Circulatory Effects of Chronic Administration of Angiotensin II into the Cerebrolateral Ventricles of Dogs

Studies on the Development of an Experimental Model of Hypertension

BHAGAVAN S. JANDHYALA, PH.D., MUSTAFA F. LOKHANDWALA, PH.D., PREMANAND NANDIWADA, M.S., AND JOSEPH P. BUCKLEY, PH.D.

SUMMARY Intraventricular administration of angiotensin II (All), 1 µg, twice a day for 2 weeks, together with saline as the drinking fluid during the second week, resulted in moderate but significant elevation of the arterial pressures of mongrel dogs. This increase in arterial pressure was accompanied by a significant elevation in femoral and renal vascular resistances. However, resting neurogenic tone to the hind limb vasculature and peripheral sympathetic transmission to renal and femoral vascular beds were not altered by All treatment, indicating that the elevation of intrinsic vascular tone of the smooth muscle was responsible for elevated vascular resistance in the hind limb vasculature. Furthermore, pressor responses to intravenous (I.V.) norepinephrine and angiotensin II were enhanced. Also, reflexly mediated vasodilator responses were attenuated in the hind limb, and vascular responses to intra-arterial and I.V. doses of histamine were reduced. However, blood pressure responses to bilateral carotid occlusion and intra-arterial and I.V. responses to acetylcholine and bradykinin were unaffected. The concentration of urinary sodium was significantly greater in the All-treated group. The data from this investigation indicate that chronic cerebrolateral ventricular administration of angiotensin II (plus saline) to mongrel dogs resulted in certain vascular alterations that are conducive to the development of high blood pressure. (Hypertension 1: 219-227, 1979)

KEY WORDS • angiotensin II • reflexogenic activity • blood pressure • histamine activity • norepinephrine response • angiotensin response

INVESTIGATORS have demonstrated that the administration of angiotensin II into the central nervous system (CNS) either via a vertebral artery or via the cerebrolateral ventricles results in a transient elevation of the arterial pressure in a number of animal species. This increase in arterial blood pressure appeared to be primarily due to increases in the total peripheral resistance in cats and dogs and to an increase in the cardiac output in greyhounds. These observations lead to the speculation that the central actions of angiotensin II may play a role in the etiology of hypertension. Recent studies leading to the discovery of an iso-renin angiotensin system within the CNS further strengthened the theory that the central actions of angiotensin may have a physiological or pathological role. However, it has not been demonstrated that chronic elevation of angiotensin II levels within the cerebrospinal fluid (CSF) is conducive to the development of hypertension in experimental animals. The present study was undertaken to investigate whether chronic administration of angiotensin II into the lateral ventricles of mongrel dogs would lead to the development of high blood pressure and if so, to investigate cardiovascular alterations accompanying such a hypertensive state.

Methods

Thirty-seven conditioned, worm-free, mongrel dogs of either sex were used in these studies. The animals were initially anesthetized with sodium pentobarbital (35 mg/kg, I.V.) and the skull was rigidly secured in a David-Kopf stereotaxic apparatus. A sterile unbeveled stainless steel cannula (18 gauge) equipped with a threaded head was introduced into a cerebrolateral ventricle (approximately 8 mm posterior and 7 mm lateral to the bregma) and lowered into the brain with...
Perfused Hind Limb Studies

The peripheral end of one of the femoral arteries was catheterized with polyethylene tubing and perfused with blood drawn from the central end of the same vessel. The length of the tubing used in the perfusion circuit was such that it allowed for sufficient delay to observe the reflexly elicited action of an intravenously administered drug prior to its entry into the hind limb circulation. Blood coagulation was prevented by heparinization (600 units/kg, I.V.). After endotracheal intubation, the animals were placed on positive pressure ventilation (Bird Respirator, Mark 7). Both carotid arteries were isolated for the purpose of obtaining bilateral carotid occlusion responses and arterial blood pressure recorded from a catheterized femoral artery. The following studies were conducted in different groups.

Studies of Peripheral Sympathetic Transmission to Renal and Femoral Vasculature

Following anesthesia, a lateral abdominal incision was made on the left side, the renal artery located and a 3-mm electromagnetic flow probe placed around the vessel and resting blood flow monitored by means of a Statham Squarewave electromagnetic flowmeter (SP-2202, Statham Instruments, Oxnard, CA). The renal nerve plexus was isolated and stimulated with supramaximal voltage at various frequencies (Grass stimulator, Model SD9 B, Grass Instruments, Quincy, MA) with pulse duration of 1 msec, and corresponding alterations in blood flow were recorded.

Right femoral blood flow was similarly recorded using a Statham flowmeter. After a midline abdominal incision was made, the ipsilateral lumbar sympathetic chain was isolated and sectioned at the L-5 level. The peripheral segment was stimulated at various frequencies with supramaximal voltage and changes in femoral blood flow recorded. All parameters were recorded on a Grass polygraph (Model 7D). Vascular resistance units were computed as the ratio of mean blood pressure (mm Hg) over blood flow (ml/sec).10 In some groups of animals, autonomic ganglionic transmission was interrupted by administering hexamethonium (10 mg/kg, I.V.) and atropine sulfate (0.5 mg/kg) and changes in blood pressure were recorded.

Urinary Electrolytes

Urine samples were collected from a catheterized ureter in groups of dogs under pentobarbital anesthesia and sodium and potassium concentrations estimated using a Perkin-Elmer Atomic Absorption Flame Photometer. Electrolyte levels are presented as mEq/liter.

All the data are represented as mean ± standard error of the means. A paired t test was used to determine significant changes within a group and Student's t test, to determine statistical significance between groups.

Results

Acute intraventricular administration of angiotensin II in five dogs, 1 μg, produced an increase in mean arterial pressure of 34 mm Hg with a maximum effect occurring between 6 and 10 minutes following administration of the peptide. The pressor effect was accompanied by an increase in drinking of 80-100 ml of fluid, which occurred approximately 10 minutes after the administration of angiotensin II and lasted up to 3 minutes. Chronic intraventricular administration of angiotensin II produced only a slight increase in

a vernier-controlled electrode carrier until CSF was seen at the electrode tip when the stylet was withdrawn. The needle head was screwed into the skull and firmly secured with dental cement. The skin was drawn together and sutured around the cannula with sterile surgical silk and the cannula capped with a male Luer-Lok hub cap. Surgical procedures were conducted under sterile conditions and the animals permitted 7 days to recover from surgery. They were then trained so that indirect blood pressure measurements could be obtained from the left foreleg utilizing an Arteriosonde unit (Roche Electronics, Model 1010).

The dogs were divided into two groups of 16 dogs each. One group received an intraventricular injection of a sterile solution of angiotensin II, 1 μg (approximately 60 ng/kg) dissolved in 0.05 ml of saline twice daily, while the placebo group received an equal volume of sterile saline using a Hamilton Microsyringe. After 7 days, drinking water was replaced with normal saline and intraventricular treatment with angiotensin or saline continued for an additional 7 days. During the treatment period, indirect blood pressure measurements were obtained every 2 days.

Following the administration of angiotensin II or saline for 14 days, dogs were anesthetized with sodium pentobarbital (35 mg/kg, I.V.). After endotracheal intubation, the animals were placed on positive pressure ventilation (Bird Respirator, Mark 7). Both carotid arteries were isolated for the purpose of obtaining bilateral carotid occlusion responses and arterial blood pressure recorded from a catheterized femoral artery. The following studies were conducted in different groups.
arterial blood pressure at the end of 6 days (blood pressure recorded 4 hours after intraventricular administration of angiotensin II). Since we had previously found that intraventricular infusion of angiotensin II to anesthetized cats significantly enhanced urinary output and the rate of sodium excretion, the animals were placed on a regimen in which isotonic sodium chloride solution was used as the drinking fluid in both the control and treated groups from Days 7 to 14. There was a significant increase in systolic, diastolic and mean arterial blood pressures in the treated animals at the end of 10, 12 and 14 days, whereas there was actually a slight reduction in arterial pressures of the control group during this period (fig. 1). Mean arterial pressure increased by 11 mm Hg at the end of 10 days, 15 mm Hg at the end of 12 days and 19 mm Hg at the end of 14 days of treatment.

The effects of centrally administered angiotensin II on peripheral sympathetic transmission are summarized in figure 2. These data indicate that increasing the angiotensin II levels in the cerebrospinal fluid for periods of 14 days did not significantly alter peripheral sympathetic transmission to the renal and femoral beds. However, both femoral and renal resting blood flows were significantly lower and the vascular resistance in both beds significantly greater in the angiotensin-treated dogs (fig. 3). In addition, the mean blood pressure was significantly greater in the animals treated with angiotensin II than in the placebo group.

The femoral pressure-flow curves obtained from both groups of dogs are shown in figure 4. Chronic intraventricular administration of angiotensin II produced a marked shift of the curves to the left, indicating an increase in resistance in the hind limb vasculature. Furthermore, this shift remained significantly to the left of the pressure-flow curves of the control group following acute denervation and denervation plus phentolamine (fig. 5). These data suggest that the increase in resistance was not due to an increase in the neurogenic tone or to enhanced tone contributed by circulating catecholamines acting on alpha-adrenergic receptors, indicating that there was...
an increase in the intrinsic tone of vascular smooth muscle of the dogs receiving chronic intraventricular angiotensin II and saline drinking fluid.

Intravenous administration of norepinephrine, 0.125 to 0.5 μg/kg, produced dose-related pressor effects accompanied by a reflexly mediated reduction in perfusion pressure (fig. 6). The pressor effects produced by the two higher doses of norepinephrine and angiotensin II were significantly greater in the angiotensin II-treated group, whereas reflexly mediated vasodilator responses were attenuated (fig. 6, table 1). The depressor responses to histamine phosphate administered intravenously were significantly attenuated in the angiotensin II-treated group (table 2), whereas the bilateral carotid occlusion response and the effects of I.V. bradykinin and acetylcholine were not significantly altered. Furthermore, the decrease in perfusion pressure produced by the intra-arterial administration of histamine, 0.5 and 1 μg, were significantly less in the angiotensin II-treated animals than in the controls, whereas the effects induced by intra-arterial bradykinin and acetylcholine were not significantly altered by chronic intraventricular administration of angiotensin II (fig. 7). Autonomic ganglion blockade with hexamethonium and atropine resulted in essentially similar reductions in the arterial pressure in both groups. The decrease in the pressure for the treated group was 56.2 ± 5.9 mm Hg and for the control group, 62.5 ± 6.1 mm Hg.

The mean urinary sodium levels obtained in the treated and placebo groups were 196.5 ± 10.4 and 128.3 ± 14.5 (mEq/liter), respectively (p < 0.01). The urinary potassium levels for the treated and placebo groups were 88.6 ± 11.2 and 123.3 ± 3.5 mEq/liter, respectively (p < 0.01).

<table>
<thead>
<tr>
<th>Drug</th>
<th>I.V. dose (μg/kg)</th>
<th>Increase in mean blood pressure (mm Hg, mean ± SEM)</th>
<th>Reflex decrease in hind limb perfusion pressure (mm Hg, mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Placebo (n = 8)</td>
<td>All treated (n = 8)</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>0.1</td>
<td>16.8 ± 1.8</td>
<td>21.2 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>23.4 ± 2.1</td>
<td>34.1 ± 2.2*</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>32.5 ± 2.4</td>
<td>45.0 ± 2.6*</td>
</tr>
</tbody>
</table>

*p < 0.05.
Figure 3. Effects of chronic intraventricular administration of angiotensin II or saline on the resting mean blood pressure, femoral and renal blood flows and on the vascular resistance in dogs under pentobarbital anesthesia. An asterisk indicates that the values are significantly different at p < 0.05 (n = 8 in each group).

Figure 4. Pressure-flow curves obtained from perfused hind limb preparations of angiotensin-treated and control dogs under pentobarbital anesthesia, before and after acute denervation and after alpha-adrenergic blockade. All the curves of the treated dogs shifted significantly to the left, indicating increase in vascular resistance (n = 8 in each group).
FIGURE 5. Pressure-flow curves obtained from perfused hind limb preparations of eight treated and eight control dogs after denervation and alpha-adrenergic blockade. Pressure-flow curves of the treated dogs remained significantly to the left of the control group, indicating marked increase in the intrinsic vascular resistance of the smooth muscle. An asterisk indicates values from the treated group are significantly different from the control group at \( p < 0.05 \).

TABLE 2. Blood Pressure Responses to Bilateral Carotid Occlusion (BCO) and to Several Pharmacological Agents in Angiotensin-Treated and Placebo Groups Under Pentobarbital Anesthesia

<table>
<thead>
<tr>
<th>Test or drug</th>
<th>I.V. dose (µg/kg)</th>
<th>Change in mean blood pressure (mm Hg, mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Placebo (n = 8)</td>
</tr>
<tr>
<td>BCO</td>
<td></td>
<td>+29.6 ± 5.9</td>
</tr>
<tr>
<td>Acetycholine</td>
<td>0.5</td>
<td>-22.8 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>-29.0 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>-41.2 ± 4.8</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>0.1</td>
<td>-21.5 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>-33.2 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>-39.6 ± 5.4</td>
</tr>
<tr>
<td>Histamine</td>
<td>0.5</td>
<td>-24.3 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>-30.4 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>-33.6 ± 3.1</td>
</tr>
</tbody>
</table>

\*\( p < 0.05 \).

FIGURE 6. Pressor and reflex vasodilator responses to various doses of I.V. norepinephrine in perfused hind limb preparations of treated (solid circles) and control (open circles) dogs. Asterisk indicates significant difference between treated and control groups (n = 8 in each group) at \( p < 0.05 \) for pressor responses and \( p < 0.01 \) for reflex vasodilator responses.

Discussion

The intraventricular administration of angiotensin II for 2 weeks (together with saline as the drinking fluid during the second week) produced moderate but consistent increases in the arterial blood pressures of mongrel dogs. Blood pressure levels of the treated group were significantly greater than the control dogs, which received only saline. There was a slight increase in heart rate in both groups. However, fluid intake was
not monitored in the present study and since angiotensin II is reported to increase fluid consumption, it is not entirely clear whether these effects are due to the direct effects of the peptide and/or secondary to an increase in fluid and sodium consumption. The data suggest that the elevation in the arterial blood pressure in the treated dogs appears to be due to an increase in peripheral vascular resistance. Such a postulation is consistent with the observation that in both the vascular beds studied (renal and femoral) there was a marked increase in vascular resistance with resulting reductions in blood flows in the treated dogs in comparison with the placebo group. In this context, it should be noted that in a previous study, acute intraventricular administration of angiotensin II resulted in an elevation of resistance in the renal and mesenteric vasculature but not in the femoral bed of anesthetized cats. Changes occurring in the mesenteric vasculature are not available in the present study, but it appears that, unlike the acute studies, femoral vasculature was also affected during chronic administration. The increase in renovascular resistance noted in the angiotensin II-treated animals may prove to be a significant finding. Guyton et al. observed that an increase in the peripheral vascular resistance alone cannot result in sustained elevation of arterial pressure unless there is a concomitant increase in the renovascular resistance. Thus, the reduction in renal blood flow and increase in resistance could minimize renal compensation to increased arterial pressure noted in the AII-treated dogs. However, the increase in vascular resistance following chronic central administration of angiotensin II is not due to an enhancement of neurogenic tone, despite the fact that increased responsiveness to norepinephrine has been noted. There were no significant changes in peripheral sympathetic transmission to either renal or hind limb vasculature. Pressure-flow curves obtained from the perfused hind limb studies indicated that the neurogenic tone to the hind limb vasculature or the tone contributed by circulating catecholamines in the treated dogs were not significantly different from those of the placebo group. Similar reductions in the arterial blood pressure following ganglionic blockade also indicate that resting neurogenic tone was essentially identical in both groups. Thus, an increase in the intrinsic vascular smooth muscle tone as reflected in the pressure-flow curves was evidently responsible for elevated arterial pressure.

The mechanisms responsible for the increase in intrinsic vascular resistance following chronic central intraventricular administration of angiotensin II cannot be adequately explained from the available data. However, two phenomena noted in the responsiveness of the vasculature should be taken into consideration; namely, enhanced responsiveness to intravenous angiotensin and norepinephrine and the attenuation of the reflex vasodilator responses to intravenous norepinephrine. Investigators have previously demonstrated that reflex vasodilation in the hind limb vasculature is mediated in part via histaminergic components. In the current study, systemic blood pressure responses to intravenous histamine, as well as the hind limb vascular responses to intra-arterial histamine were significantly inhibited in the treated dogs, which could explain the attenuation of reflex vasodilation. It could be of utmost significance to note that the inhibition of vasodilator actions of histamine appears to be very specific, since intra-arterial or intravenous responses to acetylcholine and bradykinin were essentially of similar magnitude in both the treated and placebo groups.

The vascular effects noted in the dogs treated with angiotensin II and NaCl are generally consistent with observations made by other workers in several types of hypertension. Investigators have reported an increased reactivity to various vasoconstrictor stimuli such as norepinephrine and angiotensin II in patients.
with essential hypertension. Similar observations have been made in rats with renal, DOCA-NaCl or genetic hypertension. Further, this increased responsiveness has been demonstrated in various isolated preparations, indicating that it is independent of circulating neurohumoral agents and neurogenic control. Lais et al. observed that in spontaneously hypertensive rats, vasoconstrictor responses in the hind limb to lumbar sympathetic stimulation were unchanged or reduced, and the responses to norepinephrine, epinephrine, tyramine, angiotensin and barium chloride were enhanced. In addition, vascular resistance remained significantly higher in spontaneously hypertensive rats after bilateral lumbar sympathectomy. Based on these studies, the authors concluded that hypertension in these animals does not derive from either enhanced central adrenergic discharge or altered central integration of afferent information from peripheral sensory receptors but may result from humoral (increased reactivity to vasoconstrictors) or structural factors. One could reach similar conclusions from the data obtained in the present study. The increase in the pressor responsiveness to angiotensin II and norepinephrine in the treated dogs as observed in the present study could also have been due to the inability of the baroreceptor reflex to restore blood pressure to normal levels. This is supported by the observation that reflex vasodilator responses to pressor stimuli were significantly inhibited in the angiotensin-treated dogs.

Cohen and Berkowitz investigated vascular relaxation in aortic strips obtained from spontaneously hypertensive rats and renal hypertensive rats in vitro. Aortic relaxation was attenuated in response to cyclic nucleotides, isoproterenol, nitroglycerin and adenosine. Based on these data, the authors proposed that defects in vascular relaxation may contribute to hypertension. However, Deragon et al. could not demonstrate any impairment of vasodilator activity to isoproterenol and nitroprusside in in vivo perfused hind limbs of spontaneously hypertensive rats. The discrepancies between these two studies cannot be easily explained. None of these studies used histamine as a vasodilator agent. In the present study, increases in the vascular resistance of the treated dogs was associated with reduced responsiveness, specifically to histamine, and enhanced responsiveness to norepinephrine and angiotensin, perhaps indicating certain structural or functional alterations in the vascular smooth muscle. However, it is not clear whether these three effects are specific and independent or interrelated. Studies by Lais and Brody conducted in spontaneously hypertensive rats seemed to suggest that increased sensitivity to norepinephrine is specific and independent of any changes in the responsiveness to angiotensin. However, according to Collis and Alp, endogenous angiotensin may be involved in early supersensitivity to norepinephrine, while a structural change in the vessel wall becomes the dominant factor contributing to hyperactivity in the later stages of renal hypertension. Several reports suggest a close relationship between pressor responses to angiotensin and catecholamines and urinary sodium excretion; increases in urinary sodium excretion is associated with enhanced responsiveness to these agents in patients with benign essential hypertension. In the present study, the concentration of urinary sodium was significantly greater in the treated animals, which may have contributed to the changes noted in the vascular responsiveness. Increased excretion of sodium was also noted in cats after acute intraventricular administration and what has been observed following chronic angiotensin is consistent with the observations of exaggerated natriuresis in patients with essential hypertension.

Another aspect to consider is the possible interrelationship between inhibition of histamine effects and potentiation of norepinephrine responses. It has been recently demonstrated that activation of H2 receptors could result in attenuation of exogenous norepinephrine-induced vasoconstriction in the dog hind limb vasculature. Thus, it is possible that impairment of the H2 receptor would not only reduce vasodilator responses to histamine, but also enhance vascular reactivity to norepinephrine. Thus, it may be that chronic angiotensin II administration resulted in specific structural changes in the vasculature involved in mediating the activity of histamine, norepinephrine and/or angiotensin.

The data obtained in this study demonstrated that chronic intraventricular administration of angiotensin II together with saline (as drinking fluid) for 2 weeks, results in cardiovascular alterations conducive to the development of high blood pressure; administration of angiotensin or saline alone failed to precipitate these changes. Increased renovascular resistance, inhibition of reflex vasodilation, attenuation of vasodilator actions of histamine, potentiation of the vasoconstrictor responses to angiotensin II and norepinephrine are consistent with the altered vascular responsiveness noted in various types of hypertension in animals as well as essential hypertensive patients. Mechanisms by which increasing the CSF concentration of angiotensin II triggered these changes are not yet clarified. It is possible that increased sympathetic discharge in the earlier stages could have precipitated these vascular alterations. One may raise the possibility that centrally administered angiotensin may be leaking into the peripheral circulation. However, increased concentration of urinary sodium and enhanced responsiveness of vascular angiotensin II receptors are not consistent with increased angiotensin II levels in the blood. In conclusion, we propose that the experimental protocol used in this study could be very useful in developing a new model of experimental hypertension, the characteristics of which may simulate certain types of essential hypertension noted in humans.

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