Central angiotensin receptor blockade impairs thermolytic and dipsogenic responses to heat exposure in rats

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DURING EXPOSURE to a hot environment, mammals regulate their core temperature by a number of mechanisms that include increased circulation of warm blood from the body core to the surface and evaporative cooling from the body surface. In the rat, cutaneous vasodilation redirects heat to the body surface and evaporative cooling is achieved by spreading of saliva onto the skin and fur. If drinking water is not available during heat exposure, the rats become dehydrated over time (27). There is evidence that the water drinking response to thermal dehydration is correlated with the increase in plasma sodium and osmolality (3). The major forebrain sites regulating these body functions are in the preoptic and hypothalamic regions (7, 15, 25). Different inputs from thermal and osmotic sensors are integrated there with the subsequent neural output modulating autonomic and neuroendocrine pathways influencing thermoregulatory and osmoregulatory effector organs (9, 13, 22, 23).

The role of angiotensin has been investigated as a signaling molecule for both thermoregulatory and fluid balancing effector pathways. In the rat, there is evidence that ANG II may be a central transmitter inducing heat-loss mechanisms (29, 31). Fregly and Rowland (10) showed that central administration of the AT₁ receptor antagonist losartan inhibited the hypothermic effect of intracerebroventricular ANG II. Brain structures in the lamina terminalis, such as the subfornical organ (which lacks a blood-brain barrier) and the median preoptic nucleus, may have a role in this function (9). ANG II receptors of the AT₁ subtype are present in these regions, and blockade of AT₁ receptors with systemically administered losartan prevented tail vasodilation induced by systemic administration of ANG II (10). However, Horowitz et al. (14) showed that neither systemic nor central AT₁ receptor blockade had any effect on the tail vasodilatory response to heat exposure or the onset of salivation in naive rats. These investigators suggested rather that ANG II signaling is directed at acclimatization to a hot environment by accelerating the onset of evaporative heat loss.

The lamina terminalis also has a crucial role in regulating angiotensin-induced drinking behavior and other aspects of body fluid homeostasis (22, 23, 30). Thermogenic drinking induced in cold-acclimated rats transferred back to room temperature has been partly attributed to the central action of circulating ANG II (9). Barney et al. (4) used captopril-induced blockade of systemic ANG II to investigate a role for ANG II in drinking after thermal dehydration. However, they found no difference in the drinking response, suggesting that peripherally formed ANG II is not involved in thermogenic drinking. The aim of the present study was twofold: to investigate the possible involvement of ANG II in the drinking response to thermally induced dehydration, concentrating in particular on angiotensinergic mechanisms within the brain, and second, to investigate a role for central angiotensinergic mechanisms in heat-defense pathways during exposure to a hot environment.
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EXPERIMENTAL PROCEDURES

All experimental protocols received prior approval from the Animal Ethics Committee of the Howard Florey Institute, which adheres to the Australian code of practice for the care and use of animals for scientific purposes.

Animal preparation. Male Sprague-Dawley rats (315–355 g body wt) were used in this study. Animals were anesthetized with Nembutal (60 mg/kg ip) and underwent surgical implantation of a metal cannula (23 gauge) into the right lateral cerebral ventricle. The cannula was anchored to the skull with screws embedded into dental acrylic. Two weeks later, the patency of the ventricular probe was established by a positive drinking response to an injection of 10 ng ANG II in 2 μl of artificial cerebrospinal fluid (aCSF). Experiments involving heat exposure commenced 3 days after the drinking test.

In preliminary experiments, all rats treated with intracerebroventricular aCSF survived heat exposure (39°C) for up to 120 min, but some unexpected deaths occurred in the intracerebroventricular losartan-treated group when the dosage of losartan was 100 μg and the period of heat exposure was 90 min. Consequently, the dose of losartan was decreased 10-fold and the time of exposure to heat exposure was reduced to 60 min for both experimental groups. In addition, we imposed a condition based on ethical considerations that heat exposure was stopped if colonic temperature reached 42°C. After these changes to the protocol, all animals survived the procedure in good condition, displaying alert responses and free movement.

Plasma data. To provide data on changes that occur in plasma ions and protein concentration during heat exposure, a blood sample (300 μl) was collected from rats treated with either losartan (10 μg in 5 μl aCSF; n = 5) or aCSF (5 μl; n = 5) by cutting the distal tip of the tail (0.5–1 mm). Once bleeding had stopped, the rats were returned to their home box without food or water access and placed in a chamber with an ambient temperature of 39 ± 1°C. After 1 h, the rats were removed from the heated chamber and another blood sample was collected. These rats were not used in the experiments that monitored body temperature and drinking responses to heat exposure. Plasma ion and protein concentrations were measured using a Beckman Synchron CX-5 (Beckman-Coulter). Plasma osmolality was measured using a Fiske one-ten osmometer (Fiske Associates).

Central AT1 receptor blockade and short-term heat exposure. On the day of the experiment at 1100, rats were given an injection into the lateral ventricle of either 5 μl aCSF or 10 μg of the ANG II AT1 receptor antagonist losartan (DuPont-Merck) in 5 μl of aCSF. Temperature was measured by K-type thermocouples connected to a dual channel Fluke 52 (John Fluke MFG) electronic thermometer that was calibrated to two decimal places against a conventional glass mercury thermometer. To measure deep body temperature, a thermocouple (coated in silicon at the tip) was inserted 5 cm into the anal sphincter of each rat. The tip of the thermocouple and connecting wires were coated with 5% lidocaine gel (Xylocaine, Astra Pharmaceuticals) as a local anesthetic and lubricant. To indirectly measure active cutaneous vasodilation via temperature changes of the tail skin, a second thermocouple was attached to the skin 3 cm distal to the root of the tail. The insulated wires from both thermocouples were secured to the tail using cloth tape, taking care to further insulate the tail skin thermocouple. The thermocouple wires were very flexible and light (0.5-mm diameter), allowing the rat free movement around its home cage. The body weights of the rats were measured, and they were returned for the next hour to their home cages. Recording of rectal and tail skin temperature began 10 min before the rat (while in its home cage) was put into the heat chamber for baseline measurements. The floor of the cage was lined with blotting paper that had been treated with cresyl red (11). This paper stained pink on contact with saliva (compared with yellow-brown on contact with urine), and this indicator, together with spreading of the saliva on the skin and fur, allowed visual confirmation that evaporative cooling behavior was intact. After transfer into the preheated heating chamber, the wires connected to the thermocouples were brought out of the chamber through a small hatch in the top cover. This experimental procedure was well tolerated by all animals. The temperature of the heat chamber was maintained at 39 ± 1°C by a thermostat connected to a ceramic-coated heating element. Air was circulated around the chamber by a fan situated underneath the heating element to provide an even distribution of heat.

During heat exposure (1 h), colonic and tail skin temperature were continuously monitored and recorded at 2-min intervals for 60 min. At the end of this period, the rat was taken from the heat chamber and the thermocouples were removed before it was weighed again and then returned to a fresh cage at room temperature (22°C). Each rat was then given access to water using a 20-ml graduated glass cylinder connected to a drinking spout, and water intake was monitored at 15-min intervals for a total of 120 min.

Effect of losartan on intracerebroventricular carbachol-induced drinking. In another set of experiments, the specificity of the effect of losartan on the drinking response to short-term heat exposure was tested using intracerebroventricular injection of carbachol (carbamylcholine chloride, Sigma-Aldrich), a cholinergic agonist, that is known to induce drinking via a central mechanism that is independent of angiotensin (5). As in the first set of experiments, rats were treated with intracerebroventricular injections of either losartan (10 μg in 5 μl of aCSF) or 5 μl of aCSF vehicle alone 1 h before heat exposure. However, after 1 h of heat exposure, animals were given an additional intracerebroventricular injection of carbachol (300 ng or 1.25 μg in 2 μl of aCSF) and water intake was measured for 120 min.

Statistical analysis. Plasma ionic and protein data in animals treated with losartan or aCSF before and after heat exposure were analyzed by a paired t-test.

In animals where body temperatures were continuously measured, colon and tail skin temperatures were collated for each 2-min interval and expressed as means ± SE. Data for the aCSF-treated and the losartan-treated groups of animals were then tested by repeated-measures analysis of variance with a post hoc Bonferroni correction (SigmaStat, Jandel Scientific). Similarly, water-intake data were compared between aCSF- and losartan-treated groups, with and without the intracerebroventricular injection of carbachol after heat exposure.

For each animal, the volume of saliva that was collected on the cresyl red-treated paper was determined by calculating the total surface area of the stains over 1 h and dividing the total area by a known volume-to-surface area constant (200 μl = 12.5 cm²). These data were collected for both aCSF- and losartan-treated groups, and statistical significance was determined by a Student’s t-test (SigmaStat, Jandel Scientific).

RESULTS

Table 1 shows plasma measurements in rats that received an intracerebroventricular injection of either aCSF (n = 5) or losartan (n = 5) before and after they
were exposed to a 39°C environment. In both groups, equivalent increases in plasma sodium, chloride, and osmolality were measured after 1 h of heat exposure. Changes in plasma protein were not statistically significant in either group.

Effect of central AT<sub>1</sub> receptor blockade on temperature regulation and body weight loss during short-term heat exposure. In the control group that received intracerebroventricular aCSF, colonic temperature increased steadily during heat exposure from 37.22 ± 0.21 to 40.68 ± 0.31°C (Fig. 1). In the intracerebroventricular losartan-treated group, colonic temperature increased to a significantly higher level compared with the controls, rising from 37.41 ± 0.27 to 41.72 ± 0.28°C (P < 0.03) by the end of the period of heat exposure (Fig. 1). Tail skin temperature increased rapidly in both groups of animals during heat exposure, rising from 25.40 ± 0.41°C to a maximum of 39.45 ± 0.37°C at the end of heat exposure in the control group. In losartan-treated animals, tail temperature increased from 26.4 ± 0.47 to a maximum of 41.12 ± 0.37°C, which was significantly higher than in the control group (P < 0.05). This result was consistent with the higher rectal temperature that was observed in losartan-treated animals relative to controls.

Both losartan- and aCSF-treated groups lost 13.0 ± 2.8 g of body weight during exposure to heat exposure. Grooming and licking behavior was observed in all animals exposed to the heat. In addition, increased salivation was present in both experimental groups as shown by the saliva records (cresyl red-incubated filter papers). Area analysis of the saliva records revealed that an equivalent of 2.7 ± 0.3 ml of saliva was collected in the group treated with intracerebroventricular losartan compared with 2.2 ± 0.2 ml (not significantly different) in the group treated with intracerebroventricular aCSF. These values are only approximations, because all the rats intermittently spread the saliva from their mouths onto their skin during grooming. Furthermore, the spread of the saliva spots would have been inhibited by the high rate of evaporation in the heat chamber. Although this method substantially underestimates the actual volume of saliva produced, it does provide an indication of the relative rate of salivation between the two experimental groups.

Effect of central AT<sub>1</sub> receptor blockade on water intake after short-term heat exposure. In the control group, rats initiated drinking within 30 min after the end of the period of heat exposure. They drank a total of 5.9 ± 0.7 ml by 120 min after heat exposure (Fig. 2). In rats treated with losartan, the drinking response to heat exposure was inhibited and the volume of water drunk was reduced to 1.1 ± 0.3 ml (P < 0.002) at the end of the 120-min observation period.

To test whether the blockade of the drinking response to heat exposure was specific to the pharmacological antagonism of central angiotensin rather than a consequence of the rats being debilitated, an additional experiment was performed. Both aCSF- and losartan-pretreated rats initiated drinking within 8 min after having received an intracerebroventricular injection of carbachol (300 ng or 1.25 μg in 2 μl). Both groups of rats also drank similar volumes of water during the 120-min period after the carbachol injection in a dose-dependent manner (Fig. 3).

DISCUSSION

The major finding of this study is that a central angiotensin AT<sub>1</sub> receptor-dependent pathway is involved in thermoregulatory cooling mechanisms and in the drinking response after heat exposure. Previous studies in the rat and rabbit have suggested that central injection of ANG II can reduce core temperature (19, 28, 29, 31) and have cited evidence of decreased metabolic rate and increased cutaneous heat loss. These reports are consistent with the current observation that core temperature of rats increases at a faster rate during pharmacological blockade of central AT<sub>1</sub>
receptors during short-term heat exposure. The exact location and the precise mode of this central disruption to normal thermoregulation remains to be elucidated; however, hypothalamic sites expressing AT$_1$ receptors, such as the lamina terminalis or the hypothalamic paraventricular nucleus, are possible sites of losartan influences on thermoregulation (1).

There was no difference in body weight loss between the control and losartan-treated groups and also no difference in grooming behavior, suggesting that there was no change in evaporative heat loss arising from the spreading of saliva on the skin and fur. Indeed, area analysis of stains on saliva records did not reveal a difference between the two experimental groups, confirming that this aspect of thermoregulation was not disrupted by centrally administered losartan. Similarly, tail skin vasodilation during heat exposure was not inhibited by losartan treatment, in agreement with previously reported data in the rat (17). The data in the present study show ostensibly that despite the activation of major thermoregulatory cooling mechanisms (such as cutaneous vasodilation and/or saliva spreading), core temperature increased to a higher level in losartan-treated rats compared with control animals. An increase in core temperature has been shown to be associated with increases in lumbar, renal, and splanchnic sympathetic nerve activity (16). Thus, during heat exposure, sympathetically mediated vasoconstriction might be important for blood flow redistribution to areas with a high surface area-to-volume ratio (such as the tail) and also to the vascular bed of the salivary glands to facilitate heat-loss mechanisms. In rats subjected to a similar heating protocol, Kregel et al. (17) showed that central AT$_1$ receptor blockade causes a reduction in blood pressure. In addition, by measuring regional nerve activity, these workers demonstrated that losartan treatment prevented the usual increase in splanchnic nerve activity and they suggest that this would reduce the redistribution of blood flow to the skin during heat exposure. These results fit well with our data during short-term heat exposure that show that central losartan treatment resulted in an increased core temperature. It is possible that when splanchnic vasoconstriction is attenuated (17) during heat exposure, pooling of blood in the viscera might reduce the efficacy of cutaneous heat-loss mechanisms, eventually leading to the observed increase in colonic temperature.

During heat exposure, water is lost during evaporative cooling, which leads to thermal dehydration and consequent thirst (2, 12, 27). Our experimental protocol increased plasma sodium and osmolality without significantly changing the protein concentration. Thus it is more likely that thirst arising from heat exposure is driven by osmotic signaling rather than by hypovo...
lemia. Other studies have also shown that thermogenic thirst in rats has a smaller volemic component than water deprivation-induced thirst (3, 24, 26), and, therefore, plasma ANG II levels should also be less elevated. In agreement, systemic blockade of ANG II formation with captopril failed to significantly alter thermogenic water intake after heat exposure (4). The present study demonstrated, however, that water intake after heat-induced fluid loss is attenuated by blockade of central AT₁ receptors. This is a novel finding that has several parallels in the literature relating to central angiotensinergic influences on water drinking in response to intracerebroventricular hypertonic NaCl (6, 20) and feeding (21) in a number of mammals. Therefore, we hypothesize that thermal dehydration does not activate water drinking via the peripheral renin-angiotensin system, but that neural pathways, using centrally generated ANG II, are involved in drinking after heat exposure.

The experiment using carbachol as a central dipsonogen after a 1-h period of heat exposure was performed to eliminate the possibility that the inhibitory effect of losartan on water intake might have been due to the rats feeling unwell. This possibility was investigated because of our observations in preliminary experiments, which showed that a 10-fold higher dose of losartan increased the mortality rate of rats subjected to a twofold longer period of heat exposure. Barney and West (3) also reported that rats sometimes appeared “semi-comatose” after a long-term period (3–4 h) of exposure at 40°C, which delayed their drinking response. Because of the higher rectal temperature observed in the present study in losartan-treated rats, we were careful to check that all rats were alert and exhibited free movement after heat exposure. This experiment clearly showed that even with the combined treatment of heat exposure and losartan, rats responded to intracerebroventricular carbachol with appropriate drinking behavior, suggesting that this combination of higher body temperature and central losartan treatment did not nonspecifically depress drinking behavior. In addition, the fact that losartan treatment did not inhibit the dipsonogenic response to intracerebroventricular injection of carbachol after heat exposure is consistent with earlier studies showing that carbachol-induced drinking is independent of neurochemical pathways using angiotensin (5, 8).

**Perspectives**

Central blockade of ANG II AT₁ receptors not only significantly reduced the drinking response to short-term heat exposure, but also led to a significantly higher body temperature. Therefore, a central angiotensinergic pathway appears to be important in mediating thermogenic drinking and the thermoregulatory response to heat exposure in the rat. Barney and West (3) reported a 13.6% mortality rate for rats subjected to long-term heat exposure (3–4 h, 40°C), with no deaths occurring before this time period. It is likely that pharmacological blockade of central angiotensin inhibits thermoregulatory cooling and may eventually increase mortality as well as disrupt fluid replacement in response to thermal dehydration. Whether this general impairment of heat defense is a specific effect on central body temperature control or secondary to alterations of body fluid distribution has yet to be clarified.

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