Baroreceptor Reflex Modulation by Vasopressin Microinjected into the Nucleus Tractus Solitarii of Conscious Rats

LISETE C. MICHELINI AND LENI G.H. BONAGAMB

SUMMARY To determine whether the central vasopressinergic system at the level of nucleus tractus solitarii (NTS) modulates the reflex control of heart rate, we employed a new method for microinjection into the brainstem of conscious, freely moving rats. Baroreceptor reflex function was assessed during pressure changes induced by intravenous administration of phenylephrine (0.25–8 µg/kg) and sodium nitroprusside (0.5–16 µg/kg) in rats microinjected, through a permanent cannula into the brainstem, with saline, arginine vasopressin (AVP), or an AVP blocker. Baseline levels of pressure and heart rate were not changed by either peptide pretreatment. Restricted injection of AVP (20 ng–0.2 µl) into the NTS attenuated the reflex bradycardia during pressure increases, with an upward displacement of the baroreceptor reflex function line (p<0.01) without change in the sensitivity. Local blockade of endogenous AVP, d(CH₃)₅Tyr(Me)AVP (1 µg–0.2 µl), depressed baroreceptor reflex sensitivity with intense bradycardia to either small or large pressure increases. Baroreceptor reflex control of heart rate in response to decreases in pressure was preserved during pretreatment with AVP, whereas endogenous blockade of AVP increased baroreceptor reflex sensitivity. These effects were specific to the NTS, since in another four rats there were no effects when the injections were made 1 mm above, into the cerebellum. The changes in baroreceptor reflex control of heart rate in conscious, unrestrained rats caused by administration of AVP and its endogenous blockade provide evidence that central vasopressinergic synapses at the NTS are important physiological modulators of baroreceptor reflex function. (Hypertension 11 [Suppl I]: I-75–I-79, 1988)

KEY WORDS • central vasopressinergic system • arginine vasopressin antagonist • heart rate reflex control • long-term cannulation of dorsal brainstem areas • microinjection into conscious rats • nucleus tractus solitarii

IT has been shown that circulating arginine vasopressin (AVP) potentiates the sensitivity of the arterial baroreceptor reflex for a given increase in pressure. 1–5 Several studies also indicated that systemic AVP interacts with the central nervous system to modify the reflex control of heart rate5–7 and vascular resistance. 1, 4, 5, 7 Neuroanatomical studies have shown that the brain AVP-synthesizing nuclei, the paraventricular (PVN), supraoptic (SON), and suprachiasmatic nuclei send AVP-containing projections to a number of neural target areas, including the nucleus tractus solitarii (NTS) and dorsal vagal complex (DMV) in the brainstem and the intermediolateral column of the spinal cord, which are directly involved in cardiovascular regulation. 8–10 Little is known about the functional significance of these projections. As the PVN and SON vasopressin-containing projections make axosomatic and axodendritic connections with NTS and DMV in the brainstem,9 one function for the central AVP system may be to modulate the specific neuronal systems involved in reflex control of heart rate (HR) and vascular resistance.

To test this hypothesis, we compared the baroreceptor reflex control of HR during increases and decreases of arterial pressure (AP) in conscious, unrestrained rats microinjected into the NTS with saline, exogenous AVP, and an antipressor blocker of AVP. To evaluate the physiological significance of the results further, we performed the microinjection studies in conscious, freely moving rats. It was therefore necessary to develop and validate a nontraumatic technique for long-term cannulation of the NTS.

Materials and Methods

Cannulation and Microinjection

Male Wistar rats (220–260 g) were anesthetized with nembutal (Abbott Laboratories, Chicago, IL, USA), 40 mg/kg i.p., and placed in a stereotaxic appa-
ratus (David Kopf, Tujunga, CA, USA) with the head in a horizontal position. After exposing the skull, a small window was opened just caudal to the lambda. A unilateral stainless steel guide cannula (17 mm long, 0.6 mm outside diameter) with an angle of 24 degrees was then introduced 1 mm caudal to the interaural line, 0.4 to 0.6 mm lateral (right or left) to the midline, and 8.9 mm below the skull surface. The deep tip of the cannula lay in the cerebellum (Figure 1). The cannula was fixed with fast polymerizing methacrylate and closed by an occluder (17 mm long, 0.4 mm outside diameter). The rats received 60,000 U of penicillin (Pentabi6tico Veterinario, Fontoura Wyeth, Sao Paulo, Brazil) and were allowed to recover for 7 to 10 days.

Microinjection of small volumes (0.1–0.2 μl) of peptides in conscious, unrestrained rats during continuous recording of AP was performed by introducing a 33-gauge needle (18 mm long) connected by PE-10 tubing to a microliter syringe (Model 701-N, Hamilton, Reno, NV, USA) into the guide cannula (see Figure 1). Microinjections lasted 15 to 20 seconds. The needle was then removed and replaced by the occluder.

Arterial Pressure and Heart Rate Measurements and Baroreceptor Stimulation

One day before the experiment, with the rats under ether anesthesia, venous and arterial catheters (PE-10 fused to PE-50 polyethylene tubing, Clay Adams, Parsippany, NJ, USA) were implanted through femoral vessels in order to inject drugs and measure AP (Statham P23DB transducer, Hato Rey, PR, USA). The catheters were tunneled subcutaneously to the back of the neck. Baroreceptors were stimulated by progressive intravenous injections (0.1 ml) of phenylephrine (PE), 0.25 to 8 μg/kg, to raise AP, or unloaded with sodium nitroprusside (NP), 0.5 to 16 μg/kg, to decrease AP. Both AP and HR were recorded continuously (Model 7754A, Hewlett-Packard, San Diego, CA, USA), and control and peak changes for each response were measured. Instantaneous HR was determined by the number of pulses of AP in 1 second. Injections of PE and NP were made in random order; subsequent injections were not made until the recorded parameters returned to preinjection levels. The injection of vehicle (saline) alone did not change the recorded parameters.

Experimental Protocol

In the first experimental session an initial period of 30 to 45 minutes of continuous recording was allowed for the stabilization of AP and HR of the conscious rat. Vehicle, 0.2 μl saline, was injected into the NTS, and the baroreceptor control of HR was evaluated from the 5th to 30th minute (Saline-1 test). Twenty to 30 minutes afterward, AVP (Arg8-vasopressin, Vega Biochemicals, Tucson, AZ, USA), 20 ng in 0.2 μl saline, was microinjected into the NTS, and the baroreceptor reflex response to PE and NP was again tested. Recording was then discontinued.

In the second experimental session, performed at least 24 hours later, we sought to determine the effect of endogenous AVP on modulation of baroreceptor reflex functions. The procedure was exactly the same as the first, except that an AVP antagonist (AVPa) specific to V1 receptors, d(CH2)5Tyr(Me)AVP (Bachem Inc., Torrance, CA, USA), 1 μg in 0.2 μl of vehicle, was injected at the same site after a second saline test (Saline-2). The dose of AVP that had been administered centrally in the first session was injected intravenously before and after the AVPa pretreatment. Two successive microinjections, 40 to 60 minutes apart, of 0.2 μl saline into the NTS did not change the baroreceptor control of HR (n = 3).

In other rats, the same amount of AVP was injected into lobule 9 of the cerebellum (CER group), less than 1 mm above the NTS (see Figure 1).

Histological Analysis

To determine the exact injection site, 0.2 μl of Evans blue was microinjected at the end of the experiment. The rat then received a transcardiac perfusion with 20 ml of saline followed by 10% buffered forma-
lin. The brain was removed and 50-µm serial, frozen, coronal sections of the brainstem were cut for histological verification.

Statistical Analysis

The data are reported as mean ± SE. For each microinjection session the relationship between changes in mean AP (MAP) and associated changes in HR was assessed by linear regression analysis. The regression coefficient (slope) was taken as an index of the sensitivity of the reflex. The slopes of all ΔHR × ΔMAP regression lines were significant except for the PE-induced increases in pressure after AVP injections in the NTS group. Significance of the differences between treatments and groups was evaluated by paired or unpaired t tests as appropriate. A level of p < 0.05 was considered significant.

Results

Efficacy of the Long-term Cannulation Technique

Using this technique we successfully microinjected drugs in 17 (81%) of 21 cannulated rats, 13 of which were used in this study (NTS + CER groups). Four rats were not included because of different sites of injection. Of the four rats not microinjected, two died during recovery period, one had problems with arterial cannulation, and in one the guide cannula became loose.

Baseline MAP and HR Before and After AVP or AVP Antagonist Administration

Mean baseline MAP and HR for the two groups of freely moving cannulated rats are shown in Table 1. The MAP and HR were similar for the NTS and CER groups. Microinjection of AVP or AVPα restricted to the medial part of the NTS (mNTS; see Figure 1) did not change baseline levels of MAP and HR, although a transient decrease in HR (on average 10.3%) was observed in six of nine rats 2 minutes after AVP administration. When injected into cerebellum, AVP caused a small but persistent increase in MAP (8 ± 1 mm Hg; p < 0.05).

Baroreceptor Reflex Responses to Phenylephrine-Induced Increases in MAP

Intravenous injections of PE that increased MAP resulted in dose-related decreases in HR (Figure 2). Comparable increases in MAP after restricted injections of AVP into the mNTS resulted in smaller decreases in HR, thus causing an upward displacement of the regression line for changes in HR as a function of changes in MAP. As shown in Table 1, the intercept of ΔHR × ΔMAP increased significantly (p < 0.01) with no change of the slope (p > 0.05). For the small pressure changes (up to 15 mm Hg; see Figure 2) there was no reflex bradycardia, which had been observed in saline-treated rats (3 ± 5 and −2 ± 6 beats/min vs −13 ± 2 and −27 ± 2 beats/min during the control period, for increases of 6 ± 1 and 15 ± 1 mm Hg, respectively).

In contrast, when the same rats were microinjected with AVPα, small MAP increases caused greater bradycardia (−44 ± 7 beats/min for 15 ± 1 mm Hg) than in the AVP or saline-treated rats. The increase in bradycardia was small as the changes in pressure became larger. Thus both the intercept and the slope of the ΔHR × ΔMAP regression line were significantly decreased. The AVPα in the mNTS also blocked the effect of exogenous AVP at the same site in four rats further microinjected with AVP 40 to 60 minutes after AVP pretreatment. When applied intravenously, AVP (20 ng in 0.1 ml of saline) caused hypertension and bradycardia (14 ± 3 mm Hg and −23 ± 10 beats/min, n = 5), which were not affected by central blockade of AVP since similar values were obtained after administration of AVPα into mNTS (14 ± 1 mm Hg and −23 ± 4 beats/min, respectively).

The specificity of the effect of AVP at the mNTS was confirmed in four other rats by AVP microinjection into the cerebellum (see Figures 1 and 2). Baroreceptor reflex function of the heart was the same before and after AVP treatment. No significant changes of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>ΔMAP (beats/min)</th>
<th>ΔHR (beats/min)</th>
<th>Intercept</th>
<th>Slope (beats/min)</th>
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<tr>
<td>AVP</td>
<td>127 ± 5</td>
<td>376 ± 12</td>
<td>−2.17 ± 0.38</td>
<td>−10.18 ± 12.87</td>
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<td>−10.18 ± 12.87</td>
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<td>−10.18 ± 12.87</td>
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<td>CER group</td>
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<tr>
<td>Saline-1</td>
<td>119 ± 6</td>
<td>340 ± 22</td>
<td>−1.78 ± 0.26</td>
<td>−3.04 ± 0.58</td>
<td>−1.53 ± 0.27</td>
<td>−5.59 ± 5.74</td>
<td>−3.40 ± 0.58</td>
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Values are means ± SE. PE = phenylephrine; NP = nitroprusside; MAP = mean arterial pressure; HR = heart rate; NTS = nucleus tractus solitarii; AVPα = AVP antagonist; CER = cerebellum. *p < 0.05, †p < 0.01, compared to the appropriate saline treatment.
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either the intercept or the slope were observed in the CER group (see Table 1).

Baroreceptor Reflex Responses to Nitroprusside-Induced Decreases in MAP

The sensitivity of baroreceptor reflex-mediated increases in HR was comparable during saline and AVP treatment in both groups of rats (see Figure 2). The slope and the intercept of the regression lines of increases in HR as a function of decreases in MAP after AVP microinjection into the mNTS were similar to those of the control period (see Table 1). We could not calculate the mean slope and mean intercept of the CER group because we had only few responses to NP for each rat. However, for the group as a whole, the average slope and intercept (calculated using all responses obtained in the four rats) were -2.79 beats·min⁻¹·mm Hg⁻¹ and 11.72 beats/min, which were similar to the mean values obtained during saline injection. In contrast, blockade of AVP restricted to mNTS caused a significant change in the baroreceptor reflex function of the heart during decreases in pressure, thus presenting a steeper slope for the ΔHR × ΔMAP relationship. Both slope and intercept were significantly changed after AVPa administration.

Discussion

In this study we demonstrated a physiological role of central vasopressinergic synapses at the NTS in reflex control of the heart in conscious rats. We made four new observations. First, increased AVP concentration at the mNTS (mimicking increased discharges of AVP neurons at this level) attenuates the baroreceptor reflex-mediated bradycardia during increases in pressure. Second, this modulation has physiological relevance, since blockade of endogenous AVP at the same level decreases the sensitivity of the reflex while increasing the brady cardiac responses to the small pressure changes. Third, administering exogenous AVP into the mNTS does not change the baroreceptor reflex-mediated tachycardia, although its sensitivity is increased by endogenous blockade of the vasopressinergic neurons. Finally, long-term cannulation of NTS (or other dorsal brainstem areas) is a viable technique and opens new opportunities to study the modulation of baroreceptor function by central neuropeptides in conscious animals.

Our finding that baroreceptor reflex inhibition of heart rate is attenuated during increased levels of AVP at the mNTS contrasts with the potentiation of reflex bradycardia13 or arterial baroreceptor reflex25 reported for increased levels of plasma AVP, but agrees with the finding of Ciriello and Calaresu.11 These authors reported that bradycardic response elicited by carotid sinus nerve stimulation was inhibited during simultaneous stimulation of either PVN or SON nuclei, and that bilateral lesions of these areas increased the magnitude of the response. Our results, showing not only buffering of the brady cardiac reflex response by locally applied AVP but also the increase of this response to small pressure changes (associated with suppression of baroreceptor reflex sensitivity) by local blockade of endogenous AVP, demonstrated that the inhibitory influence of the PVN and SON on the cardiac component of the reflex11 is performed by the long descending projections from the hypothalamus8-11 by way of its vasopressinergic synapses at the NTS.8-10 Moreover, the present results emphasize the fact that the level of activity of the baroreceptor reflex of the heart is dependent on the amount of AVP present in the mNTS. It is noteworthy that AVP administration into the mNTS did not change the sensitivity of the reflex (i.e., parallel shift of the ΔHR × ΔMAP relationship; see Figure 2), but appeared to influence the bradycardic response by subtracting to the phasic vagal output (or by adding to the phasic sympathetic output). The increased discharge of AVP neurons at the mNTS may be relevant to the problem of the genesis of the centrally mediated tachycardic response during exercise.

The present data showing different patterns of cardiovascular response to stimulation of the central versus peripheral AVP system are in accordance with other studies.12-16 Hypertension and tachycardia, rather than bradycardia, were observed in response to AVP administered into the NTS of anesthetized rats12 or into the cisterna magna of anesthetized dogs.13 These responses were not abolished by intravenous blockade of AVP,13 and the standard response to intravenously ad-
ministered AVP was reversed to hypotension and tachycardia after an AVP antagonist was infused into the cerebral circulation through the vertebral arteries. The central versus peripheral divergent responses are not unique for AVP; a recent report described hypotension and bradycardia after microinjection of angiotensin II confined to the DMV.

The baroreceptor reflex control of HR to NP-induced decreases in pressure was preserved during administration of AVP into the mNTS. The lack of effect of exogenous AVP in modulating reflex tachycardia was also reported for plasma AVP. This does not indicate that vasopressinergic terminals at the NTS had no action upon HR control during sudden decreases in pressure, since endogenous blockade increased baroreceptor reflex sensitivity; however, its physiological implication remains to be determined.

Unilateral injections of AVP or AVPa into the mNTS did not change baseline AP and HR, although transient changes (2–5 min) were seen. The difference between these results and the MAP and HR changes observed for urethane-anesthetized rats is probably due to the absence of anesthesia, and reinforces the validity of the present technique for the study of cardiovascular reflex control in conscious rats.

In summary, these data demonstrate for the first time that the central vasopressinergic system at the level of mNTS is a physiological modulator of baroreceptor reflex control of heart rate in response to increases in pressure by tonically maintaining the sensitivity of the reflex and by phasically attenuating (during increased discharges of AVP neurons) the reflex bradycardia without changing the reflex sensitivity. Moreover, the feasibility of these experiments in conscious rats opens new opportunities to study the central modulation of reflex control of AP and HR by specific peptidergic neurons.

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L C Michelini and L G Bonagamba

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