Peptides, Vol. 7, pp. 679–687, 1986. ' Ankho International Inc. Printed in the U.S.A. 0196-9781/86 $3.00 + .00

Quantitative Distribution of Angiotensin II Binding Sites in Rat Brain by Autoradiography

JUAN M. SAAVEDRA, ANITA ISRAEL, LAURA M. PLUNKETT, MASAKI KURIHARA, KAZUTO SHIGEMATSU AND FERNANDO M. A. CORREA

Unit on Preclinical Neuropharmacology, Section on Clinical Pharmacology Laboratory of Clinical Science, National Institute of Mental Health 9000 Rockville Pike, Building 10, Room 2D46 Bethesda, MD 20892

Received 26 November 1985

SAAVEDRA, J. M., A. ISRAEL, L. M. PLUNKETT, M. KURIHARA, K. SHIGEMATSU AND F. M. A. CORREA. *Quantitative distribution of angiotensin II binding sites in rat brain by autoradiography.* PEPTIDES 7(4) 679–687, 1986.—Angiotensin II binding sites were localized and quantified in individual brain nuclei from single rats by incubation of tissue sections with 1 nM ^{125}I-[Sar^1]-angiotensin II, [^{3}H]-Ultrofilm autoradiography, computerized microdensitometry and comparison with ^{125}I-standards. High angiotensin II binding was present in the circumventricular organs (organon vasculosum laminae terminalis, organon subfornicalis and area postrema), in selected hypothalamic nuclei (nuclei suprachiasmatis, periventricularis and paraventricularis) and in the nucleus tractus olfactorii lateralis, the nucleus preopticus medianus, the dorsal motor nucleus of the vagus and the nucleus tractus solitarii. High affinity (K_A from 0.3 to 1.5×10^9 M^{-1}) angiotensin II binding sites were demonstrated in the organon subfornicalis, the nucleus tractus solitarii and the area postrema after incubation of consecutive sections from single rat brains with ^{125}I-[Sar^1]-angiotensin II in concentrations from 100 pM to 5 nM. These results demonstrate and characterize brain binding sites for angiotensin II of variable high affinity binding both inside and outside the blood-brain barrier.

Quantitative autoradiography   Computerized microdensitometry   Image analysis
Circumventricular organs   Hypothalamic nuclei   Nucleus suprachiasmatis   Nucleus paraventricularis
Eminentia mediana   Brainstem nuclei   Nucleus tractus solitarius   Angiotensin II receptors
Neuropeptide receptors

PERIPHERAL angiotensin II (AT_{II}) acts in the brain to stimulate drinking, increase blood pressure, and release pituitary hormones [17, 35, 52, 69]. These effects are mediated, at least in part, through stimulation of AT_{II} receptors located in the circumventricular organs, areas accessible to blood-borne peptide [27–29, 44, 54, 64, 75]. AT_{II} binding sites have also been described and localized in areas of the brain protected by the blood-brain barrier, and inaccessible to blood-borne hormone [19, 25, 27–29, 44]. These observations in conjunction with the presence of all components of the peripheral AT_{II} system in brain [18,39] suggested that this peptide could be formed in brain tissue and may act as a neurotransmitter or neuromodulator in the central nervous system.

Early studies into the neural actions of AT_{II} localized peptide binding sites in large brain areas and characterized the binding in membrane fractions [3,65]. Preliminary, semiquantitative studies of rat brain AT_{II} binding sites using autoradiography were recently reported [44]. We present the complete quantitative localization of AT_{II} binding sites in individual rat brain nuclei through the use of autoradiographic methods with image analysis, coupled to computerized microdensitometry. This methodology represented an advance in both the sensitivity and the anatomical specificity of receptor binding techniques and can also be used to localize binding sites for other neuropeptides, drugs and biogenic amines [26, 36, 74, 76]. The use of ^{125}I-ligands of high specific activity and comparison with ^{125}I-standards allowed for short film exposure times and fast, precise quantitation [27–29, 37]. Further, complete binding analysis could be performed in individual rat brain nuclei [27,28], single pituitary glands [30], and single rat sympathetic ganglia [49].

In this study, we utilized ^{125}I-[Sar^1]-AT_{II}, a high-affinity agonist which binds to a single class of high affinity, saturable sites in brain tissue [44], and has a ligand specificity very similar to that reported by others using ^{125}I-labeled AT_{II} [3,65] or [^{3}H]-AT_{II} [71]. In autoradiographic studies of brain and peripheral tissues, non-specific binding using ^{125}I-[Sar^1]-AT_{II} was less than 10% of total binding [27–29, 55]. Both AT_{II} and [Sar^1]-AT_{II} showed complete cross-displacement in hypothalamic membrane binding experiments [43,44] indicating that both ligands interact with the

same binding site, as has been reported for the vascular AT_{II} receptor [23]. These characteristics made ^{125}I-[Sar^1]-AT_{II} a suitable ligand for quantitation of brain AT_{II} binding sites.

METHOD

Male Sprague Dawley rats (250 g) were obtained from Zivic Miller, Allison Park, PA. Animals were given tap water and rat chow ad lib and housed in groups of 4 at a constant temperature of 24°C, with lights on from 06.00 to 18.00 hr.

Rats were sacrificed by decapitation between 09.00 and 11.00 and tissues were immediately removed and frozen by immersion in isopentane (−30°C). Within 24 hr of sacrifice, frozen 16 μm thick sections were cut in a cryostat at −14°C, thaw-mounted onto gelatin-chrome alum coated glass slides, and dessicated under vaccuum at 4°C for at least 2 hr.

Ligand Binding

Within 48 hr of section preparation, AT_{II} binding sites were labeled in vitro by incubation of sections with ^{125}I-[Sar^1]-AT_{II}. For determination of saturation curves and Scatchard plots in the organon subfornicalis (SFO), area postrema (AP) and nucleus tractus solitarius (NTS), consecutive tissue sections from single rat brains were preincubated for 15 min at 20°C in 10 mM sodim phosphate buffer, pH 7.4, containing NaCl (120 mM), Na_2EDTA (5 mM), bacitracin (0.1 mM) and bovine serum albumin (0.2%), and then incubated for 60 min in fresh buffer with concentrations of ^{125}I-[Sar^1]-AT_{II} ranging from 100 pM to 5 nM. Nonspecific binding was determined in the presence of 0.1 to 5 μM unlabeled AT_{II} [27–29]. For analysis of AT_{II} receptor density and localization throughout the brain, additional sections were incubated under the same conditions with 1 nM ^{125}I-[Sar^1]-AT_{II} and nonspecific binding determined in the presence of 1 μM AT_{II}. After incubation, all slides were washed 4 times (60 sec each) in ice-cold 50 mM Tris-HCl buffer (pH 7.56) and dried under a cold stream of air.

Preparation of ^{125}I-Standards

Sets of ^{125}I-standards were prepared as originally described for ^3H-standards [74]. Known amounts of increasing concentrations of ^{125}I-AT_{II} were thoroughly mixed with rat brain tissue aliquots previously ground to a paste and degassed by repeated mixing under vacuum. The aliquots were placed as blocks of tissue on microtome specimen holders and frozen on dry ice. Tissue sections, 16 μm thick, were cut in a cryostat at −14°C and thaw-mounted onto subbed glass slides. Parallel sets of standards obtained from consecutive sections were used for determination of protein content [40] and radioactivity.

Autoradiography

Tissue sections were placed in cassettes (CGR Medical Corp., Baltimore, MD) along with ^{125}I-standards and opposed against [^3H]-Ultrofilm (LKB Industries, Rockville, MD) at room temperature. The films were developed at 4°C for 4 min with undiluted D19 Kodak developer. A complete set of ^{125}I-standards was processed with every cassette and developed with every film. Optical densities were measured by computerized microdensitometry [20] for both ^{125}I-standards and specific areas of brain sections from each film.

Data Analysis

After determination of the standard curve for each film (ln optical densities × 100 vs ln dpm in standards), the optical densities of the brain areas studied were interpolated from the straight line of the standard curve to obtain the corresponding dpm bound to the tissue [27–29]. Protein concentrations were assumed to be uniform in 16 μm sections throughout the brain. Results were corrected for the decay of ^{125}I. Calculation of the molar quantities of ligand bound to the tissue, saturation curves and Scatchard analysis were performed by linear regression followed by correction for the protein content of the standards.

Drugs

Angiotensin II (AT_{II}) (Sigma Chemical Co., MO) was dissolved in 0.01 N acetic acid and kept in aliquots at −70°C until the day of the experiment. [Sar^1]-AT_{II} (Peninsula Laboratories, Belmont, CA) was kept frozen until iodination by a modified Chloramine-T method (Meloy Laboratories, Inc., Springfield, VA). The specific activity of the ^{125}I-[Sar^1]-AT_{II} was 2000 Ci/mmol. ^{125}I-AT_{II} (specific activity 1280 Ci/mmol) was obtained from New England Nuclear, Boston, MA.

RESULTS

AT_{II} binding sites were highly localized in the rat brain with binding sites concentrated in 24 areas (Table 1, Fig. 1 and 2). A 20-fold difference in relative binding site concentration was noted between the areas with highest concentration (organon vasculosum laminae terminalis, OVLT) and the lowest concentration of AT_{II} binding sites (subiculum) (Table 1).

In the rostral limbic system, AT_{II} binding sites were located in the nucleus tractus olfactorii lateralis, and at all levels of the tractus olfactorius lateralis (Table 1 and Fig. 1). High levels of AT_{II} binding sites occurred in the circumventricular organs which include the OVLT, the organon subfornicalis (SFO), and the area postrema (AP) (Table 1, Fig. 1 and 2). In the hypothalamus, AT_{II} binding sites were located in a few nuclei. The highest relative density of binding sites was located in the nucleus suprachiasmatis, followed by the nucleus periventricularis. High relative concentrations of binding sites also occurred in the nucleus paraventricularis, both in its pars parvocellularis and pars magnocellularis, and in the eminentia mediana. The nucleus dorsomedialis contained a somewhat lower concentration of AT_{II} binding sites, similar in its pars dorsalis and pars ventralis (Table 1 and Fig. 1). The number of AT_{II} binding sites was also high in the nucleus medianus (Table 1 and Fig. 1).

Among the limbic cortical areas, the cortex piriformis was the only area containing AT_{II} binding sites (Table 1 and Fig. 1). AT_{II} binding site distribution was discrete in the subthalamic areas, where the nucleus subthalamicus was the only area showing AT_{II} binding, and also in the mesencephalic areas, where AT_{II} binding sites were restricted to the stratum griseum superficiale colliculi superioris and the subiculum (Table 1 and Fig. 1).

In the pons, the only area showing a measurable number of AT_{II} binding sites was the locus coeruleus (Table 1 and Fig. 2). A number of brainstem areas contained high levels of AT_{II} binding sites with the highest relative concentrations present in the nucleus tractus solitarii (NTS), the dorsal motor nucleus of the vagus and the nucleus tractus commissuralis

TABLE I

QUANTITATIVE DISTRIBUTION OF ANGIOTENSIN II BINDING SITES IN THE RAT BRAIN

Area	Coordinates*	Density (fmol/mg protein)
Rostral Limbic System		
Nucleus tractus olfactorii lateralis	A 5910 μ	35.8 ± 4.3
Tractus olfactorius lateralis	A 11050 μ	43.7 ± 8.2
	A 8920 μ	45.1 ± 3.6
	A 6790 μ	44.7 ± 9.2
Circumventricular Organs		
Organon subfornicalis	A 6280/5660 μ	227.8 ± 33.0
Organon vasculosum laminae terminalis	A 6790 μ	257.9 ± 22.2
Area postrema	Bregma-13.8 mm	119.5 ± 17.7
Hypothalamus		
Nucleus periventricularis	A 6280/5780 μ	96.7 ± 13.4
Nucleus paraventricularis	A 5660/5340 μ	62.5 ± 7.0
Nucleus suprachiasmatis	A 6280/5780 μ	161.1 ± 8.9
Nucleus dorsomedialis	A 4230 μ	21.8 ± 4.5
Eminentia mediana	A 4230 μ	55.0 ± 9.9
Nucleus medianus	A 7020 μ	98.3 ± 6.8
Limbic Cortex		
Cortex piriformis	A 10500/6790 μ	36.2 ± 4.9
Subthalamus-Metathalamus		
Nucleus subthalamicus	A 3430 μ	39.9 ± 4.4
Mesencephalic Regions		
Stratum griseum superficiale colliculi superioris	A 1610 μ	16.5 ± 3.2
Subiculum	A 1610 μ	13.5 ± 0.4
Pons		
Locus coeruleus	Bregma-9.8 mm	25.9 ± 6.3
Brainstem		
Nucleus motoris dorsalis vagii	Bregma-13.3/14.3 mm	140.6 ± 17.9
sp 5 (trigeminal tract)	Bregma-13.3/14.3 mm	28.8 ± 3.8
Nucleus tractus solitarii		
anterior	Bregma-13.3 mm	140.2 ± 17.8
intermediate	Bregma-13.8 mm	171.6 ± 17.8
posterior	Bregma-14.3 mm	72.8 ± 5.9
Nucleus tractus commissuralis	Bregma-14.3 mm	135.1 ± 12.1
Inferior olivary nuclei	Bregma-13.3/13.8 mm	31.9 ± 4.6

Autoradiographic determinations were performed in tissues from single animals after incubation with 1 nM ^{125}I-[Sar^1]-AT$_{II}$, as described in the Method section. Results are expressed as mean ± S.E.M., for values obtained from 5 different animals, assayed individually.

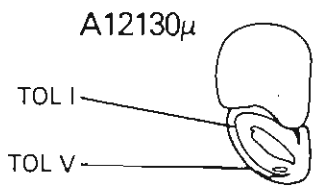
*Nomenclature and coordinates are according to König and Klippel [34] and Paxinos and Watson for the pons and brainstem areas [50].

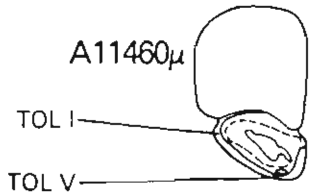
(Table 1 and Fig. 2). Binding sites were also present, although in lower relative concentrations, in the trigeminal tract and in the inferior olivary nuclei (Table 1 and Fig. 2).

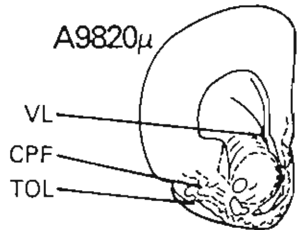
In addition to localization of specific AT$_{II}$ binding sites, these sites were characterized in the SFO, the NTS, and the AP by incubation of consecutive tissue sections obtained from single rat brains with increasing concentrations of ^{125}I-[Sar^1]-AT$_{II}$. All three areas showed binding sites with high variable affinity binding K_A 1.5×10^9 M^{-1}, 0.56×10^9 M^{-1}, and 0.36×10^9 M^{-1} for the SFO, AP, and NTS, respectively). The maximal binding capacity was 265, 552 and 1127 fmol/mg protein, for the SFO, AP, and NTS, respectively (Fig. 3).

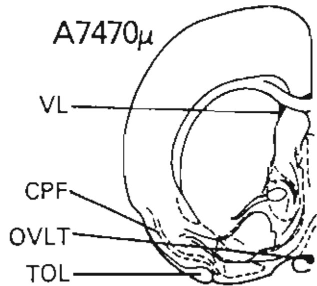
DISCUSSION

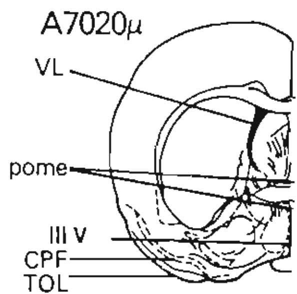
Our results showed a distribution of AT$_{II}$ binding sites similar to that observed after binding of AT$_{II}$ to membrane

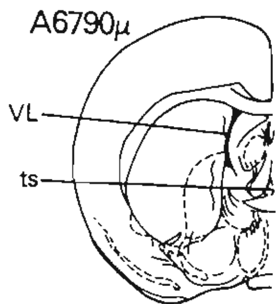

A12130μ
TOL I
TOL V

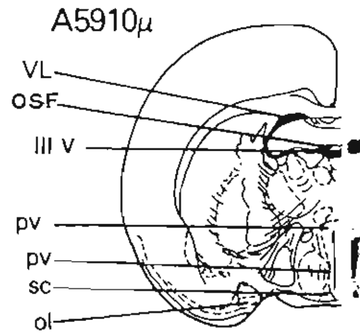

A11460μ
TOL I
TOL V

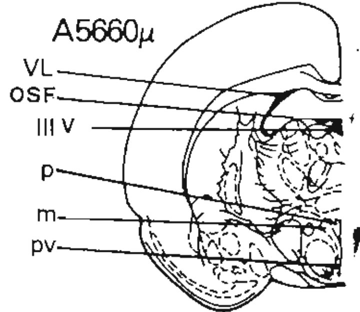

A9820μ
VL
CPF
TOL

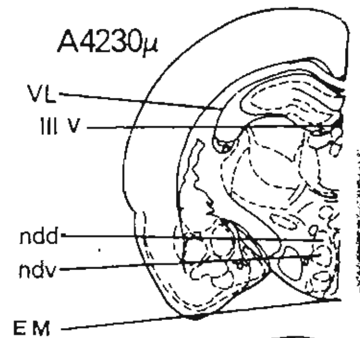

A7470μ
VL
CPF
OVLT
TOL

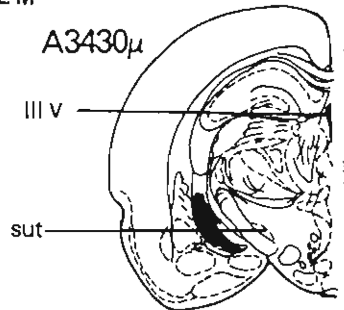

A7020μ
VL
pome
III V
CPF
TOL

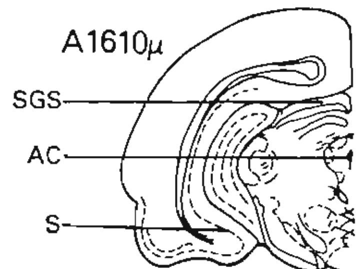

A6790μ
VL
ts


A5910μ
VL
OSF
III V
pv
pv
sc
ol


A5660μ
VL
OSF
III V
p
m
pv


A4230μ
VL
III V
ndd
ndv
EM

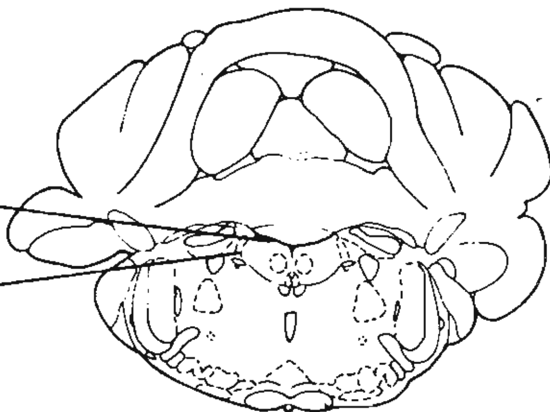

A3430μ
III V
sut


A1610μ
SGS
AC
S

B −9.8

IV V

LC



B − 13.3

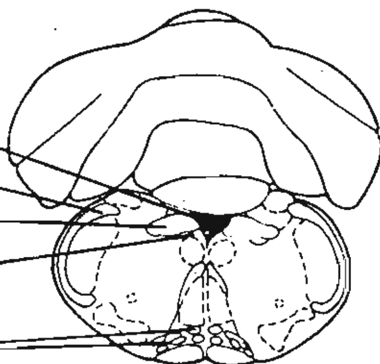
IV V

p5

NTS

NMDV

IO



B − 13.8

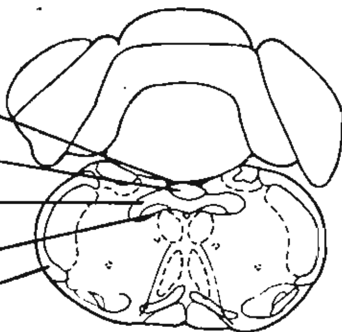
V V

AP

NTS

NMDV

p5



fractions obtained from gross dissection of the brain. With these methods, AT_{II} binding sites were reported to be concentrated in the thalamus-hypothalamus region, midbrain, and septum [3]. We found high AT_{II} binding site concentrations in discrete areas within these regions. Our results agree with and expand an earlier study on the semiquantitative distribution of AT_{II} sites in the rat brain by autoradiography [44]. However, the AT_{II} binding site distribution shown in Fig. 1 and 2 is quite different from that reported for other neuropeptides or amines [36, 56, 57, 63, 76].

High levels of AT_{II} binding sites were found in the circumventricular organs, structures lacking a blood-brain barrier and exposed to blood-borne peptides [25]. In the SFO, a structure recognized as a target site for blood-borne AT_{II} [64], there were high concentrations of high affinity AT_{II} binding sites ([25] and Fig. 1). Physiological, biochemical and behavioral evidence strongly suggest that these sites could be considered AT_{II} receptors. Neurons within the SFO are sensitive to iontophoretically applied AT_{II} [48]. Peripheral administration of AT_{II} increases drinking and blood pressure by stimulation of the SFO [64], effects which are concommitant with increased metabolic activity in this structure [21] and which are blocked by lesions of the organ or by disruption of its afferent connections [38]. The number of AT_{II} receptors in the SFO increases after water deprivation [43], a state characterized by high levels of circulating AT_{II} [41] and demonstrating an upregulation of AT_{II} binding sites.

The AT_{II} binding sites in another circumventricular organ, the OVLT, may also be physiologically active AT_{II} receptors [27, 54, 64, 67, 75]. This structure contains AT_{II}-sensitive neurons [33] and lesions of the OVLT resulted in a blockade of the effects of intracerebroventricularly administered AT_{II} [52].

Both the SFO and OVLT areas are structurally associated with the anterior wall of the third ventricle (anteroventral-third-ventricle, AV3V), an area involved in cardiovascular control and fluid regulation [7]. Neural connections have been described between the SFO and OVLT and other AV3V areas, such as the nucleus preopticus medianus [39,45]. The nucleus preopticus medianus also contains neurons sensitive to AT_{II} [33, 52, 54]. With immunohistochemical techniques, at least part of these connections have been shown to contain AT_{II} [39]. We report high concentrations of AT_{II} binding sites in areas along the wall of the third ventricle, including the nucleus preopticus medianus. It is of interest to note that the metabolic activity in the nucleus preopticus medianus ([22], Kadekaro, Gross, Saavedra, and Sokoloff, unpublished results) increased during dehydration in parallel to the increased metabolic activity in the SFO. These AV3V associated structures also possess high angiotensin converting enzyme activity [15, 59, 61], indicating that AT_{II} may be formed endogenously in the SFO and related areas. Lesions of the AV3V area block the actions of circulating as well as centrally administered AT_{II} [7,8]. Therefore, evidence shows that AT_{II} may be considered a neuromodulator in specific structures within the AV3V area and that this region may be a target site for interactions between the peripheral and central AT_{II} systems.

AT_{II} binding sites also occur in brain areas protected by a blood-brain barrier and which contain all of the components of the angiotensin system. For example, binding sites for AT_{II} were concentrated in a few hypothalamic areas, such as the nucleus paraventricularis. This nucleus is involved in the formation of the antidiuretic hormone vasopressin [70], and sends axons to the posterior pituitary where the hormone is released into the general circulation. AT_{II} and vasopressin were shown to be colocalized within the same magnocellular neurons [32]. The AT_{II} binding sites within the nucleus paraventricularis may form part of a cental pathway where AT_{II} regulates the secretion of vasopressin [58] in addition to the well established SFO neuronal circuit which responds to circulating AT_{II} [6]. A physiological link between the hypothalamic AT_{II} and vasopressin systems was provided by the demonstration of altered angiotensin converting enzyme activity in hypothalamic nuclei and the posterior pituitary of Brattleboro rats, animals unable to synthesize vasopressin [11,60]. AT_{II} activity in the nucleus paraventricularis could also be related to the central control of anterior pituitary function, through a modulation of the formation or release of the corticotropin releasing factor [1, 5, 39].

Of particular interest was the presence of AT_{II} binding sites in the eminentia mediana, a structure outside the blood-brain barrier, which contains AT_{II}-immunoreactive fibers [39]. The sites described here could represent another site of action in addition to anterior pituitary sites [29,47] for circulating AT_{II} in the control of anterior pituitary function [35,69].

We found a high concentration of AT_{II} binding sites in the nucleus suprachiasmatis, suggesting an unsuspected role of central AT_{II} in the regulation of circadian rhythms [46,48]. The olfactory areas were also identified as containing AT_{II} binding sites (Fig. 1) [13,24]. In addition, infusion of AT_{II} into the olfactory bulb elicits drinking with a temporal pattern different from that obtained after the stimulation of the SFO [13]. These findings indicate that brain AT_{II} might play a physiological role in olfactory functions.

The brainstem had high levels of AT_{II} binding but only in a few areas. Very high concentrations of single class, high affinity AT_{II} binding sites were present in the NTS [55], an area important in cardiovascular control and baroreflex regulation [16]. Alterations in central AT_{II} activity have been associated with spontaneous (genetic) hypertension [4, 10, 14, 67]. Angiotensin converting enzyme antagonists and angiotensin blockers lower blood pressure in spontaneously

FACING PAGE

FIG. 1. ^{125}I-[Sar^1]-AT_{II} binding in brain areas of the rat. Right side: drawings according to König and Klippel [34]. Left side: autoradiographs after incubation of sections with 1 nM ^{125}I-[Sar^1]-AT_{II}. Numbers represent rostro-caudal coordinates according to König and Klippel [34]. TOL I: tractus olfactorius lateralis, pars intermedia. TOL V: tractus olfactorius lateralis, pars ventralis. CPF: cortex piriformis. VL: lateral ventricle. TOL: tractus olfactorius lateralis. OVLT: organon vasculosum laminae terminalis. pome: nucleus preopticus medianus. VIII: third ventricle. SFO: organon subfornicalis. pvs: nucleus periventricularis (hypothalami). hpv: nucleus periventricularis. sc: nucleus suprachiasmatis. ol: nucleus tractus olfactorii lateralis. fp: nucleus paraventricularis pars parvocellularis. fm: nucleus paraventricularis pars magnocellularis. hdd: nucleus dorsomedialis pars dorsalis. hdv: nucleus dorsomedialis pars ventralis. ME: eminentia mediana. sut: nucleus subthalamicus. SGS: stratum griseum superficiale colliculi superioris. AC: aqueductus cerebri. S: subiculum.

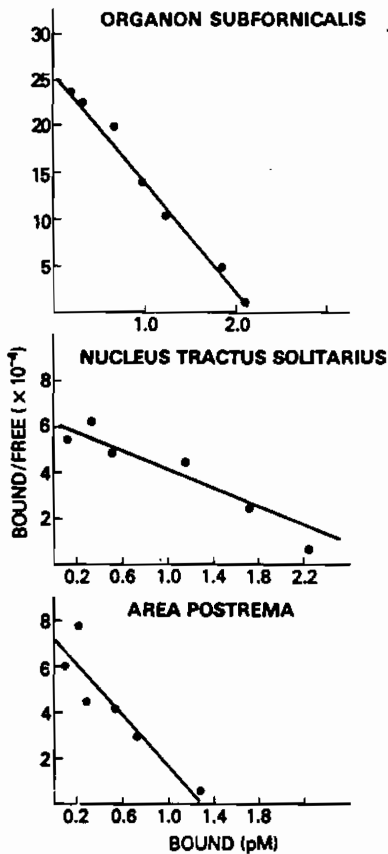


FIG. 3. Scatchard analysis of specific ^{125}I-[Sar[1]]-AT_{II} binding to the SFO (upper panel) NTS (middle panel) and AP (lower panel). Tissue sections from single animals were incubated with ^{125}I-[Sar[1]]-AT_{II} concentrations ranging from 100 pM to 5 nM (see the Method section). Each curve represents one typical experiment which was repeated with at least 4 different rats. Each point represents the average of at least 3 optical density readings at that concentration of bound ligand. Organon subfornicalis: r=0.93; nucleus tractus solitarii: r=0.88; area postrema: r=0.89.

hypertensive rats [41, 52, 53, 66, 73]. Additionally, microinjections of AT_{II} into the NTS were shown to produce dose-dependent increases in blood pressure which were similar to increases seen after intraventricular injection of AT_{II} [9]. AT_{II} immunoreactive cells and fibers have been identified in the NTS [39] as well as high levels of the angiotensin converting enzyme [12]. AT_{II} binding affinity was increased in the NTS of spontaneously hypertensive rats [55]. These observations support the hypothesis of a functional local AT_{II} system in the NTS and suggest that binding sites for AT_{II} could represent physiologically active AT_{II} receptors in this nucleus.

The AP, a circumventricular organ, is another brainstem area rich in AT_{II} binding sites. This area contains AT_{II}-positive fibers [39], and is one of the brain's AT_{II}-sensitive sites [72]. The AP also has very high angiotensin converting enzyme activity [59]. Thus, a local AT_{II} system may also function in this area and as seen with the other circumventricular organs, the AP may represent a site for interactions between the peripheral and central AT_{II} systems.

The demonstration of AT_{II} binding sites in the locus coeruleus ([44] and Fig. 2) in conjunction with the NTS and AP demonstrates that a large portion of the central actions of AT_{II} occurs in areas involved in the central regulation of sympathetic activity [2,16]. In these areas, as well as the nuclei paraventricularis and periventricularis, AT_{II} binding sites may be associated with catecholamine cell bodies and terminals. AT_{II} has been shown to stimulate norepinephrine release from peripheral sympathetic nerves [62] and catecholamine release from the adrenal medulla [51], a tissue rich in AT_{II} binding sites [28,31].

In conclusion, our results indicate that there is a very specific distribution of AT_{II} binding sites in the rat brain. Some of these areas showed variable high affinity binding, suggesting that there may be differences in classes of receptors. Some areas such as the circumventricular organs and discrete areas of the hypothalamus and brainstem contain AT_{II} binding sites as well as immunoreactive fibers and cell bodies [39] and large amounts of angiotensin converting enzyme [10, 15, 61]. These areas could be considered sites of action for a local AT_{II} system as well as target sites for peripheral-central AT_{II} interactions. Other brain areas such as the nucleus supraopticus, stria terminalis or some amygdaloid and thalamic nuclei have AT_{II} cell bodies or fibers but no AT_{II} binding sites [39]. The apparent lack of correlation between the presence of neurotransmitters or neuropeptides and their binding sites is not uncommon in the brain and has previously been reported for neurotensin, substance P, and atrial natriuretic factor [56, 57, 63]. The distribution of AT_{II} binding sites as well as AT_{II} immunoreactivity and converting enzyme suggest that AT_{II} may be an important neuromodulator with selective and precise functions within the central nervous system.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Pauline Cover for technical assistance and Ms. Sue O'Harra for editorial assistance.

REFERENCES

1. Akaishi, T., H. Negoro and S. Kobayasi. Electrophysiological evidence for multiple sites of actions of angiotensin II for stimulating paraventricular neurosecretory cells in the rat. *Brain Res* **220**: 386–390, 1981.
2. Barnes, K. L. and C. M. Ferrario. Location of the area postrema pressor pathway in the dog brainstem. *Hypertension* **6**: 482–488, 1984.
3. Bennett, J. P., Jr., and S. H. Snyder. Angiotensin II binding to mammalian brain membranes. *J Biol Chem* **251**: 7423–7430, 1976.
4. Bickerton, R. K. and J. P. Buckley. Evidence for a central mechanism of angiotensin induced hypertension. *Proc Soc Exp Biol Med* **106**: 834–836, 1961.
5. Bloom, F. E., E. L. F. Battenberg, J. Rivier and W. Vale. Corticotropin releasing factor (CRF): immunoreactive neurones and fibers in rat hypothalamus. *Regul Pept* **4**: 43–48, 1982.
6. Bonjour, J. P. and R. L. Malvin. Stimulation of ADH release by the renin angiotensin system. *Am J Physiol* **218**: 1555–1559, 1970.

7. Brody, M. J. and A. K. Johnson. Role of the anteroventral third ventricle (AV3V) region in fluid and electrolyte balance, arterial pressure regulation and hypertension. In: *Frontiers in Neuroendocrinology*, vol 6, edited by W. F. Ganong and L. Martini. New York: Raven Press, 1980, pp. 249–292.
8. Buggy, J. and A. K. Johnson. Angiotensin induced thirst: effects of third ventricle obstruction and periventricular ablation. *Brain Res* **149**: 117–128, 1978.
9. Casto, R. and M. I. Phillips. Cardiovascular actions of microinjections of angiotensin II in the brainstem of rats. *Am J Physiol* **246**: R811–R816, 1984.
10. Chevillard, C., M. Niwa and J. M. Saavedra. Angiotensin-converting enzyme in discrete forebrain areas of spontaneously hypertensive rats. *Brain Res* **309**: 389–392, 1984.
11. Chevillard, C. and J. M. Saavedra. High angiotensin-converting enzyme activity in the neurohypophysis of Brattleboro rats. *Science* **216**: 646–647, 1982.
12. Chevillard, C. and J. M. Saavedra. Distribution of angiotensin-converting enzyme activity in specific areas of the rat brain stem. *J Neurochem* **38**: 281–284, 1982.
13. Chen, F. C. M., D. P. Healy, R. Hawkins and M. P. Printz. Chronic infusion of angiotensin II into the olfactory bulb elicits an increase in water intake. *Brain Res* **259**: 335–339, 1983.
14. Cole, F. E., E. D. Frohlich and A. A. MacPhee. Angiotensin binding affinity and capacity in the midbrain area of spontaneously hypertensive and normotensive rats. *Brain Res* **154**: 178–181, 1978.
15. Correa, F. M. A., L. M. Plunkett, J. M. Saavedra and M. Hichens. Quantitative autoradiographic determination of angiotensin-converting enzyme (kininase II) kinetics in individual rat brain nuclei with ^{125}I-351A, a specific enzyme inhibitor. Brain Res **347**: 192–195, 1985.
16. Doba, N. and D. J. Reis. Acute fulminating neurogenic hypertension produced by brainstem lesions in the rat. *Circ Res* **23**: 584–593, 1973.
17. Fitzsimmons, J. T. Angiotensin stimulation of the central nervous system. *Rev Physiol Biochem Pharmacol* **87**: 117–167, 1980.
18. Ganten, D., G. Speck, P. Schelling and T. Unger. The brain renin-angiotensin system. *Adv Biochem Psychopharmacol* **28**: 359–372, 1981.
19. Gehlert, D. R., R. C. Speth, D. P. Healy and J. K. Wamsley. Autoradiographic localization of angiotensin II receptors in the rat brainstem. *Life Sci* **34**: 1565–1571, 1984.
20. Goochee, C., W. Rasband and L. Sokoloff. Computerized densitometry and color coding of [^{14}C] deoxyglucose autoradiographs. *Ann Neurol* **7**: 359–370, 1980.
21. Gross, P. M., M. Kadekaro, D. W. Andrews, L. Sokoloff and J. M. Saavedra. Selective metabolic stimulation of the subfornical organ and pituitary neural lobe by peripheral angiotensin II. *Peptides* **6**: Suppl 1, 145–152, 1985.
22. Gross, P. M., M. Kadekaro, L. Sokoloff, H. H. Holcomb and J. M. Saavedra. Alterations of local cerebral glucose utilization during chronic dehydration in rats. *Brain Res* **330**: 328–336, 1985.
23. Hall, M. M., M. C. Khosla, P. A. Khairallah and F. M. Bumpus. Angiotensin analogs: the influence of sarcosine substituted in position 1. *J Pharmacol Exp Ther* **188**: 222–228, 1974.
24. Harding, J. W., L. P. Stone and J. W. Wright. The distribution of angiotensin II binding sites in rodent brain. *Brain Res* **205**: 265–274, 1981.
25. Healy, D. P. and M. P. Printz. Localization of angiotensin II binding sites in rat septum by autoradiography. *Neurosci Lett* **44**: 167–172, 1984.
26. Herkenham, M. and C. B. Pert. Light microscopic localization of brain opiate receptors: A general autoradiographic method which preserves tissue quality. *J Neurosci* **2**: 1129–1149, 1982.
27. Israel, A., F. M. A. Correa, M. Niwa and J. M. Saavedra. Quantitative determination of angiotensin II binding sites in rat brain and pituitary gland by autoradiography. *Brain Res* **322**: 341–345, 1984.
28. Israel, A., F. M. A. Correa, M. Niwa and J. M. Saavedra. Quantitative measurement of angiotensin II (AII) receptors in discrete regions of rat brain, pituitary and adrenal gland by autoradiography. *Clin Exp Hypertens [A]* **6**: 1761–1764, 1984.
29. Israel, A., L. M. Plunkett and J. M. Saavedra. Quantitative autoradiographic characterization of receptors for angiotensin II and other neuropeptides in individual brain nuclei and peripheral tissues from single rats. *Cell Mol Neurobiol* **5**: 211–222, 1985.
30. Israel, A., J. M. Saavedra and L. Plunkett. Water deprivation upregulates angiotensin II receptors in rat anterior pituitary. *Am J Physiol* **248**: E264–E267, 1985.
31. Israel, A., M. Niwa, L. M. Plunkett and J. M. Saavedra. High-affinity angiotensin receptors in rat adrenal medulla. *Regul Pept* **11**: 237–243, 1985.
32. Killcoyne, M. M., D. L. Hoffman and E. H. Zimmerman. Immuno-cytochemical localization of angiotensin II and vasopressin in rat hypothalamus: evidence for production in the same neuron. *Clin Sci* **59**: 57S–60S, 1980.
33. Knowles, W. D. and M. I. Phillips. Angiotensin II responsive cells in the organon vasculosum laminae terminalis (OVLT) recorded in hypothalamic brain slices. *Brain Res* **195**: 256–259, 1980.
34. König, J. F. R. and R. A. Klippel. *The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Baltimore: Williams and Wilkins, 1963.
35. Kraft, K., R. R. Lang, W. Gaida, T. Unger and D. Ganten. Angiotensin stimulates β-endorphin release from anterior pituitary gland cell cultures of rats. *Neurosci Lett* **46**: 25–29, 1984.
36. Kuhar, M. J. Autoradiographic localization of drug and neurotransmitter receptors. In: *Methods in Chemical Neuroanatomy*, edited by A. Bjorklund and T. Hökfelt. Amsterdam: Elsevier, 1983, pp. 398–415.
37. Kuhar, M. J. and J. R. Unnerstall. Quantitative receptor mapping by autoradiography: some current technical problems. *Trends Neurosci* **8**: 49–53, 1985.
38. Lind, R. W., L. E. Ohman, M. B. Lansing and A. K. Johnson. Transection of subfornical organ neuronal connections diminishes the pressor response to intravenously infused angiotensin II. *Brain Res* **275**: 361–364, 1983.
39. Lind, R. W., L. W. Swanson and D. Ganten. Organization of angiotensin II immunoreactive cells and fibers in the rat central nervous system. An immunohistochemical study. *Neuroendocrinology* **40**: 2–24, 1985.
40. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
41. McDonald, W., C. Wickre, A. Aumann, D. Ban and B. Moffit. The sustained antihypertensive effect of chronic cerebroventricular infusion of angiotensin antagonist in spontaneously hypertensive rats. *Endocrinology* **107**: 1305–1308, 1980.
42. Mann, J. F. E., A. K. Johnson and D. Ganten. Plasma angiotensin II: dipsogenic levels and angiotensin-generating capacity of renin. *Am J Physiol* **238**: R372–R377, 1980.
43. Mendelsohn, F. A. O., G. Aguilera, J. M. Saavedra, R. Quirion and K. J. Catt. Characteristics and regulation of angiotensin II receptors in pituitary, circumventricular organs and kidney. *Clin Exp Hypertens [A]* **5**: 1081–1097, 1983.
44. Mendelsohn, F. A. O., R. Quirion, J. M. Saavedra, G. Aguilera and K. J. Catt. Autoradiographic localization of angiotensin II receptors in rat brain. *Proc Natl Acad Sci USA* **81**: 1575–1579, 1984.
45. Miselis, R. R. The efferent projections of the subfornical organ of the rat: a circumventricular organ within a neural network subserving water balance. *Brain Res* **230**: 1–23, 1981.
46. Mosko, S. and R. Y. Moore. Neonatal suprachiasmatic nucleus lesions: effects on the development of circadian rhythms in the rat. *Brain Res* **164**: 17–38, 1979.
47. Mukherjee, A., P. Kulkarni, S. M. McCann and A. Negro-Vilar. Evidence for the presence and characterization of angiotensin II receptors in rat anterior pituitary membranes. *Endocrinology* **110**: 665–667, 1982.

48. Nicolaidis, S., S. Ishibashi, B. Gueguen, S. N. Thorton and R. deBeaurepaire. Iontophoretic investigation of identified SFO angiotensin responsive neurons firing in relation to blood pressure changes. *Brain Res Bull* **10:** 357–363, 1983.
49. Niwa, M., K. Shigematsu, L. Plunkett and J. M. Saavedra. High affinity substance P binding sites in rat sympathetic ganglia. *Am J Physiol* **249:** H694–H697, 1985.
50. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press, 1982.
51. Peach, M. J. Adrenal medullary stimulation induced by angiotensin I, angiotensin II and analogues. *Circ Res* **28-29:** Suppl II, 107–117, 1971.
52. Phillips, M. I. Biological effects of angiotensin in the brain. In: *Enzymatic Release of Vasoactive Peptides*, edited by F. Gross and H. G. Vogel. New York: Raven, 1980, pp. 337–364.
53. Phillips, M. I., J. F. E. Mann, H. Haebara, W. E. Hoffman, R. Dietz, P. Schelling and D. Ganten. Lowering of hypertension by central saralasin in the absence of plasma renin. *Nature* **270:** 445–447, 1977.
54. Phillips, M. I., J. F. Stamler and M. K. Raizada. Visualization of specific angiotensin II binding sites in the brain by fluorescent microscopy. *Science* **210:** 791–793, 1980.
55. Plunkett, L. M. and J. M. Saavedra. Increased angiotensin II binding affintiy in the nucleus tractus solitarius of spontaneously hypertensive rats. *Proc Natl Sci USA* **82:** 7721–7724, 1985.
56. Quirion, R., M. Dalpé, A. DeLeau, J. Gutkowska, M. Cantin and J. Genest. Atrial natriuretic factor (ANF) binding sites in brain and related structures. *Peptides* **5:** 1167–1172, 1984.
57. Quirion, R., P. Guidreau, S. St Pierre, F. Rioux and C. B. Pert. Autoradiographic distribution of [³H]neurotensin receptors in rat brain: Visualization by tritium-sensitive film. *Peptides* **3:** 757–763, 1982.
58. Ramsay, D. J., L. C. Deil, M. D. Sharpe and J. Shinsako. Angiotensin II increases vasopressin, ACTH, and 11-hydrocorticosteroid secretion. *Am J Physiol* **234:** R66–R71, 1978.
59. Saavedra, J. M. and C. Chevillard. Angiotensin-converting enzyme is present in the subfornical organ and other circumventricular organs of the rat. *Neurosci Lett* **29:** 123–127, 1982.
60. Saavedra, J. M. and C. Chevillard. Vasopressin-reversible increase in angiotensin-converting enzyme in specific hypothalamic nuclei of Brattleboro rats. *Brain Res* **246:** 157–160, 1982.
61. Saavedra, J. M., F. Fernandez Pardal and C. Chevillard. Angiotensin-converting enzyme in discrete areas of the rat forebrain and pituitary gland. *Brain Res* **245:** 317–325, 1982.
62. Schölkens, B. A., W. Jung, W. Rascher, A. Schömig and D. Ganten. Brain angiotensin II stimulates release of pituitary hormones, plasma catecholamines and increases blood pressure in dogs. *Clin Sci* **59:** 53S–56S, 1980.
63. Shults, C. W., R. Quirion, B. Chronwall, T. N. Chase and T. L. O'Donohue. A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system. *Peptides* **5:** 1097–1128, 1984.
64. Simpson, J. B. The circumventricular organs and the central actions of angiotensin. *Neuroendocrinology* **32:** 248–256, 1981.
65. Sirett, N., A. S. McLean, J. T. Bray and J. I. Hubbard. Distribution of angiotensin II receptors in rat brain. *Brain Res* **122:** 299–312, 1977.
66. Stamler, J. F., M. J. Brody and M. I. Phillips. The central and peripheral effects of captopril (SQ 14,225) on the arterial pressure of the spontaneously hypertensive rat. *Brain Res* **186:** 499–503, 1980.
67. Stamler, J. F., M. K. Raizada, R. E. Fellows and M. I. Phillips. Increased specific binding of angiotensin II in the organum vasculosum of the lamina terminalis of the spontaneously hypertensive rat brain. *Neurosci Lett* **17:** 173–177, 1980.
68. Stephan, F. K. and I. Zucker. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc Natl Acad Sci USA* **69:** 1583–1586, 1972.
69. Steele, M. K., A. Negro-Vilar and S. M. McCann. Effect of angiotensin II on in vivo and in vitro release of anterior pituitary hormones in the female rat. *Endocrinology* **109:** 893–899, 1981.
70. Swanson, L. W. and P. E. Sawchenko. Paraventricular nucleus: a site for the integration of neuroendocrine and anatomic mechanisms. *Neuroendocrinology* **31:** 410–417, 1980.
71. Tonnaer, J. A. D. M., G. M. H. Engels, K. Voshart, V. M. Wiegant and W. De Jong. Binding of angiotensins to rat brain tissue: Structure activity relationships. *Brain Res Bull* **10:** 295–300, 1983.
72. Ueda, H., S. Katayama and R. Kato. Area postrema angiotensin sensitive site in brain. *Adv Exp Med Biol* **17:** 109–116, 1972.
73. Unger, T., I. Kaufman-Buhler, B. Schölkens and D. Ganten. Brain converting enzyme inhibition: a possible mechanism for the antihypertensive action of captopril in spontaneously hypertensive rats. *Eur J Pharmacol* **70:** 467–478, 1981.
74. Unnerstall, J. R., D. L. Niehoff, M. J. Kuhar and J. M. Palacios. Quantitative receptors autoradiography using [³H]Ultrofilm: application to multiple benzodiazepine receptors. *J Neurosci Methods* **6:** 59–73, 1982.
75. Van Houten, M., E. I. Schiffrin, J. F. E. Mann, B. I. Posner and R. Boucher. Radioautographic localization of specific binding sites for blood-borne angiotensin II in the rat brain. *Brain Res* **186:** 480–485, 1980.
76. Young, W. S., III and M. J. Kuhar. A new method for receptor autoradiography: [³H]opioid receptors in the rat brain. *Brain Res* **179:** 255–270, 1979.