Effects of right atrial distension on the activity of magnocellular neurons in the supraoptic nucleus

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Grindstaff, Regina R., Ryan J. Grindstaff, and J. Thomas Cunningham. Effects of right atrial distension on the activity of magnocellular neurons in the supraoptic nucleus. Am J Physiol Regulatory Integrative Comp Physiol 278: R1605–R1615, 2000.—A small balloon placed at the junction of the superior vena cava and right atrium was used to stimulate cardiac volume receptors in pentobarbital sodium-anesthetized male rats. Extracellular recordings were obtained from antidromically identified vasopressinergic and oxytocinergic neurosecretory cells of the supraoptic nucleus. Cells were considered sensitive to the stimulus if balloon inflation resulted in a 30% change in firing frequency. Balloon inflation that did not stretch the caval-atrial junction had no significant effect on vasopressin neurons (n = 51, P > 0.05). Stretch of the caval-atrial junction decreased the firing activity in 64 of 83 putative vasopressin neurons (P < 0.01 compared with control). Stretch of the caval-atrial junction influenced the firing activity of only 3 of 26 antidromically activated oxytocinergic neurons, an effect not statistically different from control (P > 0.05). When bilateral vagotomy was performed while recording from vasopressin neurons (n = 5), sensitivity to stretch of the caval-atrial junction was eliminated. Cardiac receptors located at the junction of the superior vena cava and right atrium may be important in regulating the activity of vasopressinergic but not oxytocinergic neurons of the supraoptic nucleus.

vasopressin; oxytocin; cardiac receptor

COMPLEX NEURAL NETWORKS interact with humoral and behavioral systems to maintain body fluid and electrolyte balance. The importance of vasopressin in supporting this balance is well established. Vasopressin is a peptide hormone produced by neurons in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus and secreted into the bloodstream from the posterior pituitary (2, 5, 35). It has been demonstrated that changes in the patterns and firing frequency of magnocellular neurons regulate circulating levels of the hormones they secrete (2, 35). Vasopressin is a potent antidiuretic that acts to increase water reabsorption and sodium chloride excretion at the kidney (5). In addition to acting as a vasoconstrictor, vasopressin also decreases heart rate and cardiac output while altering baroreflex control of sympathetic nerve activity (16).

A separate population of magnocellular neurons of the SON and PVN produce another peptidergic hormone, oxytocin, which also has been linked to body fluid regulation. Although oxytocin is a peptide traditionally associated with lactation (5), plasma oxytocin concentrations increase after hemorrhage (20) and decreased blood pressure (36). Oxytocin also has been shown to have natriuretic properties in the rat (42). It has been suggested that oxytocin may be further involved in mediating a diuresis-natriuresis to volume expansion. McCann and colleagues (14) suggest that increased plasma oxytocin following blood volume expansion acts at the atria to facilitate release of atrial natriuretic peptide. This hypothesis is further supported by evidence that oxytocin applied to an isolated atria increases secretion of atrial natriuretic peptide, a phenomenon blocked by application of an oxytocin antagonist (13).

In the rat, a number of physiological manipulations have been shown to alter the activity of putative vasopressin and oxytocin neurosecretory cells in the hypothalamus (2, 35). Several of the central pathways mediating some of these effects have been identified (2, 35, 38). The receptors and neural pathways responsible for inhibiting vasopressin release following an increase in blood volume are not well understood (38). Often, an increase in blood volume is paralleled by an increase in blood pressure, making the relative importance of cardiac and arterial baroreceptors difficult to distinguish. Even less understood is the effect of an increase in blood volume on the activity of oxytocinergic neurons. Previous electrophysiology experiments that examined the effects of cardiac receptor stimulation or volume expansion on magnocellular neurons did not clearly differentiate between oxytocin and vasopressin neurons (26, 29, 33). Furthermore, none of these studies addressed the central pathways that mediate these effects on hypothalamic neurosecretory cells.

Several laboratories have utilized Fos immunoreactivity, a commonly used indicator of neuronal activity (10), to determine what regions in the central nervous system are affected by volume expansion (3, 9, 30, 34). These studies have yielded conflicting results regarding the effects of volume expansion on SON magnocellular neurons. Previous work in our laboratory suggests that volume expansion is associated with the selective activation of oxytocinergic neurons in the SON (34).
The volume expansion protocol used in that study significantly increased central venous pressure, indicating that cardiac receptors were activated by the stimulus. This led us to hypothesize that activation of cardiac receptors may produce differential effects on hypothalamic magnocellular SON neurons so that the excitability of vasopressin cells is decreased, whereas the excitability of oxytocin cells is increased. Experimental procedures that can more precisely assess the response of both vasopressinergic and oxytocinergic neurons in the SON to activation of cardiac receptors are required to address this question.

In an attempt to directly determine the influence of cardiac receptors that are stimulated by volume expansion, the present study used in vivo electrophysiology techniques to obtain extracellular recordings from identified putative vasopressinergic and putative oxytocinergic neurons of the supraoptic nucleus in the rat. A small latex balloon catheter positioned at the junction of the superior vena cava and right atrium was inflated to stretch cardiac receptors located in that region without altering arterial blood pressure (21). If volume expansion activates cardiac receptors located at the junction of the superior vena cava and right atrium that depress vasopressin and activate oxytocin neurons, selective stimulation of those receptors should yield similar results. Stretch of atrial receptors has been shown to increase release of atrial natriuretic peptide (22), elicit diuresis and natriuresis (22, 23, 37), inhibit drinking (21), decrease vasopressin release, decrease plasma renin activity (37), and inhibit sympathetic nerve activity (25). The present study measured changes in the firing activity of antidromically activated supraoptic neurons in response to an acute balloon inflation. Putative vasopressinergic neurons were identified based on their sensitivity to increases in blood pressure and firing patterns. Neurons that fired continuously and were insensitive to increases in blood pressure were classified as putative oxytocinergic cells (2, 35). We hypothesized that the excitability of vasopressin-secreting cells would be decreased by stretch of the cardiac receptors, whereas the excitability of oxytocin-secreting cells would be increased in response to cardiac receptor activation. Additional experiments were conducted using bilateral vagotomy to determine whether the responses of SON neurons to stretch of the veno caval-atrial junction are vagally mediated.

**MATERIALS AND METHODS**

Adult male Sprague-Dawley rats (Harlan; Indianapolis, IN) weighing 250–350 g were used for electrophysiology experiments. Rats were housed in a temperature and humidity controlled room that was maintained on a 12:12-h light-dark cycle with food and water available ad libitum. Experiments were conducted in accordance with the guidelines of the American Physiological Society and the Society for Neuroscience. All protocols were approved by the University of Missouri Institutional Animal Care and Use Committee.

Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). Catheters [polyethylene (PE)-50, A-M Systems] were placed in the right femoral vein for administration of supplemental anesthesia sufficient to suppress withdrawal reflexes (2–5 mg pentobarbital sodium as needed) and in the left femoral vein for bolus injections of phenylephrine. Blood pressure was recorded via a catheter in the left femoral artery using a pressure transducer connected to preemptifiers (Dalton Electronics Core) and a Pentium computer running Spike2 data acquisition software (Cambridge Electronic Design). Balloons were made from fine latex spread over the tip of PE-10 tubing and sealed with a short piece of Silastic tubing (21). For tests, balloons were inflated using a saline-filled syringe attached to a three-way stopcock. Before the balloon was inserted into the jugular vein, the amount of saline required to yield a 2.5– to 3.0-mm diameter inflation of each balloon was determined. This volume was subsequently used to inflate a balloon during each test. Balloons were inserted in the right jugular vein and advanced to the junction of the superior vena cava and right atrium (21). Inflation of a balloon in the superior vena cava does not affect venous return in a rat inasmuch as the left jugular vein, which is unobstructed, drains into the inferior vena cava rather than the superior vena cava (21).

After catheterization, each rat was tracheotomized and the ventral surface of the brain was exposed using a transpharyngeal surgical approach (6, 7, 31). Once the pituitary and SON were exposed, a bipotential stimulating electrode was placed in the posterior pituitary to antidromically activate neurosecretory neurons in the SON. Neurons were identified as supraoptic magnocellular neurosecretory cells if they were antidromically activated from posterior pituitary stimulation, showing a constant latency at threshold of activation and collision cancellation with spontaneously occurring action potentials (Fig. 1 and Refs. 6, 7, and 31). The bipolar stimulating electrode discharged single 1-Hz suprathreshold current pulses of 1-ms duration with an intensity ≤10 mA to identify single units. Once a unit was identified, the current intensity was reduced to determine the threshold for antidromic activation. Extracellular action potentials from SON neurons were recorded using glass micropipettes (World Precision Instrument) filled with 3 M sodium chloride (resistance 15–45 MΩ). Signals were amplified (DAGAN 2400 extracellular Preamplifier, Minneapolis, MN), low-pass and high-pass filtered, and relayed through a window discriminator to an analog-digital converter (CED 1401, Cambridge Electronic Design). The signal for the window discriminator and the raw signal from the preamplifier were both sent to a Pentium computer running Spike2 data acquisition software.

Antidromically activated supraoptic neurons were classified as either vasopressinergic or oxytocinergic based on their firing characteristics and blood pressure sensitivity (35). Neurons with phasic patterns of activity that were completely inhibited by increases in blood pressure of at least 40 mmHg (phenylephrine, 5 µg/µl) were characterized as vasopressin secreting. All phasic cells recorded were inhibited by increases in blood pressure. Continuously active cells were classified as either vasopressinergic or oxytocinergic based on their response to a similar increase in blood pressure. Neurons whose continuous spontaneous activity was interrupted by an increase in blood pressure of at least 40 mmHg were classified as vasopressinergic. Continuously active neurons whose activity was not affected by a comparable increase in blood pressure were defined as oxytocinergic (35). Cells were tested for baroreceptor sensitivity no more than three times.

Each cell was tested for its sensitivity to stretch of the vena caval junction using ratemeter records to compare the spontaneous firing rate (in spikes/s) before, during a 15-s balloon inflation, and after the stimulus. For phasic neurons, baseline
intravenous 2-methyl-5-hydroxytryptamine maleate (20 µg/kg, the Bezold-Jarish reflex mediated depressor response to vagotomy was confirmed in three rats with the elimination of recordings from the same animal prior to vagotomy. Bilateral were compared with the responses of vasopressin neurons their sensitivity to balloon inflation, and those responses Furthermore, additional vasopressin neurons were tested for blood pressure sensitivity after both vagi were severed. The same cell was then retested for balloon and phenyl- the vagi. The vagi were cut during the recording of a 5 rats (n = 5). Recordings from vasopressin neurons that were sensitive to balloon inflation were obtained before sectioning of the vagi. The vagi were cut during the recording of a vasopressin neuron, following successful balloon and phenylephrine tests. The same cell was then retested for balloon and blood pressure sensitivity after both vagi were severed. Furthermore, additional vasopressin neurons were tested for their sensitivity to balloon inflation, and those responses were compared with the responses of vasopressin neurons recorded from the same animal prior to vagotomy. Bilateral vagotomy was confirmed in three rats with the elimination of the Bezold-Jarish reflex mediated depressor response to intravenous 2-methyl-5-hydroxytryptamine maleate (20 µg/kg, RBI; Natick, MA). This reflex is produced by the activation of cardiac chemoreceptors and is eliminated by bilateral vagotomy (28).

On completion of the experiment, the chest of the rat was opened and placement of the balloon was visually verified. Each balloon was inflated at least three times with the same volume of saline used during the experiment. This volume of inflation produced an obvious and unmistakable distension of the blood vessel and/or caval-atrial junction. If the balloon could not be visually located because it was in the atrium, brachial vein, or broken, balloon position was confirmed by touch. Inflation of the balloon with the same volume of saline used during the experiment had to stretch the junction of the superior vena cava and right atrium for an animal to be included in the experimental group. If there was no distension of the caval-atrial junction, the position of the balloon was determined visually and by touch. Balloon position was noted and if the balloon was not at the junction of the superior vena cava and right atrium, or if the balloon did not inflate, then the animal was placed in the control group.

Statistics

All statistical tests were made using commercially available software (SigmaStat 2.03, Jandel Scientific). The effect of balloon inflation on each cell type was determined using a 2 × 2 Fisher exact test, which compared the sensitivity of a cell to stretch of the junction of the superior vena cava and right atrium to a theoretical distribution. This distribution represented the null hypothesis that none of the cells would be affected by balloon inflation (39). The P value for statistical significance for these tests was adjusted for the number of tests using a Bonferroni correction (27). The effect of balloon inflation before and after bilateral vagotomy was determined using a 2 × 2 χ² test.

Baseline firing characteristics, blood pressure sensitivity, balloon sensitivity, and baseline blood pressures were analyzed by one-way ANOVA followed by Tukey test for multiple comparisons when necessary. When statistical analysis indicated the assumption of normality was violated, the nonparametric Kruskal-Wallis one-way ANOVA was performed followed by Dunn’s method of all pairwise multiple comparison procedures.

RESULTS

Data were obtained from a total of 193 spontaneously active, antidromically identified SON neurons. Latencies of antidromic activation ranged from 6 to 28 ms, and thresholds for antidromic activation ranged from 0.7 to 9.7 mA with a mean antidromic activation of 3.3 ± 0.3 mA. Seventy-seven of the antidromic neurons were phasically active, blood-pressure-sensitive vasopressin cells, 71 were continuously active, blood pressure-sensitive vasopressin cells, and 45 were continuously active, blood pressure-insensitive oxytocin cells. As many as 12 neurons were recorded from a single animal.

Rats were divided into two groups for all statistical analyses. The first group was composed of all animals with a balloon catheter positioned so that inflation stretched the junction of the superior vena cava and right atrium (n = 26). The second group consisted of all rats whose balloon catheters were placed so that the caval-atrial junction was not stretched by a comparable inflation (n = 23). In the latter group, 5 rats had
baloons in the superior vena cava, 4 rats had balloons in the right brachial vein, 12 rats had balloons below the caval-atrial junction in the right atrium, and 2 rats had balloons that would not inflate.

As previously reported (21), inflation of a balloon at the junction of the superior vena cava and right atrium has no significant effect on arterial blood pressure. Baseline, stimulus, and recovery blood pressure readings for all balloon tests were 79.9 ± 1.7, 75.3 ± 1.8, and 78.4 ± 1.7 mmHg, respectively (n = 109, ANOVA P > 0.05).

Rats With Balloon Placement at Junction of Superior Vena Cava and Right Atrium

Extracellular recordings from 37 phasically active and 46 continuously active, antidromically identified putative vasopressin neurons in the SON were obtained from rats that had correct balloon placement. Twenty-six continuously active oxytocinergic neurons in the SON were recorded from the same animals.

Phasic vasopressin neurons. Inflation of a latex balloon to stretch the caval-atrial junction decreased the firing of 32 of 37 phasic vasopressin neurons. Based on our statistical analysis, the number of phasic neurons affected by balloon inflation at the caval-atrial junction was significantly greater than the expected percentage predicted by the null hypothesis (Table 1, Fisher exact, P < 0.01). Seventy-eight percent (25 of 32) of the phasic vasopressin neurons that were sensitive to the balloon were completely shut off by stretch of the caval-atrial junction (Fig. 2A, Table 2). The activity of the remaining phasic neurons (7 of 32) were partially decreased by balloon inflation by an average of 53.0 ± 6.5% (Table 2).

Phasic vasopressin neurons were grouped based on their response to balloon stimulation, and their baseline firing characteristics were examined (Table 2). The baseline firing frequency of neurons completely shut off by balloon inflation was significantly lower than the baseline firing frequency of neurons that were unresponsive to balloon inflation. Firing rates of phasic vasopressin cells that were only partially affected by balloon inflation were not significantly different from the other groups. There were no other significant differences in baseline firing characteristics. Baseline blood pressures during recording of the different cell types did not significantly differ among groups (83.7 ± 3.9 mmHg completely sensitive to caval-atrial stretch, 76.2 ± 7.8 mmHg partially sensitive to caval-atrial stretch, and 85.3 ± 4.4 mmHg insensitive to caval-atrial stretch).

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Balloon at Junction of Superior Vena Cava and Atrium (n = 26)</th>
<th>Balloon not at Junction of Superior Vena Cava and Atrium (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Insensitive</td>
</tr>
<tr>
<td>Phasic vasopressin</td>
<td>32*</td>
<td>5</td>
</tr>
<tr>
<td>Continuous vasopressin</td>
<td>32*</td>
<td>14</td>
</tr>
<tr>
<td>Continuous oxytocin</td>
<td>3</td>
<td>23</td>
</tr>
</tbody>
</table>

Number of animals (n) is indicated with the group label. *Number of cells sensitive to caval-atrial stretch is significantly different from the null hypothesis and from the cells recorded from rats where the balloon was not placed at the caval-atrial junction (P < 0.01, Fisher exact).

Fig. 2. Ratemeter record (top) and corresponding blood pressure tracing (bottom) for 2 vasopressin neurons with phasic firing patterns. The activity of each cell was inhibited by a transient increase in blood pressure (phenylephrine 5 µg iv; solid box). A: recorded from an animal with a latex balloon positioned at the junction of the superior vena cava and right atrium. On distension of the balloon (open box), the firing activity of the neuron was completely inhibited. B: recorded from an animal with a latex balloon positioned inside the right atrium. Balloon distension (open box) did not affect the firing frequency of this cell.
Continuous vasopressin neurons. Similar results were observed from continuous vasopressin neurons. Stimulation of the caval-atrial junction with balloon inflation decreased the excitability of 32 of 46 vasopressin neurons with continuous firing patterns. The number of continuous vasopressinergic neurons affected by balloon inflation at the junction of the superior vena cava and right atrium was significantly greater than the expected percentage predicted by the null hypothesis (Table 1, Fisher exact, \( P < 0.01 \)). Fifty-nine percent (19 of 32) of the continuous vasopressin neurons that were sensitive to balloon inflation were completely shut off by stretch of the atrial junction (Fig. 3A). Firing frequencies of the remaining continuous vasopressin neurons (13 of 32) decreased by 44.3 ± 3.2% (Fig. 3B).

Table 3 provides a summary of baseline firing activity of continuous neurons that are subdivided by balloon sensitivity and the blood pressures during the recordings. The baseline firing of cells partially depressed by balloon inflation was significantly higher than the firing of cells unresponsive to balloon inflation. The firing rate of continuous vasopressin cells that were shut off by balloon inflation was not significantly different from either of the two other groups. Baseline blood pressures did not statistically differ among these groups.

Continuous oxytocin neurons. Stretch of the caval-atrial junction affected the firing frequency of only 3 of 26 continuously active oxytocinergic neurons (Fig. 4). All of these neurons were insensitive to increases in blood pressure of at least 40 mmHg. Two of the oxytocin neurons that were affected by balloon inflation increased their activity (average increase of 39.2 ± 3.9%), whereas the activity of the remaining neuron was decreased (30.5%). Stretch of the caval-atrial junction did not have a statistically significant effect on the firing of oxytocinergic cells compared with the expected distribution (Table 1, Fisher exact, \( P > 0.05 \)).

Blood pressure sensitivity to caval-atrial stretch. In an effort to determine whether there was a relationship between sensitivity of a vasopressinergic neuron to stretch of the atrial junction and its response to an increase in blood pressure, phasic and continuous vasopressin neurons were divided based on the magnitude of their response to a balloon inflation as previously described. The sensitivity of neurons to an increase in blood pressure, as described by the time necessary for a cell to shut off, the time a cell was inhibited, blood pressure at shut off, and blood pressure at recovery, was compared among groups for phasic and continuous vasopressin cells (Table 4). No significant differences were found among the groups of phasic cells. For the continuous vasopressin cells, the level of blood pressure at recovery for the neurons shut off by caval-atrial stretch was significantly lower than the other two groups of cells (Table 4). Continuous vasopressin

<table>
<thead>
<tr>
<th>Response to Stretch</th>
<th>Firing Rate, spikes/sec</th>
<th>Blood Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completely Inhibited</td>
<td>6.1 ± 0.6</td>
<td>70.9 ± 4.4</td>
</tr>
<tr>
<td>Partially Inhibited</td>
<td>7.0 ± 0.6*</td>
<td>75.1 ± 4.4</td>
</tr>
<tr>
<td>Unresponsive</td>
<td>5.1 ± 1.1</td>
<td>80.8 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \) = no. cells. All of these measurements were taken from ratemeter recordings of spontaneously active neurons prior to any stimuli. *Significant difference from the unresponsive cells (\( P < 0.05 \), Kruskal-Wallis one-way ANOVA and Dunn’s method for pairwise multiple comparisons).
neurons that were depressed by caval-atrial stretch also had a significantly lower blood pressure at shut off than cells that were not sensitive to caval-atrial stretch (Table 4). Continuous vasopressin neurons that were partially sensitive to caval-atrial stretch had acceleration latencies that were significantly greater than neurons insensitive to balloon inflation (Table 4).

It should be noted that individual neurons of the same type (phasic vasopressin, continuous vasopressin, and oxytocin) recorded from the same animal often responded differently to balloon inflation.

### Table 4. Description of baroreceptor sensitivity of vasopressin neurons from rats with balloons at the right vena caval-atrial junction

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>Sensitive to Balloon Inflation</th>
<th>Partially Sensitive to Balloon Inflation</th>
<th>Insensitive to Balloon Inflation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phasic vasopressin neurons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cells</td>
<td>25</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Acceleration latency, s</td>
<td>4.6 ± 0.7</td>
<td>4.6 ± 1.1</td>
<td>6.3 ± 1.1</td>
</tr>
<tr>
<td>Time of inhibition, s</td>
<td>60.1 ± 8.2</td>
<td>53.6 ± 13.3</td>
<td>65.8 ± 12.9</td>
</tr>
<tr>
<td>Blood pressure at shut off, mmHg</td>
<td>126.0 ± 6.2</td>
<td>143.3 ± 6.5</td>
<td>153.7 ± 7.0</td>
</tr>
<tr>
<td>Blood pressure at recovery, mmHg</td>
<td>107.4 ± 5.3</td>
<td>99.6 ± 10.6</td>
<td>96.6 ± 5.5</td>
</tr>
<tr>
<td><strong>Continuous vasopressin neurons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cells</td>
<td>19</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Acceleration latency, s</td>
<td>6.3 ± 1.1</td>
<td>3.5 ± 0.3†</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>Time of inhibition, s</td>
<td>63.2 ± 18.5</td>
<td>39.3 ± 6.5</td>
<td>43.5 ± 20.4</td>
</tr>
<tr>
<td>Blood pressure at shut off, mmHg</td>
<td>111.2 ± 8.6†</td>
<td>128.0 ± 6.4</td>
<td>145.4 ± 6.6</td>
</tr>
<tr>
<td>Blood pressure at recovery, mmHg</td>
<td>89.0 ± 5.2‡</td>
<td>113.8 ± 8.5</td>
<td>129.5 ± 7.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. cells. Acceleration latency is the time from the beginning of the increase in blood pressure to when the neuron stops firing. *significant difference between cells completely sensitive to balloon inflation and cells not sensitive to balloon inflation (P < 0.05, one-way ANOVA and Tukey test for pairwise multiple comparisons). †Significant difference between cells completely sensitive to balloon inflation and cells partially sensitive to balloon inflation and ‡significant difference between cells partially sensitive to balloon inflation and cells insensitive to balloon inflation (P < 0.05; Kruskal-Wallis one-way ANOVA and Dunn’s method for pairwise multiple comparisons).

Bilateral vagotomy. Vagotomy was performed on five rats that had correct placement of a latex balloon at the junction of the superior vena cava and right atrium. Prior to vagotomy, 20 of the 22 vasopressin neurons recorded (10 phasic, 12 continuous) were sensitive to balloon inflation. During the recording of five phasic neurons that were sensitive to caval-atrial stretch, bilateral vagal stimulation was performed. The vagi were severed during the extracellular recording of either a phasic (n = 3) or continuous vasopressin neuron (n = 2); this eliminated the balloon sensitivity of the neurons while leaving the phenylephrine sensitivity intact (Fig. 5). Fourteen vasopressin neurons (6 phasic, 8 continuous) were recorded from after vagotomy. All of those vasopressin cells were insensitive to balloon inflation but were still inhibited by an increase in blood pressure induced by phenylephrine infusion. There was a significant effect of vagotomy to eliminate the sensitivity of vasopressin cells to balloon inflation ($\chi^2$, P < 0.01). Vagotomy did not significantly alter the baseline firing rates of vasopressin neurons (5.6 ± 0.4 spikes/s before vagotomy vs. 6.8 ± 1.3 spikes/s after vagotomy). There was no significant effect of bilateral vagotomy on resting blood pressure (77.5 ± 9.9 mmHg before vagotomy vs. 79.3 ± 12 mmHg after vagotomy).

Rats With Balloon Placement Not at Caval-Atrial Junction

The percentages of phasic (Fig. 2B) and continuous vasopressin neurons (Fig. 3C) affected by balloon inflation outside of the vena caval-atrial junction were not significantly different from the null hypothesis (Fisher exact, P > 0.05). Furthermore, the percentage of phasic and continuous vasopressin cells affected by balloon inflation at the junction of the superior vena cava and right atrium was significantly different from the percentage of vasopressin cells affected by balloon inflation outside of this junction (Table 1, Fisher exact, P < 0.05). The effect of balloon inflation outside of the caval-atrial junction on the activity of continuous oxytocin neurons did not statistically differ from the null hypothesis (Fisher exact, P > 0.05) or the effect of stretch of the caval-atrial junction on the activity of continuous oxytocin neurons (Table 1, Fisher exact, P > 0.05). There was likewise no significant effect of balloon inflation on systemic blood pressure (baseline 81.3 ± 1.7, inflation 76.6 ± 1.9, and recovery 79.7 ± 1.8 mmHg; n = 89; Kruskal-Wallis, P > 0.05).

Of the antidromic neurons recorded from control animals, 5 of 34 phasic vasopressin neurons and 1 of 17 continuous vasopressin neurons were sensitive to balloon inflation. The firing frequencies of four phasic neurons were decreased (51.9 ± 16.2%). The remaining phasic neuron and the continuous vasopressin neuron showed increases in activity (37.5 ± 7.2%) in response to balloon inflation that were accompanied by decreases in blood pressure (Δ17.5 ± 3.5 mmHg). The aforementioned control cells that showed a decrease in firing activity following balloon inflation were recorded from three animals who each had a balloon placed inside the right atrium, below the vena caval junction.
Firing frequencies of other vasopressin neurons (n = 9) from the same rats were unaffected by the same magnitude of balloon inflation.

DISCUSSION

The ability of atrial stretch receptors to modulate circulating vasopressin levels has been known for decades (38). Although cardiac receptors may not solely account for the physiological response to an increase in volume, volume expansion stimulates receptors at the caval-atrial junction and those receptors may mediate a portion of the aforementioned responses. The results of our study demonstrate that stretch of the junction of the superior vena cava and right atrium decreases the excitability of a significant number of both continuously and phasically firing vasopressinergic supraoptic neurosecretory cells. This demonstrates that the activity of both types of vasopressin neurons is regulated by atrial receptors. The same stretch does not affect the firing rates of a significant number of supraoptic oxytocinergic neurosecretory cells. This signal is neurally mediated because bilateral vagotomy completely abolishes the effect of caval-atrial stretch on vasopressin neurons.

We examined the baseline firing characteristics and blood pressure sensitivities of vasopressin neurons to determine whether these parameters were related to the response to caval-atrial stretch. Phasic vasopressin cells that are shut off by stretch of the right caval-atrial junction have a significantly lower firing rate than cells...
that are unresponsive to caval-atrial stretch. This would suggest that phasic neurons with a lower basal activity are more sensitive to cardiac receptor activation. In contrast, continuous vasopressin neurons that are partially sensitive to caval-atrial stretch have a higher firing rate than continuous vasopressin neurons that are unaffected by caval-atrial stretch. Surprisingly, there is no significant difference in the firing rate of continuous vasopressin neurons completely depressed by cardiac stimulation and the firing rates of neurons in the other two groups. Although significant differences exist in the blood pressure sensitivity of continuous vasopressin neurons with different balloon sensitivities, those differences are not consistent among all groups. The continuous vasopressin cells that are more sensitive to increases in blood pressure appear to be more sensitive to caval-atrial stretch. This does not hold true for phasic neurons, perhaps because of the relatively small sampling of cells in the groups that are partially sensitive and insensitive to caval-atrial stimulation. Therefore we cannot conclude that the response of vasopressin neurons to caval-atrial stretch is related to baseline firing characteristics, resting blood pressure, or blood pressure sensitivities of the cells.

Previous studies have examined the effects of cardiac receptor stimulation (26, 29) and volume expansion (33) on the activity of magnocellular hypothalamic neurons in different animal models. These studies report that activation of atrial receptors decreases the excitability of magnocellular neurosecretory cells in both the dog and cat (26, 29). It is not clear whether the decreased excitability of magnocellular neurons was selective to vasopressin releasing neurons. The earlier studies in the dog (26) and cat (26, 29) did not find differences in the firing patterns of magnocellular neurons like those described in the rat. It subsequently has been determined in vitro that magnocellular neurosecretory cells in the cat lack the regenerative Ca²⁺ currents that are associated with the ability to generate the phasic activity seen in rat magnocellular neurons (11). Whereas similar in vitro experiments have not been conducted on magnocellular neurosecretory cells from the dog, it may not be possible to characterize magnocellular neurons based on their spontaneous activity in either the cat or dog because of differences in cellular properties. The studies by Koizumi and Yamashita (26) and Menninger (29) did test magnocellular neurons with other stimuli. In both of these studies magnocellular neurons that were activated by carotid occlusion, which selectively stimulates the release of vasopressin (2), were decreased by atrial stretch. This suggests that the decreased excitability of magnocellular neurons produced by atrial stretch involved vasopressin neurons in both studies. These data are consistent with the response of vasopressin neurons reported in this paper. Koizumi and Yamashita (26) reported that the activity of nearly all of the cells that they tested was decreased by atrial stretch. This is inconsistent with the response of oxytocin neurons, which we found to be unresponsive to caval-atrial stretch. We would predict that Koizumi and Yamashita would have found more neurons unresponsive to atrial stretch. They may have recorded from portions of the SON and PVN containing primarily vasopressin cells, which would explain why atrial stretch decreased the activity of almost all of the magnocellular neurons that were tested.

Pendlebury et al. (33) recorded changes in the extracellular activity of rat SON neurons after an intragastric volume expansion of 10 ml isotonic saline. The stimulus transiently increased and then decreased the activity of continuously firing neurons, and it produced a delayed decrease in the activity of phasically active neurons. This particular study did not identify continuously firing neurons as secreting either oxytocin or vasopressin. Because Pendlebury et al. (33) recorded cells for several minutes following saline injection, they were measuring changes in activity of neurons in response to gastric distension initially and later to blood volume expansion. Intragastric volume expansion had significantly decreased hematocrit by 50 min, the point when both phasic and continuous neurons exhibited a significant decrease in activity. The existence of two different stimuli may account for the different effects of intragastric saline injection on continuous neurons. It would be expected for gastric distension to increase the activity of oxytocin-secreting cells without changing the activity of vasopressin-secreting cells (2, 35). If the group of continuously firing neurons is composed of both oxytocin and vasopressin cells, it may be that oxytocin neurons are excited by the initial gastric distension, whereas the activity of vasopressin neurons is decreased by the later volume expansion. A decrease in the activity of vasopressin neurons following volume expansion is consistent with our findings.

Our results do not suggest that the cardiac receptors located at the caval-atrial junction are the only carotid pulmonary receptors capable of inhibiting vasopressin neurons. We did not test cardiac receptors associated with the left atrium or ventricle in the present study. We focused on receptors located at the caval-atrial junction because previous studies have demonstrated that these receptors in the rat can be stimulated without interfering with cardiovascular function (21). Furthermore, the activation of caval-atrial receptors in the rat has been shown to inhibit drinking behavior (21), stimulate atrial natriuretic peptide release (22), and elicit diuresis, natriuresis, and kaliuresis (22, 23, 37). Although our data show that caval-atrial stretch decreases the activity of vasopressinergic supraoptic neurons, the diuretic, natriuretic, and kaliuretic responses to activation of these receptors occurs in rats with central diabetes insipidus (23). Thus, whereas the decreased vasopressin release may contribute to the diuretic response to caval-atrial stretch in the rat, these receptors can activate other mechanisms to produce diuresis in the absence of vasopressin.

Stretch of cardiac receptors located at the junction of the superior vena cava and right atrium did not affect the activity of oxytocinergic neurons, although oxytocinergic neurons were clearly activated in the previous
Fos study from this laboratory (34). Narvaez et al. (30) report an increase in Fos expression in vasopressinergic, but not oxytocinergic, cells of the SON, data that also contradict the current study because Fos is expressed following neuronal activation and not inhibition. Still other Fos studies report no difference in Fos expression in the SON in response to a volume stimulus (3, 9). The discrepancies among the aforementioned studies can be explained by a number of factors, including different animal models, different physiological stimuli, anesthetic effects, and/or limitations of the Fos technique (10). The aforementioned diuretic and natriuretic role of oxytocin in the rat is consistent with activation of oxytocin neurons by a volume expansion.

If, as the literature suggests, oxytocin is involved in the response to a volume expansion (13, 14, 34, 42), the lack of activation of oxytocin neurons in this study can most easily be explained by differences in stimuli. Whole body volume expansion would activate a wide variety of receptors that alter release of oxytocin and vasopressin, not only cardiac receptors at the caval-atrial junction. These may include all of the cardiopulmonary receptors, arterial baroreceptors, renal receptors, gastric receptors, and circumventricular organs activated by hormonal changes. An increase in central venous pressure would activate cardiopulmonary receptors located not only at the caval-atrial junction, but also receptors of the atria, ventricles, lungs, and veins, which may account for activation of oxytocinergic neurons. Additionally, afferent renal nerve stimulation has been shown to increase plasma oxytocin levels (40). However, renal nerve activation does not affect the firing activity of SON oxytocinergic neurons (8), instead increasing the activity of PVN oxytocinergic neurons (4). Circumventricular organs may be more likely candidates for activating oxytocin-secreting SON cells, the area postrema (34) showed a significant increase in Fos expression following volume expansion. These regions may be activated by circulating factors that are increased in response to volume expansion such as the peptide hormones atrial natriuretic peptide and adrenomedullin that may in turn influence release of neurohypophysial hormones. Experiments in the rabbit indicate that plasma atrial natriuretic peptide does not significantly increase until right atrial pressure has been increased for 2 min (24). The relatively short duration of caval-atrial stretch used in the current study may be insufficient to adequately increase the circulating levels of these hormones if they are indeed involved in activating oxytocin neurons.

Even if a brief stretch of the caval-atrial junction was sufficient to increase circulating levels of atrial natriuretic peptide, the hormone is not likely responsible for the decreased activity of vasopressin cells observed in the current study. Bilateral vagotomy eliminated the sensitivity of vasopressinergic supraoptic cells to caval-atrial stretch, indicating that the signal is neurally mediated. This finding is consistent with that of Koizumi and Yamashita (26) who also showed that bilateral vagotomy abolished the effect of atrial stretch on magnocellular neurosecretory cells in dogs and cats.

Interestingly, sectioning the vagi in our experiment did not produce an increase in the firing of vasopressin neurons or a significant increase in blood pressure. This is in contrast to findings of other investigators who found vagotomy to significantly increase circulating vasopressin levels as well as arterial pressure in the dog (41). Even 30 min following vagotomy, when Thames and Schmid (41) took blood samples for vasopressin assay, we saw no significant change in the baseline firing rate of vasopressin neurons or in blood pressure, findings similar to those of other investigators in pentobarbital sodium-anesthetized rats (28). Tonic inhibition of both vasopressin and blood pressure may be removed by vagotomy, but because it is compensated for by other mechanisms, the disinhibition is not apparent. Harris (15) reports that the carotid sinus baroreceptors, which remained intact in our experiment, are responsible for mediating the inhibitory effects of an increase in blood pressure on vasopressin cells. This finding suggests that removal of carotid baroreceptors would be required before vagotomy to cause a significant increase in vasopressin and blood pressure in the rat. Indeed, removal of the arterial baroreceptors in the rat acutely increases mean arterial blood pressure and plasma vasopressin (1). Alternatively, vagotomy may have failed to produce an increase in blood pressure because of the anesthetic pentobarbital sodium, a barbituate known to depress cardiovascular reflexes.

This study is a necessary step in defining the neural pathway responsible for decreasing the activity of vasopressin-secreting cells of the SON after stretch of the caval-atrial junction. Several regions of the central nervous system have been suggested to be activated following volume expansion, some of which could be involved in mediating the inhibition of supraoptic vasopressin neurons. An increase in right atrial pressure has been shown to increase the firing of the majority of neurons tested in the nucleus of the solitary tract, which receives all primary visceral afferents (17). The results of Fos studies support the activation of the nucleus of the solitary tract following a volume expansion (3, 30, 34). Although lesions of the parabrachial nucleus affect vasopressin release (32), the extracellular activity of neurons in the parabrachial nucleus does not appear to be altered by right atrial stretch (18). Yet the locus ceruleus, another brain region in the pons that is suggested to be important in arterial baroreceptor regulation of vasopressin release (12), may be involved in mediating the response to atrial stretch because right atrial stretch increases the activity of neurons of the locus ceruleus (19). The locus ceruleus has also been shown to receive projections from medullary neurons that are sensitive to right atrial stretch (43). Another region that showed an increase in Fos after the isotonic saline volume expansion was the perinuclear zone of the SON (34). This area, a GABAergic region surrounding the SON, has been shown to be responsible for a majority of the inhibition of vasopressin neurons following an acute increase in blood pressure (31). It is possible that the perinuclear zone is also responsible for decreasing the excitability of vasopres-
sin neurons following volume expansion or caval-atrial stretch.

Our results indicate that caval-atrial stretch activates a neural pathway resulting in a decrease in the activity of supraoptic vasopressin neurons. Interestingly, Deng and Kaufman (9) do not see an increase in Fos expression in brain regions commonly associated with inhibition of vasopressin. Because large stimuli are necessary to produce a consistent pattern of Fos expression in the brain (10), a discrete stretch of the atrium may be insufficient to produce labeling in all brain regions that are activated. Further studies are needed to determine which brain regions are necessary to relay information from the caval-atrial junction to vasopressinergic neurons of the supraoptic nucleus and ultimately decrease their activity. What, if any, overlap exists between arterial and cardiac baroreceptor regulation of vasopressin release remains to be determined.

Perspectives

In in vivo electrophysiological experiments, vasopressinergic magnocellular neurosecretory cells are characterized by their sensitivity to increases in blood pressure (2, 35). In normal rats, an increase in blood pressure of at least 40 mmHg will inhibit 90–100% of phasic vasopressin neurons (6–8, 12, 31). The current study shows that activation of cardiac receptors at the junction of the superior vena cava and right atrium decreases the activity of most, but not all, vasopressin neurons in the SON. It is clear that a greater percentage of vasopressin neurons is sensitive to an increase in blood pressure than to caval-atrial stretch. Whereas the firing activity of vasopressin neurons is completely inhibited by an increase in blood pressure, the activity of vasopressin neurons that are sensitive to activation of caval-atrial cardiac receptors can be completely or partially decreased by that stimulus. The fact that caval-atrial stretch has a less consistent effect on vasopressin neurons does not necessarily indicate that cardiac receptors are less potent regulators of vasopressin release than arterial baroreceptors because we do not know whether the stimuli used to activate these different receptors are equivalent. An increase in blood pressure is a systemic stimulus, whereas stretch of the caval-atrial junction can only be expected to activate a portion of cardiac receptors. Therefore it becomes difficult to compare the relative influence of pressure and volume stimuli in this model.

Nonetheless, we can conclude that stimulation of cardiac receptors at the caval-atrial junction decreases the activity of most vasopressin neurons in the SON. Whether there is overlap in the central pathways by which blood pressure and blood volume control vasopressin release remains to be determined. Experiments suggest the potential for overlap in the pathways in regions such as the nucleus of the solitary tract (3, 17, 30, 34) and the perinuclear zone of the SON (34), although there is no evidence to date that the decreased activity of vasopressin neurons following caval-atrial stretch is mediated by GABA. The diagonal band of Broca does not show a significant increase in Fos expression following an increase in volume (34), although the diagonal band is necessary for arterial baroreceptor-mediated inhibition of vasopressin (6, 7). Considerable work remains to be done before any conclusion can be made concerning similarities and differences between the two pathways. The possibility that two separate pathways are able to influence vasopressin release could be advantageous for the animal. If the two pathways diverge, they would allow for more precise nonosmotic regulation of circulating vasopressin. For example, during states of volume overload, such as congestive heart failure, pregnancy, and cirrhosis, vasopressin levels could be regulated by acute changes in blood pressure. Conversely, in states of hypertension, vasopressin would still be sensitive to changes in blood volume and thus contribute to body fluid homeostasis.

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REFERENCES


