ENDOGENOUS BRAIN ANGIOTENSIN II–MEDIATED SYMPATHETIC NERVE RESPONSES ARE NOT AUGMENTED IN MATURE SPONTANEOUSLY HYPERTENSIVE RATS

HAKUO TAKAHASHI, M.D., SEIICHI YONEDA, M.D., HIROSHI ASHIZAWA, M.D.
ATSUSHI INOUE, M.D., KAZUO TAKEDA, M.D.
MANABU YOSHIMURA, M.D., AND HAMAO IJICHI, M.D.

Conjugated estrogens injected into the lateral brain ventricle in awake rats elicited behavioral excitation and vasopressor responses. Magnitude of pressor responses was greater in spontaneously hypertensive rats (SHR) than in normotensive Kyoto Wistar rats (WKY). Pressor responses in SHR were abolished by central pretreatments of either captopril or angiotensin II analog. Under urethane anesthesia, conjugated estrogens still produced greater pressor responses in SHR, but accompanying increases in sympathetic nerve firings were the same in both WKY and SHR. These results suggest that while centrally-administered estrogens may activate the brain renin-angiotensin system to increase sympathetic nerve firing and thereby elevated blood pressure, SHR have larger pressor responses only because peripheral vascular reactivity has been increased.

ANGIOTENSIN II (Ang II) injected into the lateral ventricle elevates blood pressure by increasing sympathetic nerve outflow and releasing pituitary hormones. Since similar effects can be produced with renin, other components of renin-angiotensin (R-A) system are likely to exist in the central nervous system. Despite considerable technical difficulties, Hirose et al have isolated brain renin, but still little is known about how this endogenous system operates. Intracerebroventricular (ICV) infusions of an Ang II antagonist with captopril or Ang II analog produce vasodepression only in SHR but not in normotensive controls thus suggesting that alterations of the brain R-A system could be involved in spontaneous hypertension.

We recently found that cerebroventricular infusions of conjugated estrogens produce vasopressor responses and sympathetic hyperactivity which were abolished by central pretreatment with captopril or Ang II analog in rats. Sympathetic nerve outflow is increased and augmented pressor responses accompanied by increased sympathetic nerve firing are produced by electrical stimulation of the posterior hypothalamus in SHR. The present study aimed to examine the role of the brain R-A mechanism on the sympathetic outflow by infusing conjugated estrogens into lateral ventricles of SHR.

Key Words:
Blood pressure
Conjugated estrogens
Sympathetic nerve activity
Intracerebroventricular injection
Brain renin-angiotensin system

(Received September 21, 1981; accepted April 13, 1982)
The Second Department of Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan
Address for correspondence: Hakuo Takahashi, M.D., The Second Department of Medicine, Kyoto Prefectural University of Medicine, Kamikyo-ku, Kyoto 602, Japan

1082 Japanese Circulation Journal Vol. 46, October 1982
METHODS

Two groups of 16-week old female rats were used: 32 spontaneously hypertensive rats (SHR) weighing 224 ± 6 g and 26 normotensive Wistar Kyoto rats (WKY) weighing 236 ± 8 g derived from the strain originally described by Okamoto and Aoki. For experiments in awake rats, cannulas were implanted into a lateral ventricle and the abdominal aorta, and then after allowing a few days for postoperative recovery, each awake rat was placed in a plastic holder while blood pressure was recorded continuously. In other experiments, rats were anesthetized with urethane (100 mg/100g, i.p.) and cannulas were inserted into the left lateral ventricle for injecting drugs and into the femoral artery for recording blood pressure; concurrently a bipolar electrode was placed over the abdominal sympathetic nerve bundle for recording nerve activity.

Implantation of Arterial Catheters

Rats were anesthetized with ketamine hydrochloride (40 mg/kg, i.p.), while an arterial catheter made of PE-20 and PE-10 tubings according to a method modified from the one originally described by Weeks and Jones was inserted into the lower abdominal aorta. The other end of the catheter was exteriorized by pulling it subcutaneously through a cut in the skin on the back of the neck. The catheter was held in place with wound clips and the tip was plugged with a stylet. During experiments, arterial pressure was recorded continuously by connecting the catheter tip to a pressure transducer while saline was being infused (0.3 ml/hr) through a side arm of the catheter.

Intracerebroventricular (ICV) Injections

In rats anesthetized with ketamine hydrochloride, a guide cannula (ga 23, stainless steel tubing, 1.5 cm long with a ga 30 stylet) was inserted into the left lateral brain ventricle (at stereotaxic coordinates 5.6 anteroposterior, 1.6 lateral and +2.0 dorsoventral, with the upper incisor bar set 5 mm above the interaural line) and fixed to the skull with screws and dental cement (acrylic resin, Shofu Dental MFG. Co., Kyoto, Japan). Drugs were injected by inserting an injection cannula (ga 30 stainless steel tubing) connected to a 10 μl syringe into the guide cannula. The whole system was filled with the solution to be injected and each injection had a volume of 5 μl delivered manually in 10 sec. Injections were given at least 24 hours apart and were not performed more than three times in each rat. After each experiment, methylene blue was injected through the injection cannula to verify correct placement within the lateral ventricle.

Recording of Sympathetic Nerve Activity

The abdominal plexus was exposed through a left lateral abdominal transverse incision and the inferior nerve bundle emerging from a celiac ganglion was placed over a bipolar stainless steel electrode ( uninsulated tips one mm apart). Nerves and electrode tips were immersed in mineral oil to prevent tissue drying. To reduce noise during nerve recording, spontaneous respiratory movements were abolished by paralyzing skeletal muscle with decamethonium bromide (0.2 mg/100g, i.v.) and the rat was ventilated with room air connecting it to a ventilator (Ealing Corp., South Natick, M.A.). Spike potentials were amplified (p-15 preamplifier, Grass Instrument Co., Quincy Mass., and biophysical amplifier, Sanei Instrument Co., Tokyo, Japan), monitored on a storage oscilloscope (Kikusui Electronics, Tokyo, Japan) and recorded continuously on magnetic tape together with blood pressure (TEAC Corp., Tokyo, Japan). Tapes were later played back into an amplitude analyzer to delete background noise and resulting pulses were fed to a spike counter (Dia Medical System Co., Tokyo, Japan) whose output was digitalized and printed out, while it was recorded on a recorder (Rectigraph, Sanei Instrument Co.). Five min after the drug injection, integrated nerve activity per min was compared to the baseline activity before the injection and the percent increase of the activity was calculated.

Drug Injections and Statistics

For ICV injections, conjugated estrogens (Premarin, Ayerst Lab., New York, N.Y.) were dissolved in physiological saline with 2% benzyl alcohol (final concentration, 10 mg/ml). This solution was diluted 5- and 10-fold in saline. Converting enzyme inhibitor (captopril, E.R. Squibb and Sons Inc., Princeton, N.J.) was dissolved into saline (100 mg/ml) immediately before the injection. Ang II analog (1-Sar, 8-Ile angiotensin II, Peninsula Lab. Inc., San Carlos, C.A.) was dissolved into saline (20 mg/ml). Whenever sympathetic nerve activity was recorded, pentolimium tartrate (0.5 mg/100g, i.v., Sigma Chemical Co., Saint Louis, Mo.) was injected at
the end of each experiment to determine residual activity and setting of the low window discriminator during playback.

Data expressed as average ± SEM was analyzed using a two-tailed t-test for comparing means of independent samples and differences at a 5% level (p < 0.05) or less were considered significant.

RESULTS

ICV Injections of Conjugated Estrogens in Awake Rats

Blood pressure was consistently increased following ICV injections of conjugated estrogens. After injecting a dose of 10 µg, blood pressure began to rise within one min to attain peak elevations 7–10 min later in either WKY or SHR (Fig. 1). Besides blood pressure changes, behavioral excitation was observed, namely initially a sedation for a few min and then suddenly they started to move actively sniffing and screaming restlessly for about 5 min corresponding to the rapid increasing phase of blood pressure, which was followed by a prolonged sedation for more than 20 min. In some rats, drinking behavior was observed when water was given. Magnitude of the pressor responses elicited was bigger in SHR than in WKY (p < 0.01, Fig. 2). Similar injections of the vehicle without the estrogens were ineffective. Heart rate was consistently increased, but tachycardia elicited had no significant differences between WKY and SHR (Fig. 3).

Is There Involvement of Brain Renin-angiotensin Axis in Spontaneous Hypertension?

After infusing either captopril (500 µg/rat, n = 5) or Ang II analog (100 µg/rat, n = 6) into the cerebral ventricles in awake SHR, blood pressure was recorded continuously for 30 min. Both drugs increased blood pressure transiently for less than 5 min with peak elevations of +16 ± 3 and +8 ± 2 mmHg, and then those changes returned to the level close to their baseline, −2 ± 1 and +4 ± 2 mmHg, respectively. Vasodepression was never recorded.

To determine whether pressor responses to ICV injections of the estrogens are mediated by activation of the brain R-A mechanism, Ang II blockers with captopril or Ang II analog were injected intracerebroventricularly 3 min preceding to the injection of conjugated estrogens, 10 µg, in awake SHR. Captopril or Ang II analog itself increased blood pressure transiently as
described above, and conjugated estrogens injected following it elicited neither blood pressure nor behavioral excitation (Fig. 4).

**Sympathetic Nerve Responses to ICV Injections of Conjugated Estrogens**

To determine whether increased output of sympathetic nerve activity could account for the central pressor responses to the estrogens, abdominal sympathetic nerve activity was recorded from both WKY and SHR anesthetized with urethane. Because pressor responses to centrally-administered conjugated estrogens become much smaller by urethane-anesthesia, the dose of the estrogens was increased to 50 μg. It produced marked vasopressor responses which were accompanied by corresponding increases in peripheral sympathetic nerve activity (Figs. 5 and 6). Sympathetic hyperactivity elicited showed increases in both the amplitude and frequency of the nerve spikes, which often had grouping of spikes followed by a complete suppression, regularly or irregularly. On the other hand, the vehicle without the estrogens affected neither the blood pressure nor the sympathetic nerve activity. Magnitude of the pressor responses was greater (p < 0.05) in SHR than WKY, but neither tachycardia nor increases in sympathetic nerve outflow differed significantly between SHR and WKY (Figs. 5, 6 and 7).

**DISCUSSION**

The results obtained here clearly show that ICV injections of conjugated estrogens produce augmented pressor responses in SHR, and that the augmentation is exclusively due to the peripheral mechanism because sympathetic nerve responses elicited simultaneously were the same extent as those in WKY. Since both the pressor and sympathetic nerve responses to the estrogens were abolished by pretreating rats with Ang II blockers, these responses may be attributed to the brain R-A axis activation. It seems unlikely that it is dependent upon a peripheral action of the estrogens because intravenous injections of it of doses up to 2 mg/kg affected neither the blood pressure nor the sympathetic nerve outflow in anesthetized rats. Ang II injected intracerebroventricularly similarly produces sympathetic hyperactivity leading to vasopressor responses in rats. Not only because the sympathetic nerve responses to centrally administered estrogens were not augmented but also because Ang II blockers injected intracerebroventricularly did not affect blood pressure in SHR, the brain R-A mechanism does not seem to be involved in this stage of the spontaneous hypertension.
Against these findings, hypotensive effects of an Ang II blocker are reported indicating possible involvement of a brain R-A system in SHR by Mcdonald et al. They infused an Ang II analog intracerebroventicularly continuously by using osmotic minipumps measuring blood pressure by a tail-cuff method, and they got depressor responses. Since preheating for the blood pressure measurement is more stressful in SHR to increase the sympathetic outflow than in WKY results obtained there inevitably include stress-induced responses. As we found that Ang II blockades injected centrally suppressed the sympathetic nerve activity transiently in urethane anesthetized rats vasodepression may be induced by the blockades in certain circumstances, but not in awake and stress-free SHR. On the other hand, Elghozzi et al. found, as we did, that the Ang II analog injected into the ventricle did not affect the blood pressure in awake SHR. Again, Ganten et al. could not demonstrated any vasodepressor activity of central saralasin in SHR available commercially. However, small, but significant vasodepression was found in stroke-prone SHR thereby suggesting that rats of this strain may have additional alterations in central angiotensin-mediated pressor mechanisms. While, Hoffman et al. injected Ang II into a lateral brain ventricle, which produced augmented pressor responses in SHR. However, the augmentation was ascribed to peripheral mechanism because they have demonstrated an increased vascular reactivity to infused norepinephrine, but both amounts of vasopressin released and of water drunk were the same as those of WKY. Similarly, augmentation of the vasopressor responses to injected norepinephrine systemically or in perfused hindquarter preparations in SHR have been well documented.

In conclusion, it seems reasonable that a brain R-A mechanism can be stimulated by physiologic stimuli as conjugated estrogens and that the resultant pressor responses are augmented in SHR not via the central mechanism but via...
peripheral mechanisms of increased vascular responsiveness.

REFERENCES

4. REID JA, RAMSEY DJ: The effects of intracerebroventricular administration of renin on drinking and blood pressure. Endocrinology 97: 536, 1975

Japanese Circulation Journal Vol. 46, October 1982