Acute inhibition of the hypothalamic paraventricular nucleus decreases renal sympathetic nerve activity and arterial blood pressure in water-deprived rats

Sean D. Stocker, Kimberly J. Keith, and Glenn M. Toney
Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78239

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Stocker, Sean D., Kimberly J. Keith, and Glenn M. Toney. Acute inhibition of the hypothalamic paraventricular nucleus decreases renal sympathetic nerve activity and arterial blood pressure in water-deprived rats. Am J Physiol Regul Integr Comp Physiol 286: R719–R725, 2004. First published December 11, 2003; 10.1152/ajpregu.00494.2003.—The present study was performed to determine whether sympathetic outflow and arterial blood pressure in water-deprived rats are dependent on the ongoing neuronal activity of the hypothalamic paraventricular nucleus (PVN). Renal sympathetic nerve activity (RSNA), mean arterial blood pressure (MAP), and heart rate were recorded in urethane-α-chloralose-anesthetized rats that were deprived of water but not food for 48 h before experiments. Acute inhibition of the PVN by bilateral microinjection of the GABAA agonist muscimol (100 pmol/side) significantly decreased RSNA in water-deprived rats (−26.7 ± 4.7%, n = 7) but was without effect in control rats (1.3 ± 6.3%, n = 7). Similarly, injection of muscimol produced a greater decrease in MAP in water-deprived rats than in control rats (−46 ± 3 vs. −16 ± 3 mmHg, respectively), although baseline MAP was not different between groups (105 ± 4 vs. 107 ± 4 mmHg, respectively). Neither bilateral microinjection of isotonic saline vehicle (100 nl/side) into the PVN nor muscimol (100 pmol/side) outside the PVN altered RSNA or MAP in either group. In addition, ganglionic blockade with hexamethonium (30 mg/kg iv) significantly decreased MAP in both groups; however, the decrease in MAP was significantly greater in water-deprived rats than in control rats (62 ± 2 vs. 48 ± 2 mmHg, respectively). Collectively, these findings suggest that sympathetic outflow contributes more to the maintenance of blood pressure in the water-deprived rat, and this depends, at least partly, on the ongoing activity of PVN neurons.

WATER DEPRIVATION DEPLETES the intracellular and extracellular compartments of water and consequently increases plasma osmolality (P(osmol)) and decreases intravascular volume. Despite the contraction of blood volume, arterial blood pressure (ABP) is maintained within a normal range by both hormonal and neural factors. Indeed, studies have reported increased activity of the peripheral renin-angiotensin system (31, 43, 49), increased circulating levels of vasopressin (5, 46, 49), and increased sympathoadrenal activity (14, 40, 41, 49) in water-deprived rats. The latter is reflected by the increased levels of circulating catecholamines (49), tachycardia (14, 40, 41), and elevated sympathetic nerve activity when expressed as a percentage of the baroreflex maximum (40). Collectively, these observations suggest that sympathetic outflow is elevated in water-deprived rats; however, the neural circuits that underlie this response are not known.

Neuroanatomical and functional studies suggest the hypothalamic paraventricular nucleus (PVN) plays an important role in the regulation of ABP and sympathetic outflow. The PVN receives input from neuronal populations within the lamina terminalis that detect changes in P(osmol) and circulating ANG II levels as well as neurons in the hindbrain that respond to changes in ABP and/or blood volume (38, 48, 50). In turn, parvocellular PVN neurons project to dorsomedial and ventrolateral medulla and intermediolateral cell column of the spinal cord to influence sympathetic outflow (35–37, 42, 48, 50). In this regard, it has been recently shown that blockade of ANG II type 1 receptors in the PVN attenuates both thepressor and sympathoexcitatory responses to acute central hyperosmotic stimulation (7). Similarly, several lines of evidence suggest that peripheral ANG II acting within the subfornical organ activates a descending pathway via the PVN to increase ABP and presumably sympathetic outflow (13, 15, 16). Finally, the activity of spinally projecting PVN neurons is reduced by acute volume expansion (29) and the renal vasodilatory and sympathoinhibitory responses during acute volume expansion or atrial balloon inflation depend on the integrity of the PVN (17, 30, 52). Collectively, these observations suggest that the PVN is one site in the central nervous system that plays an important role in the regulation of ABP and sympathetic outflow during changes in plasma osmolality, circulating ANG II levels, and/or blood volume.

The present study sought to determine whether the activity of PVN neurons contributes to sympathetic outflow and ABP in water-deprived rats. To test this hypothesis, rats were water deprived for 48 h, and the PVN was inhibited by bilateral microinjection of the GABAA agonist muscimol. If sympathetic outflow and ABP in water-deprived rats depends, in part, on the ongoing activity of PVN neurons, then bilateral inhibition of the PVN should decrease renal sympathetic nerve activity (RSNA) and ABP to a greater extent in water-deprived rats than in control rats. In addition, ganglionic blockade with hexamethonium was performed to confirm whether the maintenance of ABP is more dependent on sympathetic outflow in water-deprived rats than in control rats.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (Charles River Laboratories) weighing 250–375 g were housed in a temperature-controlled room (22–23°C) with a 14:10-h light-dark cycle (lights on at 7 AM). Tap water and laboratory chow (Harlan Teklad LM-485, 0.3% NaCl) were available ad libitum except where noted.

Experimental procedures. Rats were assigned randomly to one of two experimental groups. One group was deprived of water but not...
food for 48 h, whereas control rats had continuous access to both food and water before experiments.

On the day of experiments, water-deprived and control rats were anesthetized with a mixture of α-chloralose (80 mg/kg) and urethane (800 mg/kg) given intraperitoneally. Catheters were implanted in the femoral artery and vein (PE-50 tubing) for recording ABP and administration of drugs, respectively. After tracheal cannulation, the rats were paralyzed with gallamine triethiodide (25 mg/kg iv) and artificially ventilated with oxygen-enriched room air. End tidal Pco2 was maintained between 4.5–5.5% by adjusting ventilation rate (70–90 breaths/min) and/or tidal volume (2–3 ml). Body temperature was maintained at 37 ± 1°C with a water-circulating pad. An adequate depth of anesthesia was assessed by absence of a withdrawal reflex or a pressor response to foot pinch. Supplemental doses of anesthetic (10% of initial dose) were given as necessary. All experimental and surgical procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Through a retroperitoneal incision, a left renal sympathetic nerve was isolated and placed onto a stainless steel wire electrode (A-M systems, 0.005-in. OD). The renal nerve and electrode were covered with a silicon-based impression material (Super-Dent Light, Carlisle Laboratories). Nerve signals were obtained using a high-impedance probe connected to an AC amplifier (Tucker-Davis) with high-pass filters (band pass: 30–3,000 Hz) and a 60-Hz notch filter. The signal was amplified (10,000–20,000), rectified, integrated (30 ms time constant), and digitized at a frequency of 1,000 Hz using a 1401plus analog-to-digital converter and Spike 2 software (Cambridge Electronic Design). At the end of each experiment, background noise was determined by the average value of integrated voltage over 5 min after a bolus injection of hexamethonium (30 mg/kg iv) to block ganglionic transmission.

Rats were placed into a stereotaxic head frame, and the skull was leveled between bregma and lambda. A small craniotomy was performed to remove bone overlying the cortex to allow a glass micropipette to be lowered into the PVN. Baseline variables were allowed to stabilize for at least 1 h before any microinjection was performed. Then a blood sample (0.4 ml) was collected from the arterial line into microcentrifuge tubes containing heparin (6 U) for determination of Pmean and hematocrit. The blood sample was replaced by an equal volume of isotonic saline. Variables were allowed to stabilize for an additional 15 min before microinjections were performed.

Microinjections were performed bilaterally into the PVN using a pneumatic picopump (WPI) at the following stereotaxic coordinates: 1.6–2.0 mm caudal to bregma, 0.5–0.7 mm lateral to midline, and 7.8 mm ventral to the dura. Injections were made on one side, and the pipette was removed and then lowered into the contralateral PVN. The two injections were ∼2–3 min apart. Muscimol (100 pmol dissolved in isotonic saline) or isotonic saline was microinjected over 30 s in volumes of 100 nl/side with the use of single-barrel glass micropipettes with tips of 30–50 μm (OD). Each rat received both muscimol and isotonic saline in a randomized order separated by at least 2 h with four rats receiving isotonic saline first. There was no difference in any variable with regard to the order of microinjections within a group. In addition, microinjections of muscimol (100 pmol) were performed in euhydrated and dehydrated rats lateral to the PVN at the following coordinates: 1.6–2.0 mm caudal to bregma, 2.0–2.2 mm lateral to the midline, and 7.8 mm ventral to the dura. Baseline variables were allowed to stabilize for at least 20 min between microinjections.

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**Ganglionic blockade.** To determine the contribution of the sympathetic nervous system to the maintenance of ABP, additional experiments were performed in a separate group of water-deprived and control rats. Rats were anesthetized, artificially ventilated, and prepared for recording of ABP and RSNA as described above. At least 1 h after all surgical procedures were completed, baseline ABP and RSNA were recorded for a minimum of 15 min. Then, water-deprived and control rats were given a bolus injection of hexamethonium (30 mg/kg iv). Again, blood samples (0.4 ml) were collected from the arterial line for determination of Pmean and hematocrit as described above ∼15 min before administration of hexamethonium.

**Determination of Pmean and hematocrit.** Pmean was determined from the average of duplicate plasma samples using a vapor pressure osmometer (model 5100C, Wescor, Logan, UT). A single hematocrit tube was filled with arterial blood and centrifuged, and hematocrit was determined with a Lancer microhematocrit tube reader (St. Louis, MO).

**Data analysis.** All values of RSNA, MAP, and HR were measured as a 60-s average of each variable. RSNA responses are expressed as a percent change from an average baseline value, which was calculated from values averaged at −5, −2.5, and −1 min before the first microinjection. All RSNA values were analyzed by subtracting background noise taken from a 5-min average after hexamethonium injection. The peak change in RSNA, MAP, and HR was averaged from a 60-s segment within 60 min from the first microinjection. In addition, the latencies of the peak changes were calculated from the start of the first microinjection.

The effects of bilateral microinjection of muscimol and isotonic saline into the PVN on RSNA, MAP, and HR were analyzed by a one-way ANOVA (Systat 10.2, Systat Software). Response latencies in water-deprived and control rats were analyzed by a two-way ANOVA. When significant F values were obtained, an independent t-test or paired t-test with layered Bonferroni corrections was performed to compare differences between the response to PVN microinjection of muscimol and isotonic saline in water-deprived and control rats. For ganglionic blockade experiments, MAP values at baseline and after hexamethonium as well as the peak change in MAP observed in water-deprived and control rats were compared by a directional t-test. For all experiments, Pmean and hematocrit were analyzed by independent directional t-tests. All values are expressed as means ± SE, except for Fig. 1. A P value <0.05 was considered statistically significant for all comparisons.

**RESULTS**

**Effect of bilateral microinjection of muscimol into the PVN on RSNA, MAP, and HR in water-deprived and control rats.** If renal sympathetic outflow during water deprivation is partly dependent on the activity of PVN neurons, inhibition of the PVN by bilateral microinjection of muscimol should decrease RSNA and ABP to a greater degree in water-deprived rats than in control animals. Figure 1 shows examples of the response observed in both groups. After microinjection of muscimol, ABP and RSNA decreased promptly in the water-deprived rat, whereas no obvious change occurred in the control animal (Fig. 1). Group data for these responses are summarized in Fig. 2. RSNA decreased significantly after injection of muscimol in water-deprived rats but did not change in control rats. Moreover, the peak decrease in RSNA was significantly greater in water-deprived rats than in control rats (Fig. 2). In addition, microinjection of muscimol significantly decreased MAP in both groups; however, the peak decrease was significantly greater in water-deprived vs. control rats (Fig. 2). Microinjec-
tion of isotonic saline vehicle did not affect RSNA or ABP in either group (Fig. 2). Moreover, neither baseline MAP nor the latency to the peak change in RSNA or MAP differed between groups or drug treatments (Tables 1 and 2). Whereas RSNA and MAP of control rats and RSNA of water-deprived rats typically returned to baseline values within 120 min after muscimol, MAP of water-deprived rats was more variable and did not always fully return to baseline values.

In contrast, bilateral microinjection of muscimol lateral to the PVN did not alter RSNA or MAP in control (n = 8) and water-deprived rats (n = 6; Fig. 2). These responses were not significantly different from those after microinjection of isotonic saline into the PVN. In marked contrast, muscimol injected into the PVN produced a significantly greater decrease in RSNA and MAP than muscimol injected lateral to the PVN (Fig. 2).

As previously reported (14, 40, 41), HR of water-deprived rats was significantly higher than HR of control rats (Table 1). Bilateral injection of muscimol into the PVN produced similar and significant peak decreases in HR in both water-deprived and control rats (−49 ± 6 vs. −36 ± 8 beats/min, respectively; P < 0.05). Microinjection of isotonic saline vehicle did not significantly alter HR in either group (control: 9 ± 7 beats/min; water deprived: 9 ± 2 beats/min). Moreover, muscimol injected lateral to the PVN did not alter HR in control and water-deprived rats (−7 ± 4 vs. −8 ± 8 beats/min, respectively), and these results are not significantly different from isotonic saline.

All microinjection sites in the PVN are similar to those of previous studies published by our laboratory (6, 9). The spread of the dye impacted the dorsal cap region, magnocellular subnucleus, and the ventrolateral subnucleus throughout the rostral-caudal extent of the PVN without rupturing the wall of the third ventricle (Fig. 3). Spread of the dye was not consistently observed in a nucleus outside of the PVN. In addition, lateral microinjections of dye did not impact any region of the PVN (data not shown).

As expected, P-osmol and hematocrit of water-deprived rats were elevated significantly above values of control rats (Table 1).

Effect of ganglionic blockade on MAP in water-deprived and control rats. To determine whether the maintenance of ABP was more dependent on sympathetic outflow in water-deprived vs. control rats, additional experiments were performed in separate groups of rats given hexamethonium. As expected, hexamethonium significantly decreased MAP in both water-deprived and control rats; however, the peak decrease in MAP was significantly greater in water-deprived rats compared with control rats (Fig. 4). Baseline MAP did not statistically differ between groups (103 ± 3 vs. 98 ± 4 mmHg, respectively). Again, P-osmol and hematocrit was significantly elevated in this group of water-deprived vs. control rats (P-osmol: 311 ± 1 vs. 290 ± 1 mosmol/l, respectively; hematocrit: 60 ± 1 vs. 50 ± 1%, respectively; both P < 0.01).

DISCUSSION

Several lines of evidence suggest that water deprivation activates the sympathetic nervous system (14, 40, 49), but the neural circuits mediating this effect have not been examined previously. Because the PVN is one site in the central nervous system that plays an important role in regulating sympathetic activity and ABP during changes in P-osmol, circulating ANG II levels, and blood volume (13, 17, 30, 50), we hypothesized that acute inhibition of the PVN with bilateral microinjection of the GABA_A receptor agonist muscimol would decrease RSNA and ABP in water-deprived rats. The present findings confirm the notion that sympathetic outflow contributes more to the main-
tenance of blood pressure in anesthetized water-deprived rats than in control rats and suggest that sympathetic activity after water deprivation depends, at least partly, on the ongoing activity of PVN neurons.

Because currently available methods cannot directly assess differences in basal sympathetic nerve activity between animals, several studies have relied on indirect measurements that have yielded disparate results regarding differences in the basal level of sympathetic nerve activity between water-deprived and control rats. For example, plasma norepinephrine levels have been reported to either not change (12, 25) or increase (49) after water deprivation. On the other hand, direct nerve recording studies have reported that lumbar but not renal sympathetic nerve activity is significantly increased in water-deprived rats when values are normalized to a percentage of the baroreflex-mediated maximum (40, 41). In the present study, ganglionic blockade produced a significantly greater fall in ABP in water-deprived rats than in control rats, thereby indicating that sympathetic nerve activity contributes more to the maintenance of ABP in anesthetized water-deprived rats. Therefore, it seems likely that basal sympathetic nerve activity in the present study was elevated in water-deprived rats compared with control rats.

In the present study, acute inhibition of the PVN with bilateral microinjection of muscimol decreased RSNA to a greater extent in water-deprived rats compared with control rats. These findings suggest that muscimol reduced the ongoing activity of parvocellular PVN neurons to reduce RSNA. However, it is not clear whether the reduction in RSNA that occurred was due to an inhibition of PVN neurons that innervate the spinal intermediolateral cell column, the dorsomedial or ventrolateral medulla, or a combination of these pathways (35–37, 42, 50). Although the reduction in RSNA by microinjection of muscimol into the PVN suggests that the activity of PVN neurons may be elevated in water-deprived rats, it is also possible that the activity of these neurons could contribute more to the level of RSNA without being overtly increased. For example, downstream targets such as the rostral ventrolateral medulla could be more responsive to PVN inputs in water-deprived rats. Such augmented responses could occur because volume-sensitive cardiopulmonary receptors may be unloaded in water-deprived rats, and vagal inputs have been demonstrated to affect brain regions that also receive inputs from the PVN (19, 37, 47). Furthermore, brain regions excluding the PVN may participate in the changes of sympathetic

Table 1. Baseline MAP, HR, P\textsubscript{osmol}, and hematocrit of water-deprived and control rats that received bilateral microinjection of muscimol or isotonic saline

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Baseline MAP, mmHg</th>
<th>Baseline HR, beats/min</th>
<th>P\textsubscript{osmol}, mosmol/l</th>
<th>Hematocrit, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>107±4</td>
<td>447±12</td>
<td>292±3</td>
<td>50±1</td>
</tr>
<tr>
<td>Muscimol</td>
<td>7</td>
<td>104±3</td>
<td>431±10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isotonic saline</td>
<td>7</td>
<td>105±4</td>
<td>496±10*</td>
<td>309±2†</td>
<td>59±1†</td>
</tr>
<tr>
<td>Water deprived</td>
<td>7</td>
<td>102±6</td>
<td>480±9*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE. MAP, mean arterial blood pressure; HR, heart rate; P\textsubscript{osmol}, plasma osmolality. *Significant difference from control rats receiving the same treatment (P < 0.05). †Significant difference from control rats (P < 0.001).

Table 2. Latency of peak changes in RSNA, MAP, and HR of water-deprived and control rats that received bilateral injections of muscimol or isotonic saline into the PVN

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>RSNA</th>
<th>MAP</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>17.7±4.2</td>
<td>25.7±4.0</td>
<td>27.9±3.3</td>
</tr>
<tr>
<td>Muscimol</td>
<td>7</td>
<td>22.6±4.3</td>
<td>16.0±3.5</td>
<td>16.4±3.4</td>
</tr>
<tr>
<td>Isotonic saline</td>
<td>7</td>
<td>29.9±5.6</td>
<td>22.9±2.1</td>
<td>27.0±5.7</td>
</tr>
<tr>
<td>Water deprived</td>
<td>7</td>
<td>20.4±2.9</td>
<td>28.0±6.4</td>
<td>17.6±3.3</td>
</tr>
</tbody>
</table>

Values are mean ± SE. RSNA, renal sympathetic nerve activity; PVN, paraventricular nucleus. There were no significant differences in the latencies of any variable across groups.
outflow and ABP in water-deprived rats. Clearly, additional experiments are required to test these possibilities.

In contrast to the significant fall in RSNA and ABP observed in water-deprived rats, the present study found that bilateral microinjection of muscimol into the PVN in control rats produced a small decrease in ABP without a significant change in RSNA. These findings in control rats are consistent with previous studies in which a similar dose of muscimol was used to inhibit this region (21, 44, 45). However, a few studies using larger doses of muscimol reported decreases in both ABP and RSNA (1, 2, 54). Regardless, the present study demonstrates that the activity of PVN neurons contributes more to ongoing RSNA and ABP in water-deprived rats than in control rats. Although these findings suggest that activity of sympathetic-regulatory neurons of the PVN provides greater support for the maintenance of ABP in water-deprived rats, several studies also indicate that vasopressin aids in the maintenance of ABP during water deprivation (5, 40, 41). Therefore, microinjection of muscimol likely inhibited vasopressin release by the PVN but not by the supraoptic nucleus. Because the half-life of circulating vasopressin is a few minutes (10), it seems likely that the large reduction in ABP observed in water-deprived rats was due to the inhibition of both PVN-mediated vasopressin release and the activity of PVN sympathetic-regulatory neurons.

Because water deprivation increases $P_{osmol}$, stimulates the peripheral renin-angiotensin system, and decreases intravascular volume, one or a combination of these signals could underlie the enhanced contribution of the PVN to the level of RSNA and ABP. In this regard, recent data from our laboratory demonstrate that intracarotid injection of hyperosmotic NaCl increases the discharge of PVN neurons with axonal projections to the rostral ventrolateral medulla and/or spinal cord (8, 50). Furthermore, blockade of ANG II type 1 receptors within the PVN attenuates sympathoexcitatory and pressor responses to central hyperosmotic stimulation (7). Similarly, PVN neurons projecting to the spinal cord or medulla are excited by inputs arising from subfornical organ, a central site of action for peripheral ANG II (13), and PVN lesions attenuate pressor responses originating from subfornical organ (15, 16). Finally, volume expansion reduces the activity of PVN neurons projecting to the spinal cord (29), and renal vasodilatory and sympathoinhibitory responses during acute volume expansion or atrial balloon inflation are dependent on the integrity of the PVN (17, 30, 52). Thus one or more of these signals may underlie the enhanced role of PVN neuronal activity observed in water-deprived rats.

It is noteworthy that restoration of $P_{osmol}$ in awake rats that were water-deprived for 48 h reduced lumbar but not renal

![Figure 3](image3.png)

**Fig. 3.** Schematic drawings of microinjection sites targeted at the hypothalamic PVN in control (left) and water-deprived (right) rats. Shaded regions represent the largest possible distribution of dye for all rats within the respective group rather than a typical injection site of 1 rat. Coordinates are in reference to bregma using standard sections from the atlas of Paxinos and Watson (34). f, Fornix; AH, anterior hypothalamus; 3V, third ventricle; VMH, ventromedial hypothalamus

![Figure 4](image4.png)

**Fig. 4.** Peak decrease in MAP of water-deprived and control rats after a bolus injection of hexamethonium (30 mg/kg iv). Decrease in MAP in response to hexamethonium was significantly greater in water-deprived rats than in control rats, although baseline MAP was not different between groups (103 ± 3 vs. 98 ± 4 mmHg, respectively). In fact, absolute MAP values after hexamethonium were significantly lower in water-deprived rats than in control rats (41 ± 3 vs. 50 ± 3 mmHg, respectively; $P < 0.05$). *Significant difference from control rats ($P < 0.01$).
sympathetic nerve activity (40, 41). Whether a similar restoration of $P_{\text{osmol}}$ would eliminate the decrease in RSNA observed in response to microinjection of muscimol into PVN remains to be determined. Whereas acute increases in $P_{\text{osmol}}$ produced by peripheral venous infusions of hyperosmotic solutions have been reported to increase lumbar but reduce RSNA in baroreceptor-intact rats (51), intracarotid injections of hyperosmotic NaCl, mannitol, or glucose increase RSNA (7, 39), and this response is enhanced in baroreceptor-denervated rats (7, 39). However, water deprivation decreases blood volume and activates several hormonal systems that may interact with the increase in $P_{\text{osmol}}$ to influence RSNA. Thus several factors known to influence PVN neuronal activity may ultimately act in concert to determine the extent to which the PVN contributes to ongoing cardiovascular and sympathetic control during water deprivation.

To the extent that PVN neuronal activity becomes elevated during water deprivation, it remains to be determined whether this reflects reduced inhibitory or increased excitatory input. The PVN contains a large number of GABAergic terminals (4, 11), and acute interruption of local GABAergic transmission increases ABP, HR, and sympathetic nerve activity in awake and anesthetized rats (24, 32, 33, 54). Because cardiovascular and sympathetic responses to GABA$_{A}$ receptor blockade in the PVN are altered in cardiovascular diseases in which sympathetic outflow is elevated such as chronic hypertension and heart failure (21, 33, 53), it has been suggested that GABAergic transmission in the PVN undergoes a corresponding change (18, 21, 33). At present, it is not known whether water deprivation leads to similar changes in GABAergic function within the PVN. On the other hand, water deprivation may increase excitatory input to the PVN. In this regard, recent observations from our laboratory indicate that activation of ANG II type 1 (9) and ionotropic excitatory amino acid (6) receptors in the PVN is required for the sympathoexcitatory and pressor responses to blockade of local GABA$_{A}$ receptors. These data indicate that excitatory and inhibitory inputs to PVN may interact to regulate the discharge of PVN autonomic neurons.

Insight into the cellular mechanism(s) that may underlie such interactions is provided by recent in vitro studies. In PVN slices, both ANG II and NMDA were reported to depolarize the membrane potential of magnocellular neurons while also increasing the frequency of inhibitory postsynaptic potentials (3, 26). These findings indicate that a local inhibitory “feedback” mechanism may limit the level of excitation produced by ANG II and NMDA. Alternatively, increased neuronal discharge may result from disinhibition mediated by presynaptic actions to reduce GABA release. Such an effect has been reported recently for ANG II-evoked excitation of spinoally projecting neurons of the PVN (27). Whether similar mechanisms influence the excitability of sympathetic regulatory neurons of the PVN in vivo has not been established. If such mechanisms do regulate the activity of PVN autonomic neurons, it remains to be determined whether their modulation contributes to an increase in PVN neuronal activity during water deprivation. Clearly, the cellular mechanisms that regulate the excitability of autonomic neurons of the PVN warrant further investigation.

**Perspectives**

Together with previous observations (14, 40, 49), the present findings suggest that water deprivation increases the activity of the sympathetic nervous system. Furthermore, the observation that inhibition of the PVN with GABA$_{A}$ receptor agonist muscimol decreases RSNA and ABP in water-deprived rats closely resembles the response observed in several models of chronic hypertension (20, 22, 23, 28). Therefore, it appears that under these varied conditions elevated sympathetic nerve activity is maintained, in part, by the ongoing activity of PVN neurons. However, very little is known about how the basal discharge of PVN neurons is affected under these conditions. Moreover, it is not known to what extent PVN neurons with identified projections to cardiovascular regions are subject to modulation. Clearly, future studies are needed to establish the cellular processes that control the activity of PVN sympathetic-regulatory neurons during cardiovascular disease states. Whether similar or distinct cellular mechanisms underlie PVN neuronal plasticity under various physiological and pathophysiological conditions is a key to understanding the neural mechanisms contributing to cardiovascular disease.

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