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Activation of spinally projecting and nitrergic neurons in the PVN following heat exposure

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Cham, Joo Lee, Rudi Klein, Neil C. Owens, Michael Mathai, Michael McKinley, and Emilio Badoer. Activation of spinally projecting and nitrergic neurons in the PVN following heat exposure. Am J Physiol Regul Integr Comp Physiol 291: R91–R101, 2006; doi:10.1152/ajpregu.00675.2005.—The present study investigated the effect of acute thermal stimulation in conscious rats on the production of Fos, a marker of increased neuronal activity, in spinally projecting and nitrergic neurons in the hypothalamic paraventricular nucleus (PVN). The PVN contains a high concentration of nitrergic neurons, as well as neurons that project to the intermediolateral cell column (IML) of the spinal cord that can directly influence sympathetic nerve activity (SNA). During thermal stimulation, the PVN is activated, but it is unknown whether spinally projecting PVN neurons and the nitrergic neurons are involved. Compared with controls, rats exposed to an environmental temperature of 39°C for 1 h had a 10-fold increase in the number of cells producing Fos in the PVN (133 ± 23 vs. 1,336 ± 43, respectively, \( P < 0.0001 \)). Of the spinally projecting neurons in the PVN of heated rats (98 ± 10), over 20% expressed Fos. Additionally, of the nitrergic neurons (NADPH-diaphorase positive) located in the paravascular PVN (723 ± 17), ~40% also expressed Fos (\( P < 0.0001 \) compared with controls). Finally, there was a significant increase in the number of spinally projecting neurons in the PVN that were nitrergic and expressed Fos after heat exposure (12%) compared with controls (0.1%) (\( P < 0.0001 \)). These results suggest that spinally projecting and nitrergic neurons in the PVN may contribute to the central pathways activated by thermal stimulation.

Fos immunohistochemistry; spinally projecting.

EXPOSURE TO A HOT TEMPERATURE challenge elicits responses mediated in part by the autonomic nervous system to promote heat loss and maintain body fluid homeostasis. Such responses include sweating, an increase in heart rate, increased respiration rate, skin vasodilation, and visceral vasoconstriction in humans; increased salivary secretion in rodents; and tail vaso-dilation in rats (25, 27). The latter autonomic cardiovascular responses often involve changes in sympathetic nerve activity (SNA) and result in the redistribution of blood flow from the viscera to the skin. These changes are mediated by the central nervous system (CNS) (24, 25, 40, 43, 45, 52, 65).

It has been well established that the CNS is essential in the regulation of body temperature. There are several brain regions that are likely to contribute to the CNS pathways that mediate the thermoregulatory responses. Studies using the marker of neuronal activation, Fos, or electrophysiological recordings have shown that several forebrain areas are activated after the elevation in body temperature (2, 5–7, 21, 28, 34, 38, 39, 42, 52, 53). These forebrain areas include the preoptic area, anterior hypothalamus, and the paraventricular nucleus (PVN) of the hypothalamus. The preoptic area and anterior hypothalamus are well known key thermoregulatory sites within the brain; however, a role of the PVN in thermoregulation has largely been ignored. This is surprising given the circumstantial evidence, suggesting that it plays an important role in the cardiovascular changes elicited by the disturbances in body temperature. In particular, the PVN contains 1) thermosensitive neurons (23) 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 (BAT) and the vasculature of the rat tail, salivary gland, as well as kidney and gut (22, 41, 44). Furthermore, increases in body temperature activate neurons within the PVN (2, 7, 20, 28, 38).

The PVN consists of several subgroups of neurons, including those that can directly influence SNA via projections to the intermediolateral cell column (IML) of the thoracolumbar spinal cord, where the sympathetic preganglionic motor neurons are located (3, 26, 55). These projections are likely to mediate the effects of the PVN on SNA and may contribute to the cardiovascular changes induced by elevations in body temperature. However, this has not been examined to date. Therefore, the first aim of this study was to determine whether spinally projecting neurons in the PVN are activated by thermal stimulation in the conscious rat.

Studies investigating the nature of the neurochemical content of spinally projecting neurons in the PVN have revealed that numerous neurochemicals may be present in this population (10, 51). Of interest, there is a dense concentration, in the PVN, of neurons containing nitric oxide synthase (NOS), the enzyme responsible for the production of nitric oxide (NO). Current evidence suggests that NO in the central nervous system is important in the thermoregulatory pathways mediating heat dissipation (13–15, 17, 54, 56). For example, thermal stimulation induces enhanced secretion of saliva, which is spread on the fur as a means of heat defense in rats (11, 24). Inhibition of NO production reduces saliva production during body warming (11). Blockade of central NO production has
Animals and Housing

Male Sprague-Dawley rats (obtained from Monash University Animal Services, Melbourne, Victoria, Australia) weighing 200–250 g were housed in the Animal Facility (RMIT University, Melbourne, Victoria, Australia) where rat Chow and tap water were available ad libitum. All experimental protocols used in this study were performed in accordance with the Prevention of Cruelty to Animals Act 1986, 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 (Australia), and were approved by the RMIT University Animal Ethics Committee. Every attempt was made to minimize animal suffering, discomfort, and reduce the number of animals needed to obtain reliable results. Animals were handled on a daily basis before the experimental day to minimize stress.

All surgical procedures were performed under general anesthesia (pentobarbital sodium 60 mg/kg ip; Boehringer Ingelheim, North Ryde, New South Wales, Australia). buscopan compositum (0.03 ml/kg/h) during the heat exposure. This amount of fluid replacement was verified histologically at the end of the experiment. Only animals in which the injected tracer covered the IML were used in this study (see Fig. 1 for a representative example of an injection site). The injection site also spread to parts of the tractus rubrospinalis, tractus corticohalamicus lateralis, and the tractus corticohalamicus lateralis (Fig. 1).
Detection of Fos by Immunohistochemistry

Serial sections of the hypothalamus and spinal cord (40 μm) were cut on a cryostat and 1:3 sections were collected. To identify activated neurons, immunohistochemistry to detect Fos was performed on sections from the hypothalamus encompassing the PVN. The sections were incubated and processed using standard immunohistochemical procedures, as previously described (26). Briefly, the floating sections underwent washes in PBS before incubation with 10% normal horse serum (NHS) in PBS for 1 h at room temperature. This was followed by an overnight incubation with a primary antibody raised in rabbit against a conserved region of the human Fos (Ab5, 1:20,000; Oncogene Research Products, Cambridge, MA) containing 2% NHS (JRH Biosciences, Brooklyn, Victoria, Australia) and 0.3% Triton X-100 (Sigma Aldrich, Castle Hill, Sydney, Australia). After washes in PB, the sections were incubated for 1 h with biotinylated anti-rabbit secondary antibody (diluted to 1:600 in PB, Sigma-Aldrich) that was raised in goat. After washes in PB, the sections were incubated for 1 h using Extravidin (Sigma-Aldrich) diluted to 1:400 in PBS. Subsequently, the sections were then washed in Tris buffer (0.05 M, pH 7.6) and incubated for 10 min in 0.05% 3,3′-diaminobenzidine hydrochloride (Sigma-Aldrich) and 0.05 M Tris buffer. The reaction was initiated by the addition of 5 μl of 17.5% hydrogen peroxide (H2O2) (Biotech Pharm, Laverton North, Melbourne, Australia) and terminated by washes with fresh Tris buffer.

NADPH-Diaphorase Staining

NADPH-diaphorase (NADPH-d) staining was used as a marker of NOS in cells. Immediately after the immunohistochemistry procedure to detect Fos, the sections were incubated in a mixture of 2.5 mg Nitroblue Tetrazolium (Sigma-Aldrich), 10 mg β-NADPH (Sigma Aldrich) and 0.2% Triton X-100 in 10 ml of 0.05 M Tris buffer. The reaction was then allowed to proceed for 30–40 min at room temperature (23°C). The intensity of staining was examined before terminating the reaction with Tris buffer washes.

Sections were then mounted onto gelatin-subbed slides and dried before a brief wash in water, and redrying. The slides were then dipped in Xylene (Analar; Merck, Kilsyth, Victoria, Australia) before being coverslipped using DePex mounting medium (BDH, Poole, UK).

Analysis

Rats with intraspinal injections. Both Fos-positive cell nuclei and NADPH-d-positive neurons were identified under normal bright field illumination. Retrogradely labeled neurons were detected by using a fluorescent light source on a microscope fitted with a Rhodamine filter. Double-labeled neurons containing retrogradely transported tracer and either a Fos-positive nucleus or NADPH-d positive cytoplasm were detected by rapidly switching between the two light sources. Double-labeled neurons containing both a Fos-positive nucleus and NADPH-d-positive cytoplasm were detected under normal bright field illumination. Triple-labeled neurons were detected by rapidly switching between the bright field and fluorescent light sources.

On the side of the PVN ipsilateral to the injection site, labeled neurons were counted (using ×200 magnification) in 10 sections (processed in 5 lots of 2), which represented five different levels encompassing the rostral-caudal extent of the PVN. The data were expressed as the average number per section at each level. The average values for Fos-positive cell nuclei, NADPH-d positive neurons and retrogradely labeled neurons for each group of animals were then calculated and compared between the heated and control groups. The average numbers of double-labeled and triple-labeled neurons were also calculated.

Rats without intraspinal injections. In rats that were not injected intraspinally, Fos-positive nuclei were counted unilaterally in two sections of the PVN. The average values per section were determined and compared statistically. The approximate rostral caudal level of the PVN analyzed is shown in Fig. 5.

Statistical Analysis

The overall mean values in the heated and control groups of rats were compared using the unpaired Student’s t-test. For comparisons between the groups at the five different levels of the PVN, the means were compared using Student’s t-test and applying Bonferroni’s modification to compensate for multiple comparisons. The statistical software package used was GB-STAT version 7.0 (Dynamic Microsystems, Silver systems, Silver, MD), and the level of significance was set at P < 0.05.

Mapping

For illustration of the distribution of labeled neurons in the different levels of the PVN, maps were drawn from representative sections in each of the five rostral to caudal levels. The digital files were generated using the software package MD Plot (version 4.0) and a MD3 microscope digitizer stage (Minnesota Datametric Corporation, Shoreview, MN) attached to a Leica DMLB microscope.

Photomicroscopy

Images were acquired using a digital SPOT camera mounted on an Olympus BX60 microscope. The digital images obtained were imported into Adobe Photoshop (version 5.5, Adobe Systems, San Jose, CA) and only the contrast and brightness were modified for presentation purposes.

RESULTS

Effect of Heating on Fos Expression in the PVN

In the heated group of animals, the total number (unilateral) of Fos-positive cell nuclei (1,336 ± 43) in the PVN was significantly elevated by tenfold compared with the control group (133 ± 23; P < 0.0001) [which is similar to the numbers found in handled animals (31)]. This increase in the production of Fos occurred throughout the rostral-caudal extent of the PVN, with the maximum number of Fos-positive cell nuclei found predominantly in the middle to caudal levels of the PVN (Figs. 2 and 3). Fos-positive cell nuclei were present in both magnocellular and parvocellular regions, but only quantitated in the paraventricular region of the PVN. Within the paraventricular PVN, the Fos-positive cells were distributed in the dorsal, medial, and lateral paraventricular subnuclei of the PVN (Figs. 3 and 4). In the control group of animals, very few Fos-positive cell nuclei were observed, and these were distributed evenly throughout the rostral-caudal extent of the PVN (Figs. 2 and 4).

After the 1 h of heating, the average body weights of the rats in the heated group fell significantly to 313 ± 11 g from 324 ± 11 g before the heating (P < 0.001). In the control animals, the body weights did not change significantly during their time in the temperature chamber. The body weights of the rats in the control group before entering the chamber averaged 320 ± 11 g, which was not significantly different from the respective weight in the heated group.

Because the thermal stimulation resulted in a reduction in body weight of ~3.5%, which reflects a loss in body fluid (and a subsequent increase in osmolality), we also investigated the effect of restoring the body fluid to preheating levels by infusing hypotonic saline intravenously during the heat exposure. In the rats in which fluid loss was replaced, the number of...
Fos-positive cell nuclei in the PVN averaged 194 ± 17 per section (counted in the plane shown in Fig. 5), where maximal Fos-positive cell nuclei were observed in the heated rats (see Fig. 2). This was not significantly different from controls, in which 192 ± 14 Fos-positive cell nuclei per section were counted. The body weights of the rats in which fluid was infused did not change significantly during heating (average change = −1.6 ± 0.7 g from 293 ± 23 g before heating), but fell, as expected, in the control animals by 12 ± 3 g from 329 ± 19 g. Plasma osmolality in the hypotonic saline infused group did not increase (312 ± 6 mosmol/kg H2O vs. 302 ± 4 mosmol/kg H2O pre- and postheating, respectively).

**Distribution of Spinally Projecting Neurons in the PVN**

Spinally projecting neurons were observed at all rostral-caudal levels of the paraventricular PVN (Fig. 3). The maximum number was found in the middle to caudal levels, which was similar to the distribution of Fos-positive cell nuclei. The average number of neurons per animal in the paravascular PVN (unilateral) that projected to the spinal cord was similar in the heated (98 ± 10) and control groups (104 ± 16) (Fig. 2).

**Distribution of Spinally Projecting Neurons Containing Fos**

After the exposure of the animals to the hot environment, there was a significant increase in the number of spina-projecting neurons that contained a Fos-positive nucleus (P < 0.0001, compared with the control group). These double-labeled neurons represented 22 ± 2% of all the spina-projecting neurons in the PVN and were found in the dorsal, medial, and lateral paravascular PVN, primarily in the middle to caudal levels of the PVN (Figs. 2 and 3). In the control group of animals, there were very few spina-projecting neurons that contained a Fos-positive nucleus. These double-labeled neurons represented only 2% of all the spina-projecting neurons counted in the PVN (Fig. 2).

**Distribution of Neurons That Contained NADPH-d**

NADPH-d positive neurons were observed throughout the rostral-caudal extent of the PVN (Figs. 6 and 7). The distribution profiles of NADPH-d-positive neurons in both the control and heated group of animals were similar (Fig. 8). The total number of NADPH-d positive neurons in the heated group averaged 722 ± 17, which was significantly greater than in the control group (542 ± 26) (P < 0.0001 between groups). This increase was predominantly due to the greater number of NADPH-d positive neurons found in the middle to caudal levels of the PVN (Fig. 6).

**Distribution of Spinally Projecting Neurons Containing NADPH-d**

After the exposure of the animals to the heated environment, the number of spina-projecting neurons containing NADPH-d represented 21 ± 2% of all the spina-projecting neurons in the PVN. These double-labeled neurons were found primarily in the middle to caudal levels of the PVN (Figs. 6–8). In the control group, there was a similar distribution of spina-projecting neurons containing NADPH-d (Fig. 6). The numbers of double-labeled neurons in the control group were not significantly different to the heated group (Fig. 6).

**Distribution of Neurons Containing NADPH-d and Fos**

In the heated group, the average number of neurons in each animal, containing both NADPH-d and Fos in the PVN (282 ± 10) was significantly elevated by 15-fold, compared with the control group (P < 0.0001) (Fig. 6). These double-labeled neurons represented 20 ± 1% of all the Fos-positive cells, and...
of the NADPH-d positive neurons, counted in the PVN of the heated group. This increase occurred throughout the rostral-caudal extent of the PVN, with the maximum number found predominantly in the middle to caudal levels of the PVN (Figs. 6 and 7). In the control group of animals, there was on average a total of only $19 \pm 6$ NADPH-d positive neurons that also exhibited a Fos-positive nucleus (Fig. 6).

**Distribution of Triple-Labeled Neurons**

In the heated group of animals, the total number of neurons containing all three labels was significantly elevated compared with the control group ($P < 0.0001$). These triple-labeled neurons represented $12 \pm 1\%$ of all the spinally projecting neurons in the PVN. This increase occurred throughout the
rostral-caudal extent of the PVN, with the maximum number found predominantly in the middle to caudal levels of the PVN (Figs. 6 and 7). An example of a triple-labeled neuron is shown in Fig. 8. In the control group of animals, triple-labeled neurons were scarcely present in the PVN. On average, these neurons represented 0.1 ± 0.1% of all the spinally projecting neurons counted in the PVN (Figs. 6 and 7).

**Behavioral Responses to Heat Exposure**

In general, all animals placed in the temperature chamber usually explored the surroundings for 5 to 10 min. Subsequently, rats in the control group usually curled up and performed very little or no activity. In contrast, animals in the heated group continued to explore actively and also exhibit characteristic behaviors that included tunneling into the bedding and spreading saliva on their fur, and in some cases, scrotum licking, to increase their evaporative heat loss. A wet chin was commonly observed by the end of the 1-h exposure in the temperature chamber.

**DISCUSSION**

In the current study, we have made several novel observations. We found that following exposure of conscious animals to a heated environment of 39°C, there was 1) a significant increase in the number of spinally projecting neurons in the PVN that exhibited Fos, 2) a significant increase in the number of PVN neurons that exhibited Fos and were NADPH-d positive, and 3) a significant increase in the number of PVN neurons that contained all three markers (i.e., were Fos-positive, NADPH-d positive, and spinally projecting). Indeed, our data suggest that an environmental temperature of 39°C is a powerful stimulus that activates nitrergic neurons in the PVN, as well as spinally projecting neurons in the PVN.

The present study, for the first time, also provides a detailed quantification of Fos-positive cell nuclei and highlights their rostral-caudal distribution within the parvocellular PVN after heat exposure. In conscious rats exposed to the hot environment, there was a marked increase in the number of Fos-positive cell nuclei observed in all subdivisions of the PVN,
and the numbers peaked in the middle to caudal levels of the parvocellular PVN. The increased production of Fos after heat exposure is in agreement with earlier studies (2, 7, 20, 28, 38). In contrast, there have been previous reports that have not detected an increase in Fos production in the PVN after an elevated body temperature (28, 48). The contradictory nature of the observations has most likely contributed to the lack of studies investigating the role of the PVN in thermoregulation. The contrasting findings may be reconciled, however, by the differences in species used, the duration of heat exposure, and the degree to which body temperature was elevated (2, 7, 20, 38, 48). In general, studies that have elicited marked activation of the parvocellular PVN have used a higher environmental temperature (7, 20, 38). We have previously found that the stimulus used in the present study elevates body temperature by an average of 3.5°C (37).

One of the most important findings of the present study is that PVN neurons projecting to the spinal cord are activated after exposure to a hot environment and an increase in core body temperature. The proportion of spinally projecting neurons that were activated following heat exposure was 21%, which makes an elevated body temperature the most effective stimulus to activate this pathway observed to date. Previous stimuli that have elicited marked activation of PVN neurons include hemorrhage, dehydration, increased osmolality, and hypotension (4, 26, 49, 58). These stimuli elicited as much if not more Fos production in the PVN but were not as effective as the heating stimulus used in the present study in activating spinally projecting neurons.

In the present work, we used an ambient temperature of 39°C to elevate body temperature. Under these conditions, plasma osmolality is elevated and body fluid is reduced (37). Thus thermal stimulation involves the integration of different afferent inputs resulting in the complex behavioral, neural, and hormonal changes that characterize the response to heat defense. Because an elevation in plasma osmolality is known to activate neurons in the PVN (26, 58), one could argue that this stimulus is responsible for the activation of the spinally projecting neurons in the PVN. However, we have previously shown that an intravenous infusion of hypertonic saline does not activate spinally projecting neurons in the PVN (26). Thus it is unlikely that the increase in osmolality that accompanied thermal stimulation in the present study could account for the increased activation of spinally projecting PVN neurons.

The fluid reduction accompanying thermal stimulation may result in a reduction in blood volume. We have previously found that hemorrhage activated spinally projecting neurons in the PVN. This suggests that the fluid loss occurring during thermal stimulation could contribute to the activation of spinally projecting neurons observed in the present study. It is unlikely, however, that this stimulus is solely responsible for activating those neurons because we have observed that although severe hemorrhage can elicit more Fos production in the PVN, it is not as effective as thermal stimulation in activating spinally projecting neurons (4, 26, 49, 58). Furthermore, we have observed in the present study that the administration of hypotonic saline during the heat exposure to counteract the elevation in plasma osmolality and the reduction in
extracellular volume, had no effect on the number of Fos-positive neurons in the PVN, suggesting that under the conditions of heating used in the present study, fluid loss and hypertonicity were not the major contributors to Fos production. Taken together, we hypothesize that the activated spinally projecting PVN neurons are important in the central pathways mediating the responses initiated by thermal stimulation, most likely in response to the elevation in body temperature, although we cannot exclude a contribution to the response by a reduction in blood volume.

In the present study, we found a significant increase in the number of activated neurons in the PVN that were NADPH-d positive following exposure to a hot environment. This could occur as a result of an increase in body temperature, a decreased blood volume, an increased osmolality or a combination of all these inputs. Previous studies have found that 24–48 h of water deprivation, in which body weight fell by ~10% (after 24-h deprivation), resulted in an increase in the expression of nitric oxide synthase (the enzyme responsible for the production of nitric oxide) within the PVN (15, 16, 61). However, exposing the animals to a warm external environment of 34°C for 2 days did not have such an effect (16). These results suggest that a warm external environment may not be sufficient to increase the NADPH-d positive neurons in the PVN. Thus the reduction in blood fluid and/or the accompanying increase in osmolality after exposure to a hot external environment for 1 h could contribute to the increase in NADPH-d positive neurons in the PVN, observed in the present study. It needs to be kept in mind, however, that since an environmental temperature of 34°C did not increase NADPH-d expression in the PVN, it does not imply that a further increase in temperature per se could not contribute to the observations we have made. This may be particularly pertinent to the activation of the spinally projecting nitrergic neurons as discussed later.

In the present study, we found a significant increase in the number of activated nitrergic neurons in the PVN after exposure to the hot environment. Indeed, almost 40% of the nitrergic neurons in the hypothalamic PVN were activated. These findings suggest that NO-producing neurons in the hypothalamic PVN are activated after heating. This is in agreement with the view that NO in the CNS is important in heat dissipation (15, 54). Thus the PVN may be a potential site of action within the CNS through which NO may influence the redistribution of blood flow to facilitate heat dissipation. Accordingly, the PVN may be a site in the brain in which NOS inhibitors, by attenuating this action, elicit increases in body temperature (15, 35, 36). Further investigations are needed, however, to address these issues. We also note that a reduction
in blood volume and an increase in osmolality may also contribute to the observations.

Another significant finding of the present study is the increase in the number of activated PVN neurons projecting to the spinal cord, which were also NADPH-d positive (12% of the spinally projecting neurons). We hypothesize that these neurons are activated by an elevated body temperature. This view is based on our earlier discussion in which we highlight that 1) spinally projecting neurons are not activated by an increase in plasma osmolality, and 2) intravenous fluid replacement during the thermal stimulation did not affect the number of activated neurons in the PVN. We concede, however, that we cannot entirely exclude hypovolemia contributing to the activation of nitrergic, spinally projecting neurons in the PVN.

The precise function of NO in the activated PVN neurons projecting to the spinal cord is unknown. One possibility may be that NO inhibits surrounding neurons, as it diffuses easily through cell membranes, while its action within the activated spinally projecting neurons is to dampen the excitatory drive that is being experienced by those neurons. Although highly speculative, this could be a way to elicit integrated responses, so that during thermal stimulation, PVN neurons involved in vasoconstriction in the viscera are activated while other neurons are inhibited. For example, PVN neurons influencing the sympathetic nerves to the heart could be activated while those influencing the cardiac vagal neurons could be inhibited; the PVN is well known to innervate the sites in the CNS containing the sympathetic preganglionic motor neurons and the cardiac vagal motor neurons projecting to the heart (32). It is also of interest that there is a dense innervation of sympathetic neurons projecting to BAT originating in the PVN, identified by studies using transneuronal retrograde labeling with pseudorabies virus (9, 41, 44). These PVN neurons should be inhibited during heat exposure, and it could be argued that NO within the PVN plays some role in this function. This needs further investigation.

Because ~12% of the spinally projecting neurons in the PVN that were activated by thermal stimulation also contained NADPH-d. Eighty-eight percent of the spinally projecting neurons activated must contain some other neurochemical markers. These may include vasopressin and oxytocin, as these are present in a significant proportion of spinally projecting neurons in the PVN (10, 51). Indeed, up to 40% of those neurons have been reported to contain mRNA for each of those neurochemicals (18, 19). Spinally projecting neurons in the PVN have also been reported to contain various other neurochemicals, including enkephalin, dynorphin, and CRF (10, 19, 50). ANG II might also be a possibility as inhibition of its actions in the central nervous system markedly attenuated the normal increase in mean arterial pressure, heart rate, and sympathetic nerve activity to the gut observed after exposure to heat (29).

Perspectives

The hypothalamus, particularly the rostral part, has long been recognized as a critical site in thermoregulation. The hypothalamic PVN, however, has been largely ignored, despite anatomical and electrophysiological evidence, suggesting that it could contribute to the central pathways mediating thermoregulation. The present study provides evidence for a potential role of the spinally projecting neurons of the PVN in the response to thermal stimulation. The PVN is known to contribute to the anatomical framework that influences the autonomic nervous system, which innervates important thermoregulatory organs such as the tail, salivary glands, BAT, as well as the kidney, gut, and heart (9, 22, 41, 44) Neurons in the PVN that project to the spinal cord contribute to this anatomical framework. We hypothesize that spinally projecting neurons in the PVN, by influencing sympathetic nerve activity, contribute to the cardiovascular responses elicited by exposure to a high environmental temperature. These responses could include an increased heart rate and visceral vasoconstriction that aids in shunting blood from the viscera to the skin. This suggestion is in agreement with studies showing that stimulation of the PVN can induce increases in heart rate and sympathetic nerve activity to the viscera (12).

The role of NO in thermoregulation is emerging as a major focus of investigation. The consensus view, at present, is that NO within the central nervous system is critical in heat dissipation (15, 54). One of the highest concentrations of NOS-containing neurons within the hypothalamus occurs in the PVN, and the present findings highlight that ~40% of the nitrergic neurons in the parovcellular PVN are activated by thermal stimulation, and a subpopulation of those neurons project to the spinal cord, suggesting an influence on sympathetic nerve activity that may contribute to the cardiovascular effects elicited by thermal stimulation. However, the ability of NO to diffuse easily across cell membranes suggests its production within the PVN may have more widespread actions during thermal stimulation.

There is increasing evidence suggesting that NO within the PVN may be important in pathological conditions like heart failure. In this condition, there is a reduced level of NOS in the PVN (47), and this is believed to contribute to the autonomic dysfunction that is a characteristic of this debilitating condition. It is of interest to note there have been positive clinical outcomes for heart failure patients treated with thermal therapy (60). On the basis of the present findings, together with those from Patel’s laboratory (47, 62–64), it is tempting to speculate that NO production within the PVN is enhanced by thermal therapy, and this may contribute to the positive influence of this treatment on the symptoms of heart failure.

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