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Heightened Blood Pressure Responsiveness to Intracarotid Infusion of Angiotensins in the Spontaneously Hypertensive Rat

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WRIGHT, J. W., L. L. JENSEN, L. L. CUSHING AND J. W. HARDING. *Heightened blood pressure responsiveness to intracarotid infusion of angiotensins in the spontaneously hypertensive rat.* PHARMACOL BIOCHEM BEHAV **30**(2) 343–346, 1988.—The purpose of this study was to test the hypothesis that intracarotid infusion of angiotensin via a brachial arterial catheter results in a heightened pressor response in the alert spontaneously hypertensive rat (SHR) as previously observed for intracerebroventricular (ICV) injection of angiotensin. We infused angiotensin II and III since these ligands are equivalently potent with respect to peak pressor effect when delivered ICV. We measured somewhat greater pressor responsiveness to AII than to AIII in the Wistar-Kyoto (WKY) normotensive control strain from a baselevel of 133.1±5.8 (mean±SEM) to 151.3±6.2 mmHg (+13.7%) at the 100 pmol/kg/min dose of AII, and from 132.5±5.8 to 146.0±6.1 mmHg (+10.2%) for AIII. The SHR revealed a heightened pressor sensitivity to AII, from a baselevel of 170.0±3.8 to 200.6±5.9 mmHg (+18%) while the response to AIII was less dramatic, from 171.3±2.1 to 189.8±2.4 mmHg (+10.8%). These findings suggest that a similar heightened pressor responsiveness occurs to peripheral infusion of angiotensin II in the SHR as previously observed to ICV injection.

Arterial pressure Angiotensin II Angiotensin III Intracarotid infusion Spontaneously hypertensive rat

THE Okamoto-Aoki spontaneously hypertensive rat (SHR) appears to be an acceptable animal model of human essential hypertension [3, 29, 34]. It has been proposed that a dysfunctional brain angiotensin system may contribute to this strain's hypertension [6, 8, 18], in that intracerebroventricular (ICV) injection of angiotensin receptor antagonists, or converting enzyme inhibitors, are differentially effective at lowering blood pressure in the SHR as compared with the Wistar-Kyoto (WKY) normotensive control rat [6, 19, 22, 23, 28], while intravenous infusions have been reported to be effective in the anesthetized bilaterally nephrectomized SHR [15], although ineffective in the alert bilaterally nephrectomized stroke-prone SHR [24]. Further, the ICV injection of angiotensin II (AII) produces an exaggerated pressor response in the SHR as compared with WKY rats [7, 14, 32]. Taken together these results encourage the hypothesis that central angiotensin receptor sites mediate the angiotensin-induced pressor effect and presumably a dysfunction at these sites contributes to the exaggerated pressor response seen in the SHR.

The most likely brain angiotensin sites of action in the rat include two forebrain circumventricular organs (CVO), the organum vasculosum of the lamina terminalis (OVLT) and the subfornical organ (SFO) [17,21]. However, since CVO possess a reduced blood-brain barrier [1,25] it is possible for blood-borne AII to access these sites [30]. Thus, elevations in circulating levels of AII should result in heightened pressor responses in SHR similar to those seen with ICV injection. This question has not previously been addressed although it is particularly relevant given the clinical implications of independent populations of brain angiotensin receptors that are exposed to cerebrospinal fluid and blood changes in peptide levels [12], and the likelihood that essential hypertension may be accompanied by heightened sensitivity to angiotensins in both fluid compartments.

In addition to testing AII, we included angiotensin III (AIII) given our recent findings that AII and AIII are equipotent when injected ICV at low doses in normotensive rats [31], despite a shorter half-life for AIII [11]. We have also determined that the SHR shows a heightened pressor response to ICV AIII similar to that previously reported to AII [31,32], and a greater potency than AII when microiontophoretically applied to paraventricular neurons [10].

METHOD

Mature male SHR and WKY rats (Taconic Farms), 120–

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TABLE 1

BASELEVEL MEAN ARTERIAL BLOOD PRESSURE (MABP) PRIOR TO EACH TREATMENT OF AII OR AIII IN WISTAR-KYOTO (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

Strain	N	Treatment	Baselevel MABP (mmHg) 0	1	10	100
			(pmol/kg/min)			
WKY	8	AII	131.6 ± 5.0*	134.1 ± 5.7	135.6 ± 6.4	133.1 ± 5.8
	8	AIII	131.9 ± 4.2	134.4 ± 5.5	132.1 ± 5.5	132.5 ± 5.8
SHR	8	AII	173.8 ± 3.2	173.6 ± 3.3	169.4 ± 3.8	170.0 ± 3.8
	8	AIII	175.0 ± 2.5	171.9 ± 3.5	176.3 ± 1.8	171.3 ± 2.1

*Values are mean ± SEM.

150 days of age, were adapted to single caging for a minimum of two weeks prior to surgery at 22±1°C on a 12–12 photo period, initiated at 0700 hr. Beginning 7 days before surgery, each animal was handled 5 min daily and adapted to the test chambers (20.5 cm diameter × 20 cm tall, glass bell jars) located in a sound isolated room for 10 min daily to reduce excitability and thus elevated blood pressure responses to extraneous environmental stimuli during testing.

Surgical Preparation

Each animal was anesthetized with equithesin (Jensen-Salsbury Laboratory, 3.5 ml/kg, IP) and prepared with a brachial arterial infusion catheter (PE 10, Clay Adams) according to the procedures offered by Haywood and colleagues [13]. The catheter was inserted until the tip was at, or near, the junction of the right brachial and carotid arteries and was securely sutured to the surrounding muscle tissues and tunneled subcutaneously to the point of externalization between the scapulae. Blood pressure was monitored via a femoral arterial catheter (PE 50), also externalized between the scapulae using Statham transducers (Model P23AC) and a Grass Intruments Polygraph (Model 7B).

Following surgery, each animal received procain, penicillin G and dihydrostreptomycin sulfate (20,000 U, IM, Combiotic, Pfizer, NY). The catheters were flushed daily with heparinized saline (75 U/ml) and occluded with 1 cm lengths of stainless steel wire. Each animal was allowed at least 72 hr recovery before testing.

Experimental Protocols

Eight animals from each strain received 0, 1, 10 and 100 pmol/kg/min AII and AIII on separate days in sterile 0.15 M NaCl vehicle infused via the brachial arterial catheter at 50 μl/min (Sage Instruments infuser, Model 355) for 5 min, with 30 min between doses. The treatments were counterbalanced for ascending and descending order of doses, half the animals of each strain received AII on the first day, and AIII 48 hr later, whereas the other half received AIII first. A 10 min baselevel blood pressure was recorded before each infusion. Following testing each animal was killed by anesthetic overdose and correct placement of the brachial arterial catheter was checked.

Each animal was tested while alert and free-moving in the chambers. The infusion catheter was connected via 2 cm of 30 gauge stainless steel tubing to a 15 cm length of PE 10 tubing that had been heat-bonded to a 70 cm length of PE 50. The PE 50 tubing was in turn fitted with a 23 gauge hypodermic needle that was leur-locked to a 3 ml syringe. Prior to being connected to the test animal the infusion syringe was loaded and placed in the infuser and air bubbles were cleared. The blood pressure catheter was treated similarly, however, a 2 cm length of 23 gauge stainless steel, inserted in a 70 cm length of PE 50, was used to connect with the transducer.

Statistical Analyses

The initial baselevel mean arterial blood pressures (MABP) for members of each strain were evaluated by a 2 (strain) × 4 (level prior to each dose) analysis of variance (ANOV) for AII and AIII, with repeated measures on the second factor [2]. The magnitudes of the pressor response to each treatment were calculated by subtracting the corresponding MABP baselevel from the maximum pressor change induced by each dose of AII or AIII. These data were analyzed by a 2 (strain) × 4 (doses) ANOV with repeated measures on the second factor. Significant effects were further evaluated by Newman-Keuls post hoc tests at a significance level of 0.01.

RESULTS

As expected there were differences between the members of each strain with respect to baselevel MABP prior to the infusion of AII and AIII, $F(1,14)=34.68$ and 56.35, respectively, $p<0.001$ (Table 1). However, within each strain, SHR and WKY, the baselevels prior to each dose did not differ, $F(3,42)=0.70$ and 0.58, respectively, indicating that sufficient time was allowed between treatments to recover baselevel blood pressures.

Change in MABP during the 5 min intra-arterial infusion of each dose of AII and AIII for SHR and WKY rats are presented in Fig. 1. The maximum MABP (±SEM) changes from baselevel in the SHR group during the infusion of 1, 10 and 100 pmol/kg/min doses of AII were 5.0±1.3, 14.0±1.2, and 30.6±4.4 mmHg, respectively. Comparable MABP changes from baselevel in the WKY animals were 3.1±0.6, 7.8±0.9, and 18.1±2.3 mmHg, respectively. The infusion of AIII at doses of 1, 10 and 100 pmol/kg/min resulted in corresponding MABP changes from baselevel in the SHR animals

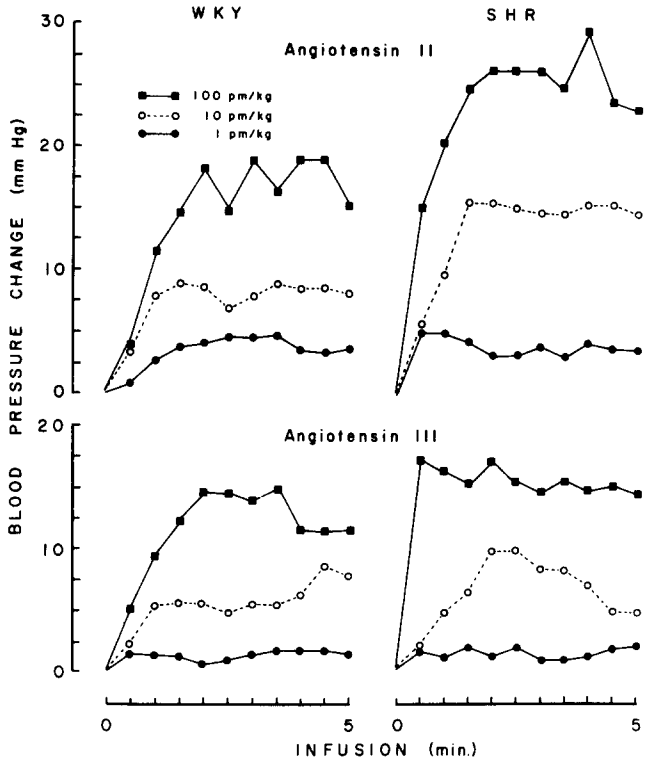

FIG. 1. Changes in mean arterial blood pressure during the 5 minute infusion of angiotensin II (top panel) and angiotensin III (bottom panel) in Wistar-Kyoto (WKY) normotensive and spontaneously hypertensive rats (SHR). Eight animals were used from each strain. The doses of 1, 10 and 100 pmol/kg/min were administered via a brachial arterial catheter in sterile 0.15 M NaCl at 50 μl/min. The blood pressure changes resulting from the control infusions of 0.15 M NaCl did not statistically differ from the 1 pmol/kg/min dose of either AII or AIII and were therefore not included in this figure.

of 1.9±0.9, 9.1±1.7, and 18.5±2.1 mmHg, respectively. And in the WKY group these values were 2.4±0.8, 6.8±1.1, and 13.5±1.1 mmHg, respectively. With respect to AII (Fig 1, upper panel), there was an overall dose effect, $F(3,42)=62.16$, $p<0.001$, with significant differences among the doses 1, 10, and 100 pmol; the 0 and 1 pmol doses did not differ. There were strain differences for the 10 and 100 pmol doses of AII which induced significantly greater pressor effects in the SHR than the WKY animals. Similar analyses of AIII (Fig. 1, bottom panel) failed to indicate a strain effect, but there was an overall dose effect, $F(3,42)=59.72$, $p<0.001$. Once again there were significant differences among the 1, 10, and 100 pmol doses, while the 0 and 1 pmol doses were not different. Comparing the SHR and WKY animals at each dose, only at 100 pmol did the SHR indicate greater responsiveness than the WKY rats. The threshold dose of both AII and AIII, defined as the lowest dose yielding a significant elevation in MABP above baselevel, was at the 10 pmol for both strains.

A final set of analyses compared the elevation in MABP to AII and AIII for each strain. The SHR evidenced a significant difference between angiotensins, $F(1,14)=8.96$, $p<0.01$, with greater overall responsiveness to AII. Post hoc analyses of the ligand × dose effect, $F(3,42)=3.26$, $p<0.05$, indicated greater responsiveness at the 10 and 100 pmol doses of AII as compared with AIII. There was no overall difference between AII and AIII for the WKY group, nor was there a ligand × dose effect, although there was an overall dose effect as expected, $F(3,42)=59.14$, $p<0.001$.

There were no differences in the rates of recovery of baselevel blood pressure comparing SHR and WKY animals following infusion of AII or AIII.

DISCUSSION

The present results, which indicate a heightened pressor response to systemically infused AII in the SHR, are not surprising given the access of circulating peptides to CVO angiotensin receptors via fenestrated capillary beds. What is not clear is whether this effect is due to angiotensin's action directly on the peripheral vasculature or whether it is solely the result of interaction with CVO receptors. If it is in fact centrally mediated, the defect responsible for the enhanced response could reside at the CVO receptors or within the central angiotensinergic pathways involved with transmitting information from the CVO to the hypothalamus and ultimately to cardiovascular centers in the hindbrain. Two primary defects in the angiotensinergic system seem plausible. These include an alteration in the angiotensin receptor and its associated transduction system, and/or a decrease in the efficiency of signal termination. To date, findings to support the possibility of changed receptor number and/or affinity have not been convincing due to contradictory results [9, 16, 26, 27], however our ignorance of central angiotensin transduction mechanisms leaves this possibility viable. Recent studies from our laboratory have directly tested the second possibility of reduced angiotensin degradation. Using microwave fixation to rapidly stop peptidase activity, we have determined that both AII and AIII are degraded at a much slower rate in SHR than in WKY and Sprague-Dawley normotensive rats [33]. These results are further supported by recent electrophysiological data that demonstrate that SHR have a tremendously extended duration of response to iontophoretically applied AII and AIII in the paraventricular nucleus [10].

Although the SHR appears to have a decreased ability to degrade angiotensins, it is not clear whether this defect is specific to angiotensin degradation or more generalized to include metabolism of other peptides. Ganten *et al.* [7] and Bunag and coworkers [4] have shown that sensitivity to other peptides, most notably Leu-enkephalin, is enhanced in SHR. Related to this observation, it has been established that the SHR evidences an increased pain threshold which is reducible with the opiate antagonist, naloxone [20].

In summary, the present results demonstrate that the heightened pressor response observed in the SHR to ICV injections of AII and AIII can also be induced by peripheral infusion of AII. Although the discrepancy concerning the influence of intravenously infused saralasin upon blood pressure in the SHR and stroke-prone SHR [15,24] must be resolved, the present observations support the notion of a central defect in members of these rat strains, and emphasize the importance of the central angiotensinergic system in blood pressure control.

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