The Ventrolateral Medulla
A New Site of Action of the Renin-Angiotensin System

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SUMMARY High-affinity binding sites for angiotensin II (Ang II) in the ventrolateral medulla suggest that Ang II may act at cell groups that are known to modulate blood pressure. This hypothesis was investigated by the topical application of angiotensin I (Ang I), Ang II, the Ang II antagonist [Sar1, Thr1] Ang II, and the Ang I converting enzyme inhibitor MK 422 to a restricted region of the ventral medullary surface known as the glycine-sensitive area. Both Ang I (100 pmol) and Ang II (100 pmol) produced significant (p<0.01) increases in blood pressure (+20 ± 4 and +31 ± 5 mm Hg, respectively) that were associated with no change in heart rate. Furthermore, the relationship between the peak increases in blood pressure and Ang II was dose-dependent. Blockade of endogenous Ang II by [Sar1, Thr1] Ang II (13 nmol) produced a significant decrease in baseline blood pressure (-8 ± 1 mm Hg; p<0.001). Similarly, topical application of MK 422 prevented the pressor effect of Ang I. Taken together, these experiments indicate that at least some components of the renin-angiotensin system exist in the ventrolateral medulla and they may modulate vasomotor outflow. (Hypertension U [Suppl I]: I-163-I-166, 1988)

KEYWORDS • blood pressure • vasomotor center • ventrolateral medulla • angiotensin II antagonist • angiotensin converting enzyme • angiotensin II

ANGIOTENSIN II (Ang II) acts at a number of sites in the central nervous system to influence circulatory homeostasis. At the subfornical organ it provokes drinking and elicits a pressor response,1 2 and at the area postrema it increases blood pressure through activation of sympathetic nervous system outflow.3 When injected into superficial structures of the dorsomedial medulla of rats and dogs, Ang II affects blood pressure and heart rate through alterations in the activity of the sympathetic and parasympathetic nervous system.4 6

Ang II binding sites have been identified in these regions by quantitative autoradiography.7 8 In the dog, high-affinity binding for Ang II has also been localized in the rostral ventrolateral medulla.7 8 In many species besides the dog this region of the ventrolateral medulla is known to have a facilitatory action on spinal sympathetic preganglionic neurons.9 Guertzenstein10 showed that topical application of various neurochemicals to a specific region significantly affects the activity of the sympathetic nervous system. On the basis of the depressor response evoked by topical application of glycine, Guertzenstein and Silver11 proposed that this area be referred to as the glycine-sensitive area (GSA). This location of the GSA coincides with the C1 catecholamine cell group of the ventrolateral medulla.9 Because of the juxtaposition of high-affinity binding for Ang II with neural structures known to influence sympathetic nerve activity, we characterized the action of Ang II applied topically to the GSA.

Materials and Methods
Experiments were performed on 24 cats (body weight, 3.4 ± 0.3 kg) initially sedated with ketamine hydrochloride (20 mg/kg i.m.) and anesthetized with α-chloralose (60 mg/kg i.v.). Catheters were inserted into a femoral artery and vein for the measurement of arterial blood pressure and the administration of fluids and drugs, respectively. The trachea was cannulated, and the animal was artificially ventilated. The head of each cat was fixed in a stereotaxic frame and the ventral aspect of the medulla was exposed as described by Feldberg and Guertzenstein.12 A pair of Perspex rings10 was positioned at the GSA, which corresponded to a site between the rostral hypoglossal rootlets and the caudal border of the trapezoid bodies. All surgical procedures were performed in accordance with our
in institutional guidelines on animal use in research.

Drugs, applied in a 25-μl volume into each ring, included Ang I (Bachem, Torrance, CA, USA), Ile2-Ang II and the Ang II antagonist [Sar1, Thr5]Ang II (synthesized by M. Khosla, Cleveland Clinic, Cleveland, OH, USA), and the angiotensin converting enzyme (ACE) inhibitor MK 422 (enalaprilat; gift of C.S. Sweet, Merck Institute for Therapeutic Research, West Point, PA, USA).

To determine the cell groups of the ventrolateral medulla that were influenced by Ang II applied topically. 125I-Ang II (New England Nuclear, Boston, MA, USA) was delivered into the Perspex rings in one experiment. Venous blood was collected before and 5 minutes after application of the labeled Ang II. The labeled Ang II was then removed from the rings, the animal was killed, and the brainstem was removed quickly. The brainstem was sectioned coronally (50 μm), tissue was apposed to x-ray film (SB-5, Kodak, Rochester, NY, USA) for 3 to 6 weeks, and the distribution of labeled Ang II was assessed.

All results are expressed as means ± SEM. Blood pressure and heart rate before and during drug intervention were compared using a paired t test. Differences were considered significant for a p value below 0.05.

Results

Application of Ang II to the GSA produced dose-dependent effects on blood pressure over a dose range of 12.5 to 100.0 pmol of Ang II (Figure 1), whereas delivery of 0.0125 and 6.25 pmol of the peptide had no effect on blood pressure. The time course of the pressor response to topical application of 100 pmol of Ang II is shown in Figure 2. In this animal, blood pressure increased within 15 seconds and reached a maximum between 2 and 3 minutes after application of the peptide. On average, the peak pressor response occurred at 2.0 ± 0.3 minutes (n = 6). Both Figures 1 and 2 illustrate that Ang II, acting at the GSA, had little effect upon heart rate. To evaluate whether some of the Ang II applied to the GSA may have gained access to the general circulation, we injected similar amounts (100 pmol) of Ang II intravenously in three cats. In contrast to the responses described above, this produced a rapid increase in blood pressure (+24 ± 5 mm Hg) associated with bradycardia (−10 ± 7 beats/min).

To test the specificity of action of Ang II at the GSA, the Ang II antagonist [Sar1, Thr5]Ang II was delivered into the Perspex rings. In seven cats Ang I evoked a pressor response that averaged +20 ± 4 mm Hg (from 122 to 142 mm Hg; p < 0.01). Delivery of the ACE inhibitor MK 422 (100 nmol) in the rings had no effect on baseline blood pressure or heart rate. However, placement of Ang I in the rings immediately after removal of MK 422 did not elicit an increase in blood pressure (121 ± 8 mm Hg before Ang I vs 124 ± 8 mm Hg after Ang I).

The sites at which Ang II might act were evaluated in one cat by placing 125I-Ang II (100 fmol; specific activity, 2.2 μCi/pmol) in the Perspex rings. Venous blood samples obtained when the labeled Ang II had been in the rings for 5 minutes showed no increase in counts above background levels. The autoradiograms showed that the greatest depth of penetration of labeled Ang II below the ventral surface was no more than 0.5 mm. The spread of the labeled peptide in the lateral direction was 5 to 6 mm off the midline. Autoradiograms from coronal sections had detectably increased density for sections between 3.75 mm caudal and 3.75 mm rostral to the most rostral hypoglossal rootlets, and measurably greater density between 2.25 mm caudal.
and 3.75 mm rostral to the most rostral hypoglossal rootlets. These measurements were similar to the dimensions of the Perspex rings, which were 3.5 mm in the rostrocaudal direction and 5 mm lateral to the midline.

Discussion

The data presented here constitute the first demonstration (to our knowledge) in the cat that Ang II acts at the ventral medulla to increase blood pressure. Our data support the previous observation of Punnen et al.\textsuperscript{13} that microinjection of Ang II into the ventrolateral medulla of rats evokes pressor responses. Furthermore, the decrease in blood pressure observed during application of the Ang II antagonist suggests that Ang II may act at this site upon neural elements that contribute to the maintenance of blood pressure. The results of these experiments also suggest that components of the renin-angiotensin system may be endogenous to this brainstem region, since MK 422 effectively blocked the pressor response associated with the local application of Ang I.

The effects of topically applied Ang I, Ang II, and the Ang II antagonist provide evidence for a direct action of Ang II upon structures in the ventrolateral medulla. It has been shown in a number of species that cells in the nucleus paragigantocellularis lateralis (NPGCL) have axonal projections to sympathetic preganglionic neurons of the spinal cord.\textsuperscript{9} Cells of the NPGCL are found dorsal to the site where the Perspex rings were placed; this cell group may also consist of neurons containing phenylethanolamine-N-methyl transferase.\textsuperscript{9} However, the present data do not allow us to state whether Ang II and angiotensinlike peptides acted directly upon cells of the NPGCL or indirectly by way of more superficial neurons. The autoradiograms obtained with labeled Ang II demonstrated that the topically applied peptide did not penetrate the underlying tissue deeper than 0.5 mm. This result would suggest that the peptide acted upon a small number of NPGCL neurons or more superficially located neurons having axonal projections to the intermediolateral cell column.\textsuperscript{14}

Several considerations suggest that the major mode of action for topically applied Ang II (or angiotensinlike peptides) was not due to diffusion of the peptide into the general circulation. First, application of radiolabeled Ang II in the Perspex rings did not result in detectable radioactivity in peripheral blood 5 minutes after the labeled peptide had been placed in the rings. Second, intravenous administration of the Ang II antagonist did not modify the pressor response to topically applied Ang II. Third, topical application of the Ang II antagonist did not alter the pressor response to intravenous injection of Ang II.

Although the effector pathway responsible for the hemodynamic effects associated with application of Ang II to the GSA was not investigated, the characteristics of the pressor response suggest a sympathetic-mediated increase in vascular resistance. The absence of any change in heart rate is consistent with this possibility. McAllen\textsuperscript{15} showed that neurochemical stimulation of the superficial ventral medulla can provoke pressor responses often associated with no change in heart rate. Gatti et al.\textsuperscript{16} observed that, with the vagi intact, topical application of L-glutamic acid to the GSA evoked an increase in blood pressure that was not accompanied by alteration in heart rate. After bilateral vagotomy, L-glutamic acid elicited increases in both blood pressure and heart rate. These data suggest that the absence of a consistent change in heart rate may be due to either a direct action of Ang II on

\begin{figure}[h]
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\caption{Pressor response provoked by topical application of Ang II (100 pmol) to the glycine-sensitive area. Within 15 seconds after delivery of Ang II into the Perspex rings, arterial blood pressure increased; by 3 minutes after placement of the peptide in the rings blood pressure had plateaued (increase of 30 mm Hg). Heart rate was not altered during the increase in blood pressure.}
\end{figure}
vasomotor neurons or a reflexly mediated suppression of the heart rate component.

In addition to the demonstration that Ang II applied to the ventral medulla can provoke changes in blood pressure, the results of this study provide evidence that endogenous Ang II may act to modulate the activity of vasomotor neurons. The most direct piece of evidence in this regard is the observation that topical application of the antagonist [Sar¹, Thr⁸] Ang II produced a significant decrease in arterial blood pressure. Furthermore, this observation provides a functional correlate for Ang II binding in the ventrolateral medulla of the dog.²,³

The possibility of a local action by in situ generation of Ang II is suggested by the observation that ACE inhibition by MK 422 prevented the pressor response provoked by local application of Ang I. Chevillard and Saavedra in the rat and, recently, Santos et al. in the dog showed higher levels of ACE activity colocalized with the A1 and C1 catecholamine cell groups of the ventrolateral medulla. Finally, Bridle et al. showed that renin activity is concentrated in the ventrolateral medulla of the dog at sites that are also rich in catecholamines.

In conclusion, this study demonstrated that Ang II acts in the rostral ventrolateral medulla to influence the level of arterial pressure. This action of Ang II is probably mediated by neuronal structures in the ventrolateral medulla that project to sympathetic preganglionic neurons.

Acknowledgments

The authors acknowledge the advice of Drs. Debral and Maria J. Campagnole-Santos. In addition, the technical assistance of Mark Chappell, Michele Flasher, and Lisana Mann is appreciated.

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The ventrolateral medulla. A new site of action of the renin-angiotensin system.
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Hypertension. 1988;11:I163
doi: 10.1161/01.HYP.11.2_Pt_2.I163

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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