AT₁ and glutamatergic receptors in paraventricular nucleus support blood pressure during water deprivation

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Freeman KL, Brooks VL. AT₁ and glutamatergic receptors in paraventricular nucleus support blood pressure during water deprivation. Am J Physiol Regul Integr Comp Physiol 292: R1675–R1682, 2007. First published December 21, 2006; doi:10.1152/ajpregu.00623.2006.—Water deprivation activates sympathoexcitatory neurons in the paraventricular nucleus (PVN); however, the neurotransmitters that mediate this activation are unknown. To test the hypothesis that ANG II and glutamate are involved, effects on blood pressure (BP) of bilateral PVN microinjections of ANG II type 1 receptor (AT1R) antagonists, candesartan and valsartan, or the ionotropic glutamate receptor antagonist, kynurenate, were determined in urethane-anesthetized water-deprived and water-replete male rats. Because PVN may activate the sympathetic nervous system via the rostral ventrolateral medulla (RVLM) and because PVN disinhibition increases sympathetic activity in part via increased drive of AT1R in the RVLM, candesartan was also bilaterally microinjected into the RVLM. Total blockade of the PVN with bilateral microinjections of muscimol, a GABAₐ agonist, decreased BP more (P < 0.05) in water-deprived (−29 ± 8 mmHg) than in water-replete (−7 ± 2 mmHg) rats, verifying that the PVN is required for BP maintenance during water deprivation. PVN candesartan slowly lowered BP by 7 ± 1 mmHg (P < 0.05). In water-replete rats, however, candesartan did not alter BP (1 ± 1 mmHg). Valsartan also produced a slowly developing decrease in arterial pressure (−6 ± 1 mmHg; P < 0.05) in water-deprived but not in water-replete (−1 ± 1 mmHg) rats. In water-deprived rats, PVN kynurenate rapidly decreased BP (−19 ± 3 mmHg), and the response was greater (P < 0.05) than in water-replete rats (−4 ± 1 mmHg). Finally, as in PVN, candesartan in RVLM slowly decreased BP in water-deprived (−8 ± 1 mmHg; P < 0.05) but not in water-replete (−3 ± 1 mmHg) rats. These data suggest that activation of AT₁ and glutamate receptors in PVN, as well as of AT1R in RVLM, contributes to BP maintenance during water deprivation.

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WATER DEPRIVATION IS ASSOCIATED with regional activation of sympathetic nerves (8, 37, 38). Recent studies indicate that sympathoexcitatory neurons in the paraventricular nucleus (PVN) contribute to this activation, since acute blockade of the PVN results in profound decreases in arterial blood pressure (BP) and sympathetic activity (42, 43). Moreover, PVN neurons that project to the spinal cord, which houses sympathetic preganglionic neurons, or to the rostral ventrolateral medulla (RVLM), a hindbrain region responsible for the basal activity of many sympathetic nerves, express c-fos after water deprivation (41, 42), indirectly suggesting activation. However, the neurotransmitters that drive PVN sympathoexcitatory neurons during water deprivation have not been identified.

Considerable indirect evidence implicates ANG II or a related peptide. First, water deprivation increases circulating ANG II levels, and increased binding of ANG II to type 1 receptors (AT1R) in circumventricular organs, such as the subfornical organ, increases the release and activity of an ANG II-like peptide in PVN (for a review, see Ref. 17). Second, both water deprivation and increases in osmolality trigger release of ANG II in PVN (21, 34). Third, administration of an AT1R antagonist in PVN attenuates the increases in BP and renal sympathetic nerve activity in response to intracarotid injections of hypertonic saline (11). Therefore, one purpose of these experiments was to test the hypothesis that stimulation of AT1R contributes to PVN activation by determining whether microinjection of AT1R antagonists into PVN decreases BP more in water-deprived than in water-replete rats.

Another excitatory neurotransmitter that may participate in the increased activity of PVN sympathoexcitatory neurons during water deprivation is glutamate. Indeed, glutamate is involved in hypertonicity-induced activation of magnocellular neurons (5, 14, 40). To test this hypothesis, we aimed to determine whether PVN bilateral microinjection of the non-specific ionotropic excitatory amino acid antagonist kynurenate decreases BP more in water-deprived than in water-replete rats.

PVN may activate the sympathetic nervous system via a pathway that includes the RVLM. As described above, PVN neurons that project to the RVLM are activated during water deprivation (41, 42), and the vast majority of these neurons are glutamatergic (44). Moreover, previous work indicates that the RVLM is tonically excited by glutamatergic inputs during water deprivation (6, 7). However, the role of other neurotransmitters has not been previously investigated. Activation of AT1R in the RVLM has been shown to contribute to hypertension in spontaneously hypertensive rats (1, 23), Dahl salt-sensitive rats (22), and transgenic TGR(mREN2)27 rats (18), as well as to maintenance of normal BP in rats on a low-salt diet (15). Therefore, a final purpose of these experiments was to test the hypothesis that activation of AT1R in RVLM underlies maintenance of basal BP during water deprivation by determining whether bilateral AT1R blockade of RVLM decreases BP more in water-deprived than in water-replete rats.

METHODS

Animals. We used male Sprague-Dawley rats (Sasco, Wilmington, MA) weighing ~275–375 g for all experiments. All rats were housed in a room with a 12:12-h light-dark cycle for a minimum of 5 days before experimentation. Rats had free access to food (LabDiet 5001,
An intravenous infusion of urethane (1.2 g/kg in 1 ml saline) was then administered, volume depletion and increased tonicity induced by water deprivation. L-glutamate [100 nl, 1 nmol/100 nl (24, 26, 46)]. Injections were made via a micropipette angled 20° rostrally and the following coordinates (camera: 1.6 – 2.0 mm caudal to the bregma; 500 m caudal to the lambda; x– y– z range: 10 – 15 mmHg) to each injection. The latency of the maximal response was defined as the elapsed time between completion of all bilateral injections to the mediolateral position of the facial nucleus and ventral to nucleus ambiguus (see Fig. 5 (6)). Experimental protocols. After the PVN or RVLM injection, the rats were allowed to stabilize for 30 – 60 min before experiment. After completion of all surgical manipulations, a blood sample (400 µl) was collected in most animals to document the volume depletion and increased toxicity induced by water deprivation. An intravenous infusion of urethane (1.2 g/kg in 1 ml saline) was then administered over ~30 min. Beginning 10 min after the urethane infusion was initiated, the gas anesthetic was slowly withdrawn, but artificial ventilation with 100% oxygen was maintained throughout the experiment. After completion of surgery and the urethane infusion, the rats were allowed to stabilize for ~30–60 min before experiment. Depth of anesthesia was periodically assessed by confirming the lack of response to tail or paw pinch. Additional urethane (0.2 g/kg) was occasionally administered intravenously as needed.

In water-replete rats, acute inhibition of PVN by bilateral microinjection of muscimol (0.2 g/kg) was occasionally administered intravenously as needed. RVLM microinjections, a midline incision was made on the back of the head to allow exposure of the dorsal surface of the medulla. The atlanto-occipital membrane was then removed via a limited craniotomy. After completion of all surgical manipulations, a blood sample (400 µl) was collected in most animals to document the volume depletion and increased toxicity induced by water deprivation. Additional urethane (0.2 g/kg) was occasionally administered intravenously as needed.

PVN and RVLM microinjections. The rat was positioned in the stereotaxic apparatus with the incisor bar set at –11 mm. We positioned single-barreled glass micropipettes (20- to 40-µm tip diameter) containing bicuculline in PVN using the following coordinates (using bregma and the dorsal surface of the dura as zero and the pipette angled caudally at 11.5° so that it entered the brain perpendicularly to the skull): 1.6 – 2.0 mm posterior, 0.5 mm lateral, and 7.4 – 7.6 mm ventral. PVN was identified functionally by noting pressor responses (>10 – 15 mmHg) to unilateral microinjection of bicuculline (20 – 50 nl of 1 mM). The RVLM was functionally identified by observing >15 mmHg pressor responses to t-glutamate [100 nl, 1 nmol/100 nl (24, 26, 46)]. Injections were made via a micropipette angled 20° rostrally and the following coordinates (camera: 1.6 – 2.0 mm anterior, 1.8 mm lateral, and 2.4 – 3.2 mm ventral. Injections were conducted over ~3–7 s with a Picopump (World Precision Instruments, Sarasota, FL); the successful microinjection of drugs was verified by watching, through a microscope reticle, the movement of the fluid meniscus a distance calibrated to be 20 – 100 µl. At the conclusion of the experiment, ~50 nl of 2.5% Alcian blue in 0.5 M sodium acetate were injected into the PVN or RVLM with the same pipette and coordinates as for injections. The brain was removed and placed in 4% paraformaldehyde in PBS for at least 48 h. We subsequently cut the hypothalamus and brain stem into 25-µm sections using a cryostat; sections were mounted on glass microscope slides and counterstained with neutral red. Correct placement into PVN was indicated by dye centered just dorsally or within the nucleus, ~1.8 to 2.2 mm caudal to bregma (see Figs. 1–4). RVLM injection sites were verified against those previously published, within an area ~500 µm caudal to the caudal end of the facial nucleus and ventral to nucleus ambiguus (see Fig. 5) (6).

RESULTS

Basal values. As expected, water-deprived rats exhibited evidence of volume depletion, including increased hematocrit and plasma protein concentration, as well as increased plasma osmolality and concentrations of sodium and chloride (Table 1). Basal values were different between water-deprived and water-replete rats.

Role of the PVN in BP maintenance during water deprivation. In water-replete rats, acute inhibition of PVN by bilateral microinjection of muscimol tended to decrease arterial pressure, although the response was not significantly different from the response to aCSF (Fig. 1). In contrast, as previously reported (42, 43), muscimol administration in water-deprived rats promptly decreased BP, and the depressor response ex-
Table 1. Effect of water deprivation on basal blood and hemodynamic values

<table>
<thead>
<tr>
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<th>Water-Deprived Rats</th>
<th>Water-Replete Rats</th>
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<tbody>
<tr>
<td>No.</td>
<td>37–44</td>
<td>14–17</td>
</tr>
<tr>
<td>Plasma sodium concen.</td>
<td>143.2±0.4*</td>
<td>138.7±0.3</td>
</tr>
<tr>
<td>Plasma chloride concen.</td>
<td>109.6±0.2*</td>
<td>104.9±0.5</td>
</tr>
<tr>
<td>Plasma osmolality</td>
<td>312.7±0.5*</td>
<td>303.5±0.5</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>49.2±0.4*</td>
<td>43.3±0.5</td>
</tr>
<tr>
<td>Plasma protein concen.</td>
<td>6.2±0.1*</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>115±2</td>
<td>111±3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>342±7</td>
<td>325±9</td>
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Values are means ± SE. *P < 0.05 compared with water-replete rats.

Nevertheless, because of the rather modest depressor response, a higher dose of candesartan (1 nmol) was also microinjected in a separate group of water-deprived rats. Again, a slow fall in pressure was observed; however, the maximal depressor response did not differ from that after injection of 100 pmol, although the latency to reach the nadir was increased (P < 0.05; see legend to Fig. 2).

To establish the specificity of the depressor actions of candesartan at AT1R, another nonpeptide AT1R antagonist, valsartan, was also microinjected bilaterally into the PVN. As with candesartan, a slowly developing depressor response was observed in water-deprived rats that exceeded the response observed in water-replete rats (Fig. 3). Again, heart rate was not significantly altered (data not shown). Collectively, these data suggest that an endogenous ANG II-like peptide acting at AT1R tonically drives PVN pressor neurons during water deprivation.

Role of tonic PVN glutamatergic input in BP maintenance during water deprivation. To investigate the role of PVN ionotropic glutamatergic receptors, kynurenate (7.2 nmol) was microinjected bilaterally in water-deprived and water-replete rats. Although kynurenate administration had little effect in water-replete rats, in water-deprived rats it elicited a prompt and profound decrease in BP and heart rate (Fig. 4). Therefore, water deprivation-induced PVN activation appears to also depend on increased glutamatergic drive of PVN pressor neurons.
Role of tonic RVLM AT1R activation in BP maintenance during water deprivation. As in PVN, bilateral microinjection of candesartan (100 pmol) into RVLM produced a slowly developing decrease in BP in water-deprived rats that was greater than that observed in water-replete rats (Fig. 5). Consistent or significant changes in heart rate were not observed (data not shown). These results suggest that AT1R activation in RVLM also contributes to BP maintenance during water deprivation.

DISCUSSION

PVN pressor neurons are activated during water deprivation; however, the neurotransmitters that drive this activation have...
not been identified. The major new findings of this study are that bilateral PVN microinjection of AT1R antagonists and the ionotropic glutamate receptor antagonist, kynurenate, and bilateral microinjection of candesartan in RVLM decrease arterial BP in water-deprived but not water-replete rats. These results suggest for the first time that PVN AT1 and glutamatergic receptors are tonically excited during water deprivation and that this excitation allows BP maintenance in the face of volume depletion. We further conclude that RVLM AT1R activation is also required for basal BP support.

As previously reported (42, 43), we observed that acute inhibition of PVN after microinjection of muscimol rapidly decreased arterial BP in water-deprived rats. The rapid nature of the depressor response, as well as previous simultaneous measurements of renal and lumbar sympathetic activity (42, 43), indicates that the fall in pressure is largely mediated by decreases in sympathetic activity. The mechanisms for activation of PVN sympathoexcitatory neurons by water deprivation have not been directly investigated, but significant previous research indirectly implicates a role for increased stimulation of AT1R. In support of this hypothesis, we found that PVN administration of two AT1R antagonists, candesartan and valsartan, significantly decreased arterial BP in water-deprived rats. In contrast, AT1R blockade had little effect in water-replete animals, in agreement with previous reports (12, 49). Interestingly, the depressor response was slowly developing, reaching its nadir only after 15–20 min. This slow response is not specific to PVN; a similar gradual BP decline has been...
reported after AT1R blockade in RVLM (1, 22, 23). The slow response is consistent with electrophysiological studies showing that the reversal of ANG II-induced activation of PVN sympathoexcitatory neurons following washout of the peptide requires several minutes (9, 30). Alternatively, the slow time course could be explained by PVN AT1R blockade decreasing vasopressin secretion and its subsequent clearance from plasma, due to reversal of the known stimulatory effect of ANG II on vasopressin release (39, 48). However, our finding that the greatest depressor responses to candesartan were observed in the more caudal regions of PVN, which house sympathoexcitatory neurons that project to the spinal cord and/or to RVLM, but contain few neurohypophysial magnocellular vasopressin neurons (20, 35), argues against this possibility.

Recent studies provide a mechanistic framework by which AT1R activation excites PVN neurons. AT1R have been described throughout the PVN (19, 28, 32, 33) but are notably absent on vasopressin magnocellular neurons (28). Moreover, immunocytochemical visualization of AT1R in PVN failed to detect receptors on neurons that project to RVLM or spinal cord (33). Therefore, the action of ANG II in PVN to stimulate parvocellular sympathoexcitatory (9, 30) or magnocellular vasopressinergic neurons may be, in part, indirect. This idea is supported by the recent studies of Latchford and Ferguson (27), which suggested that ANG II stimulates PVN magnocellular neurons via a glutamatergic interneuron. Similarly, Li and coworkers (29, 30) propose that ANG II-induced excitation of PVN sympathoexcitatory neurons is via inhibition of local GABAergic synaptic activity but is independent of glutamatergic input. On the other hand, Cato and Toney (9) provided support for a parallel direct or indirect excitatory effect of ANG II through AT1R-mediated activation of a mixed cation current. Collectively, therefore, during water deprivation, an endogenous ANG II-like peptide may excite PVN sympathoexcitatory neurons indirectly by inhibiting GABA release, but also possibly by direct depolarization (Fig. 6).

Another key finding of the present study was that blockade of ionotropic glutamate receptors produced rapid, large decreases in arterial BP and heart rate in water-deprived but not water-replete rats. The brisk time course indicates that the fall in pressure is secondary to decreases in sympathetic activity. Therefore, these data suggest for the first time that tonic activation of PVN sympathoexcitatory neurons, in particular during water deprivation, also includes increased excitation of glutamate receptors (Fig. 6). Interestingly, the sum of responses to PVN AT1R and glutamatergic blockade was equivalent to that of complete blockade with muscimol. If ANG II acts distinctly from glutamatergic inputs (29, 30), then this result would suggest that excitation of AT1 and glutamatergic receptors can completely explain the sustained activation of PVN observed during water deprivation.

The present experiments do not reveal whether the tonically increased drive of PVN AT1 and glutamate receptors is secondary to increased release of these neurotransmitters or to increases in receptor expression or functional efficacy. Moreover, the modalities that mediate the increased activation of PVN AT1 and glutamate receptors were not investigated. Water deprivation, at least in conscious rats, increases arterial pressure (4, 37, 38); therefore, it is unlikely that arterial baroreceptor unloading is involved. Because it is presently unclear whether volume-sensing cardiac stretch receptors are active at rest or whether their unloading leads to sympathoexcitation (36), a potential role for cardiac receptors remains uncertain. On the other hand, water deprivation increases circulating levels of ANG II and osmolality, each of which could contribute. It is well established that ANG II activates neurons in circumventricular organs, such as the subfornical organ, which through monosynaptic or polysynaptic (e.g., via median preoptic nucleus (MnPO)) pathways. Subsequently, sympathetic activity increases, mediated by direct projections to the spinal cord (IML) and also indirectly via synapses in the RVLM. Details of proposed synaptic mechanisms in PVN (dashed box) are expanded in bottom dotted box. Bottom: increased OSM/ANG II excites PVN sympathoexcitatory neurons in part via ANG II type 1 receptors (AT1R). A portion of the AT1R activation may be indirect, via local inhibition of GABA release. AT1R on PVN sympathoexcitatory neurons may also be directly excited. Finally, a parallel direct excitation via glutamate receptors is also proposed.

Fig. 6. Model by which water deprivation leads to excitation of PVN sympathoexcitatory neurons (see text for details). Top: increases in osmolality (OSM) and/or ANG II stimulate neurons in sensory circumventricular organs (CVOs), including the subfornical organ, organum vasculosum of the lamina terminalis, and area postrema. CVO stimulation then activates PVN neurons via monosynaptic and polysynaptic [e.g., via the median preoptic nucleus (MnPO)] pathways. Subsequently, sympathetic activity increases, mediated by direct projections to the spinal cord (IML) and also indirectly via synapses in the RVLM. Details of proposed synaptic mechanisms in PVN (dashed box) are expanded in bottom dotted box. Bottom: increased OSM/ANG II excites PVN sympathoexcitatory neurons in part via ANG II type 1 receptors (AT1R). A portion of the AT1R activation may be indirect, via local inhibition of GABA release. AT1R on PVN sympathoexcitatory neurons may also be directly excited. Finally, a parallel direct excitation via glutamate receptors is also proposed.
very recently reported that PVN microinjection of kynurenate reverses the increase in lumbar sympathetic nerve activity triggered by an acute increase in osmolality. Finally, acute and chronic increases in osmolality have been shown to increase AT1R activity in PVN (11, 21, 34). Therefore, we speculate that the increase in osmolality underlies at least in part the activation of AT1 and glutamate receptors observed during water deprivation. If so, and if ANG II acts independently of glutamate as suggested by in vitro electrophysiological studies (29, 30), then osmoreceptor-mediated activation of PVN sympathoexcitatory neurons may be conveyed via two separate parallel pathways, which include AT1 and glutamate receptors, respectively (Fig. 6). In support of this scenario, Bains and Ferguson (3) noted that PVN neurotransmission after electrical stimulation of subfornical organ-PVN-spinal cord pathways was mediated by both a slowly acting ANG II-like peptide and a rapid (presumably glutamatergic) chemical messenger.

In addition to increased activity of AT1R in PVN, the present study also documented that water deprivation induces parallel activation of AT1R in RVLM, similarly to that reported in rat models of hypertension and sodium deprivation (1, 15, 18, 22, 23). As in the PVN and as previously described in the RVLM (1, 22, 23), the depressor response was slowly developing. The slow nature of this response suggests that the rapid falls in arterial pressure after PVN administration of muscimol or kynurenate are not mediated by decreased drive of RVLM AT1R. In addition, as described above, ANG II excites PVN sympathoexcitatory neurons, at least in part, by attenuating GABA release (29, 30), and disinhibition of PVN by microinjection of the GABA_A antagonist bicuculline excites RVLM pressor neurons by activation of AT1R in RVLM (46). Therefore, increased AT1R activity in PVN may be linked to the increased AT1R activation in RVLM, similarly to the previously proposed linkage between ANG II activation of the subfornical organ and the release of ANG II-like peptides in PVN (17). Indirect support of this idea is that the magnitude of the depressor responses to AT1R blockade in PVN and RVLM was similar to that shown in the present study. Nevertheless, future experiments are required to test this hypothesis.

In summary, although significant previous work indicated that acute increases in systemic osmolality and/or ANG II activate PVN pressor neurons via AT1 or glutamate receptors, the present results demonstrate that this rapid activation can be sustained during water deprivation. We also show that RVLM AT1Rs are required for BP maintenance in this hypovolemic state, as in sodium deprivation (16), in parallel with tonic excitation of RVLM glutamate receptors (6, 7).

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REFERENCES


