Contributions of Baroreceptor Reflex to the Hypothermic Effect of Intraventricular Angiotensin II in Rats

Osamu Shido, Akira Kifune, and Tetsuo Nagasaka

Department of Physiology, School of Medicine, Kanazawa University, Kanazawa, 920 Japan

Abstract The mechanisms of the hypothermic effect of angiotensin II (AII) injected into the lateral ventricle were investigated in unanesthetized rats at an ambient temperature of 18°C. Mean blood pressure (BP), heart rate (HR), metabolic rate (M), colonic temperature (T_co1), and temperatures of the interscapular brown adipose tissue (T_BAT), and the tail skin (T_sk) were continuously monitored. AII at a dose of 5 µg produced a sharp and marked elevation in BP accompanied by bradycardia, and a decrease of M and T_co1 in the sinoaortic baroreceptor intact rats. The difference between T_BAT and T_co1 decreased significantly, which suggests a suppression of nonshivering thermogenesis of the BAT. T_sk was not changed by the AII injection. After sinoaortic denervation, however, the decrease in T_co1 and M with AII injection was significantly reduced despite a marked elevation in BP. In addition, intravenous arginine-vasopressin antagonist pretreatment suppressed the elevation in BP and the decrease in HR, T_co1, and M after AII injection. From these results, it is concluded that the hypothermia which occurred after AII injection into the lateral ventricle can be largely attributed to the baroreflexive suppression of M, and to some extent to the direct effect on the thermoregulatory center in rats.

Key words: angiotensin II, baroreceptor reflex, brown adipose tissue, (arginine) vasopressin, hypothermia, metabolic suppression.

Centrally administered angiotensin II (AII) has been shown to produce hypothermia in conscious monkeys (Sharpe and Swanson, 1974), rabbits (Lin, 1980; Sharpe et al., 1978) and rats (Lin et al., 1980). In rabbits and rats, the AII-induced hypothermia was dose-dependent and particularly consistent in cold environments. Sharpe et al. (1978) observed a vasodilatation of the ear with AII-induced hypothermia in rabbits, and Lin (1980) reported that, in the same species, AII decreased heat production and increased skin blood flow and respiratory evaporative heat loss. On the other hand, Lin et al. (1980) reported...
that AII decreased both heat production and heat loss in rats. Thus, metabolic suppression seems a main cause of hypothermia following central AII administration, although the effect of AII on heat loss is not clear.

It is well known that centrally administered AII elevates the systemic blood pressure via the stimulation of vasopressin release (Gregg and Malvin, 1978; Haack and Möhring, 1978; Keil et al., 1975; Mouw et al., 1971) and the stimulation of the sympathoadrenal system (Buckley, 1972; Ferrario et al., 1970, 1972; Lappe and Brody, 1984; Severs et al., 1966). It has been reported that an elevation of the systemic blood pressure suppresses shivering and nonshivering thermogenesis through the sinoaortic baroreceptor reflex (Hohtola et al., 1980; Nunomura, 1983; Shibata, 1982; Wasserstrom and Herd, 1977). We have recently observed that an intravenous injection of vasopressin produces a fall in body temperature with a marked elevation in blood pressure in rats (Shido et al., 1984). This fall in body temperature was not produced, however, after the sinoaortic baroreceptor deafferentation. Thus, the present study is an attempt to clarify the mechanisms of the AII-induced hypothermia in connection with the vasopressin-mediated elevation of blood pressure and the arterial baroreflex in the conscious rat.

METHODS

Animals and preparations. A total of 26 male Wistar rats, initially weighing 280-300 g, were used. They were housed individually in stainless steel wire-mesh cages in an animal room at 24±1°C with a 12:12 LD cycle and were provided laboratory rat chow and tap water ad libitum. In all rats, a 22-gauge stainless steel cannula was stereotaxically implanted into the left lateral ventricle under pentobarbital sodium (50 mg/kg, i.p.) anesthesia. The correct placement of the cannula was confirmed by spontaneous diffusion of cerebrospinal fluid. A stainless steel stylet was inserted into the cannula to prevent obstruction. Two days after surgery, the rats were loosely restrained in a cylindrical wire-mesh cage for 4 hr every day for over 12 days to get them accustomed to the experimental procedures. One day before the experiment, the rats were subjected to a second operation under pentobarbital anesthesia. A polyethylene catheter (o.d. 0.6 mm, i.d. 0.3 mm) filled with heparinized saline for measuring the mean arterial blood pressure (BP) was inserted into the abdominal aorta via the right femoral artery, and a Cu-Ct thermocouple was placed underneath the interscapular brown adipose tissue (BAT) next to the Sulzer's vein for monitoring the temperature of BAT \( T_{BAT} \). The sinoaortic baroreceptor deafferentation (SAD) was performed in 4 of the rats, as described by Krieger (1964). In the other 11 rats, another polyethylene catheter for drug injection was positioned in the inferior vena cava via the right femoral vein (for experiment 2). The catheter(s) and the lead-wire of the thermocouple covered with a polyethylene tube were inserted subcutaneous-
ly and brought out through the back skin of the neck. On the next day the animals were used for experiments. After each experiment, the rats were sacrificed with a large dose of anesthetics for a gross examination of the location of the ventricular cannula.

**Experiment 1.** Eleven sinoaortic innervated rats and 4 SAD rats were used. Innervated rats were divided into two groups; 4 rats were for intraventricular saline injection (control) and 7 were for AII injection (SAI). The rats were loosely restrained in the cylindrical wire-mesh cage at an ambient temperature of $18 \pm 1\degree C$. BP was monitored with a pressure transducer (MPU-0.5, Toyo Baldwin, Tokyo) and the heart rate (HR) was counted every minute from the ECG recordings. The rat with the cage was covered with a plastic hood, and the ambient air was drawn through the hood (2 l/min) and into an oxygen analyzer (Model 755, Beckman, USA) for the measurement of metabolic rate ($W/m^2: M$). RQ was assumed to be 0.83. Colonic temperature ($T_{col}$) was measured with a thermistor inserted 6 cm into the colon. $T_{BAT}$ was measured in the interscapular BAT by a thermocouple and the tail skin temperature ($T_{sk}$) was monitored by another thermocouple attached to the tail 5 cm distal from its root except in the control rats. All the variables except HR were continuously recorded on potentiometers (MC 6735, Watanabe Sokki, Tokyo; SP-H6P, Riken Denshi, Tokyo). Saline and angiotensin II (Peninsula Laboratories, San Carlos, USA) were administered with a stainless steel injection needle connected to a micro-syringe. After a 90 min rest period, the injection needle was inserted into the implanted guide cannula 0.5 mm beyond the tip of the cannula. To avoid the spontaneous diffusion of the drug from the needle, a very fine air bubble had been sucked into the tip of needle. When all the records were stabilized, 3 µl of saline was injected into the lateral ventricle of control rats and 5 µg of AII, a sufficient dose to elevate BP and to lower $T_{col}$ (LIN et al., 1980; HAACK and MOHRING, 1978), dissolved in 3 µl saline was injected in SAI and SAD rats. The injection needle was left in the guide cannula after injection. The observations continued for the following 30 min.

**Experiment 2.** Eleven rats which received both aortic and venous catheters were used. The experimental procedures were the same as in experiment 1 except for the pretreatment with a vasopressin-antagonist. The competitive arginine-vasopressin (AVP)-antagonist, (CH₃)₃(OMe)-Tyr-AVP (Senn chemicals, Dielsdorf, Switzerland) (KRUSZYN斯基 et al., 1980), at a dose of 20 µg/kg dissolved in 0.25 ml/kg saline was injected into the inferior vena cava via the femoral vein catheter in 5 rats and 0.25 ml/kg of saline was injected into 6 rats. Five min later, 5 µg of AII in 3 µl saline was administered into the lateral ventricle and the changes of all variables were observed for the following 30 min.

**Statistics.** The results are presented as the mean±S.E. Statistical evaluation of the data was performed by a one-way analysis of variance (ANOVA) and the statistical significance of difference between mean values was assessed by
paired and unpaired Student's t-test. The level of significance was set at $p<0.05$.

RESULTS

Experiment 1

The mean resting values of BP, HR, $M$, $T_{co1}$, the difference between $T_{BAT}$ and $T_{co1}$ ($(T_{BAT}-T_{co1})$), and $T_{sk}$ are summarized in Table 1. BP and HR were 13.6 mmHg and 62.7 beats/min (bpm), respectively, higher in SAD rats than in SAT ones. $T_{co1}$ was significantly lower in SAD rats. However, there were no significant differences in $M$, $(T_{BAT}-T_{co1})$, and $T_{sk}$ between the two groups.

The responses of these variables to the intraventricular injection of All in the SAT and SAD rats are shown in Fig. 1. An injection of 3 μl saline did not affect BP, HR, $M$, or $T_{co1}$ in the control rat. In the SAT rat, intraventricular injection of All promptly elevated BP and decreased HR by about 100 bpm. $M$ was greatly suppressed and reached the minimum level within a few minutes after the injection. Within 20 min, all these changes were restored to the original levels. All injection in SAD rats also resulted in an immediate rise in BP, which was significantly greater than that in SAT rats. In spite of such a marked elevation of BP, no bradycardia was observed. All injection produced only a slight

<table>
<thead>
<tr>
<th></th>
<th>Control ($n=4$)</th>
<th>SAI ($n=7$)</th>
<th>SAD ($n=4$)</th>
</tr>
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<tr>
<td>BP (mmHg)</td>
<td>120.0</td>
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<td>142.4</td>
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<tr>
<td>HR (bpm)</td>
<td>432.4</td>
<td>421.5</td>
<td>484.2</td>
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<tr>
<td>$M$ (W/m²)</td>
<td>±2.0</td>
<td>±1.8</td>
<td>±6.4*</td>
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<tr>
<td>$T_{co1}$ (°C)</td>
<td>±17.2</td>
<td>±12.8</td>
<td>±7.0*</td>
</tr>
<tr>
<td>$(T_{BAT}-T_{co1})$ (°C)</td>
<td>±8.0</td>
<td>±2.9</td>
<td>±2.5</td>
</tr>
<tr>
<td>$T_{sk}$ (°C)</td>
<td>±0.08</td>
<td>±0.07</td>
<td>±0.26*</td>
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</table>

Table 1. The resting values and the maximal changes following intraventricular All injection in mean blood pressure (BP), heart rate (HR), metabolic rate ($M$), the difference between the temperature of BAT and $T_{co1}$ ($(T_{BAT}-T_{co1})$), and tail skin temperature ($T_{sk}$) in the control, sinoaortic innervated (SAI) and denervated (SAD) rats at 18°C.

Resting values (mean±S.E.) are obtained as the means of 6 data before All injection (from −5 to 0 min). Maximal changes (mean±S.E.) indicate the largest changes from resting values within 30 min after All injection. The numbers in the parentheses mean the numbers of rats. * Significantly different ($p<0.05$) from the corresponding values of control rats who received saline injection. ns, no significant change.

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Fig. 1. Changes in mean blood pressure (ΔBP), heart rate (ΔHR), metabolic rate (ΔM), colonic temperature (ΔTco1), difference between the temperature of BAT and Tco1 (Δ(TBAT - Tco1)), and tail skin temperature (ΔTek) from the resting levels following an injection of All (5 µg) into the lateral ventricle in the sinoaortic innervated (SAI) (n=7) and denervated (SAD) (n=4) rats. Time 0 indicates the time of All injection. Values are means and vertical bars are S.E. Black circles, values of SAI rats; white circles, values of SAD rats.

Table 2. The resting values and the maximal changes following intraventricular All injection in mean blood pressure (BP), heart rate (HR), metabolic rate (M), colonic temperature (Tco1), difference between the temperature of BAT and Tco1 ((TBAT - Tco1)), and tail skin temperature (Tek) in the intravenous saline and AVP-antagonist pretreated rats at 18°C.

<table>
<thead>
<tr>
<th></th>
<th>BP (mmHg)</th>
<th>HR (bpm)</th>
<th>M (W/m²)</th>
<th>Tco1 (°C)</th>
<th>(TBAT - Tco1) (°C)</th>
<th>Tek (°C)</th>
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<tr>
<td>Control (n=6)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>values</td>
<td>±2.2</td>
<td>±4.2</td>
<td>±2.1</td>
<td>±0.10</td>
<td>±0.12</td>
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<tr>
<td>Maximal changes</td>
<td>±35.0</td>
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<td>-17.8</td>
<td>-0.56</td>
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<tr>
<td>AVP-antagonist (n=5)</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>values</td>
<td>±1.1</td>
<td>±4.2</td>
<td>±3.6</td>
<td>±0.13</td>
<td>±0.13</td>
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<td>Maximal changes</td>
<td>±19.1</td>
<td>-58.8</td>
<td>-13.0</td>
<td>-0.33</td>
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</table>

Legends are the same as in Table 1.
and insignificant decrease in $M$ in SAD rats. $T_{\text{co1}}$ fell gradually after AII injection and reached a minimum within 20 min in both SAI and SAD rats. This fall in $T_{\text{co1}}$ was statistically significant, but the changes in SAD rats were significantly smaller than those in SAI rats. In SAI rats, AII injection significantly decreased ($T_{\text{BAT}}-T_{\text{co1}}$), an index of BAT thermogenesis. The decrease in ($T_{\text{BAT}}-T_{\text{co1}}$) occurred immediately after AII injection in SAI rats, but not in SAD rats. ($T_{\text{BAT}}-T_{\text{co1}}$) in SAD rats remained at the resting level for nearly 4 min after injection, and gradually but insignificantly decreased thereafter. AII injection did not produce any significant change in $T_{sk}$ in either group. The maximal changes from the resting levels within 30 min after AII injection are summarized in Table 1.

**Experiment 2**

Table 2 summarizes the mean resting values in BP, HR, $M$, $T_{\text{co1}}$ ($T_{\text{BAT}}-T_{\text{co1}}$), and $T_{sk}$ in both saline and AVP-antagonist pretreated rats. There were no significant differences in these variables between the two groups. The differences of the maximal responses to the intraventricular injection of AII are shown in Table 2. By the AVP-antagonist treatment, the elevation of BP was suppressed by 45.5%, and the decreases in HR, $M$, $T_{\text{co1}}$ and ($T_{\text{BAT}}-T_{\text{co1}}$) were also suppressed, by 47.9, 27.9, 41.8, and 29.6%, respectively.

**DISCUSSION**

The present study has shown that a 5 μg dose of AII administered intraventricularly elevates blood pressure and decreases heat production and body temperature in conscious rats at an ambient temperature ($T_a$) of 18°C. In the SAD rat (experiment 1), the reduction of $M$ and $T_{\text{co1}}$ induced by central AII was significantly attenuated, although a more marked elevation of BP with no reflexive bradycardia was observed (Fig. 1 and Table 1). This indicates that the sinoaortic baroreflex contributes to the decrease in heat production and hypothermia following intraventricular administration of AII. It has been known that centrally administered AII elevates the systemic blood pressure via stimulation of vasopressin (AVP) release (Gregg and Malvin, 1978; Haack and Möhring, 1978; Keil et al., 1975; Mouw et al., 1971). In experiment 2, we observed that both the pressor and hypothermic effect of central AII were reduced after treatment with an AVP-antagonist (Table 2). There was a close correlation between the extent of baroreflexive bradycardia ($JHR$) and that of hypothermia ($JT_{\text{co1}}$) in the present 22 rats including SAD and AVP-antagonist pretreated rats after central injection of the same amount of AII (Fig. 2). Thus, the present data suggest that the sinoaortic baroreflex plays an important role in the hypothermic response to AII in rats, at least, in a cold environment.

Recent observations (Höltöla et al., 1980; Nunomura, 1983; Shibata, 1982; Shido et al., 1984; Wasserstrum and Herd, 1977) indicate that elevation

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of blood pressure by physical or pharmacological means suppresses shivering and nonshivering thermogenesis through the sinoaortic baroreflex in various animals. The suppression of heat production observed in the present study seemed to be due largely to the reduction of nonshivering thermogenesis, because the rats scarcely shivered in the present experimental conditions and showed an immediate decrease of \(T_{BAT} - T_{col}\), an index of nonshivering thermogenesis of BAT. Since BAT is under the control of the sympathetic nervous system (HIMMS-HAGEN, 1967; SEYDOUX and GIRARDIER, 1977), the reduction of heat production after AII might be a consequence of attenuated sympathetic nervous activity in the metabolic tissues.

The central AII produced a slight but significant fall in body temperature even after sinoaortic deafferentation in rats (Fig. 1). This fall in body temperature cannot be explained only by the baroreflexive suppression of the sympathetic nervous activity. Direct injection of AII into the preoptic and anterior hypothalamus produced a fall in body temperature in rats (KIYOHARA et al., 1984) and in rabbits (SHARPE et al., 1978). KIYOHARA et al. (1984) observed that warm-sensitive and cold-sensitive neurons in the medial preoptic area increased and decreased the firing rate, respectively, in response to an iontophoretic application of AII in anesthetized rats. These findings may suggest a direct action of AII in the hypothalamic control mechanisms of thermoregulation. The findings that the preoptic area contains AII (FUXE et al., 1976) or has specific AII binding sites

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in the rat (Sirett et al., 1977) may favor this conclusion. Therefore, it can be said that All induced hypothermia may be explained largely by baroreflexive suppression of metabolism, and to some extent by the direct effect of this hormone on the thermoregulatory center.

Concerning the effect of All on heat loss, a rise in tail skin temperature was not observed following the central injection of All in the present study (Fig. 1). However, Lin (1980) and Sharpe et al. (1978) observed an All-induced activation of heat loss mechanisms in rabbits. On the contrary, Lin et al. (1980) reported a fall in tail skin temperature in rats after intraventricular All. Therefore, a further study is needed to clarify the effect of All on the cutaneous circulation in rats at various ambient temperatures.

As shown in Fig. 1 and Table 2, intraventricular injections of All at a dose of 5 µg significantly elevated blood pressure, and AVP-antagonist pretreatment reduced the pressor effect of central All by 45.5%. Meanwhile, it has been known that centrally administered All raises BP through enhancement of the sympathoadrenal activity (Buckley, 1972; Ferrario et al., 1970, 1972; Lappe and Brody, 1984; Sever et al., 1966). Therefore, our result suggests that about half of the BP response to All is mediated by AVP secretion, but the other half may be elicited by increasing central adrenergic outflow to the cardiovascular effectors.

In the rats whose baroreceptor afferents were interrupted, the resting body temperature was significantly lower (by 0.66°C) than that of the control rats (Table 1). The changes in some vital functions such as cardiovascular (Krieger, 1964) and water balance (Werber and Fink, 1981) may be partly responsible for this lower resting body temperature.

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CENTRAL ANGIOTENSIN II AND BAROREFLEX


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