Is osmolality a long-term regulator of renal sympathetic nerve activity in conscious water-deprived rats?

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Scrogin, Karie E., Donogh F. McKeogh, and Virginia L. Brooks. Is osmolality a long-term regulator of renal sympathetic nerve activity in conscious water-deprived rats? Am J Physiol Regulatory Integrative Comp Physiol 282: R560–R568, 2002; 10.1152/ajpregu.00780.2000.—Acute increases in osmolality suppress renal sympathetic nerve activity (RSNA). However, it is not known whether prolonged physiological increases in plasma osmolality chronically inhibit RSNA. To address this hypothesis, mean arterial blood pressure (MAP), heart rate (HR), and RSNA were measured during acute normalization of plasma osmolality in conscious rats made hyperosmotic by 48 h of water deprivation. Water deprivation significantly elevated MAP (120 ± 1 vs. 114 ± 3 mmHg, P < 0.05) and plasma osmolality (306 ± 1 vs. 293 ± 1 mosmol/kgH2O, P < 0.01). When plasma osmolality was subsequently lowered to normal (−17 ± 1 mosmol/kgH2O) with a 2-h (0.12 ml/min) infusion of 5% dextrose in water (5DW), MAP decreased (−11 ± 1 mmHg), and RSNA increased (25 ± 10% baseline). To assess the role of circulating vasopressin in these changes, rats were pretreated with a V1-vasopressin receptor antagonist before infusion of 5DW. The antagonist lowered MAP (−4 ± 1 mmHg) and raised RSNA (31 ± 3% baseline) and HR (25 ± 5 beats/min) in water-deprived rats (all changes P < 0.05). However, V1-vasopressin receptor blockade did not increase RSNA or HR independently of baroreflex responses to decreases in arterial pressure. After V1 blockade, infusion of 5DW lowered blood pressure (−8 ± 1 mmHg) but did not further affect HR or RSNA. An isotonic saline infusion that produced the same volume expansion as 5DW lowered MAP (−5 ± 2 mmHg) and HR (−68 ± 2 beats/min or 1 vs. 11 vs. 29 vs. 3 vs. 1 vs. 8 vs. 11 vs. 0.05). However, V1-vasopressin receptor blockade did not increase RSNA or HR independently of baroreflex responses to decreases in arterial pressure. After V1 blockade, infusion of 5DW lowered blood pressure (−8 ± 1 mmHg) but did not further affect HR or RSNA. An isotonic saline infusion that produced the same volume expansion as 5DW lowered MAP (−5 ± 2 mmHg) and HR (−68 ± 2 beats/min) but had no effect on osmolality or RSNA in water-deprived rats. Finally, 5DW infusion had negligible effects in water-replete animals. In conclusion, these results fail to support the hypothesis that sustained increases in plasma osmolality, either directly or via increased vasopressin, tonically suppress RSNA.

METHODS

Animals

Male Sprague-Dawley rats weighing between 350 and 400 g (Simonson, Gilroy, CA) were housed individually in Plexiglas cages in the Animal Care Unit with ad libitum access to a 1% NaCl diet (Harlan Teklad, Madison, WI) for at least 1 wk before surgery. The housing facility was main-

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tained at a constant temperature of 22 ± 2°C with a 12:12-h light-dark cycle. All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional (Oregon Health & Science University) Animal Care and Use Committee.

Surgery

Twenty-four hours before the experiment, the rats were anesthetized (pentobarbital sodium, 25 mg ip) for placement of vascular catheters and a renal sympathetic nerve recording electrode as described in detail elsewhere (19). Briefly, animals were implanted with bilateral femoral arterial catheters (PE-50 heat welded to a length of PE-10) for direct measurement of mean arterial pressure (MAP) and arterial blood sampling. In addition, a single femoral vein catheter (Tygon, Norton Performance Plastics, Akron, OH) was inserted for infusion of drugs and solutions. Finally, a stainless steel bipolar recording electrode was implanted to enable direct measurement of RSNA. The bipolar stainless steel electrode (bare diameter 0.003 in., A-M Systems, Everett, WA) was implanted through a left flank incision. A multifiber nerve bundle that projected to the kidney was isolated, placed on the electrode leads, and assessed for viability by qualitative assessment of the signal-to-noise ratio viewed on an oscilloscope. When a viable nerve bundle was isolated, it was embedded in a quick-drying, lightweight dental silicone (Bisico, Bielefeld, Germany). The electrode connector was externalized subcutaneously along with the vascular catheters at the nape of the neck. The flank incision was sutured closed in two layers with the electrode leads coiled within the subcutaneous space. The rats were allowed to recover overnight in their home cages.

Chemicals

Pentobarbital sodium for anesthesia was obtained from Abbott Laboratories (North Chicago, IL). Sodium nitroprusside (Elkins-Sinn, Cherry Hill, NJ) was used for determination of baroreflex-mediated maximum RSNA. The V1-receptor antagonist [1-(β-mercapto-β,β-cyclopentamethylene propionic acid),2-(O-methyl)tyrosine]-Arg-8 vasopressin was obtained from Peninsula Laboratories (Belmont, CA) or from Sigma (St. Louis, MO). Hexamethonium chloride (Sigma) was used to block autonomic ganglion transmission to determine the noise level of the recording electrode.

Data Acquisition

An arterial line was connected to a Grass bridge amplifier (7P1; Grass Instruments, Quincy, MA) for determination of arterial pressure. The pulse pressure signal was fed through a Grass tachograph amplifier (7P4) for determination of heart rate (HR). The recording electrode was connected to a Grass differential preamplifier (P511). Raw nerve activity was directed through band-pass filters that transmitted frequencies between 100 and 3,000 Hz, was amplified (20,000–50,000×), and the resulting signal was monitored visually with an oscilloscope (model 2212, Tektronics, Beaverton, OR) and simultaneously fed through a Grass integrator (7P10). The signal was whole-wave rectified and integrated over 1-s intervals. MAP, HR, and integrated RSNA signals were recorded on a Grass polygraph (7D). In most cases, nerve activity was averaged over a 12-s stable or quasistable period. Determination of the baroreflex maximum was performed by averaging the nerve signal over a 2-s period measured during the peak response to a bolus dose of nitroprusside (70 μg iv) that lowered pressure by >50 mmHg. At the end of the experiment, background noise was determined after blockade of postganglionic nerve activity with hexamethonium chloride (30 mg/kg iv). Background noise was subtracted from values obtained during the experiment to provide a measurement of RSNA. RSNA was normalized to baseline nerve activity determined before treatment (%baseline). All data were obtained from visibly healthy animals that demonstrated viable nerve preparations, i.e., greater than 50% rise in nerve activity after a maximal depressor dose of nitroprusside (70 μg iv).

Experimental Protocols

The day after surgery, the vascular catheters and electrode leads were connected to the recording equipment between 8 and 9 AM while the rats rested unrestrained in their home cages. The rats were allowed at least 2 h habituation to the instrumentation, after which one of the following six protocols was performed.

Protocol 1 was performed to test the hypothesis that a sustained increase in plasma osmolality tonically suppresses renal sympathetic outflow. This was done by assessing the renal sympathetic response to an acute decrease in osmolality that had been previously elevated by 48-h water deprivation. Rats were deprived of drinking water beginning 24 h before surgery and during the intervening 24-h recovery period. During the experiment, basal MAP, HR, and RSNA were determined continuously. After a 30-min recording period of basal measurements, MAP, HR, and RSNA responses to the bolus injection of nitroprusside were determined. Approximately 30 min later, a 350-μl blood sample was taken for determination of plasma osmolality, hematocrit, and plasma protein. The withdrawn blood was immediately replaced with an equal volume of 0.9% saline. When recorded parameters had stabilized after blood sampling (5–10 min), 5% dextrose in water (5DW) was infused (0.12 ml/min) for 120 min. Osmolality, hematocrit, and plasma protein were determined from additional 350-μl blood samples taken 30, 60, and 120 min after initiation of the infusion, and each sample was replaced with an equal volume of saline. After termination of the infusion, background noise was determined after ganglionic blockade with hexamethonium (30 mg/kg iv).

Protocol 2. Vasopressin and osmolality are tightly coupled. Moreover, vasopressin infusion can decrease RSNA (2). Thus any increase in RSNA that occurs during normalization of plasma osmolality could result from a reversal of vasopressin-dependent inhibition of RSNA. Therefore, this protocol was performed to test the hypothesis that a sustained increase in osmolality tonically suppresses RSNA independent of vasopressin. The same procedure was used as described in protocol 1 except that animals were pretreated with the selective vasopressin V1-receptor antagonist (5 μg iv) 30 min before collection of the initial blood sample.

Protocols 3 and 4. These protocols were designed to determine whether the volume load provided by 5DW influenced RSNA independently of the resulting change in plasma osmolality and in vasopressin levels. In this experiment, water-deprived rats were treated exactly as in protocol 2 except that they were infused with isotonic saline (0.9%) rather than 5DW to increase volume without altering plasma osmolality. Preliminary experiments showed that an infusion of isotonic saline given at the same rate as the 5DW infusion produced a larger absolute decline in plasma protein, whereas isotonic saline infused at three-fourths the rate of 5DW infusion produced a similar degree of hemodilution. Therefore, separate groups of water-deprived, vasopressin antagonist-treated rats were given an isotonic saline infusion equivalent
to three-fourths (protocol 3) or the full rate (protocol 4) of the 5DW infusion.

Protocol 5. In previous experiments (19), intravenous infusion of 5DW did not significantly alter plasma osmolality or hematocrit/protein in water-replete animals, suggesting that the fluid is rapidly excreted in normally hydrated animals. Therefore, this protocol was performed to determine whether 5DW infusion had indirect effects unrelated to changes in plasma osmolality, the volume load, or vasopressin acting at V1 receptors. In this protocol, the identical procedure was carried out as in protocol 2 except that hydrated rats given ad libitum access to drinking water were used.

Protocol 6. V1-vasopressin blockade decreased MAP and increased HR and RSNA. Therefore, to determine if this carried out as in V1 receptors. In this protocol, the identical procedure was plasma osmolality, the volume load, or vasopressin acting at water-deprived rats. It was reasoned that if vasopressin chronically suppressed sympathetic activity, then blockade of V1 receptors would result in elevated RSNA and/or HR relative to MAP over the entire baroreflex range.

Animals were instrumented and set up for experimentation as described in protocol 1. After the stabilization period, RSNA and HR were measured during ramp increases and decreases in arterial pressure induced by intravenous infusion of 1 mg/ml phenylephrine or nitroprusside, respectively. Drugs were infused at an increasing rate (1–30 μl/min), such that both the full pressor and depressor responses occurred at a relatively linear rate of ~0.2 mmHg/s. The order of exposure to phenylephrine and nitroprusside was randomized between rats but was kept consistent within an experiment. Sufficient time for reestablishment of baseline MAP, HR, or RSNA was always allowed between pressor and depressor infusions (normally about 30 min). After generation of the first curve, animals were given the vasopressin antagonist (5 μg/100 μl iv). Beginning 30 min after antagonist injection, the second set of pressor and depressor ramps were performed. Hexamethonium chloride was injected (30 mg/kg iv), and noise was subtracted from RSNA determined during the experiment. MAP, HR, and RSNA were recorded with a MacIntosh (G3 Powerbook) computer via Chart data acquisition software (v. 4.01, ADInstruments, Grand Junction, CO). Raw data were averaged and integrated as described previously for infusion experiments, except that movement artifacts were excluded and all the data for a given drug infusion period were averaged into consecutive 5-s bins using Chart software. All nerve activity signals were normalized to baseline values, which were calculated by averaging across 5 min of quiet recordings. Control baseline values were determined just before curve generation. Curves produced after antagonist administration were normalized to data obtained 5 min before antagonist administration. Curves were generated by comparing simultaneously averaged MAP, RSNA, and HR data points.

Data Analysis

All data were presented as group means ± SE. One- and two-way analyses of variance (ANOVA) with repeated measures were used to assess MAP, HR, and RSNA responses to 5DW or saline infusion. Separate ANOVAs were used to answer specific questions; therefore, the data were grouped so as to determine whether 1) 5DW increases RSNA in untreated water-deprived rats (one-way ANOVA), 2) the response to V1 antagonist differs between water-deprived and water-replete rats (two-way ANOVA), 3) the responses to 5DW infusion are different in V1 antagonist-treated vs. untreated rats (two-way ANOVA), 4) saline infusion produces the same effects as 5DW in V1-blocked rats (two-way ANOVA), and 5) responses to 5DW infusion can be detected in water-replete rats (one-way ANOVA). Post hoc Tukey-Kramer tests were used to determine specific between- and within-group differences.

Baroreflex curves were constructed for each animal by fitting the 5-s averages of HR and RSNA responses observed during MAP changes to the following four-parameter logistic curve function using a least-squares regression analysis (Sigma Plot 2000 for Windows, v. 6.10): HR or RSNA = A + D/[1 + exp[(B(MAP − C))]], where A equals the response range, B equals the gain coefficient, C is MAP50, or MAP at the midpoint of the curve, and D is the asymptotic minimum. Maximal gain was also calculated from the resulting parameters as (−B/A)/4.56. The resulting values of the parameters for both pre- and postantagonist curves for each rat were averaged to obtain group means and were compared using paired t-tests.

P values <0.05 were considered significant. All analyses were performed using GB-STAT software (v. 7, Dynamic Microsystems, Silver Spring, MD).

Analytical Assays

Plasma osmolality was measured by freezing point depression using a Micro-osmometer (model 3MO, Advanced Instruments, Norwood, MA). Values were obtained by averaging the results from at least three 20-μl samples. Hematocrit was determined from duplicate blood-filled hematocrit tubes that were spun and read with an Adams Micro-hematocrit reader (New York, NY). The tubes were subsequently broken, and the remaining plasma was used for duplicate determinations of plasma protein with a Hitachi protein refractometer (National Instruments, Baltimore, MD).

RESULTS

Effects of Water Deprivation on Baseline Parameters

Water deprivation produced a significant contraction of plasma volume as indicated by the increased hematocrit, plasma osmolality, and plasma protein concentration found among water-deprived rats compared with rats given unlimited access to water (Table 1). Water-deprived rats also had elevated MAP compared with water-replete animals. Neither HR nor baseline RSNA (expressed as a percentage of arterial baroreflex

Table 1. Control values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Water Deprived</th>
<th>Water Replete</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>120 ± 1</td>
<td>114 ± 3</td>
<td>0.04</td>
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<tr>
<td>HR, beats/min</td>
<td>444 ± 7</td>
<td>434 ± 9</td>
<td>0.15</td>
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<tr>
<td>RSNA, %max</td>
<td>32 ± 2</td>
<td>36 ± 8</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH2O</td>
<td>306 ± 1</td>
<td>293 ± 1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Protein, g/dl</td>
<td>8.1 ± 0.1</td>
<td>7.6 ± 0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>48.1 ± 0.4</td>
<td>43.7 ± 1.4</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity. P indicates significant difference among water-deprived (n = 25–26) and water-replete (n = 6 or 7) rats.
maximum) was significantly affected by water deprivation.

Effects of 5DW Infusion in Water-Deprived Rats

Over the duration of the 2-h 5DW infusion, hematocrit, plasma osmolality, and plasma protein values of water-deprived rats (protocol 1) decreased progressively to levels similar to that observed in water-replete rats before infusion (Fig. 1 and Table 2). RSNA rose slowly from the onset of the infusion and became significantly elevated above baseline after 50 min of infusion. At this point, nerve activity reached a plateau at which it remained for the duration of the infusion (Fig. 1). Blood pressure fell progressively throughout the experiment and became significantly lower than baseline after 20 min of infusion (Fig. 2). HR tended to rise slightly during the first half of the infusion. However, the effect did not achieve statistical significance. Thus normalization of plasma osmolality in water-deprived rats increased RSNA.

### Table 2. Hematocrit and plasma protein concentration

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Time, min</th>
<th>Hematocrit, %</th>
<th>Protein, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Protocol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>49.0 ± 0.6</td>
<td>47.4 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>47.7 ± 0.5</td>
<td>45.7 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>47.1 ± 0.9</td>
<td>45.5 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>48.5 ± 0.8</td>
<td>45.7 ± 0.9*</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>43.6 ± 1.4</td>
<td>42.0 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number of rats. **Protocols 1–5 as described in METHODS. *P < 0.05 compared with time 0 within group.**

Fig. 1. Effect of 5% dextrose in water (5DW) infusion on plasma osmolality (A) and renal sympathetic nerve activity (RSNA, B) in water-deprived rats either untreated (•, n = 6) or pretreated (○, n = 7) with a V1-vasopressin receptor antagonist. RSNA is expressed as a percentage of baseline determined before any treatment. 5DW infusion was begun at time zero and continued throughout the 120-min period shown. *P < 0.05 compared with time zero within group.

Fig. 2. Effect of 5DW infusion on mean arterial pressure (MAP, A) and heart rate [HR in beats/min (b/min), B] in water-deprived rats either untreated (•, n = 6) or pretreated (○, n = 7) with a V1-vasopressin receptor antagonist. *P < 0.05 compared with time zero within group.
To determine whether the sympathoexcitatory effect of lowering osmolality was due to decreases in vasopressin, water-deprived rats were pretreated with a V₁-vasopressin receptor antagonist before osmolality was lowered (protocol 2). The antagonist caused a significant fall in blood pressure that was accompanied by a simultaneous increase in HR and RSNA (Fig. 3). Subsequent infusion of 5DW produced changes in osmolality, plasma protein concentration, and hematocrit similar to those seen in intact rats (protocol 2; Fig. 1, Table 2). However, the infusion failed to produce a further increase in RSNA (Fig. 1). As a result, there was no correlation between the fall in plasma osmolality and RSNA ($r^2 = 0.04; P > 0.2$). 5DW infusion also lowered blood pressure in water-deprived rats that had received the V₁ antagonist, although the depressor response was slower in onset than that observed in intact water-deprived rats; i.e., pressure became consistently lower than baseline only after 80 min of infusion (Fig. 2). HR remained unchanged initially but then showed a slight but significant fall by the end of infusion. Thus the increase in RSNA produced by decreases in osmolality in water-deprived rats was prevented by prior V₁-vasopressin blockade, suggesting that the sympathoexcitatory effect of 5DW infusion was due to decreased vasopressin.

**Effect of Isotonic Saline Infusion in Water-Deprived Rats**

The slower infusion of isotonic saline, i.e., three-fourths the rate of the 5DW infusion (protocol 3), decreased plasma protein and hematocrit to the same degree as did the 5DW infusion but had no effect on plasma osmolality (Table 2 and Fig. 4). The lower volume saline infusion had no significant effect on RSNA (Fig. 4) but lowered both HR and MAP significantly after 60 and 100 min, respectively (Fig. 5). When infused at the same rate as 5DW (protocol 4), isotonic saline lowered plasma protein to a significantly greater extent than did 5DW (Table 2). Likewise, the larger saline infusion tended to lower hematocrit to a greater extent than did 5DW (Table 2). The larger saline infusion produced a delayed fall in RSNA that began 50 min after the start of infusion and became significantly lower than baseline 40 min after the start of infusion.
later (Fig. 4). Blood pressure also showed a progressive fall in response to the larger saline infusion, becoming significantly lower than baseline after 50 min (Fig. 5). HR rate fell precipitously throughout the infusion, becoming significantly lower than baseline after 50 min of infusion. Thus a volume load similar to that produced by the 5DW infusion did not significantly affect RSNA in water-deprived rats, although the larger expansion was sympathoinhibitory.

**Effect of 5DW Infusion in Water-Replete Animals**

The 5DW infusion in water-replete, V₁ receptor antagonist-treated rats (protocol 5) produced a small fall in osmolality that became significant only after the full 120-min infusion (Fig. 6). Although there was a tendency for RSNA to rise towards the end of the infusion, the increase never achieved significance. Neither MAP nor HR was affected by 5DW infusion (Fig. 6).

**Effect of Blockade of V₁-Vasopressin Receptors in Water-Deprived Rats on Baroreflex Control of RSNA and HR**

Group means for each of the logistic function parameters determined before and after the V₁ antagonist are shown in Table 3. In contrast to the expectation, V₁-receptor blockade shifted baroreflex control of RSNA towards lower pressures (Fig. 7), as indicated by a significant decrease in the \( \text{MAP}_{50} \) (\( P < 0.05 \)). However, the trend for a similar shift in HR was not significant (\( P = 0.2 \)). No other baroreflex parameter was influenced by V₁-receptor blockade. Thus loss of vasopressin activity due to acute V₁-vasopressin blockade did not increase RSNA or HR independently of the decrease in pressure and baroreflex responses in conscious, water-deprived rats. Therefore, the increase in RSNA after V₁-vasopressin blockade in water-deprived rats can be completely explained by a reflex response to hypotension.

**DISCUSSION**

The present study was performed to investigate whether sustained increases in osmolality can contrib-

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Fig. 5. Effect of isotonic saline infusion on MAP (A) and HR (B) in water-deprived rats. Saline was infused at a rate that produced the same hemodilution as 5DW infusion in protocol 2 (protocol 3; •, \( n = 7 \)) or at the same absolute rate as the 5DW infusion in protocol 2 (protocol 4; ○, \( n = 6 \)). *\( P < 0.05 \) compared with time zero within group.

![AJP-Regulatory Integrative Comp Physiol](http://ajpregu.physiology.org/)
ute to the tonic activity of the renal sympathetic nerve. To address this question, changes in RSNA were evaluated during the acute normalization of chronically elevated plasma osmolality. The novel findings in this study are that 1) infusion of 5DW sufficient to normalize elevated plasma osmolality significantly increased RSNA in water-deprived rats, 2) systemic blockade of vasopressin V1 receptors decreased arterial pressure and increased RSNA in water-deprived rats and prevented any further increase in RSNA during subsequent normalization of plasma osmolality with 5DW infusion, 3) blockade of V1-vasopressin receptors in water-deprived rats did not significantly increase RSNA or HR relative to changes in arterial pressure, and 4) an isotonic volume expansion, equivalent to that produced by 5DW infusion, had no effect on RSNA in water-deprived rats given a V1 antagonist. Collectively, these data fail to document a chronic action of increased osmolality on RSNA, either dependent on, or independent of, vasopressin.

In water-deprived rats, RSNA rose during normalization of elevated plasma osmolality. These data supported the initial hypothesis that increased plasma osmolality tonically suppresses RSNA. However, given the potential sympathoinhibitory effects of vasopressin (7), it was necessary to determine whether the rise in RSNA during normalization of plasma osmolality was related to a decline in circulating vasopressin. Indeed, when rats were treated with a vasopressin V1-receptor antagonist before 5DW infusion, the rise in RSNA no longer occurred. Moreover, the magnitude of the rise in RSNA that resulted from V1-receptor blockade was similar to that seen during 5DW infusion in rats that did not receive the antagonist. These data indicated that RSNA increased when osmolality fell because of decreases in vasopressin; however, they did not reveal whether the mechanism of the increase in RSNA was due to loss of a central sympathoinhibitory effect (2, 6, 23) or due to a reflex increase in RSNA secondary to the depressor effect of V1-receptor blockade. Further experiments indicated that blockade of the actions of endogenous vasopressin at V1 receptors in water-deprived rats did not shift the relationship between arterial pressure and RSNA/HR to the right. More specifically, vasopressin blockade did not elevate RSNA or HR relative to arterial pressure. This finding suggests that the increase in RSNA and HR after V1 blockade or after 5DW infusion in untreated rats is mediated via hypotension-induced reflex effects, rather than the loss of a chronic sympathoinhibitory action of vasopressin.

The possibility that a sympathoinhibitory effect of the volume load produced by 5DW infusion may have masked a direct sympathoexcitatory effect of falling osmolality was also considered. Studies were performed to determine if the same volume load produced by 5DW infusion inhibited RSNA when osmolality was held constant. It was reasoned that if falling osmolality

| Table 3. Effects of V1-vasopressin blockade on baroreflex parameters in water-deprived rats |
|-----------------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                          | RSNA            | HR              | RSNA            | HR              |
|                                          | Control         | V1 antagonist   | Control         | V1 antagonist   |
| Range, %baseline or bpm                  | 289 ± 28        | 255 ± 23        | 263 ± 32        | 270 ± 25        |
| Gain coefficient                         | 0.12 ± 0.03     | 0.11 ± 0.01     | 0.077 ± 0.014   | 0.054 ± 0.004   |
| MAP50, mmHg                              | 112 ± 1         | 100 ± 3*        | 132 ± 4         | 127 ± 4         |
| Asymptotic minimum, %baseline or bpm     | 13 ± 5          | 25 ± 7          | 262 ± 28        | 261 ± 31        |
| Gain, %baseline or bpm/mmHg              | -7.9 ± 1.9      | -6.3 ± 0.9      | -4.2 ± 0.6      | -3.1 ± 0.3      |

Values are means ± SE. bpm, Beats per minute; MAP50, MAP at the midpoint of the curve. n = 6 rats. *P < 0.05.

Fig. 7. Effect of V1-vasopressin receptor blockade on baroreflex control of RSNA (A) and HR (B) (n = 6) in conscious water-deprived rats. Control points (± SE) just before reflex curve generation are represented on the curves. Solid line, before antagonist; dashed line, after antagonist.
had a direct renal sympathoexcitatory effect that was counteracted by a concurrent sympathoinhibitory effect of the volume load, then an equivalent isotonic volume expansion should visibly inhibit RSNA. However, RSNA was not affected by an infusion of isotonic saline that provided the same degree of hemodilution and thus presumably the same degree of intravascular volume expansion, as 5DW. In contrast, when isotonic saline was infused at the same rate as 5DW, the extent of hemodilution was larger than that observed after 5DW infusion, and RSNA was significantly reduced. The significant decrease in RSNA observed after the larger volume of saline infusion likely resulted from cardiopulmonary baroreceptor stimulation. Although RSNA was sensitive to increased intravascular volume, by deduction, these data indirectly suggest that the volume load produced by 5DW infusion was apparently not sufficient to mask a significant sympathoexcitatory effect of falling plasma osmolality. As such, the data do not support the hypothesis that either increased osmolality per se or hypertonicity-induced elevations of vasopressin directly and tonically inhibit RSNA, at least via a short-term reflex mechanism, such as by stimulation of osmoreceptors or vasopressin receptors to activate neuronal pathways. These experiments do not rule out the possibility, however, that chronic increases in osmolality influence the tonic level of RSNA via a mechanism with a longer half-life, such as through changes in protein expression.

The present results confirm that 5DW infusion fails to influence HR, despite the profound bradycardia induced by an equivalent saline load (19). Indirectly, these results suggest that decreases in osmolality from elevated levels increase HR, but this effect is counteracted by the concurrent volume expansion. Therefore, we conclude that a sustained increase in osmolality, independent of effects on vasopressin, tonically suppresses HR.

Results from a previous study using similar experimental protocols as in the present study suggested that water-deprivation increases sympathetic drive to the hindlimbs (19). Specifically, baseline lumbar sympathetic nerve activity (LSNA) in water-deprived rats was found to be a higher proportion of baroreflex maximum compared with water-replete rats. It was concluded that a portion of the sympathoexcitatory effect of dehydration was due to a direct effect of increased osmolality, because normalization of plasma osmolality with 5DW infusion reduced LSNA in both vasopressin-blocked and intact dehydrated rats, whereas isotonic volume expansion had no effect (19). In contrast, water deprivation did not appear to significantly influence baseline RSNA in the present study despite evidence of significant volume contraction. Definitive conclusions about between-group differences in basal nerve activity cannot be made from these multifer recording, because of differences between animals in the number of viable fibers and in nerve-electrode contact. Nevertheless, the fact that elevated osmolality and vasopressin do not seem to chronically influence RSNA and that RSNA as a function of reflex maximum is not altered by water deprivation indirectly suggests that the basal level of RSNA may not be significantly altered. In addition, natriuresis is observed after dehydration and is thought to be an important physiological mechanism to help maintain normal osmolality when water intake is limited (14, 15, 22). However, the natriuretic response is apparently not due to suppression of RSNA, since it is not prevented by renal denervation (18). Thus these data also suggest that RSNA is not significantly suppressed during water deprivation. Nevertheless, the apparent lack of increased RSNA despite volume contraction could be an important mechanism to allow for the excretion of excess sodium during water deprivation. Protection against an excessive increase in RSNA may permit appropriate sodium excretion mediated by some other natriuretic factors such as oxytocin (8, 9).

If acute increases in osmolality decrease RSNA, then why is this action apparently not sustained when osmolality is increased for several hours to days? First, previous studies suggest that the acute decrease in RSNA is mediated, in part, indirectly via increases in vasopressin and/or in arterial pressure, which activate the baroreflex (11, 16, 24). However, the large pressor effect produced by acute hypertonic saline infusion is absent during water deprivation; therefore, RSNA may not be significantly altered during more slowly developing increases in osmolality. Second, under some circumstances, such as during intracerebroventricular or intracarotid infusion of hypertonic solutions in anesthetized animals, it appears that RSNA can be increased by hyperosmolality (3, 21, 25). There is also evidence that increased osmolality in blood perfusing the liver decreases RSNA (16, 17). Therefore, during water deprivation, the actions of these individual sympathoexcitatory and sympathoinhibitory effects may be cancelled with no net change. Finally, because conclusions about the role of osmolality in control of RSNA in the present study must be deduced from the comparison of more than one experimental protocol, it remains possible that a modest effect of chronic increases in osmolality to tonically inhibit (or support) RSNA was not detected.

In summary, the results of the present study do not support the hypothesis that, in conscious rats, increases in either osmolality or vasopressin directly and tonically suppress RSNA.

Perspectives

Data from this study together with our previous work indicate that sympathetic drive to various organs or regions differs dramatically in sensitivity to reflex and circulating sympathoregulatory factors such as vasopressin, plasma osmolality, and the cardiopulmonary reflex. Evidence from the present study indicates that sympathetic drive to the kidney is relatively insensitive to sustained increases in vasopressin and osmolality but is suppressed by acute increases in intravascular volume. Results of our previous study (19) indicate that sympathetic drive to skeletal muscle

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is less sensitive to acute cardiopulmonary reflex activation but is highly sensitive to the stimulatory effects of increased plasma osmolality. At the same time, HR appears to be highly sensitive to cardiopulmonary baroreceptor reflex activation and may also be chronically suppressed by increased osmolality. Anatomical data also support the view that sympathetic outflow to different organs is selectively controlled (for review, see Ref. 20), although much more information is required to specifically delineate the neuronal circuits involved. Nevertheless, from a teleological perspective, the present data suggest that water deprivation chronically stimulates vasopressin secretion and LSNA, while not influencing RSNA, so that near maintenance of arterial pressure and plasma osmolality can occur simultaneously.

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