Role of the hypothalamic PVN in the reflex reduction in mesenteric blood flow elicited by hyperthermia

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Chen F, Dworak M, Wang Y, Cham JL, Badoer E. Role of the hypothalamic PVN in the reflex reduction in mesenteric blood flow elicited by hyperthermia. Am J Physiol Regul Integr Comp Physiol 295: R1874–R1881, 2008. First published October 22, 2008; doi:10.1152/ajpregu.90384.2008.—The hypothalamic paraventricular nucleus (PVN) is a key integrative center in the brain. Within the forebrain, the hypothalamic paraventricular nucleus (PVN) contains thermosensitive neurons (19); neurons in the PVN are labeled 1) the PVN contains thermosensitive neurons (19); 2) neurons in the PVN are activated by elevations in core body temperature (3, 7, 11, 16, 29); and 3) neurons in the PVN are labeled following injections of retrogradely transported transsynaptic viral neuroanatomical tracers into important thermoregulatory effector organs, such as the brown adipose tissue, the vasculature of the rat tail, and salivary gland, as well as kidney and gut (18, 36, 39, 42). This indicates that sympathetic nerves innervating those tissues are connected polysynaptically to the PVN and, thereby, can be influenced by the PVN. In support of a role of the PVN in temperature regulation, we have found that neurons in the PVN projecting to the spinal cord or to the pressor region of the RVLM are activated by an increase in the thoracolumbar spinal cord. Within the forebrain, the hypothalamic paraventricular nucleus (PVN) is a key integrative site involved in hormonal, endocrine, and neural control. The PVN is composed of different neuronal subgroups subserving different functions, including cardiovascular regulation. The PVN is one of the small number of premotor nuclei present in the brain (4, 47). 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 (8, 40, 48). In addition to the spinal projecting neurons present in the PVN, there are neuronal subgroups that project to the pressor region of the rostral ventrolateral medulla (RVLM), a critical region in the maintenance of normal sympathetic nerve activity (40). Thus, this connection enables the PVN to indirectly influence sympathetic nerve activity (40).

These neuroanatomical connections underlie the influence of the PVN on blood pressure, heart rate (HR), and blood flow mediated via the sympathetic nervous system (4, 13, 17, 20). Indeed the changes in blood flow obtained following stimulation of the PVN resemble the responses observed during the defense response (14). However, the role of the PVN in mediating the cardiovascular components of the defense reaction elicited by stress has been challenged by DiMicco and colleagues (12, 35, 45, 46) who argue that it is the neighboring dorsomedial hypothalamic nucleus that is the critical site for the cardiovascular responses initiated by stress. By contrast, there is strong evidence for a critical role of the PVN in the cardiovascular responses to various physiological stimuli, such as changes in blood volume and osmolality (5, 22, 23, 38, 44). Current evidence suggests a role of the PVN in the responses elicited by changes in body temperature. The evidence includes 1) the PVN contains thermosensitive neurons (19); 2) neurons in the PVN are activated by elevations in core body temperature (3, 7, 11, 16, 29); and 3) neurons in the PVN are labeled following injections of retrogradely transported transsynaptic viral neuroanatomical tracers into important thermoregulatory effector organs, such as the brown adipose tissue, the vasculature of the rat tail, and salivary gland, as well as kidney and gut (18, 36, 39, 42). This indicates that sympathetic nerves innervating those tissues are connected polysynaptically to the PVN and, thereby, can be influenced by the PVN. In support of a role of the PVN in temperature regulation, we have found that neurons in the PVN projecting to the spinal cord or to the pressor region of the RVLM are activated by an increase in

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core body temperature (9, 11). Thus, these central pathways may contribute to the central thermoregulatory pathways elicited by hyperthermia.

More recently we have provided strong evidence indicating that the PVN is essential for the reflex vasoconstriction of the renal vasculature induced by an elevation in core body temperature (10), indicating that the hypothalamic PVN is functionally important in the cardiovascular reflex responses of the renal vasculature elicited by hyperthermia. Whether the role of the PVN is confined to the renal vasculature or whether the PVN contributes to the decrease in mesenteric blood flow that also accompanies an increase in core body temperature is not known. Thus, the aim of the present study was to determine the effect of inhibition of neuronal activity within the PVN on the reflex reduction in mesenteric blood flow that occurs in response to an increased core body temperature. In the present study, we have used the GABAA receptor agonist, muscimol, to inhibit neuronal function within the PVN (15, 32, 38, 44).

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 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”), were approved by the 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, Melbourne, Australia) weighing 300–350 g were housed in the Animal Facility (RMIT University, Melbourne, 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 regimen.

Surgical Preparations

All animals were anesthetized initially with Equithesin [pentobarbital sodium (0.5 g)– chloral hydrate (2.219 g/100 ml) mixture (0.6 ml/100 g ip)] 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 iv initially followed by supplemental doses of ~0.05 g/kg, as required). The depth of anesthesia was monitored every 15 min and 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). Mean arterial pressure (MAP) and HR were determined electronically from the phasic arterial pressure.

Throughout the surgical procedures, the body temperature was maintained at ~37°C with a custom-made water-circulating blanket through which either cold water (4 to 8°C measured directly at source) or warm water (48 to 52°C measured directly at source) were pumped through at a rate of 16–26 ml/min. A small animal thermometer inserted into the colon was used to measure the core body temperature.

Microinjection into the Hypothalamic PVN

Each animal was placed prone and the head was mounted in a Stoelting 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.

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 bregma, 0.5 mm lateral to midline, and 7.5 mm ventral to the surface of the brain) or into the brain regions adjacent to the PVN. Microinjection volumes were 100 nl/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 were included in the microinjected solution (LumaFluor, Durham, NC).

Mesenteric Blood Flow Measurement

The mesenteric artery was identified using a retroperitoneal approach via an incision in the left flank. The mesenteric artery was dissected free and carefully cleared from the surrounding tissue. A flow probe (model IRB449; Transonic System, Ithaca, NY) was positioned around the mesenteric artery near its junction with the aorta and connected to a T206 small animal blood flow meter (Transonic System, Ithaca, NY) to enable monitoring of the mesenteric blood flow. Following the implantation of the flow probe, ~15–20 min (rest period) were allowed to elapse to ensure that a steady basal blood flow was attained. Mesenteric vascular conductance was calculated by dividing mesenteric blood flow (ml/min) by the mean arterial pressure (mmHg).

Experimental Protocol

After the rest period, the rat’s core body temperature was lowered to 36°C by passing cold water through the water-circulating blanket. This occurred within 5–10 min. Subsequently, muscimol (1 nmol in 100 nl/side, n = 7; Sigma-Aldrich, St. Louis, MO) was microinjected into the PVN bilaterally or into brain areas adjacent to the PVN (n = 5). In another group of rats, saline vehicle (100 nl/side, n = 7) was microinjected into the brain bilaterally. After the completion of the microinjections, the core body temperature of the animal was gradually increased to 41°C at a rate of ~0.1°C every 2 min. This was performed over ~100 min. In a separate group of animals (n = 5), muscimol was injected into the PVN and the effects were followed over time. In this group of rats, core body temperature was not altered and was maintained between 37.0 and 37.5°C for 100 min.

Brain Histology

At the completion of the experiment, rats were killed with an overdose of pentobarbital sodium (300 mg/kg, Lethabarb, Virbac, NSW, Australia). The brains were then carefully removed and fixed in 4% paraformaldehyde solution for ~6 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 cover slipping 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 levels of MAP, HR, mesenteric blood flow, and mesenteric vascular conductance were compared between the four groups using one-way ANOVA, followed by a comparison of the group means using the Bonferroni modification for multiple comparisons. The absolute levels and the changes in MAP, HR, mesenteric...
blood flow, and mesenteric vascular conductance were compared between the groups using a two-way ANOVA with repeated measures. When a significant difference in the overall analysis was detected, comparisons were subsequently made between the following groups using a two-way ANOVA with repeated measures: 1) saline in the PVN 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. When there was a significant difference between groups or temperature-group interaction, comparisons at the different temperature points between the two groups 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

Resting MAP, HR, and mesenteric vascular conductance in the muscimol-treated groups were not significantly different from the saline-treated groups (Table 1). Resting mesenteric blood flow in the group administered muscimol out of the PVN was significantly greater than the resting level seen in the group administered saline (P < 0.05) (Table 1).

Effect of Increased Core body temperature on Cardiovascular Variables

Responses in rats microinjected with saline into the PVN. In animals in which saline was microinjected into the PVN, raising core body temperature from 36°C to 41°C did not significantly alter MAP (Fig. 1). Simultaneously, HR increased over time as temperature increased so that by the end of the observation period HR had increased by over 83 ± 17 beats/min (P < 0.001, Fig. 1). In these control rats, in response to the hyperthermia, mesenteric blood flow fell by a maximum of 3.8 ± 0.7 ml/min or 21% (Fig. 2), while mesenteric vascular conductance fell by a maximum of 15 ± 5% (Fig. 2).

Responses in rats microinjected with muscimol into the PVN. When muscimol was microinjected bilaterally into the PVN, the MAP and HR responses elicited by the increase in core body temperature were not significantly different to the response observed in the control group (Fig. 1). In contrast, as shown in Fig. 2, mesenteric blood flow did not fall markedly in response to the increasing core body temperature in the group microinjected with muscimol into the PVN compared with the control rat group. Following muscimol microinjection, mesenteric blood flow fell no more than 0.8 ml/min or 3 ± 5% (Fig. 2), and this was significantly different from the response observed in the control group [F(1,12) = 5.557, P < 0.05]. Thus, muscimol into the PVN virtually abolished the reflex reduction in mesenteric blood flow elicited by an increase in core body temperature. Similarly, in this group, the mesenteric vascular conductance response was different from the control group and reached statistical significance by the end of the observation period (P < 0.05, compared with the control group) (Fig. 2).

Responses in rats microinjected with muscimol out of the PVN. When muscimol was microinjected into areas outside the PVN, the changes in MAP and HR were not significantly different from those observed in the group in which saline was microinjected or the group in which muscimol was microinjected into the PVN, except when core body temperature reached 41°C (Fig. 1). When muscimol was microinjected outside the PVN, mesenteric blood flow fell by 24 ± 3%, which was not significantly different from the control group (Fig. 2). However, this response was significantly different from that seen in the group administered muscimol into the PVN [F(1,10) = 18.68, P < 0.001] (Fig. 2). Mesenteric vascular conductance fell considerably following the temperature challenge by 32 ± 6% and was significantly different from the response observed when muscimol was microinjected into the PVN [F(1,10) = 7.503, P < 0.05], but was not different from the control group and reached statistical significance by the end of the observation period (P < 0.05, compared with the control group) (Fig. 2).

Table 1. Resting mean arterial pressure (MAP), heart rate (HR), mesenteric blood flow (MBF), and mesenteric vascular conductance (MVC) in rats administered saline (control) or muscimol (1 nmol/side) bilaterally into the brain

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Muscimol into PVN</th>
<th>Muscimol out of PVN</th>
<th>Muscimol into PVN (no change in body temperature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>106.5±4.5</td>
<td>91.3±5.9</td>
<td>109.9±3.5</td>
<td>103.0±2.9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>402±13</td>
<td>350±17</td>
<td>390±20</td>
<td>387±23</td>
</tr>
<tr>
<td>MBF, ml/min</td>
<td>17.8±0.9</td>
<td>17.7±1.2</td>
<td>24.2±0.8*</td>
<td>18.9±1.8</td>
</tr>
<tr>
<td>MVC ml·min⁻¹·mmHg⁻¹</td>
<td>0.17±0.01</td>
<td>0.21±0.01</td>
<td>0.22±0.01</td>
<td>0.18±0.01</td>
</tr>
</tbody>
</table>

Rats had their body core temperature either raised or unchanged. PVN, paraventricular nucleus. *P < 0.05 compared to control.
significantly different from the control group (Fig. 2). Thus, comparing the responses observed when muscimol was microinjected into the PVN with those after muscimol was microinjected outside the PVN, there were no significant differences in MAP and HR; however, mesenteric blood flow and mesenteric vascular conductance was significantly different between the two groups.

Effect of Muscimol into the PVN Alone (Without a Change in Core Body Temperature) on the Cardiovascular Variables

Monitoring the cardiovascular variables over time after muscimol was microinjected into the PVN with those after muscimol was microinjected outside the PVN, there were no significant differences in MAP and HR; however, mesenteric blood flow and mesenteric vascular conductance was significantly different between the two groups.

Neuroanatomical Location of Injection Sites

The microinjection sites of muscimol into the PVN are shown in Fig. 3. The microinjections were centered in the PVN and ranged from 1.4 to 1.8 mm caudal to bregma. The microinjection of saline into the brain covered a similar rostral-caudal distribution. In one of the seven animals, saline was microinjected immediately dorsal to the PVN (Fig. 3). Microinjections of muscimol out of the PVN were centered caudal and dorsal of the PVN as shown in Fig. 4.

DISCUSSION

The present study has found that microinjections of muscimol, to inhibit neuronal activity in the hypothalamic PVN, prevented the reduction in mesenteric blood flow that is reflexly induced by hyperthermia. This effect was not observed when microinjections of muscimol were made into areas outside the PVN. The results suggest, therefore, that the PVN is a critical brain nucleus mediating the mesenteric vascular component of the cardiovascular responses induced by hyperthermia.
Maintaining a normal body temperature is a fundamental homeostatic function. An important mechanism brought into play in response to disturbances in body temperature is the ability to shift blood between the warm internal and the cooler peripheral environments. For example, in response to hyperthermia, blood flow is redirected from the internal vasculature, such as the mesenteric bed to the cooler peripheral skin vasculature to facilitate the dissipation of heat (25, 30). In the present study, we found that an elevation in core body temperature elicited a fall in mesenteric blood flow by 21% in the control group, which is similar to that observed by others (30). However, when neuronal function within the PVN was inhibited by the GABA_A receptor agonist muscimol, mesenteric blood flow fell by only 3%. Thus, acute inhibition of the PVN virtually abolished the reflex reduction in mesenteric blood flow elicited by an increase in core body temperature. This suggests the hypothalamic PVN is a key central nucleus contributing to the redistribution of blood flow away from the viscera to the periphery to enable heat dissipation.

Changes in mesenteric blood flow are mediated by the autonomic nervous system (30); the present findings, therefore, suggest that the PVN is critical in mediating the increased sympathetic nerve activity responsible for the mesenteric vasoconstriction initiated by hyperthermia. This conclusion is somewhat at variance with that of Kenney et al. (26, 27), who have reported that the reflex increase in splanchnic sympathetic nerve activity induced by hyperthermia does not appear to involve suprabulbar areas in young and mature F344 rats, although the response is mediated by suprabulbar regions in senescent rats. Our rats would be considered young using the definition of Kenney et al. (i.e., <3 months of age). Apart from the differences in rat strain, the reasons for the differences in the conclusions from our present work and that of Kenney et al. are not clear, but it should be noted that their experiments used knife cuts that sever all information traveling through the affected regions.

Hyperthermia is known to activate neurons in the PVN (3, 7, 11). Recently, we have found that neurons in the PVN that project to the spinal cord or to the RVLM are among the populations of neurons that are activated when body temperature is increased (9, 11). Indeed, the proportion of spinal projecting neurons in the PVN that were activated by hyperthermia was > 20% and is higher than many stimuli examined to date. For example, stimuli that induce dramatic increases in Fos production in the PVN, such as severe hemorrhage, elicit more expression of Fos than hyperthermia, but the proportion...
of spinal projecting neurons activated is not as great (5, 6, 11, 22, 23). These pathways enable the PVN directly or indirectly (via a relay in the RVLM) to influence the sympathetic nervous system. Thus, we hypothesize that in response to hyperthermia, these pathways from the PVN are activated and play a critical cardiovascular role by contributing to the reflex increase in sympathetic nerve activity. Direct nerve recording measurements, however, will be required in the future to test this hypothesis.

Abolition of the mesenteric blood flow response following muscimol in the PVN was not observed when muscimol was microinjected into areas outside the PVN (see Fig. 4), suggesting that the inhibition of neuronal function within the PVN is critical to the full expression of the cardiovascular response to hyperthermia. The resting mesenteric blood flow was significantly higher in the group in which muscimol was microinjected outside the PVN (Table 1). Although this could be considered a potentially confounding factor, this difference in resting levels did not appear to affect the response to hyperthermia, since the reduction in mesenteric blood flow in response to the elevation in core body temperature in this group was similar to that observed in the control animals (Fig. 2). We did not microinject into the rostral forebrain, as areas there (such as the medial preoptic area) are known to be essential for thermoregulation (21, 37). We deliberately avoided the caudal hypothalamus, which has also been reported to be involved in thermoregulatory responses (31, 33). Our findings, however, do not mean that other areas in the forebrain do not play important roles in the cardiovascular responses initiated by changes in core body temperature.

Splanchnic circulation contains ~25% of the circulating blood volume; thus, a reduction in central blood volume could eventuate if splanchnic vasoconstriction did not occur during an increase in core body temperature. Indeed, in heat stroke there is a dangerous reduction in blood pressure that is mediated in part by a sudden and dramatic reduction in resistance in the splanchnic circulation (30). Although, little is known of the factors contributing to the selective reduction in splanchnic vasoconstriction that occurs in heat stroke, the present study suggests that any dysfunction of the role of the PVN in mediating the changes in mesenteric blood flow in response to hyperthermia may result in a reduced capability to vasoconstrict the splanchnic vasculature, potentially predisposing the individual to heat stroke.

In a recent study, we found that the PVN also plays a critical role in the reflex reduction in renal blood flow elicited by an increase in core body temperature (10). This effect was also specific to the PVN. Taken together with the present findings, the results suggest the PVN may be the key integrative site in the hypothalamus that mediates the renal and mesenteric blood flow changes that are important in mediating heat dissipation in response to hyperthermia. When muscimol was microinjected into the PVN and the cardiovascular variables were monitored over time (without a change in body temperature), there was a significant reduction in MAP. The effects of muscimol microinjected into the PVN on blood pressure have been reported in many studies, and the results have been considerably variable. In some reports, there is little change in MAP following muscimol into the PVN, while in others a considerable change in MAP has been reported (1, 15, 32, 34, 41, 43–46). Indeed, in our earlier study, we did not detect any effect of muscimol on blood pressure following its microinjection into the PVN (10). The reasons for the differences are unclear, but may include the specific sites of injection, depth of anesthesia, rat strains, etc.
Perspectives and Significance

Temperature regulation is a fundamental physiological function of the body, and one of the critical factors in temperature regulation is the ability to dissipate heat when core body temperature begins to rise. The present study suggests that mesenteric vasoconstriction that occurs with hyperthermia is mediated via the PVN. Our recent work suggests that the PVN is also critical for the renal vasoconstriction that occurs with hyperthermia. Thus, the PVN appears to be an essential central nucleus mediating cardiovascular responses elicited by hyperthermia, at least in the mesenteric and renal vascular beds. Given these two regions receive a considerable proportion of the cardiac output, vasoconstriction of these vascular beds as core body temperature rises is important in maintaining blood pressure in the face of vasodilation of the large skin vasculature. Thus, we suspect that any dysfunction within the PVN that prevents the normal vasoconstriction induced by the increased core body temperature could predispose an individual to heat stroke.

References


