Hypothalamic paraventricular nucleus is critical for renal vasoconstriction elicited by elevations in body temperature

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Redistribution of blood from the viscera to the peripheral vasculature is the major cardiovascular response designed to restore thermoregulatory homeostasis after an elevation in body core temperature. In this study, we investigated the role of the hypothalamic paraventricular nucleus (PVN) in the reflex decrease in renal blood flow that is induced by hyperthermia, as this brain region is known to play a key role in renal function and may contribute to the central pathways underlying thermoregulatory responses. In anesthetized rats, blood pressure, heart rate, renal blood flow, and tail skin temperature were recorded in response to elevating body core temperature. In the control group, saline was microinjected bilaterally into the PVN; in the second group, muscimol (1 nmol in 100 nl per side) was microinjected to inhibit neuronal activity in the PVN; and in a third group, muscimol was microinjected outside the PVN. Compared with control, microinjection of muscimol into the PVN did not significantly affect the blood pressure or heart rate responses. However, the normal reflex reduction in renal blood flow observed in response to hyperthermia in the control group (~70% from a resting level of 11.5 ml/min) was abolished by the microinjection of muscimol into the PVN (maximum reduction of 8% from a resting of 9.1 ml/min). This effect was specific to the PVN since microinjection of muscimol outside the PVN did not prevent the normal renal blood flow response. The data suggest that the PVN plays an essential role in the reflex decrease in renal blood flow elicited by hyperthermia.

hypothermia

THE HYPOTHALAMIC PARAVENTRICULAR nucleus (PVN) is an important integrative site involved in hormonal, endocrine, and neural control. The PVN is composed of different neuronal subgroups that can influence sympathetic nerve activity and thereby contribute to cardiovascular regulation (3, 28). There are neurons in the PVN that project to regions of the spinal cord where sympathetic preganglionic neurons are located and thereby can directly influence sympathetic activity (5, 26, 29). Other subgroups project to the pressor region of the rostral ventrolateral medulla and thereby indirectly influence sympathetic nerve activity (26). Additionally, there are subgroups that send collaterals to both autonomic regions and therefore are capable of both direct and indirect influences on sympathetic nerve activity (26).

Activation of the PVN can markedly alter blood pressure, sympathetic neural outflows, and hemodynamic sequelae. Excitation of the PVN with excitatory amino acids or by using bicuculline to inhibit GABA-mediated inhibition of the PVN, and thereby allowing activation of the PVN via endogenous excitatory inputs, can result in increases in renal sympathetic nerve activity and a reduction in renal blood flow (9, 12). The functional relevance of the effects on the renal sympathetic nerve activity mediated via the PVN includes the reflex response to volume expansion. Indeed, we and others (8, 17, 21) have provided strong evidence that the PVN is essential for the reflex reduction in renal sympathetic nerve activity that occurs when blood volume is elevated.

Recently, we (6) have also shown that elevations in body core temperature strongly activated neurons in the PVN that project to the spinal cord. An elevation in body core temperature induces changes in sympathetic nerve activity that result in a redistribution of blood flow from the viscera to the periphery to enable dissipation of heat from the body. These reflex responses include vasoconstriction of the renal vasculature, which would be consistent with the observation of the activation of the spinally projecting PVN neurons. Evidence that the PVN may contribute to the circulatory responses induced by a temperature challenge comes from several sources that include the observations that the PVN contains 1) thermosensitive neurons (11), and 2) neurons that project to the spinal cord and influence sympathetic nerve activity to important thermoregulatory effector organs such as the brown adipose tissue and the vasculature of the rat tail, salivary gland, as well as kidney and gut (10, 20, 22, 27). Furthermore, increases in body core temperature activate neurons within the PVN (2, 4, 7, 16, 19). However, whether the PVN contributes to the decrease in renal blood flow that accompanies an increase in body core temperature is not known. Thus the aim of the present study was to determine the effect of inhibition of neuronal activity, using the GABA agonist muscimol, within the PVN on the renal blood flow in response to an increased body core temperature and to investigate whether the effect was specific to the PVN.

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 and conform to the “Guiding Principles for Research Involving Animals and Human Beings” (1) 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) and were approved by the Royal Melbourne Institute of Technology University Animal Ethics Committee. Every attempt was made to reduce animal suffering and discomfort and to reduce the number of
animals needed to obtain reliable results. Male Sprague-Dawley rats (obtained from Monash University Animal Services, Victoria, Australia) weighing 300–350 g were housed in the Animal Facility (Royal Melbourne Institute of Technology University) with free access to rat chow and tap water at a room temperature of 22 ± 1°C with a 12:12-h light-dark regimen. Before the experimental day, animals were handled on a daily basis to minimize stress.

Surgical Preparations

All animals were anesthetized initially with Equithesin (pentobarbitone sodium 0.5 g; chloral hydrate 2.219 g/100 ml) mixture administered intraperitoneally (0.6 ml/100 g) to enable the cannulation of the femoral artery and vein. Anesthesia was then subsequently maintained with urethane (1–1.4 g/kg iv initially followed by supplemental doses of ~0.05 g/kg as required). The depth of anesthesia was maintained 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 Mac Lab data-acquisition System (AD Instruments, Colorado Springs, CO). Mean arterial pressure (MAP) and heart rate (HR) were determined electronically from the phasic arterial pressure. The femoral vein was cannulated for the intravenous delivery of supplemental doses of urethane.

Throughout the surgical procedures, the body core temperature was maintained at ~37.0°C (range 36.5–37.5°C) with a custom-made water-circulating blanket through which either cold water (4–8°C measured directly at source) or warm water (48–52°C measured directly at source) was pumped through at a rate of 16–26 ml/min.

A thermocouple was taped onto the base of the tail (MLT409, AD Instruments) to record tail skin temperature. A second thermocouple (RET-2 rectal probe for rats, PhysiTemp Instruments, Clifton, NJ) was inserted ~2–3 cm into the rectal cavity and connected to a Thermocouple Analog converter (MLT1101, AD Instruments) to enable the measurement of body core temperature.

Microinjection Into the Hypothalamic PVN

The animals were placed prone, and the head was mounted in a Kopf stereotaxic frame such that both the bregma and lambda were positioned on the same horizontal plane. A midline reference point was marked 2 mm rostral to the bregma. This was necessary because the 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. After the drilling procedure, the hole was covered with cotton wool soaked in normal saline to prevent drying of the exposed surface.

Microinjections were made bilaterally using a fine glass micropipette (with a tip diameter of 50–70 μm) into the PVN (stereotaxic coordinates: 1.5 mm caudal to the bregma, 0.5 mm lateral to the midline, and 7.5 mm ventral to the surface of the brain) or into the hypothalamus adjacent to the PVN (stereotaxic coordinates: 2.8 mm caudal to the bregma, 0.5 mm lateral to the midline, and 7.5 mm ventral to the brain surface).

Microinjections of muscimol (E; to inhibit neuronal function) were microinjected into the paraventricular nucleus (PVN), or muscimol was microinjected outside the PVN (□) in separate groups. There was no significant difference among the groups.

Table 1. Basal MAP, HR, renal blood flow, and renal vascular conductance before microinjections of muscimol to inhibit neuronal activity into the hypothalamus

<table>
<thead>
<tr>
<th></th>
<th>Muscimol In PNV (n = 6)</th>
<th>Muscimol Out PNV (n = 5)</th>
<th>Saline Control (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>95.7 ± 8.1</td>
<td>100.5 ± 3.1</td>
<td>98.0 ± 1.8</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>360 ± 14</td>
<td>358 ± 14</td>
<td>372 ± 21</td>
</tr>
<tr>
<td>Renal blood flow, ml/min</td>
<td>9.07 ± 0.56</td>
<td>9.53 ± 0.35</td>
<td>11.47 ± 1.86</td>
</tr>
<tr>
<td>Renal vascular conductance, ml·min⁻¹·mmHg⁻¹</td>
<td>0.099 ± 0.012</td>
<td>0.095 ± 0.006</td>
<td>0.118 ± 0.021</td>
</tr>
</tbody>
</table>

Values are means ± SE; 1 nmol/side of muscimol was microinjected. MAP, mean arterial pressure; HR, heart rate.

Fig. 1. Increase in tail skin temperature after an increase in body core temperature in anesthetized rats. Saline (■; control) or muscimol (□; to inhibit neuronal function) was microinjected into the paraventricular nucleus (PVN), or muscimol was microinjected outside the PVN (△) in separate groups. There was no significant difference among the groups.

Fig. 2. Responses of mean arterial pressure (MAP) and heart rate (HR) after an increase in body core temperature in anesthetized rats. Saline (■; control) or muscimol (□; to inhibit neuronal function) was microinjected into the PVN, or muscimol was microinjected outside the PVN (△) in separate groups. There was no significant difference among the groups.
flow probe, ~15–20 min were allowed to elapse to ensure that a steady basal renal blood flow was attained. Subsequently, the body core temperature of the rat was lowered to 36.0°C by passing cold water through the water-circulating blanket. This occurred within 5–10 min. Renal vascular conductance was calculated by dividing renal blood flow (ml/min) by the MAP.

**Experimental Protocol**

When body core temperature reached 36.0°C, muscimol (Sigma-Aldrich, St. Louis, MO; 1 nmol in 100 nl, n = 6) or saline vehicle (100 nl, n = 5) was microinjected into the PVN bilaterally. In a third group of rats, muscimol was microinjected in the hypothalamus outside the PVN (n = 5). After the completion of the microinjections, the body core temperature of the animal was gradually increased to 41°C. This was performed over ~90–120 min. In another group of animals, muscimol was injected into the PVN and the effects were followed over time. In this group of rats, core body core temperature was not altered and was maintained between 37.0–37.5°C. To mark the injection sites, rhodamine-tagged microspheres (LumaFluor, New York, NY) were included in the microinjected solution.

**Brain Histology**

At the completion of the experiment, rats were killed with an overdose of pentobarbitone sodium (300 mg/kg; Lethaborb, Virbac, Milperra, Australia). The brains were then carefully removed and fixed in 4% paraformaldehyde solution for ~7 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 gelatine-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 determine anatomical structure, and the injection sites were subsequently mapped in relation to the anatomical structure.

**Statistical Analysis**

The basal resting MAP, HR, renal blood flow, and renal vascular conductance levels before microinjections into the brain were compared among the three groups using one-way ANOVA. Effects on the MAP, HR, renal blood flow, and renal vascular conductance levels as body core temperature changed were compared among all groups using two-way ANOVA with repeated measures. When the overall difference was statistically significant, the following comparisons were made: 1) control vs. muscimol in the PVN, 2) muscimol in the PVN vs. muscimol out of the PVN, and 3) muscimol out of the PVN vs. saline in the PVN groups, using two-way ANOVA with repeated measures.

When there was a significant difference among groups, comparisons among the groups at the different temperature points were made using Student’s unpaired t-test and applying Bonferroni’s modification to compensate for multiple comparisons. All data are presented as means ± SE.

**RESULTS**

**Resting Levels**

Basal MAP, HR, renal blood flow, and renal vascular conductance levels before the microinjection of muscimol or saline were not significantly different among the three groups of animals (Table 1).

![Fig. 3](http://ajprenal.physiology.org/) Responses of renal blood flow and renal vascular conductance after an increase in body core temperature in anesthetized rats. Left: absolute levels; right: percent changes from resting levels. Saline (●; control) or muscimol (○; to inhibit neuronal function) was microinjected into the PVN, or muscimol was microinjected outside the PVN (▲) in separate groups. Microinjections of muscimol into the PVN abolished the normal reflex reduction in renal blood flow (and conductance). *P < 0.001 compared with comparable time point in control group.
Effect of Increased Body Core Temperature on Cardiovascular Variables

As body core temperature increased, tail skin temperature also increased in a linear manner in each group of animals. The change was similar in each group irrespective of whether muscimol or saline was microinjected into the PVN (Fig. 1). Further, there was no difference in the tail skin temperature response when muscimol was injected in or out of the PVN. This result suggests that blood flow to the tail increased during heating as expected.

Responses in rats microinjected with saline into the PVN. In animals in which saline was microinjected into the PVN, raising body core temperature from 36.0 to 41.0°C did not greatly influence MAP or HR (Fig. 2). However, renal blood flow was markedly altered. As body core temperature increased, there was a steady reduction in renal blood flow in the control animals so that by the end of the observation period renal blood flow had fallen by 70% from the resting level (Fig. 3). A similar response was observed in the renal vascular conductance response (Fig. 3).

Responses in rats microinjected with muscimol into the PVN. In rats administered muscimol into the PVN, there was no marked change in MAP or in HR. As body core temperature increased, the MAP and HR responses after muscimol was microinjected into the PVN were not significantly different compared with the control group (Fig. 2). Renal blood flow and vascular conductance, by contrast, were dramatically affected. When muscimol was microinjected into the PVN, the renal blood flow did not decrease as body core temperature was elevated (Fig. 3). This was significantly different from the control group \[ F(1,9) = 39.22; \ P < 0.0002 \]. By the end of the observation period, renal blood flow had fallen by only 8% from the resting level.
mol in the PVN had a similar effect on renal vascular conductance [Fig. 3; \( F(1,9) = 20.87; P < 0.002 \)]. Thus muscimol microinjected into the PVN abolished the reduction in renal blood flow and vascular conductance normally observed after an increase in body core temperature. When muscimol was microinjected into the PVN and the variables were monitored over time, while maintaining a normal body core temperature, there was no change in the renal blood flow (data not shown).

Responses in rats microinjected with muscimol out of the PVN. In the group of rats in which muscimol was microinjected outside the PVN, there was no marked effect on MAP and HR (Fig. 2). Indeed, the levels of these variables observed during the time the body core temperature was increasing were similar to those seen after microinjections made into the PVN. In response to an elevation in body core temperature, renal blood flow in this group of animals fell from 9.53 to 4.74 ml/min, a reduction of 50%, by the end of the observation period (Fig. 3). This response was significantly different from the response observed when muscimol was microinjected into the PVN \([F(1,9) = 21.66; P < 0.002]\). Furthermore, the response did not differ significantly from the control group. Renal vascular conductance was also reduced during the rise in body core temperature, and this also differed significantly from the group in which muscimol was microinjected into the PVN \([F(1,9) = 13.29; P < 0.005]\) but not the control group (Fig. 3). Thus muscimol microinjected into the PVN, but not out of the PVN, prevented the normal reduction in renal blood flow and vascular conductance induced by elevating body core temperature.

Histological Analysis of Microinjection Sites

The microinjection sites were examined histologically at the conclusion of the experiments. As shown in Fig. 4, microinjections within the PVN were distributed at different rostral-caudal levels of the PVN ranging from the mid to caudal levels of the PVN. The rostral-caudal distribution of the microinjection sites in which muscimol was centered into the PVN was similar to the distribution of saline microinjection sites. An example of a microinjection site observed under fluorescent lighting conditions is shown in Fig. 5. Microinjections out of the PVN were located immediately caudal to the PVN and were centered just dorsal of the dorsomedial hypothalamic nucleus (Fig. 6).

DISCUSSION

The present work highlights several important findings: 1) inhibition of neuronal function with muscimol in the hypothalamic PVN prevented the normal reduction in renal blood flow elicited by raising body core temperature, 2) this effect was specific to the PVN as microinjection of muscimol out of the PVN did not have such an effect, and 3) the PVN does not have a tonic influence on resting renal blood flow since inhibition of the PVN did not affect this variable. The results suggest that the hypothalamic PVN is a critical central nucleus regulating reflex renal vasoconstriction in response to elevations in body core temperature.

An increase in body core temperature elicits reflex responses designed to reduce heat production and to dissipate heat so as to restore the temperature back to normal. The cardiovascular responses that are evoked are important in these thermoregulatory adjustments. The major mechanism in the cardiovascular responses involves the redistribution of blood from the hot internal environment (i.e., the viscera) to regions where it can be in close contact with the cooler external environment. Thus...
Vasoconstriction of the blood vessels supplying visceral organs and vasodilation of the skin vasculature result in a reduction in blood flow to the visceral organs and a concomitant increase in skin blood flow. In the present study, we directly investigated one component of this reflex response, the reduction in renal blood flow. We observed a dramatic 70% reduction in renal blood flow when body core temperature was elevated. We also observed an increase in tail skin temperature, suggestive of an increased flow in the tail vasculature. The tail is a major heat dissipation site in the rat; increasing blood flow to the tail facilitates heat dissipation via the tail skin, which is reflected in the increased tail skin temperature. This response in the rodent is equivalent to vasodilation of the skin vasculature in humans.

These integrated autonomic reflex responses involve the central nervous system. The regions of the brain contributing to the cardiovascular responses must involve nuclei known to regulate sympathetic nerve activity; indeed, studies (2, 4, 6, 7, 16) utilizing the distribution of the protein Fos, a marker of activated neurons, have highlighted such brain regions. When body core temperature is elevated, several forebrain areas are activated, including the hypothalamic PVN. Such studies suggest that the PVN could contribute to thermoregulatory responses elicited by heating. The present work provides evidence for a physiologically relevant role of the PVN in the cardiovascular responses initiated by an elevated body core temperature. The present work, however, contrasts with a previous report (14) in which midbrain transections reduced the increase in splanchnic nerve activity but did not appear to affect the increase in renal sympathetic nerve activity elicited by heating. The report suggested the renal vasoconstriction was primarily driven by medullary brain regions. Interestingly, the same laboratory subsequently reported that lesions of the PVN markedly attenuated the renal vasoconstriction elicited by an increased body core temperature in the coronary artery ligation model of heart failure in the rat (13), suggesting that the hypothalamic PVN plays a critical role in the renal vasoconstriction in agreement with the present hypothesis. The reasons for the contrasting findings are not clear. Perhaps, the transections used in the previous work damaged descending pathways that contribute to opposing the role of the PVN?

The efferent pathways that could contribute to the involvement of PVN in the renal vasoconstriction include the spinal projecting neurons and/or those that project to the pressor region of the rostral ventrolateral medulla (18, 26, 30). These pathways provide the anatomical framework enabling the PVN to directly and indirectly influence sympathetic nerve activity. Previous studies (2, 4, 6, 7, 16) have shown that PVN neurons are activated by elevations in body core temperature and ~22% of those project to the spinal cord (6). We suggest, therefore, that an increase in body core temperature activates the PVN to elicit renal vasoconstriction, and we hypothesize that the spinal projecting neurons in the PVN contribute to the central pathways mediating the response. Supporting this view are findings that show that activation of the PVN elicits increases in renal sympathetic nerve activity with concomitant reductions in renal blood flow, which is dependent on intact renal nerves (9).

In the present study, we found that the microinjection of muscimol into areas outside the PVN did not significantly affect the reflex renal vasoconstriction elicited by elevating body core temperature. This suggests that the abolition of the renal vasoconstriction after muscimol into the PVN is specific and that the PVN is critical to the response. Our microinjections outside the PVN were made so that we avoided the forebrain rostral of the PVN, as this region is known to be important in thermoregulatory responses. We also avoided the dorsomedial hypothalamus, as this region may also contribute to thermoregulation.

We also observed that the microinjection of muscimol into the PVN had no effect on the basal level of blood pressure, heart rate, renal blood flow, and conductance nor did the presence of muscimol in the PVN influence those variables over time when body core temperature was kept within normal limits (Fig. 7).

This suggests that inhibiting neuronal function within the PVN is not critical for renal blood flow when body core temperature is normal but upon elevation of body core temperature, the PVN assumes an essential role.

Rat tail skin temperature increased linearly with the increase in body core temperature. The rat tail skin temperature is an indicator of tail blood flow, albeit a crude one (24). Nonetheless, we found that the increase in tail skin temperature induced by hyperthermia was virtually identical in each group of experimental animals. Thus microinjection of muscimol into the PVN, or adjacent areas, did not affect this response. The increase in blood flow to the tail in response to heating involves sympathoinhibition, mediated by the raphe pallidus (23, 25, 31). It is tempting to speculate that the PVN may not play a critical role in the central pathways mediating tail blood flow, in contrast to the effects on the renal vasculature; however, we recognize that this is a highly speculative possibility, but we believe it warrants investigation in the future.

In conclusion, we have found that an elevation in body core temperature induces a reduction in renal blood flow in which the PVN plays an essential role. We hypothesize that the PVN receives information from the preoptic area, the major temperature sensing region within the central nervous system, and that the efferent pathways contributing to the essential role of the PVN in the response may involve neurons that project to the intermediolateral cell column in the spinal cord, thereby directly influencing sympathetic nerve activity, and neurons that project to the pressor region of the rostral ventrolateral medulla, thereby indirectly influencing the sympathetic outflow innervating the kidney.

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