982) Locus coeruleus projections to cortex: topography, morphology and collateralization. Brain Res Bull 9:287-294.

Loy R, Koziell DA, Lindsey JD, Moore RY (1980) Noradrenergic innervation of the adult rat hippocampal formation. J Comp Neurol 189:699-710.

Lynch KR, Simnad VI, Ben-Ari ET, Garrison JC (1986) Localization of preangiotensinogen messenger RNA sequences in the rat brain. Hypertension 8:540-543.

Lyons WE, Grzanna R (1988) Noradrenergic neurons with divergent projections to the motor trigeminal nucleus and spinal cord: A double retrograde neuronal labelling study. Neuroscience 26:681-693.

Lyons WE, Fritschy JM, Grzanna R (1989) The noradrenergic neurotoxin DSP-4 eliminates the coeruleo-spinal projection but spares projections of the A5 and A7 groups to the ventral horn of the rat spinal cord. J Neurosci 9:1481-1489.

MacDermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL (1986)

NMDA receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurons. Nature 321:519-522.

MacDonald JF, Wojtowicz JM (1980) Two conductance mechanisms activated by applications of L-glutamic, L-aspartic, DL-homocysteic, N-methyl-D-aspartic, and DL-kainic acids to cultured mammalian central neurons. Can J Physiol Pharmacol 58:1393-1397.

MacDonald JF, Porietis AV, Wojtowicz JM (1982) L-Aspartic acid induces a region of negative slope conductance in the current-voltage relationship of cultured spinal neurons. Brain Res 237:248-253.

Maclean MR, Raizada MK, Summers C (1990) The influence of angiotensin II on catecholamine synthesis in neuronal cultures from rat brain. Biochem Biophys Res Comm 167:492-497

Madison DV, Nicoll RA (1986) Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 pyramidal neurons, in Vitro. J Physiol 372:221-244.

Mann JFE, Phillips MI, Dietz R, Ganten D (1978) Effects of central and peripheral angiotensin blockade in hypertensive rats. Am J Physiol 234:H629-H637.

Mann JFE, Schiller PW, Schiffrin EL, Boucher R, Genest J (1981) Brain receptor binding and central actions of angiotensin analogs in rats. Am J Physiol 241:R124-R129.

Marie J, Jard S (1981) Angiotensin II inhibits adenylate cyclase from adrenal cortex glomerulosa zone. FEBS Lett 159:97-101.

Marshall KC, Engberg I (1980) The effects of hydrogen ion on spinal neurons. Can J Physiol Pharmacol 58:650-655.

Marshall KC (1983) Catecholamine and their actions in the spinal cord. In: *Handbook of the Spinal Cord, Vol. 1, Pharmacology,* Davidoff RA (Ed.), Marcel Dekker, Inc., New York, pp275-328.

Marshall KC, Finlayson PG (1988) Synaptic regulation of locus coeruleus neuronal activity. In: Neurotransmitters and cortical function. (Avoli M, Reader TA, Dykes RW, Gloor P, eds), pp373-390. Plenum Publishing Co.

Marshall KC, Tsai W (1988) Noradrenaline induces short and long duration potentiation of glutamate excitations of cultured Purkinle neurons. Can J Physiol Pharmacol 66:848-853.

Marshall KC and Xiong H (1991) Modulation of amino acid neurotransmitter action by other neurotransmitters: some

examples. Can J Physiol Pharmacol 69:1115-1122

Mason ST, Fibiger HC (1979) Regional topography within noradrenergic locus coeruleus as revealed by retrograde transport of horseradish peroxidase. J Comp Neurol 187:703-724.

Mattson M, Dou P, Kater SB (1988) Outgrowth-regulating actions of glutamate in isolated hippocampal pyramidal neurons. J Neurosci 8:2087-2100.

Mauzy CA, Hwang O, Egloff AM, Wu LH, Chung FZ (1992) Cloning, expression, and characterization of a gene encoding the human angiotensin type 1A receptor. Biochem Biophys Res Comm 186: 277-284.

Mayer ML, Westbrook GL (1987) The physiology of excitatory amino acids in the vertebrate central nervous system. Prog Neurobiol 28:197-276.

Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurons. Nature 309:261-263.

McKinley MJ, Allen AM, Clevers J, Denton DA, Mendelsohn FAO (1986) Autoradiographic localization of angiotensin receptors in the sheep brain. Brain Res 375:373-376.

McKinley MJ, Allen AM, Clevers J, Paxinos G, Mendelsohn FAO (1987) Angiotensin receptor binding in human hypothalamus: Autoradiographic localization. Brain Res 420:375-379.

McLennan H, lodge D (1979) The antagonism of amino acid-induced excitation of spinal neurons in the cat. Brain Res 169:83-90.

McLennan H (1976) The autoradiographic localization of L-[^{3}H] glutamate in rat brain tissue. Brain Res 115:139-144.

Meibach RC (1984) Serotonergic receptors. In: *Handbook of Chemical Neuroanatomy. Vol. 3,* A. Bjorklund, T. Hokfelt, M.J. Kuhar (Eds.), Elsevier, Amsterdam, pp304-324.

Melander T, Hokfelt T, Rokaeus A (1986) Distribution of galaninlike immunoreactivity in the rat central nervous system. J Comp Neurol 248:475-517.

Mendelsohn FAO, Quirion R, Saavedra JM, Aguilera G, Catt KJ (1984) Autoradiographic localization of angiotensin II receptors in the rat brain. Proc Natl Acad Sci USA 81:1575-1579

Mendelsohn FAO (1985) Localization and properties of angiotensin receptors. J Hypertension 3:307-316.

Mendelsohn FAO, Allen AM, Clevers J, Denton DA, Tarjan E, McKinley MJ (1988) Localization of angiotensin II receptor binding in rabbit brain by in vitro autoradiography. J Comp Neurol 270:372-384.

Meyer JM, Weyhenmeyer JA. (1986) Chemically evoked release of an angiotensin-like peptide from fetal rat brain cells in culture. Am J Physiol 251:C17-C22

Meyer JM, Felten DL, Weyhenmeyer JA (1989) Levels of immunoreactive angiotensin II in microdissected nuclei from WKY and SH rat brain. Clin Exp Hypertens 11:103-117.

Mikami H, Suzuki H, Smeby RR, Ferrario CM (1985) Cerebrospinal fluid angiotensin II immunoreactivity is not derived from the plasma. Hypertension 7:65-71.

Milner TA, Joh TH, Miller RJ, Pickel VM (1984) Substance P, neurotensin, enkephalin, and catecholamine-synthesizing enzymes: light microscopic localization compared with autoradiographic label in solitary efferents to the rat parabrachial region. J Comp Neurol 226:434-437.

Miura M, Reis DJ (1969) Termination of secondary projections of carotid sinus nerve in the cat brainstem. Am J Physiol 217:142-153.

Moises HC, Woodward DJ, Hoffer BJ, Freedman R (1979) Interactions of norepinepherine with Purkinje cell responses to putative amino acid neurotransmitter applied by microiontophoresis. Exp Neurol 64:493-515.

Monaghan DT, Yao D, Cotman CW (1985) L-[^{3}H]glutamate binds to kainate-. NMDA- and APMA-sensitive binding sites: an autoradiographic analysis. Brain Res 340:378-383.

Monaghan DT, Cotman CW (1986) Anatomical organizations of NMDA, kainate and quisqualate receptors. In: *Excitatory Amino Acids.* P. J. Roberts, J. Storm-Mathisen, H. F. Bradford (Eds.), MacMillan Press, London. pp.279.

Moore RY, bloom FE (1979) Central catecholamine neuron systems: Anatomy and physiology of the norepinephrine and epinephrine systems. Ann Rev Neurosci 2:113-168.

Moore RY, Card JP (1984) Noradrenaline-containing neuron system. in: Handbook of Chemical Neuroanatomy: Vol. 2 , A. Bjorklund and T. Hokfelt (eds.), pp123-156. New York, Amsterdam.

Morgane PJ, Jacobs MS (1979) Raphe projections to the locus

coeruleus in the rat. Brain Res Bull 4:519-534.

Mori-Okamoto J, Tatsuno J (1988) Effects of noradrenaline on the responsiveness of cultured cerebellar neurons to excitatory amino acids. Brain Res 448:259-271.

Mori-Okamoto J, Tatsuno J (1989) Participation of cyclic adenosine monophosphate and ß-adrenergic receptors in the facilitatory effect of noradrenaline on the response of cultured cerebellar neurons to glutamate. Brain Res 490:64-72.

Morris J, Reid IA (1978) The distribution of angiotensinogen in dog brain studied by cell fractionation. Endocrinol 103:492-500.

Mutch WAC, Hanssen AJ (1984) Extracellular pH changes during spreading depression and cerebral ischemia:mechanisms of brain pH regulation. J Cerebral Blood Flow and Metabolism 4:17-27.

Murphy TJ, Alexander RW, Griendling KK, Runge MS, Bernstein KE (1991) Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. Nature. 351(6323):233-236.

Nahmod VE, Finkielman S, Benarroch EE, Pirola CJ (1978) Angiotensin regulates release and synthesis of serotonin in brain. Science 202:1091-1093

Nagai T, Satoh K, Imamoto K, Maeda T (1981) Divergent projections of catecholamine neurons of the locus coeruleus as revealed by fluorescent retrograde double labelling technique. Neurosci Lett 23:117-123.

Nakamura S, Sakaguchi T (1990) Development and plasiticity of the locus coeruleus: A review of recent physiological and pharmacological experimentation. Prog Neurobiol 34:505-526

Nakamura S, Kimura F, Sakaguchi T (1987) Postnatal development of electrical activity in the locus coeruleus. J Neurophysiol 58:510-524.

Nakazato T (1987) Locus coeruleus neurons projecting to the forebrain and the spinal cord in the cat. Neuroscience 23:529-538.

Nedergaard S, Engberg I, Flatman JA (1987) The modulation of excitatory amino acid responses by serotonin in the cat neocortex in vitro. Cell Mol Neurobiol 7:367-379.

Nicoll, RA, Barker JL (1971) Excitation of supraoptic neurosecretory cells by angiotensin II. Nature 233:172-174.

Nicoll R, Malenka RC, Kauer JA (1990) Functional comparison of

neurotransmitter receptor subtypes in mammalian central nervous system. Physiol Rev 70:513-565.

Nomura S, Mizutami S, Kurauchi O, Kasugai M, Narita O, Tomoda Y (1990) Effects of low molecular weight peptides and divalent cations on degradation and binding of angiotensin II. Horm Meta Res 22:444-448

North RA, Williams JT (1983) μ-type opiate receptors on single locus coeruleus neurons. Br J Pharmacol 79:423p.

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurons. Nature 307:462-465.

Nygren LG, Olson L (1977) A new major projection from locus coeruleus: the main source of noradrenergic nerve terminals in the ventral and dorsal columns of the spinal cord. Brain Res 132:85-93.

O'Brien RJ, Fishbach GD (1986) Characterization of excitatory amino acid receptors expressed by embryonic chick motoneurons in vitro. J Neurosci 6:3275-3783.

O'Dell Tj, Christensen BN (1989a) A voltage-clamp study of isolated stingray horizontal cell non-NMDA excitatory amino acid receptors. J Neurophysiol 61:162-172.

O'Dell Tj, Christensen BN (1989b) Horizontal cells isolated from catfish retina contain two types of excitatory amino acid receptors. J Neurophysiol 61:1097-1109.

Olney JW (1984) Excitotoxins: an overview. In: *Excitotoxins,* K. Fuxe, P. J. Roberts and M. T. Schwarcz, (Eds.) Macmillan, London, pp. 82-96.

Ohkubo H, Nakayama K, Tanaka T, Nakanishi S (1986) tissue distribution of rat angiotensinogen mRMA and structure analysis of its heterogeneity. J Biol Chem 261:319-323.

Olpe HR, Baltzer V (1981) Vasopressin activates noradrenergic neurons in the locus coeruleus: a microiontophoretic investigation. Eur J Pharmacol 73:377-378.

Olpe HR, Jones RSG (1982) Excitatory effects of ACTH on noradrenergic neurons of the locus coeruleus in rat. Brain Res 251:177-179.

Olpe HR, Steinmann MW, Brugger F, Pozza MF (1989) Excitatory amino acid receptors in the rat locus coeruleus: an extracellular in vitro study. Naunyn-Schmiedeberg's Arch Pharmacol 339:312-314.

Olson L, Fuxe K (1971) On the projections from the locus coeruleus noradrenaline neurons: The cerebellar innervation. Brain Res 28:165-171.

Olson L, Fuxe K (1972) Further mapping out of central noradrenaline nervous systems: projections of the "subcoeruleus" area. Brain Res 43:389-295.

Osman MY, Smeby RR, Sen S (1979) Separation of dog brain renin-like activity from acid protease activity. Hypertension 1:53-60.

Osmanovic SS (1991) Excitatory action of thyrotropin releasing hormone on rat locus coeruleus neurons in vitro. Period Biol 93:301-302.

Osmanovic SS, Shefner SA (1990) Gamma-aminobutyric acid responses in the rat locus coeruleus neurons in vitro: a current clamp and voltage clamp study. J Physiol 421:151-170.

Ottersen OP (1981) Afferent connections to the amygdala complx of the rat with some observations in the cat. III. Afferents from the lower brain stem. J Comp Neurol 202:335-356.

Ottersen OP, Storm-Mathisen J (1984) Neurons containing or accumulating transmitter amino acids. In: *Handbook of chemical neuroanatomy. Vol. 3. Classical transmitters and transmitter receptors in the CNS.* Part II. A. Bjorklund, T. Hokfelt, M.J. Kuhar (Eds.), Elsevier, Amsterdam, pp141-224.

Palacios JM, Wamsley JK, Kuhar MJ (1981) The distribution of histamine H_1-receptors in the rat brain: An autoradiographic study. Neuroscience 6:15-37.

Pasquier DA, Gold MA, Jacobowitz DM (1980) Noradrenergic perikarya (A5-A7, subcoeruleus) projections to the rat cerebellum. Brain Res 196:270-275.

Paul M, Printz MP, Harms E, Unger T, Lang RE, Ganten D (1985) Localization of renin (EC 3.4.23) and converting enzyme (EC 3.4.15.1) in nerve endings of rat brain. Brain Res 334:315-324.

Pendleton RG, Gessner G, Horner E (1989) Studies defining minimal receptor domains for angiotensin II. J Pharmacol Exp Ther 250:31-36.

Pepper CM, Henderson G (1980) Opiates and opioid peptides hyperpolarize locus coeruleus neurons in vitro. Science 209:394-395.

Pert CB, Kuhar MJ, Snyder SH (1975) Autoradiographic localization

of the opiate receptor in the brain. Life Sci 16:1849-1854.

Pfeilschifter J (1990) Angiotensin II B-type receptor mediates phospoinositide hydrolysis in mesangial cells. Eur J Pharmacol 184:201-202.

Philiips MI (1978) Angiotensin in the brain. Neuroendocrinol 25:354-377.

Phillips MI (1987) Functions of angiotensin in the central nervous system. Ann Rev Physiol 49:413-435

Phillips MI, Stenstrom B (1985) Angiotensin II in rat brain comigrates with authentic angiotensin II in HPLC. Circ Res 56:212-219.

Pickel VM, Joh TH, Reis DJ (1977) A serotonergic innervation of noradrenergic neurons in nucleus locus coeruleus: Demonstration by immunocytochemical localization of the transmitter specific enzymes tyrosine and tryptophan hydroxylase. Brain Res 131:197-214.

Pin JP, Van Vliet BJ, Boockaert J (1989) Complex interaction between quisqualate and kainate receptors as revealed by measurement of GABA release from striatal neurons in primary culture. Eur J Pharmacol 172:81-91

Plummer TH, Ryan TJ (1981) A potent mercapto bi-product analogue inhibitor for human carboxypeptidase N. Biochem Biophys Res Comm 98:448-454

Plunkett LM, Saavedra JM (1985) Increased angiotensin II binding affinity in the nucleus tractus solitarius of spontaneous hypertensive rats. Proc Natl Acad Sci USA 82:7721-7724.

Pobiner BF, Northup Jk, Bauer PH, Fraser ED, Garrison JC (1991) Inhibitory GTP-binding regulatory protein G_{i3} can couple angiotensin II receptors to inhibition of adenylyl cyclase in hepatocytes. Mol Pharmacol 40:156-167.

Price JL, Amaral DG (1981) An autoradiographic study of the projections of the central nucleus of the monkey amygdala. J Neurosci 1:1242-1259.

Printz MP, Lewicki JA (1977) Renin substrate in the CNS: potential significance to central regulatory mechanisms. In" Central actions of angiotensin and related hormones", eds. Buckley and Ferrario. pp57-64. Pergamon Press, New York.

Proudfit HK (1988) Pharmacologic evidence for the modulation of nociception by noradrenergic neurons. In: *Progress in Brain Research,*

Vol. 77, H.L. Fields and J.M. Besson (Eds.), Elsevier, Amsterdam, pp357-370.

Pucell AG, Hodges JC, Sen I, Bumpus FM, Husain A (1991) Biochemical properties of the ovarian granulosa cell type 2-angiotensin II receptor. Endocrinol 128:1947-1959.

Qadri F, Badoer E, Stadler T, Unger T (1991) Angiotensin II-induced noradrenaline release fran anterior hypothalamus in conscious rats: a brain microdialysis study. Brain Res 563:137-141

Quirion R, Zajac JM, Morgat JL, Roques BP (1983) Autoradiographic distribution of mu and delta opiate receptor in rat brain using highly selective ligands. Life Sci 33(Suppl. I):227-230.

Quirion R, Dalpe M, DeLean A, Gutkowska J, Cantin M, Genest J (1984) Atrial natriuretic factor (ANF) binding sites in brain and related structures. Peptides 5:1167-1172.

Raabe W (1989) Ammonium decreases excitatory synaptic transmission in cat spinal cord in vivo. J Neurophysiol 62:1461-1473.

Rainey WE, Byrd WB, Sinnokrot RA, Carr BR (1991) Angiotensin II activation of cAMP and corticosterone production in bovine adrenocortical cells:effects of nonpeptide angiotensin-II antagonists. Mol Cell Endocrinol 81:33-41.

Raizada MK, Yang JW, Phillips MI, Fellows RE (1981) Rat brain cells in primary culture: Characterization of angiotensin II binding sites. Brain Res 207:343-345.

Raizada MK, Phillips MI, Gerndt JS (1983) Primary cultures from fetal rat brain incorporate ^{3}H-isoleucine and ^{3}H-valine into immunoprecipitable angiotensin II. Neuroendocrinol 36:64-67.

Raizada MK, Muther TF, Sumners C (1984) Increased angiotensin II receptors in neuronal cultures from hypertensive rat brain. Am J Physiol 247:C364-C372.

Raizada MK, Phillips MI, Crews FT, Sumners C (1987) Distinct angiotensin II receptor in primary cultures of glia cells from rat brain. Proc Natl Acad USA 84:4655-4659.

Randic M, Hecimovic H, Ryu PD (1990) Substance P modulates glutamate-induced currents in acutely isolated rat spinal dorsal horn neurons. Neurosci Lett 117:74-80.

Regoli D, Rioux F, Park WK, Choi C (1974) Role of the N-terminal amino acid for the biological activities of angiotensin and

inhibitory analogues. Can J Physiol Pharmacol 52:39-49.

Reid IA (1979) The brain renin-angiotensin system: a critical analysis. Fed Proc 38:2255-2259.

Reid IA, Ramsay DJ (1975) The effects of intracerebroventricular administration of renin on drinking and blood pressure. Endocrinol 97:536-542.

Robert PJ and Watkins JC (1975) Structural requirements for the inhibition for L-glutamate uptake by glia and nerve endings. Brain Res 85:120-125.

Robbins TW (1984) Cortical noradrenaline, attention and arousal. Psychological Medicine 14:13-21.

Roth RH, Hughes J (1972) Acceleration of protein synthesis by angiotensin. Correlation with angiotensin's effect on catecholamine biosynthesis. Biochem Pharmacol 21:3182-3187.

Rotter A, Birdsall NJM, Field PM, Raisman G (1979) Muscarine receptors in the central nervous system of the rat. II. Distribution of binding of [^{3}H]propylbenilylcholine mustard in the midbrain and hindbrain. Brain Res Rev 1:167-183.

Rowe BP, Kalivas PW, Speth RC (1990a) Autoradiographic localization of angiotensin II receptor binding sites on noradrenergic neurons of the locus coeruleus of the rat. J Neurochem 55:533-40.

Rowe BP, Grove KL, Saylor DL, Speth RC (1990b) Angiotensin II receptor subtypes in the rat Brain. Eur J Pharmacol 186:339-342.

Rowe BP, Grove KL, Saylor DL, Speth RC (1991) Discrimination of angiotensin II receptor subtype distribution in the rat brain using non-peptidic receptor antagonists. Regulatory Peptides. 33:45-53.

Russell GV (1955) The nucleus locus coeruleus (dorsolateral tegmenti). Tex Rep Biol Med 13:939-988.

Saavedra Jm, Correa FMA, Plunkett LM, Israel A, Kurihara M, Shigematsu K (1986a) Binding of angiotensin and atrial natriuretic peptide in brain of hypertensive rats. Nature 320:758-760.

Saavedra JM, Israel A, Plunkett LM, Kurihara K, Shigematsu K, Correa FMA (1986b) Quantitative distribution of angiotensin II binding sites in rat brain by autoradiography. Peptides 7:679-687.

Sakaguchi T, Nakamura S (1987) Some in vivo electrophysiological properties of locus coeruleus neurons in fetal rats. Exp Brain Res 68:122-130.

Sakanaka M, Shibansaki T, Lederis K (1987) Corticotrophin releasing factor-like immunoreactivity in the rat brain as revealed by a modified cobalt-glucose oxidase-diaminobenzidine method. J Comp Neurol 260:256-298.

Santos RAS, Brosnihan KB, Chappell MC, Pesquero J, Chernicky CL, Greene LJ, Ferrario CM (1988) Converting enzyme activity and angiotensin metabolism in the dog brain stem. Hypertension 11:I153--I157.

Saper CB, Swanson LW, Cowan WM (1979) The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J Comp Neurol 169:409-442.

Sara SJ, Devauges V (1988) Priming stimulation of locus coeruleus facilitates memory retrival in the rat. Brain Res 438:299-303.

Sasaki K, Yamano Y, Bardhan S, Iwai N, Murray HJ, Hasegawa M, Matsuda Y, Inagami T (1991) Cloning and expression of a complementary NDA encoding a bovine adrenal angiotensin II type-1 receptor. Nature 351:230-232.

Sawchenko PE and Swanson LW (1981) central noradrenergic pathways for the innervation of hypothalamic neuroendocrine and autonomic responses. Science 214:685-687.

Schelling P, Meyer D, Loos HE, Speck G, Phillips MI, Johnson AK, Ganten D (1982) A micromethod for the measurement of renin in brain nuclei: its application in spontaneously hypertensive rats. Neuropharmacol 12:455-463

Schiavone MT, Santos RAS, Brosnihan KB, Khosla MC, Ferrario CM (1988) Release of vasopressin from the rat hypothalamo-neurohypophysial system by angiotensin-(1-7) heptapeptide. Proc Natl Acad Sci USA 85:4095-4098.

Schoelkens BA, Jung W, Steinbach R (1976) Blood pressure response to central and peripheral injection of angiotensin II and 8-C-phenyl-glycine analogue of angiotensin II in rats with experimental hypertension. Clin Sci Mol Med 51:403s-406s.

Schousboe A, Larsson OM, Drejer J krogsgaard-larsen P, Hertz L (1983) Uptake and release processes for glutamine, glutamate and GABA in cultured neurons and astrocytes. In: *Glutamine, glutamate and GABA in the central nerve system.* Hertz L, Kvamme E, McGeer EG, Schousboe A (Eds.) Alan R. Liss Inc. 150:297-315.

Scriabine A, Clineschmidt BU, Sweet CS (1976) Central noradrenergic control of blood pressure. In: Annual Review of Pharmacology and Toxicology. Vol. 16, Eds. H.W. Elliott. Annual Reviews, Polo Alto.

Segal M (1982) Norepinephrine modulates reactivity of hippocampal cells to chemical stimulation in vitro. Exp Neurol 77:86-93.

Segal M, Landis S (1974) Affernts to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. Brain Res 78:1-15.

Sernia C, Reid IA (1980) Release of angiotensinogen by rat brain in vivo. Brain Res 192:217-225.

Sernia C, Mowchanuk MD (1983) Brain angiotensinogen: in vitro synthesis and chromatographic characterization. Brain Res 259:275-283.

Seutin V, Franchimont N, Massotte L, Dresse A (1990) Comparison of the effect of morphine on locus coeruleus noradrenergic and ventral tegmental area dopaminergic neurons in vitro. Life Sci 46:1879-1885.

Severs WB, Summy-Long J, Daniels-Severs A (1973) Effect of a converting enzyme inhibitor (SQ20881) on angiotensin-induced drinking. Proc Soc Exp Biol Med 142:203-204.

Shaffer MM, Moody TW (1986) Autoradiographic visualization of CNS receptors for vasoactive intestinal peptide. Peptides 7:283-288.

Shi WX and Bunney BS (1990) A simple effective method for preventing the formation of salt bridges between barrels of a multibarrel microiontophoresis electrode. J Neurosci Meth 35:89-91.

Shimizu N, Imamoto K (1970) Fine structure of the locus coeruleus in the rat. Arch Histol Jap 31:229-246.

Shimizu N, Ohnishi S, Tohyama M, Maeda M (1978) Cellular Organization of locus coeruleus in the rat as studied by golgi method. Arch Histol Jpn 41:103-112, 1978.

Shimizu N, Katoh Y, Hida T, Satoh K (1979) The fine structure organization of the locus coeruleus in the rat with reference to noradrenaline contents. Exp Brain Res 37:139-148.

Shipley MT, Halloran FJ, de la Torre J (1985) Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat. Brain Res 329:294-299.

Shults CW, Quirion R, Chronwall B, Chase TN, O'Donohue TL (1984) A comparison of the anatomical distribution of substance P and substance p receptors in the rat central nervous system. Peptides 5:1097-1128.

Simon RP, Swan JH, Griffiths T, Meldrum BS (1984) Blockade of N-methyl-D-aspartate receptors may protest ischemic damage in the brain. Science 226:850-852.

Simonnet G, Carayon A, Alard M, Cesselin F, Lagoguey A (1984) Evidence foe an angiotensin II-like material and for a rapid metabolism of angiotensin II in the rat brain. Brain Res 304:93-103

Simmonet G, Legender P, Laribi C, Allard M, Vincent JD (1988) Location of angiotensin II binding site on neuronal and glial cells of cultured mouse spinal cord: an autoradiographic study. Brain Res 443:403-406.

Sirett NE, Mclean AS, Bray JJ, Hubbard JI (1977) Distribution of angiotensin II receptors in rat brain. Brain Res 122:299-312.

Sirett NE, Thornton SN, Hubbard JI (1979) Angiotensin binding and pressor activity in the rat ventricular system and midbrain. Brain Res 166:139-148.

Sladeczek F, Pin JP, Recasen M, Bockaert J, Weiss S (1985) Glutamate stimulates inositol phosphate formation in striatal neurons. Nature 317:717-719.

Slater EE, Defendini R, Zimmerman EA (1980) Wide distribution of immunoreactive renin in the nerve cells of human brain. Proc Natl Acad Sci USA 77:5458-5460.

Sofroniew MV (1985) Vasopressin- and neurophysin0immunoreactive neurons in the septal region, medial amygdala and locus coeruleus in colchicine-treated rats. Neuroscience 15:347-358.

Song KF, Allen AM, Paxinos G, Medelsohn FAO (1992) Mapping of angiotensin II receptor subtype heterogeneity in rat brain. J Com Neurol 316:467-484.

Soubrier F, Alhenc-Gelas F, Hubert C, Allegrini J, John M, Tregear G, Corvol P (1988) Two putative active centers in human angiotensin I-converting enzyme revealed by molecular cloning. Pro Natl Acad Sci USA 85:9386-9390.

Speck G, Poulsen K, Unger TH, Rettig R, Bayer C, Scholkens B, Ganten D (1981) In vivo activity of purified mouse brain renin. Brain Res 219:371-384.

Speth RC, Kim KH (1990) Discrimination of two angiotensin II receptor subtypes with a selective agonist analogue of angiotensin II, p-aminophenylalanine^{6} angiotensin II. Biochem Biophys Res Comm 169:997-1006.

Speth RC, Grove KL (1991) Petussis toxin blocks the dipsogenic actions of carbachol, but does not block the dipsogenic pressor actions of angiotensin II. Regulat Pept 32:121-128.

Speth RC, Wamsley JK, Gehlert DR, Chernicky CL, Barnes KL, Ferrario CM (1985) Angiotensin II receptor localization in the canine CNS. Brain Res 326:137-143.

Speth RC, Rowe BP, Grove KL, Carter MR, Saylor D (1991) Sulfhyfryl reducing agents distinguish two subtypes of angiotensin II receptors in the rat brain. Brain Res 548:1-8.

Stadler T, Veltmar A, Qadri F, Unger T (1992) Angiotensin II evokes noradrenaline release from the paraventricular nucleus in conscious rats. Brain Res 569:117-122.

Stallcup WB, Buloch K, Baetge EE (1979) Coupled transport of glutamate and sodium in a cerebellar nerve cell line. J Neurochem 32:57-65.

Stamler JF, Raizada MK, Fellows RE, Phillips MI (1980) Increased specific binding of angiotensin II in the organum vasculosum of the lamina terminalis of the spontaneous hypertensive rat brain. Neurosci Lett 17:173-177.

Steindler DA (1981) Locus coeruleus neurons have axons that branch to the forebrain and cerebellum. Brain Res 223:367-373.

Steinbusch HWM (1984) Serotonin-immunoreactivity neurons and their projections in the CNS. In: *Handbook of Chemical Neuroanatomy, Vol. 3, Classical Transmitter Receptors in the CNS,* Part II. A. Bjorklund, Hokfelt T, Kuhar MJ (Eds.), Elsevier, Amsterdam, pp68-125.

Steckelings U, Leburn C, Qadri F, Veltmar A, unger T (1992) Role of brain angiotensin in cardiovascular regulation. J cardiovasc Pharmacol 19(suppl. 6):S72-S79.

Stornetta RL, Hawelu-Johnson CL, Guyenet PG, Lynch KR (1988) Astrocytes synthesize angiotensinogen in brain. Science 242:1444-1446

Stromberg C, Tsutsumi K, Viswanathan M, Saavedra JM (1991) Angiotensin II AT_1 receptor in rat superior cervical ganglian:characterization and stimulation of phosphoinositide hydrolysis. Eur J Pharmacol 208:331-336.

Sugiyama H, Ito I, Hirono C (1987) A new type of glutamate receptor linked to inositol phospholipid metabolism. Nature 325:531-533.

Summy-Long JY, Keil LC, Sells G, Kirby A, Chee O, Severs WB (1983) Cerebroventricular site for enkephalin inhibition of the central actions of angiotensin. Am J Physiol 244:R522-R529

Sumners C, Raizada MK (1986) Angiotensin II stimulates norepinepherine uptake in hypothalamus-brain stem neuronal cultures. AM J Physiol 250:C236-244.

Sumners C, Myers LM (1991) Angiotensin II decreases cGMP levels in neuronal cultures from rat brain. Am J Physiol 260:c79-c87.

Summers C, Phillips MI, Raizada MK (1983) Angiotensin II stimulates changes in the norepinephrine content of primary cultures of rat brain. Neurosci Lett 36:305-309

Sumners C, Myers CL, Kalberg CJ, Raizada MK (1990) Physiological and pharmacological comparisons of angiotensin II receptors in neuronal and astrocyte glia cultures. Prog Neurobiol 34:355-385.

Sumners C, Tang W, Zelezna B, Raizada MK (1991) Angiotensin II receptor subtypes are coupled with distinct signal-transduction mechanisms in neurons and astrocytes from rat brain. Proc Natl Acad Sci USA 88:7567-7571.

Sutin EL, Jacobowitz DM (1988) Immunochemical localization of peptides and other neurochemicals in the rat laterodosal tegmental nucleus and adjacent area. J Comp Neurol 270:243-270.

Sutin EL, Jacobowitz DM (1991) Neurochemicals in the dorsal potine tegmentum. In: *Progress in Bain Research, Vol. 88, Neurobiology of the Locus Coeruleus,* C.D.Barnes and O.Pompeiano, (Eds.), Elsevier, Amsterdam, pp3-14.

Sved AF, Felsten G (1987) Stimulation of locus coeruleus decreases arterial pressure. Brain Res 414:119-132

Svensson TH, Thoren P (1979) Brain noradrenergic neurons in the locus coeruleus: inhibition by blood volume load through vagal afferents. Brain Res 172:174-178.

Swanson LW (1976) The locus coeruleus: a cytoarchitectonic, golgi and immunohistochemical study in the albino rat. Brain Res 110:39-56.

Swanson LW, Hartman BK (1975) The central adrenergic system. an immuno-fluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-

hydroxylase as a maker. J Comp Neurol 163:467-506.

Swanson LW, Sawchenko PE (1980) Paraventricular nucleus. A site for the integration of neuroendocrine and autonomic mechanisms. Neuroendocrinol 31:410-417.

Swanson LW, Sawchenko PE, Berod A, Hartman BK, Helle KB, Vanordan DE (1981) An immunohistochemical study of the organization of catecholaminergic cells and terminal fields in the paraventricular and supraoptic nuclei of the hypothalamus. J Comp Neurol 196:271-285.

Sykova E, Svoboda J (1990) Extracellular alkaline-acid-alkaline transients in the rat spinal cord evoked by peripheral stimulation. Brain Res 512:181-189.

Szabo B, Hedler L, Schurr C, Starke K (1990) Peripheral presynaptic facilitatory effect of angiotensin II on noradrenaline release in anaesthetized rabbits. J Cardiovasc Pharmacol 15:968-975.

Takahara A, Suzuki-Kusaba M, Hisa H, Satoh S (1990) Effects of a novel Ca^{2+} entry blocker, CD-349, and TMB-8 on renal vasoconstriction induced by angiotensin II and vasopressin in dogs. J Cardiovasc Pharmacol 16:966-970.

Takigawa M, Mogneson GJ (1977) A study of inputs to antidromatically identified neurons of the locus coeruleus. Brain Res 135:217-230.

Tallant EA, Jaiswal N, Diz DI, Ferrario M (1991) Human astrocytes contain two distinct angiotensin receptor subtypes. Hypertension 18:32-39.

Talman WT, Perrone MH, Reis DJ (1980) Evidence for L-glutamate as the neurotransmitter of baroceptor afferent nerve fibers. Science 209:813-815.

Talman WT (1989) Kynurenic acid microinjected into the nucleus tractus solitarius of rat blocks the arterial baroreflexes but not responses to glutamate. Neurosci Lett 102:247-252.

Tamura CS, Speth RC (1990) Effects of angiotensin II on phosphatidylinositol hydrolysis in rat brain. Neurochem Int 17:475-479.

Tanaka J, Kaba H, Saito H, Seto K (1986) Angiotensin II-sensitive neurons in the rat lateral hypothalamic area with efferent projections to the subfornical organ. Exp Neurol 94:791-795.

Tang CM, Dichter M, Morad M (1990) Modulation of the *N*-methyl-D-

aspartate channel by extracellular H^+.
Proc Natl Acad Sci USA 87:6445-6449.

Tani M (1991) Angiotensin converting enzyme in human brain discrete localization and biochemical properties.
Jpn Heart J 32:189-201.

Tasaki, et al. (1968) A simple direct and rapid method for filling microelectrodes. Physiol Behav 3:1009-1011.

Taube HD, Starke K, Borowski E (1977) Presynaptic receptors on the noradrenergic neurons of rat brain. Naunyn-Schmiedebergs Arch Pharmacol 299:123-141.

Theoret Y, Bossu JL (1985) Effects of ammonium salts on synaptic transmission to hippocampal CA1 and CA3 pyramidal cells *in vivo*.
Neuroscience 14:807-821.

Theoret Y, Davis MF, Esplin B, Capek R (1985) Effects of ammonium chloride on synaptic transmission in rat hippocampal slice.
Neuroscience 14:798-806.

Thomas WG, Sernia C (1988) Immunocytochemical localization of angiotensinogen in the rat brain. Neuroscience 25:319-341.

Tonnaer JA, Wiegant VM, DeJong W, DeWeid D (1982) Central effects of angiotensins on drinking and blood pressure: structure-activity relationships. Brain Res 236:417-428.

Tonnaer JADM, Engels GMH, Voshart K, Wiegant VM, De John W (1983) Biding of angiotensins to rat brain tissue: structure activity relationship. Brain Res 236:417-428.

Trachte GJ, Meixner k, Ferrario CM, Khosla MC (1990) Prostagglandin production in response to angiotensin-(1-7) in rabbit isolated vasa deferentia. Prostaglandins 39:385-394.

Tschopl M, Harms L, Norenberg W, Illes P (1992) Excitatory effects of adenosine 5'-triphosphate on rat locus coeruleus neurons. Eur J Pharmacol 213:71-77.

Tse FW, Weiss S, MacVicar BA (1991) Quisqualate agonists occlude kainate-induced current in cultured striatal neurons.
Neuroscience 43:429-436

Tsutsumi K, Saavedra JM (1991a) Characterization and development of angiotensin II receptor subtypes (AT_1 and AT_2) in rat brain.
Am J Physiol 261:R209-R216

Tsutsumi K, Saavedra JM (1991b) Differential development of

angiotensin II receptor subtypes in the rat brain. Endocrinol 128:630-632.

Tsutsumi K, Saavedra JM (1991c) Increased dithiothreitol-insensitive, type 2 angiotensin II receptors in selected brain areas of young rats. Cell Mol Neurobiol 11:295-299.

Tsutsumi K, Saavedra JM (1991d) Quantitative autoradiography reveals different angiotensin II receptor subtypes in selected rat brain nuclei. J Neurochem 56:348-351.

Tsutsumi K, Saavedra JM (1992) Heterogeneity of angiotensin II AT_2 receptors in the rat brain. Mol Pharmacol 41:290-297.

Uhl G, Goodman RR, Snyder SH (1979) Neurotensin-containing cell bodies, fibers and nerve terminals in the brain stem of the rat: Immunohistochemical mapping. Brain Res 167:77-91.

Valentino RJ, Foote SL, Aston-Jones G (1983) Corticotropin-releasing factor activates moradrenergic neurons of the locus coeruleus of the locus coeruleus. Brain Res 270:363-367.

Valentino RJ, Foote SL (1988) Corticotropin-releasing hormone increase tonic but not sensory-evoked activity of noradrenergic locus coeruleus neurons in unanesthetized rats. J Neurosci 8:1016-1025.

Valentino RJ (1989) Corticotropin-releasing factor: putative neurotransmitter in the noradrenergic nucleus locus coeruleus. Psychopharmacol Bull 25:306-311.

Valentino RJ, Wehby RG (1989) Locus coeruleus discharge characteristics of morphine-dependent rats: effects of naltrexone. Brain Res 488:126-134.

Vanderhaeghan JJ, lotstra E, DeMay J, Gilles C (1980) Immunohistochemical localization of cholecystokinin- and gastrin-like peptides in the brain and hypophysis of the rat. Proc Natl Acad Sci USA 77:1190-1194.

van Houten M, Schiffrin EL, Mann JFE, Posner BI, Boucher R (1980) Radioautographic localization of specific binding sites for blood-borne angiotensin II in the rat brain. Brain Res 186:480-485.

Velly L, Cardo B, Kempf E, Mormede P, Nassif-Caudarella S, Velly J (1991) Faciltation of learning consecutive to electrical stimulation of the locus coeruleus: cognitive alteration or stress-reduction? In: *Progress in Brain Research, Vol. 88, Neurobiology of the Locus Coeruleus.* C.D. Baenes and O.Pompeiano (Eds.), Elsevier, Amsterdam, pp555-569.

Vyklicky L, Vlachova V, Krusek J (1990) The effect of external pH changes on responses to excitatory amino acids in mouse hippocampus neurons. J Physiol 430:497-517.

Walden J, Speckmann EJ, Bingmann D, Stranb H (1990) Modulation of Glu effects by GABA in neocortical and archicortical structures. J Basic & Clinical Physiol & Pharmacol 1:65-70.

Wallis CJ, Printz MP (1980) Adrenal regulation of regional brain angiotensinogen content. Endocrinol 106:337-342.

Walters DE, Speth RC (1988) Neuronal localization of specific angiotensin II binding in the rat inferior olivary nucleus. J Neurochem 50:812-817.

Wamsley JK, Herblin WF, Alburges ME, Hunt M (1990) Evidence for the presence of angiotensin II-type 1 receptors in brain. Brain Res Bull 25:397-400.

Ward DG, Baertschi AT, Gann DS (1977) Neurons in medullary areas controlling ACTH: atrial input and rostral projections. Am J Physiol 233:R116-R126

Waterhouse BD, Lin CS, Burne RA, Woodward DJ (1983) The distribution of neocortical projection neurons in the locus coeruleus. J Comp Neurol 217:418-431.

Watkins JC, Evans RH (1981) Excitatory amino acid transmitters. Ann Rev Pharmacol Toxicol 21:165-204.

Watkins JC, Olverman HJ (1987) Agonists and antagonists for EAA receptors. TiNS 10:265-272.

Watkins JC, Krogsgaard-Larsen P, Honore T (1990) Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. TiPS 11:25-33.

Watson SJ, Richard CW, Barchas JC (1978) Adrenocortiotropin in rat brain: Immunocytochemical localization in cells and axons. Science 200:1180-1182.

Wayner MJ, Ono T, Nolley D. (1973) Effects of angiotensin II on central neurons. Pharmacol Biochem Behav 1:679-691.

Weil-Malherbe H (1936) Studies on brain metabolism. I. The mechanism of glutamic acid in brain. Biochem J 30:665-676.

Welches WR, Santos RAS, Chappell MC, Brosnihan KB, Greene LJ, Ferrario CM (1991) Prolyl endopeptidase participates in the processing of brain angiotensin. J Hypertens 9:631-638.

Westlund KN, Bowker RM, Ziegler MG, Coulter JD (1983) Noradrenergic projections to the spinal cord of the rat. Brain Res 263:15-31.

Westlund KN, Sorkin LS, Carlton SM, Ferrington DG, Willcockson HH, Willis WD (1990) Serotonergic and noradrenergic projections to the ventral posterolateral nucleus of monkey thalamus. J Comp Neurol 295:197-207.

Weyhenmeyer JA, Phillips MI (1982) Angiotensin-like immunoreactivity in the brain of the spontaneously hypertensive rat. Hypertension (Dallas) 4:514-523

White SR, Neuman RS (1983) Pharmacological antagonism of facilitatory effects of serotonin and norepinepherine on excitability of spinal motoneurons. Neuropharmacology 22:489-494.

White SR, Penner JD, Speth RC, Chan JYH (1988) Angiotensin II receptors in the lumbar spinal cord of the rat. Brain Res 441:195-201

Whitebread S, Mele M, Kamber B, de Gasparo M (1989) Preliminary biochemical characterization of two angiotensin II receptor subtypes. Biochem Biophys Res Comm 163:284-291.

Whiting P, Nava S, Mozley L, Eatham H, Poat J (1991) Expression of angiotensin converting enzyme mRNA in rat brain. Mol Brain Res 11:93-96.

Wieloch T (1985) Hypoglycemia-induced neuronal damage prevented by an *N*-methyl-D-aspartate antagonist. Science 230:681-683.

Wied DDE, Jolles J (1982) Neuropeptides derived from pro-opiocortin: behavioral, physiological and neurochemical effects. Physiol Rev 62:976-1059.

Williams JT, Marshall KC (1987) Membrane properties and adrenergic responses in locus coeruleus neurons of young rats. J Neurosci 7:3687-3694

Williams JT, North RA (1984) Opiate-receptor interactions on single locus coeruleus neurons. Mol Pharmacol 26:489-497.

Williams JT, Egan T, North RA (1982) Enkephalin opens potassium channels on mammalian central neurons. Nature 229:74-77.

Williams JT, Henderson G, North RA (1984) locus coeruleus neurons, In R. Dingledine (Ed.), Brain slices, plenum, New York, pp.297-311

Williams JT, Henderson G, North RA (1985) Characterization of α_2-adrenoceptors which increase potassium conductance in rat locus coeruleus neurons. Neuroscience 14:95-101.

Williams JT, North RA, Tokimasa T (1988) Inward rectification of resting and opioid-activated potassium currents in rat locus coeruleus neurons. J Neurosci 8:4299-4306.

Woodward DJ, Moises HC, Waterhouse BD, Hoffer BJ, Freedman R (1979) Modulatory actions of norepinepherine in the central nervous system. Fed Proc 38:2109-2116.

Wright JW, Morseth SL, Abhold RH, Harding JW (1985) Pressor action and dipsogenicity induced by angiotensin II and III in rats. Am J Physiol 249:R514-R521.

Wynn PC, Huager RL, Holmes MC, Millian MA, Catt KJ, Aguilera G (1984) Brain and pituitary receptors for corticotropin releasing factor: Localization and differential regulation after adrenalectomy. Peptides 5:1077-1084.

Xiong H, and Marshall KC (1990) Angiotensin II modulation of glutamate excitation of locus coeruleus neurons. Neurosci Lett 118:261-264

Yamamoto C, Sato H (1985) Some properties of ionic channels activated by excitatory amino acids in hippocampal neurons. Exp Brain Res 57:313-320.

Yang C, Phillips MI, Renaud LP (1992) Angiotensin II AT_1 receptor activation depolarizes rat supraoptic neurons in vitro. Am J Physiol 263:R1333-R1338.

Yang HY, Neff NH (1972) Distribution and properties of angiotensin converting enzyme of rat brain. J Neurochem 19:2443-2450.

Yang HY, Neff NH (1973) Differential distribution of angiotensin-converting enzyme in the anterior and posterior lobe of the rat pituitary. J Neurochem 21:1035-1036.

Yonkov D, Georgiev V (1990) Cholinergic influence on memory facilitation induced by angiotensin II in rats. Neuropeptides 16:157-163.

Young WS III, Kuhar MJ (1980) Noradrenergic alpha 1 and alpha 2 receptors: Light microscopic autoradiographic localization. Proc Natl Acad Sci USA 77:1696-1700.

Young WS III, Kuhar MJ (1981) Noradrenergic $alpha_1$ and $alpha_2$ receptors: Light microscopic autoradiographic visualization.

Eur J Pharmacol 59:317-323.

Young WS III, Uhl GR, Kuhar MJ (1978) Iontophoresis of neuron neurotensin in the area of the locus coeruleus. Brain Res 150:431-435.

Zandberg p, Palkovits M, De Jong W (1978) Effects of various lesions in the nucleus tractus solitarii of the rat on blood pressure and heart rate, and cardiovascular reflex responses. Clin Exp Hyperten 1:355-379.

Ziogas J, Story DF, Rand MJ (1985) Effects of locally generated angiotensin II on noradrenergic transmission in guinea-pig isolated atria. Eur. J. Pharmacol. 106:11-18