Angiotensin II Receptors and Angiotensin Converting Enzyme in the Medulla Oblongata

ANDREW M. ALLEN, SIEW-YEEN CHAI, PATRICK M. SEXTON, STEPHEN J. LEWIS, ANTHONY J.M. VERBERNE, BEVYN JARROTT, WILLIAM J. LOUIS, JENNY CLEVERS, MICHAEL J. MCKINLEY, GEORGE PAXINOS, AND FREDERICK A.O. MENDELSOHN

SUMMARY Quantitative in vitro autoradiography was used to map angiotensin II (ANG II) receptors and angiotensin converting enzyme (ACE) in sections from rat, rabbit, sheep, and human medulla oblongata and to follow changes in receptor and ACE density after disruption of vagal projections by nodose ganglionectomy in the rat. ANG II receptors and ACE are both concentrated in the nucleus of the solitary tract and dorsal motor nucleus of vagus of the rat, rabbit, sheep, and human. An ANG II receptor–containing band connecting the nucleus of the solitary tract with the dorsolateral medulla was seen in rabbit and human tissue, providing evidence for association of ANG II receptors with vagal afferent fibers. ANG II receptors were found to be concentrated in the rostral and caudal ventrolateral medulla, which corresponded to the region of C1 and A1 catecholamine-containing cell groups in the rabbit. This localization was also evident in rat and human tissue. In all four species, a prominent, ANG II receptor–rich band in the intermediate reticular nucleus was found to connect the ventrolateral medulla and the dorsal vagal complex. In humans and sheep, this band contains puncta that overlie cell bodies. One week after nodose ganglionectomy in the rat, the density of ANG II receptors in the ipsilateral dorsal vagal complex fell markedly. This fall was most prominent in the rostral dorsal motor nucleus of vagus (to 46% of control density) and in the nucleus of the solitary tract (to 56% of control). ACE levels and calcitonin gene–related peptide receptor density were unchanged in both nuclei after ganglionectomy. These studies identified anatomical substrates for interactions of brain ANG II with vagal function, central catecholaminergic systems, and autonomic activity. (Hypertension 9 [Suppl III]: III-198–III-205, 1987)

KEY WORDS • angiotensin II • angiotensin converting enzyme • receptors • medulla oblongata • autonomic control • calcitonin gene–related peptide

Angiotensin II (ANG II) interacts with the autonomic nervous system at several different levels of organization. The peptide is known to stimulate sympathetic activity centrally and peripherally. In addition to its direct vasoconstrictor effects, ANG II inhibits the baroreceptor reflex by reducing cardiac vagal efferent activity through central and peripheral actions and also through central attenuation of the reflex withdrawal of sympathetic discharge.

Early investigators focused on the ability of circulating ANG II to exert these effects. However, evidence for an intrinsic angiotensin system in the brain has been strengthened by detailed mapping of ANG II–containing elements and ANG II receptors. Neurolytically released as well as circulating ANG II might therefore participate in central effects of ANG II.

ANG II microinjected into the nucleus of the solitary tract (NTS) of the rat exerts a neurogenically mediated pressor effect. At lower doses, biphasic effects occur that probably reflect both vagal and sympathetic actions of the peptide. Microinjection of ANG II into the dorsal motor nucleus of vagus (DMX) produces hypotension and bradycardia.

The first synapse of most vagal primary afferent fibers occurs in the NTS. This nucleus has intrinsic ANG II–containing neurons, receives rich ANG II innervation, and has a high density of ANG II receptors. In order to elucidate actions of ANG II in central
autonomic control), we here report the distribution of both angiotensin converting enzyme (ACE) and ANG II receptors in the medulla oblongata of several mammals, including humans. We determined the possible presence of ACE and ANG II receptor binding sites on vagal afferent fibers and efferent neurons by examining the densities of ACE and ANG II receptors following nodose ganglionectomy in the rat. The presence of calcitonin gene-related peptide (CGRP)-immunoreactive cell bodies within the nodose ganglion and terminals in the NTS suggests that CGRP might be released to activate CGRP receptors in the NTS. Therefore, we also evaluated the density of CGRP receptor binding sites within the NTS after nodose ganglionectomy in the rat.

**Methods**

**Comparative Studies of ACE and ANG II Receptors in the Medulla Oblongata**

Male Sprague-Dawley rats (> 250 g) from the Austin Hospital breeding colony were killed by decapitation and their brains rapidly removed, snap frozen in isopentane on dry ice (−40°C), and stored at −80°C. Three rabbits and five sheep were killed by a lethal dose of sodium pentobarbital and their brains removed, blocked, rapidly frozen, and stored at −80°C. Human brains were obtained 6 to 7 hours postmortem from a 71-year-old man who died of disseminated breast carcinoma and metastatic carcinoma and from a 67-year-old woman who died of bronchopneumonia and obstructive renal failure. In neither case was there pathological evidence of cerebral involvement. The human brains were blocked into 1-cm coronal sections, frozen on dry ice, and stored at −80°C until sectioning for autoradiography.

**Effects of Nodose Ganglionectomy on ACE, ANG II Receptors, and Calcitonin Gene–Related Peptide Receptors in Rat Medulla**

Female Sprague-Dawley rats (16–17 weeks old, weighing 241 ± 5 g) were anesthetized with methohexitol. In one group of rats (n = 5), the left or right nodose ganglion was excised by sectioning the vagus immediately rostral and caudal to the ganglion and the superior laryngeal and the pharyngeal nerves at their point of connection with the ganglion. The contralateral ganglion was exposed but not sectioned. In the sham-operated group (n = 4) both ganglia were exposed but not sectioned. One week after surgery the rats were killed by decapitation and medullary sections prepared for quantitative in vitro autoradiography.

**Quantitative In Vitro Autoradiography**

Twenty-μm frozen sections from the medulla of all four species were cut in a cryostat maintained at −20°C, thaw-mounted onto gelatin-coated slides, and dried at reduced pressure in a desiccator for 14 hours at 4°C. To localize ACE, the sections were incubated with 125I-351A, a derivative of the potent converting enzyme inhibitor, lisinopril, as previously described. For ANG II receptor localization, the sections were incubated with the angiotensin antagonist 125I-[Sar1, Ile8]ANG II, as previously described. To localize CGRP receptors, sections were incubated with 125I-labeled rat CGRP as previously described. After incubation, the sections were rapidly rinsed at 0°C to remove nonspecifically bound radioligand, dried under a stream of cold air, and exposed to Agfa-scopix x-ray film at room temperature for 3 to 7 days (ACE studies) or 10 to 14 days (ANG II and CGRP receptor binding studies). In each cassette, a set of 125I-radioactivity standards was included. For the ACE studies, the system was calibrated for enzyme activity using a set of ACE enzyme standards. The x-ray films were processed and the optical density quantitated using an EyeCom Model 850 image analysis system (Spatial Data Systems, Springfield, VA, USA) coupled to a DEC 11/23 LSI computer (Digital Equipment, Maynard, MA, USA). For the ANG II and CGRP receptor binding studies, the radioactivity standards were fitted to calibration curves by the computer to convert optical density values of each pixel into 125I-radioactivity (dpm/mm²) or bound radioligand (fmol/mg protein). The optical density of the autoradiographs in the ACE studies was calibrated in terms of ACE activity (pmol/min/mg protein) by using the computer to fit calibration curves to the optical densities of the enzyme standards. After autoradiography, the sections were stained with thionine for neuroanatomical localization.

**Statistical Analysis**

Analysis of receptor densities and ACE levels in the dorsal vagal complex after nodose ganglionectomy was performed on four sections from each of two levels of the rat medulla, 4.0 and 4.5 mm caudal to the interaural (IA) line from three experimental and three control rats. Values from each section were used to generate means ± SEM. Paired t-tests were carried out to assess differences between the two sides for rats grouped according to treatment.

In two other rats, separate analyses of ANG II receptor densities in the DMX and the NTS were carried out as described above. Paired t-tests were used to examine changes in ANG II receptor density between control and lesioned sides at the two levels described.

**Results**

**ANG II Receptors and ACE in Rat, Rabbit, Sheep, and Human Medulla Oblongata**

Figure 1 demonstrates ANG II receptor binding and ACE localization in serial sections from rat, rabbit, sheep, and human medulla oblongata. These sections were taken caudal to the rostral pole of the hypoglossal nucleus and rostral to the level of the area postrema, although in the human section the area postrema is still visible (see Figure 1 G). In the rat, the section is approximately 4.2 mm caudal to the IA line. Consequently, at this level, the ventrolateral reticular nucleus is composed predominantly of C1 adrenaline-containing cells.

A high density of ANG II receptors was found in
FIGURE 1. Color-coded images of ANG II receptors (left panel) and ACE (right panel) visualized by in vitro autoradiography in sections from rat (A, B), rabbit (C, D), sheep (E, F) and human (G, H) medulla oblongata. The level of section in the rat is 4.2 mm caudal to the interaural line, which is just rostral to the level of the area postrema and caudal to the rostral pole of the hypoglossal nucleus. For the other species, a similar level is shown. The color scales have been adjusted to demonstrate radioligand binding optimally for each section and are not equivalent between sections. SOL = nucleus of the solitary tract; DMX = dorsal motor nucleus of vagus; IRT = intermediate reticular nucleus; RVL = rostral ventrolateral reticular nucleus; IO = inferior olivary nucleus; IV = fourth ventricle; CHP = choroid plexus; AP = area postrema.
several regions of the medulla. First, ANG II receptors were found in high concentrations in the NTS and DMX of rat, sheep, and human medulla (Table 1; see Figure 1 A, C, E, and G). In the rabbit, the extent of ANG II receptor binding in the DMX was confined to the ventral edge of this nucleus (see Figure 1 C). Second, in the rostral and caudal ventrolateral medulla (VLM), corresponding to the C1 and A1 catecholamine-containing cell regions, respectively, ANG II receptor binding was observed in sections from all four species (see Table 1 and Figure 1). This localization was most prominent in the rabbit and human (see Figure 1 C and G). Third, in all species a band of receptor-dense tissue was seen in the region between the dorsal vagal complex and the rostral and caudal VLM. This region corresponds to the intermediate reticular nucleus of the rat. This band was very prominent in the human medulla (see Figure 1 G) and consisted of punctate binding overlaying neuronal cell bodies. In all cases, the band of receptor-dense tissue skirted and sometimes surrounded the nucleus ambiguus and was seen mostly dorsal to the lateral reticular nucleus (see Figure 1 G). In the human medulla, the solitary tract was observed to contain moderate levels of ANG II receptor binding and, at the level of entry of vagal rootlets into the medulla oblongata, ANG II receptor-positive fascicles were found in the area between the dorsolateral surface of the medulla and the solitary tract (not shown). This was also observed in the rabbit (not shown). These rootlets were located rostral to the hypoglossal nucleus in both human and rabbit medulla. The area postrema of the rat, rabbit, and sheep exhibited moderate ANG II receptor density, whereas that of the human exhibited no detectable ANG II receptor binding (see Table 1 and Figure 1 G). Finally, ANG II receptors were observed only in the inferior olivary nucleus of the rat (see Figure 1 A) and sheep (level not shown).

In the rat, moderate levels of ACE activity have been detected in the dorsal vagal complex (NTS and DMX) and also in the inferior olivary nucleus (see Table 1 and Figure 1 B). In the rabbit, sheep, and human, ACE activity was found consistently in these nuclei (see Figure 1 D, F, and H). High levels of ACE activity were found to occur in the area postrema of the sheep (not shown) and human (see Figure 1 H), but not of the rat (see Table 1). High levels of ACE activity were also observed in the ependyma of the fourth ventricle and the choroid plexus, shown here in the rat and rabbit (see Figure 1 B and D).

**ACE, ANG II Receptors, and Calcitonin Gene-Related Peptide Receptors in Rat Medulla Oblongata After Unilateral Nodose Ganglionectomy**

ANG II receptor density in the dorsal vagal complex was 12% higher caudally than rostrally ($p < 0.05$); therefore, measurements of receptor density were made at two different levels, one at the rostral pole of the hypoglossal nucleus, 4.0 mm caudal to the IA line, and another at a more caudal site, just rostral to the area postrema 4.5 mm caudal to the IA line.

In sham-operated animals, there was no significant difference in ANG II receptor density in the dorsal vagal complex between the two sides at either the rostral or caudal levels (Figure 2; mean difference, 1.4 ± 2.1%). In the ganglionectomized animals, ANG II receptors on the lesioned side were reduced to 65% of the density on the sham-operated controls. At the rostral level, sham density was 17.9 ± 0.4 and lesioned density was 11.4 ± 0.6 fmol/mg protein. At the caudal level, sham density was 19.6 ± 1.3 and lesioned density was 12.8 ± 1.3 fmol/mg protein. As shown in Figure 3, these values were significantly lower than respective values for the contralateral side at both levels (rostral, 17.1 ± 0.5; caudal, 16.3 ± 1.4 fmol/mg protein; $p < 0.02$). ANG II receptor density at the rostral level was unchanged on the side contralateral to the lesion, being 98% of that in sham-operated animals (see Figure 3). At the caudal level, the side contralateral to the lesion showed a nonsignificant decrease in ANG II receptor density to 87% of that in the sham-operated animals ($p = 0.06$; see Figures 2 and 3).

In two ganglionectomized rats, where the resolution of receptor binding between the NTS and the DMX could be clearly distinguished on the Nissl-stained sections, the pattern of changes between these nuclei was analyzed further (Figure 4). At the rostral level, the fall in ANG II receptors on the side ipsilateral to the lesion was comparable in the NTS and DMX, being 56% ($p < 0.0001$) and 46% ($p < 0.0001$) of the contralateral values, respectively. In the NTS control values were 12.9 ± 0.6 and lesioned values were 7.2 ± 0.5 fmol/mg protein. In the DMX control values were 11.4 ± 0.3 and lesioned values were 5.2 ± 0.2 fmol/mg protein. At the caudal level, the fall in ANG II receptor density on the side ipsilateral to the lesion was smaller in the NTS than in the DMX, being 82%
Figure 2. The effect of nodose ganglionectomy on ANG II receptor binding and ACE levels in the rat medulla oblongata 4.3 mm caudal to the interaural line (IA). Nissl-stained section (A) showing the nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus (DMX), and inferior olivary nucleus (IO). Computer-generated images of autoradiographs showing receptor binding of $^{125}$I-$\text{Sar}^1,\text{Ile}^8$ANG II (B). ACE localized with $^{125}$I-351A (C) and $^{125}$I-labeled rat calcitonin gene-related peptide (CGRP) receptors (D). For ANG II, red represents $>16$; yellow 6.5–16; green 1.5–6.5; and blue $<1.5$ fmol/mg protein. For CGRP, red represents $>60$; yellow 30–60; green 10–30; and blue $<10$ fmol/mg protein. For ACE, red represents $>1000$; yellow 400–1000; green 50–400; and blue $<50$ pmol/min/mg protein.

Figure 3. ANG II receptor density in the dorsal vagal complex of rats 1 week after nodose ganglionectomy. Densities were measured relative to the interaural line (IA) at a rostral (IA - 4.0 mm) and a caudal (IA - 4.5 mm) level on the ipsilateral lesioned (Ls) and contralateral (C) sides of ganglionectomized rats (lesion) compared to sham-operated rats (sham). Values are means ± SEM of quadruplicate measurements for each level from three sham-operated and three ganglionectomized rats (n = 12). Asterisk indicates $p < 0.02$. All = ANG II; L = left; R = right.

(p < 0.01) and 57% ($p < 0.0001$) of the contralateral values, respectively. In the NTS control values were 15.4 ± 0.5 and lesioned values were 12.7 ± 0.9 fmol/mg protein. In the DMX control values were 13.5 ± 0.4 and lesioned values were 7.7 ± 0.9 fmol/mg protein.

Following ganglionectomy, the DMX showed a large decrease in ANG II receptor binding on the ipsilateral side over the majority of its distribution. Along its rostrocaudal axis, the NTS showed its greatest decrease in ANG II receptor density rostral to the level of the area postrema. No change was observed in ANG II receptor binding caudal to the obex. Within the NTS, the largest reduction in receptor density was observed in the medial subnucleus, with the lateral subnucleus showing a smaller change.

ANG II receptor density in the area postrema and the inferior olivary nucleus did not change after nodose ganglionectomy. There were no differences between ACE levels from the two sides of the sham-operated animals (left, 360 ± 15 pmol/min/mg protein; right, 370 ± 10 pmol/min/mg protein; $p > 0.6$), between the
of Lind et al., who identified ANG II-containing cells in the NTS and also the DMX and area postrema. This finding was in good agreement with that of Lind et al., who identified ANG II-containing cells in the NTS and also the DMX and area postrema. These studies demonstrated a characteristic pattern of ANG II receptor and ACE distribution within the medulla oblongata that was similar among the four species studied. The distribution of ANG II receptors in the dorsal vagal complex was shown to be associated with vagal neurones, and both the medial subnucleus of the NTS and the DMX were shown to have large decreases in ANG II receptor concentration following ganglionectomy. Our results also suggested that ACE and CGRP are not associated with the same components of the vagus nerve.

These studies revealed high densities of ANG II receptors and ACE in several regions of the medulla. First, high densities of ANG II receptors were observed in the NTS and DMX of the rat, rabbit, sheep, and human medulla oblongata (see Figure 1 A, C, E, and G). This finding was in good agreement with that of Lind et al., who identified ANG II-containing cells and a plexus of ANG II-containing terminals in the NTS of the rat. We also found high concentrations of ACE in these structures in all four species (see Figure 1 B, D, F, and H). In both rabbit and human medulla, ANG II receptor–containing fascicles were observed coursing between the dorsolateral medulla and the solitary tract. In this position, these fascicles almost certainly represent ANG II receptors associated with vagal afferent fibers. In the human medulla, ANG II receptors were also observed to be moderately dense in the solitary tract itself.

Second, in all four species, ANG II receptors were found in the rostral and caudal VLM, corresponding to the C1 adrenaline and A1 noradrenaline cell groups, respectively. The C1 cells have a critical role in arterial blood pressure regulation and project to innervate cholinergic preganglionic neurones in the spinal cord, whereas the A1 cells project rostrally to innervate vasopressin-containing cells in the hypothalamus and may modulate vasopressin release. Our studies showed high concentrations of ANG II receptors in the areas occupied by C1 and A1 cells. Further studies will be needed to discover whether ANG II receptors are associated with catecholamine-containing cells in the medulla oblongata and hypothalamus. Our current findings suggest that ANG II may modulate the activity of the C1 and A1 cells, in a manner analogous to its effects on peripheral autonomic neurones and terminals.

Indeed, recently ANG II has been shown to stimulate norepinephrine uptake in cultured neurones derived from hypothalamus and brainstem, supporting the concept that ANG II interacts with central catecholamine-containing neurones.

Third, in all four species we found a band of ANG II receptors in the region between the dorsal vagal complex and the VLM in the intermediate reticular nucleus. This receptor-positive band was most prominent in the human medulla and was associated with neuronal cell bodies. We found that the receptor-positive band was present in the rostrocaudal length of the NTS. This finding is in good agreement with evidence that a prominent group of ANG II–containing fibers traverses the rat medulla mediadorsally from the VLM. Fourth, we found that the inferior olivary nucleus contained high levels of ACE in all species studied except humans, in which ACE levels were moderate. ANG II receptors were found in the inferior olivary nucleus of only the rat and sheep medulla. The role of ANG II in this precerebellar nucleus, if any, is not known.

Effect of Nodose Ganglionectomy

The present study demonstrated that unilateral nodose ganglionectomy results in a reduced density of ANG II receptor binding sites within the dorsal vagal complex of the rat. In the rat, sensory connections of the vagus are known to project to a wide extent of the NTS and also the DMX and area postrema. This afferent vagal input to the NTS is most marked in the medial and commissural subnuclei, which parallel the areas of dense ANG II receptors observed in the
present study. The results of nodose ganglionectomy and the finding of ANG II receptors associated with intramedullary vagal afferent fibers indicate that at least a component of the ANG II receptor population in the NTS and DMX appears to be associated with terminals of vagal afferent projections. However, the change in receptor density observed in the NTS and DMX is also consistent with the possibility that ANG II binding sites are present on cell bodies or dendritic processes of vagal motor neurones, which are also known to project widely throughout the NTS.25 The association of ANG II receptors with vagal afferent terminals in the dorsal vagal complex, with vagal motor neurones, or both, suggests that ANG II may act at these sites to inhibit the baroreceptor reflex centrally.

That nodose ganglionectomy did not affect ACE levels in the dorsal vagal complex is consistent with the possibility that ACE is present intrinsically in NTS neurones or arises from supramedullary projections to this nucleus. No significant change in CGRP receptor binding was observed after nodose ganglionectomy, suggesting that either CGRP present in the nodose ganglion is not released onto sites within the NTS or that a change in the density of CGRP receptor binding sites requires a different time course. However, the finding that CGRP receptors were unaltered by nodose ganglionectomy indicates that CGRP receptors in the dorsal vagal complex are unlikely to be associated with vagal afferent terminals, which are destroyed by this procedure.13 The lack of change in CGRP receptors after nodose ganglionectomy also indicates that the observed changes in ANG II receptor density are specific for the angiotensin system and are not due to a nonspecific destruction of the dorsal vagal complex by the procedure.

These results have been published in part in preliminary form.26 After the present study was completed, we became aware of other reports of reduced ANG II receptors in the dorsal vagal complex after nodose ganglionectomy in the dog.27,28 and rat.28,29

These findings demonstrate that ANG II receptors in the medulla oblongata are associated with nuclei critically involved in regulation of autonomic activity, including the NTS, DMX, and the areas of the catecholamine-containing cells in the VLM. The association of ANG II receptors with the rostral and caudal VLM raises the possibility that ANG II interacts with central catecholamine-containing cells in these areas. Overall, our findings support the hypothesis that ANG II is an important modulator of vagal function and central catecholaminergic pathways involved in sympathetic activation and vasopressin release.

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Angiotensin II receptors and angiotensin converting enzyme in the medulla oblongata.
A M Allen, S Y Chai, P M Sexton, S J Lewis, A J Verberne, B Jarrott, W J Louis, J Clevers,
M J McKinley and G Paxinos

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