AT₁ receptors in the paraventricular nucleus mediate the hyperthermia-induced reflex reduction of renal blood flow in rats

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Chen F, Liu F, Badoer E. AT₁ receptors in the paraventricular nucleus mediate the hyperthermia-induced reflex reduction of renal blood flow in rats. Am J Physiol Regul Integr Comp Physiol 300: R479–R485, 2011. First published December 1, 2010; doi:10.1152/ajpregu.00604.2010.—Increasing body core temperature reflexly decreases renal blood flow (RBF), and the hypothalamic paraventricular nucleus (PVN) plays an essential role in this response. ANG II in the brain is involved in the cardiovascular responses to hyperthermia, and ANG II receptors are highly concentrated in the PVN. The present study investigated whether ANG II in the PVN contributes to the cardiovascular responses elicited by hyperthermia. Rats anesthetized with urethane (1–1.4 g/kg iv) were microinjected bilaterally into the PVN (100 nl/side) with saline (n = 5) or losartan (1 nmol/100 nl) (n = 7), an AT1 receptor antagonist. Body core temperature was then elevated from 37°C to 41°C and blood pressure (BP), heart rate (HR), RBF, and renal vascular conductance (RVC) were monitored. In separate groups losartan (n = 4) or saline (n = 4) was microinjected into the PVN, but body core temperature was not elevated. Increasing body core temperature in control rats elicited significant decreases in RBF (−48 ± 5% from a resting level of 14.3 ± 1.4 ml/min) and MVC (−40 ± 4% from a resting level of 0.128 ± 0.013 ml/min-mmHg), and these effects were entirely prevented by pretreatment with losartan. In rats in which body core temperature was not altered, losartan microinjected into the PVN had no significant effects on these variables. The results suggest that endogenous ANG II acts on AT1 receptors in the PVN to mediate the reduction in RBF induced by hyperthermia.

THERMOREGULATION IS A VITAL, fundamental physiological function that enables the body to defend itself from external and internal temperature changes. There are a number of cardiovascular mechanisms that help do this; for example, during hyperthermia in humans, blood flow is redirected from the viscera (e.g., renal and splanchnic regions) to the skin so that the body may dissipate heat. Such responses are brought about by altering sympathetic nerve activity. Increasing sympathetic nerve activity to the vasculature of visceral organs like the kidneys and mesentery is important since failure to adequately vasoconstrict these in response to hyperthermia can cause a dramatic reduction in cardiac output and the resultant life-threatening sequelae of heat stroke (22–23).

The hypothalamus, in particular, plays a key role in thermoregulation and the hypothalamic paraventricular nucleus (PVN), located adjacent to the third ventricle in the forebrain, is an important integrative site involved in hormonal, endocrine, and peripheral neural control.

Activation of the PVN can cause a pronounced alteration in blood pressure, sympathetic neural outflows, and hemodynamic responses (5–6, 11, 21, 31). There are several lines of evidence supporting a role of the PVN in the cardiovascular responses elicited during thermoregulation: 1) the PVN contains neurons that project to areas important for thermoregulation, such as the brown adipose tissue, the tail (in the rat), kidney, and gut (19, 26, 30, 34–35); 2) the PVN can influence sympathetic nerve activity and blood flow to the gut and kidney (21); 3) some PVN neurons have an intrinsic sensitivity to changes in temperature (20); and 4) neurons in the PVN are strongly activated by hyperthermia (3, 9, 27).

More recently, we have found that the PVN is essential for the normal reflex reduction in renal and mesenteric blood flows induced by hyperthermia (6, 8, 10). The PVN is one of the few sympathetic premotor nuclei present in the brain; that is, it contains neurons that project directly to sympathetic preganglionic motor neurons in the intermediolateral cell column of the thoracolumbar spinal cord (9, 32, 36). These connections underlie the influence of the PVN on sympathetic nerve activity that contributes to the blood flow responses (4–5).

ANG II is generated in the brain and can act as a neurotransmitter or neuromodulator (12–13, 15, 29, 38). ANG II receptors are found in key brain areas that contribute to temperature and cardiovascular regulation, such as the medial preoptic area and the hypothalamic PVN (1, 6, 29, 34), suggesting ANG II may play a key role in the cardiovascular responses elicited by changes in body temperature. This is strongly supported by findings showing that ANG II is critical in regulating body temperature in the face of an increased environmental temperature (28) and that inhibition of ANG II receptors in the brain prevents the increase in splanchnic nerve activity (contributes to visceral vasoconstriction), normally induced by hyperthermia (24).

The specific site in the brain at which ANG II may act to influence visceral vasoconstriction in response to hyperthermia is unknown. Our hypothesis is that the PVN is the key site, since it has a dense concentration of ANG II type I receptors and that inhibition of the PVN prevents the reflex vasoconstriction in renal and mesenteric vasculature (1, 8, 10). In the present study, therefore, we investigated the effect of microinjection of the ANG II type I receptor antagonist, losartan, directly into the PVN on the reflex reduction in renal blood flow induced by elevating body core temperature in rats.
MATERIALS AND METHODS

Animals and Housing

All experimental protocols used in this study were performed in accordance with the Prevention of Cruelty to Animals Act 1986 (Australia) and to the guidelines set out by the National Health and Medical Research Council of Australia (Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 2007). The protocols were approved by the Royal Melbourne Institute of Technology (RMIT) University Animal Ethics Committee, and conform to the “Guiding Principles for Research Involving Animals and Human Beings” (2). Male Sprague-Dawley rats (obtained from Monash University Animal Services, Victoria, Australia) weighing 350–450 g (10–12 wk) (at the time of experiment) were housed in the Animal Facility (RMIT University, Victoria, Australia) with free access to rat chow and tap water at a room temperature of 22 ± 1°C with a 12:12-h light-dark cycle.

Surgical Preparations

All animals were anesthetized initially with 2–3% isoflurane in 100% oxygen to enable the cannulation of the femoral artery and vein. The femoral vein was cannulated for the intravenous delivery of urethane for the maintenance of anesthesia (1–1.4 g/kg, initially, followed by supplemental doses of ~0.05 g/kg as required). The depth of anesthesia was monitored every 15 min and was adjusted to ensure the absence of corneal and pedal reflexes. The femoral artery was cannulated for monitoring arterial blood pressure. The signal was recorded using a MacLab data-acquisition system (AD Instruments, Colorado Springs, CO, USA). Mean arterial pressure (MAP) and heart rate (HR) were determined electronically from the phasic arterial pressure. Throughout the surgical procedures, the body temperature was maintained at ~37.0°C with a custom-made water-circulating blanket, through which warm water (48 to 52°C measured directly at source) could be pumped through at a rate of 16–26 ml/min. The water jacket was also used to gradually raise body core temperature from 37°C to 41°C. A small animal thermometer inserted into the rectum was used to measure the body core temperature.

Microinjection into the Hypothalamic PVN

Each animal was placed prone, and the head was mounted in a Stoeling stereotaxic frame, such that both bregma and lambda were positioned on the same horizontal plane. A midline reference point was marked 2 mm rostral to bregma. This was necessary because bregma was removed during the subsequent bone-drilling procedure. To expose the dorsal surface of the brain, a hole, ~4 mm in diameter, was drilled into the skull centered 3.5 mm caudal from the reference point. Following the drilling procedure, the hole was covered with cotton wool soaked in normal saline to prevent drying of the exposed surface. The entire procedure lasted ~30 min.

Microinjections were made bilaterally using a fine glass micropipette (with a tip diameter of 50–70 μm) into the PVN (stereotaxic coordinates: 1.5–1.8 mm caudal to bregma, 0.5 mm lateral to midline and 7.5 mm ventral to the surface of the brain). Microinjection volumes were 100 nl per side and were injected over 1 min. After each microinjection, the micropipette was left in place for ~1 min. To mark the injection sites, a small amount of rhodamine-tagged fluorescent microspheres was included in the microinjected solution (LumaFluor, Durham, NC).

Renal Blood Flow Measurement

The right kidney was identified using a retroperitoneal approach. The renal artery was dissected free and carefully cleared from the surrounding tissue and from the renal vein. A flow probe (IRB449; Transonic Systems, Ithaca, NY) was positioned around the renal artery and connected to a T206 small animal blood flow meter (Transonic Systems) to enable monitoring of the renal blood flow (RBF). This procedure lasted approximately half an hour. Following the implantation of the flow probe, ~5–20 min (rest period) were allowed to elapse to ensure that a steady basal blood flow was attained. Renal vascular conductance (RVC) was calculated by dividing renal blood flow (ml/min) by the mean arterial pressure (mmHg).

Experimental Protocol

Upon completion of the surgical procedures, the rats were randomly assigned into four groups. In the first two groups, rats were bilaterally microinjected into the PVN with 100 nl/side of either saline (n = 5) or losartan (Sigma-Aldrich, St. Louis, MO), an ANG II AT1 receptor antagonist, at a dose of 1 nmol per side (n = 7). Immediately after the completion of the microinjections, the water jacket was used to gradually increase the body core temperature of the animal from 37°C to 41°C at a rate of ~0.1°C every 2 min, and this was performed over ~80 min. In the second two groups of animals (n = 4/group), losartan (1 nmol), or saline (n = 4) was microinjected into the PVN, and the effects were followed over time. In these rats, body core temperature was maintained at 37.0–37.5°C.

Brain Histology

At the completion of the experiment, the rats were killed with an overdose of pentobarbital sodium (300 mg/kg; Lethabarb, Virbac, Milperra, Australia). The brains were then carefully removed and fixed in 4% paraformaldehyde solution for 3 days and then placed into a solution of phosphate buffer containing 20% sucrose overnight. The hypothalamus was cut on a cryostat into sections (40 μm thick) and mounted onto gelatin-subbed slides. The sections were then viewed wet under fluorescent microscopy to identify the rhodamine beads at the site of injection. The sections were then dried before being counterstained with cresyl violet and coverslipped with Depex mounting medium (BDH Lab Supplies, Poole, UK). The sections were then reexamined using light microscopy to identify the PVN and to determine adjacent anatomical structures. The injection sites were subsequently mapped in relation to the PVN.

Statistical Analysis

The basal resting levels of MAP, HR, RBF, and RVC prior to the microinjections were compared between appropriate groups using a Student’s unpaired t-test. The changes in MAP, HR, RBF, and RVC from resting levels were compared within groups using a one-way ANOVA with repeated measures followed by Dunnet’s post hoc test. The comparisons between groups were analyzed using a two-way ANOVA with repeated measures followed by post hoc comparisons of the group means using the Bonferroni modification for multiple comparisons. All data are presented as means ± SE.

RESULTS

Effect of Increased Body Core Temperature on Cardiovascular Variables

Responses in rats microinjected with saline into the PVN. In animals in which saline was microinjected into the PVN (n = 5), there was a significant reduction in RBF that reached a maximum of −6.8 ± 0.9 ml/min or −48 ± 5% from the average resting level when body core temperature reached 41°C (Fig. 1) (P < 0.0001). There was an accompanying significant reduction in RVC, which reached a maximum of −0.052 ± 0.008 ml/min-mmHg or −40 ± 4% from the average resting level by the end of the observation period (Fig. 1) (P < 0.0001).

On average, when body core temperature reached 41°C, mean arterial pressure was below, and heart rate was above the
basal resting levels, but statistical significance was not reached (Fig. 1).

Responses in rats microinjected with losartan (1 nmol/side) into the PVN. In rats administered losartan into the PVN (n = 7), increasing body core temperature from 37.0–41.0°C did not elicit the reduction in RBF normally observed (Fig. 1). On average, RBF fell only by 2% by the end of the observation period. The response in the presence of losartan was significantly different from the control group [F(1,50) = 28.86, *P < 0.001]. Losartan also significantly reduced the reduction in RVC [F(1,50) = 4.58, **P < 0.05], on average, RVC fell by 7% by the time body core temperature reached 41.0°C, a dramatic attenuation of the normal response (Fig. 1). There was no statistically significant difference in the HR responses following losartan compared with the control group (Fig. 1). Blood pressure changes, however, were significantly different between the two groups (Fig. 1) [F(1,50) = 4.70, **P = 0.035]. On average, there was a slight elevation in blood pressure in the losartan-treated group by the time body core temperature reached 41.0°C compared with the control group. Resting MAP, HR, RBF, and RVC levels were not significantly different from the control group (Table 1).

Table 1. Basal levels of MAP, HR, RBF, and RVC prior to microinjections of losartan or vehicle into the hypothalamic paraventricular nucleus in rats in which body core temperature was raised from 37°C to 41°C or was maintained at 37°C

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beat/min</th>
<th>RBF, ml/min</th>
<th>RVC, ml/min × mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C–41°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>112 ± 4</td>
<td>385 ± 15</td>
<td>14.3 ± 1.0</td>
<td>0.128 ± 0.013</td>
</tr>
<tr>
<td>Losartan (1 nmol)</td>
<td>7</td>
<td>112 ± 3</td>
<td>368 ± 8</td>
<td>11.8 ± 1.2</td>
<td>0.108 ± 0.016</td>
</tr>
<tr>
<td>37°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Saline</td>
<td>4</td>
<td>93 ± 3</td>
<td>381 ± 17</td>
<td>15.5 ± 2.0</td>
<td>0.169 ± 0.026</td>
</tr>
<tr>
<td>Losartan (1 nmol)</td>
<td>4</td>
<td>91 ± 6</td>
<td>357 ± 22</td>
<td>14.6 ± 2.0</td>
<td>0.158 ± 0.016</td>
</tr>
</tbody>
</table>

All data are expressed in means ± SE. MAP, mean arterial blood pressure; HR, heart rate; RBF, renal blood flow; RVC, renal vasculature conductance. n = number of animals per group.

Effect of Losartan Microinjections into the PVN in Rats Without a Change in Body Core Temperature

In two separate groups of rats, saline (100 nl/side; n = 4) or losartan was microinjected into the PVN (n = 4), while body core temperature was kept at 37.0–37.5°C, and the cardiovascular variables were monitored over time. There was no significant difference between the two groups on any of the variables monitored over time (Fig. 2), indicating that losartan did not influence MAP, HR, RBF, and RVC in the absence of hyperthermia. Resting levels prior to losartan or saline were not significantly different between the two groups (Table 1).

Neuroanatomical Location of Injection Sites

The anatomical locations of the microinjection sites within the brain were determined histologically at the conclusion of the experiments. The microinjection sites for losartan and saline in the PVN are shown in Fig. 3. Microinjection sites were centered at rostral-caudal levels 1.8–2.2 mm caudal to bregma (Fig. 3). The rostral-caudal distribution of the losartan and the saline microinjection sites were similar (Fig. 3).
microinjection sites encompassed the rostral-caudal extent of the PVN.

**DISCUSSION**

The key finding of the present study is that blockade of ANG II receptors with losartan in the PVN prevents the reflex reduction in renal blood flow induced by elevations in body core temperature. This suggests that ANG II acting on AT1 receptors in the PVN is an important chemical mediator in the cardiovascular thermoregulatory response to hyperthermia.

Elevations in ambient temperature can increase body core temperature and induce reflex vasoconstriction of the visceral vasculature, including the renal and mesenteric vessels (6, 23–24) that are designed to help dissipate body heat. Neurons in the PVN are activated by increases in ambient temperature, including those that influence sympathetic nerve activity (3, 7, 9, 27), suggesting a role for the PVN in temperature regulation.

The present study, together with our more recent work showing that inhibition of neuronal function within the PVN prevented the hyperthermia-induced reduction in renal and mesenteric blood flows, provides direct evidence that the PVN plays a key role in the cardiovascular thermoregulatory responses (8, 10). This does not exclude other brain regions contributing to the reflex renal vasoconstriction elicited by hyperthermia.

The octapeptide, ANG II, is present in the periphery and central nervous system. Central administration of ANG II elicits marked cardiovascular effects and body temperature changes. Intracerebroventricularly administered ANG II induces hyperthermia, and the evidence suggests that a reduction in metabolic rate and redistribution of blood flow contribute (33, 37). More recent work indicates that intracerebroventricular administration of losartan into rats placed in a hot environment markedly increased the rate of increase of body core temperature and the maximum level of body core temperature reached, suggesting ANG II in the brain is important in the regulatory responses critical to maintaining body core temperature (28). There are several autonomically important regions in the brain that have dense concentrations of ANG II receptors, in particular, the lamina terminalis, PVN, and the nucleus tractus solitarius in the medulla oblongata (1). The present study suggests that the PVN is a critical integrative nucleus, in which ANG II is acting to mediate the reflex renal vasoconstriction induced by hyperthermia. This is supported by studies that show that microinjection of ANG II into the PVN can increase renal sympathetic nerve activity in normal rats and that endogenous ANG II in the PVN is important in maintaining an abnormally elevated renal sympathetic nerve activity in heart failure animals (25).

Blocking ANG II receptors in the brain using losartan has been found to inhibit the reflex increase in splanchnic sympathetic nerve activity induced by hyperthermia, suggesting that central ANG II is critically involved in the hyperthermia-induced visceral vasoconstriction (24). Because we have found in previous studies that inhibition of neuronal activity in the PVN using muscimol prevented the reduction in mesenteric blood flow that results from an increased body core temperature (10), we hypothesize that ANG II in the PVN may also mediate the mesenteric vasoconstriction elicited by hyperthermia. Direct evidence that ANG II acts within the PVN to mediate this response, however, is required to confirm this hypothesis.

Hyperthermia reflexly increases visceral sympathetic nerve activity and, because of the changes in osmolality and hypovolemia, plasma levels of vasopressin are elevated (24). These neurohumoral responses can be prevented by antagonizing ANG II receptors in the brain with losartan (24). Vasopressin, released into the blood from magnocellular neurons present in the PVN
and supraoptic nuclei, is a vasoconstrictor (16). Because central administration of ANG II can increase circulating vasopressin, it may be possible that the efferent mechanisms mediating the renal blood flow response induced by hyperthermia involves an increase in vasopressin release. We suspect, however, that its role is small since 1) vasopressin also comes from the supraoptic nucleus, which was not affected by our injections of losartan, 2) vasopressin levels in anesthetized animals following surgery are already high, and 3) the renal bed is less susceptible to the vasoconstrictor actions of vasopressin (18).

Losartan microinjected into the PVN did not affect MAP, HR, RBF, and RVC when body core temperature was maintained constant, suggesting there was no tonic effect of ANG II in the PVN on those cardiovascular variables. Similarly, losartan microinjected into the rostral ventrolateral medulla of rabbits, an area dense in ANG II receptors, had no effect on blood pressure or renal sympathetic nerve activity (17). Interestingly, intracerebroventricular administration of losartan does not affect blood pressure, although it did reduce renal sympathetic nerve activity in normal rats (14), suggesting there are sites in the brain at which endogenous ANG II acts tonically.

In the present study, we injected 1 nmol of losartan into the PVN. Previous studies have microinjected up to 50 nmol into the PVN (39). With the higher doses, small increases in blood pressure, heart rate, and renal sympathetic nerve activity have been reported (39). When smaller doses are used, as in the present study, losartan does not induce changes in basal resting cardiovascular variables, but the excitatory responses to ANG II are still blocked (25).

We have performed our studies on anesthetized rats. We acknowledge that temperature regulation is compromised under anesthesia. This is an issue faced by all research studies using anesthetized animals. Nevertheless, hyperthermia in anesthetized rats elicits reflex renal and mesenteric responses (6, 10, and present study) that are also observed in conscious rats (23, 24). The difference in thermoregulatory control between rats and humans (e.g., rats lose most heat through dilation of their tail vasculature compared with humans, who lose heat through vasodilation of the skin vasculature and sweating) is also acknowledged. Both issues need to be borne in mind by researchers when drawing conclusions from their work in anesthetized animals and applying them to human physiology.
Thermoregulation is a vital physiological function. Cardiovascular responses are essential in mediating the redistribution of blood flow that allows dissipation of heat when body temperature is elevated. The redirection of blood is managed by increasing the sympathetic nerve activity to the vasculature of visceral organs like the kidneys and mesentery and dilatation of the skin blood vessels. Failure to adequately vasoconstrict the renal and mesenteric vasculature in response to hyperthermia may cause a dramatic reduction in cardiac output and the resultant life-threatening sequelae of heat stroke (22–23). The present findings showing that ANG II in the PVN is critical in the reflex reduction in renal blood flow together with studies showing that inhibition of ANG II function in the brain results in enhanced body core temperature in response to elevation in ambient temperature suggest that central ANG II is important in heat dissipation. This may have clinical ramifications since therapeutics that interfere with the function of the renin-angiotensin system are commonly used as antihypertensive agents. Thus, we suggest that elderly hypertensive patients treated with angiotensin receptor blockers or angiotensin-converting enzyme inhibitors may be particularly susceptible to heat stroke during summer heat waves, and clinicians and care givers may need to be particularly vigilant with these patients in our community. We note that there are no reports from clinical trials that indicate patients on those therapeutics are more susceptible to heat stroke. This could indicate that heat stroke in those patients has not been a cause for concern. Alternatively, elderly patients, in whom heat stroke is more common, may have other health complications and may be receiving additional pharmacological treatments, and together, these may complicate identification of a correlation between heat stroke and angiotensin receptor blockers or angiotensin-converting enzyme inhibitors.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


