Modulation of exercise tachycardia by vasopressin in the nucleus tractus solitarii

DANIEL L. DUFLOTH, MARIANA MORRIS, AND LISETE C. MICHELINI

1Department of Physiology and Biophysics, Instituto de Ciências Biomédicas, University of Sao Paulo 05508–900, Sao Paulo, SP, Brazil; and 2Department of Physiology and Pharmacology, Hypertension Center, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27109

Modulation of exercise tachycardia by vasopressin in the nucleus tractus solitarii. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1271–R1282, 1997.—Our objective was to study the role of vasopressinergic synapses at the nucleus tractus solitarii (NTS) in the modulation of exercise-induced tachycardia. We evaluated the effect of NTS administration of vasopressin (AVP) or vasopressin antagonist (AVPant) on heart rate (HR) and mean arterial pressure (MAP) responses during dynamic exercise in male rats with chronic arterial and NTS cannulas. Sedentary (S) and trained (T) animals were tested at three or four exercise levels (from 0.4 up to 1.4 km/h) after NTS injection of AVP or AVPant 20–30 min before treadmill exercise. Plasma and regional brain levels of AVP were measured in separate groups of S and T rats at rest and immediately after acute exercise. When administered into the NTS, exogenous AVP (20 pmol) caused a small but significant decrease in baseline HR and potentiated the tachycardic response to mild to moderate exercise intensities (on average, increases of 35–46 beats/min over control tachycardia, respectively). No changes were observed in baseline MAP or the exercise-induced pressor responses. There were specific changes in brain stem AVP levels that were related to the exercise treatment. T rats showed a marked increase in dorsal and ventral brain stem AVP content after acute exercise. There were no changes in hypothalamus, median eminence, posterior pituitary, or plasma AVP. These data indicate that vasopressinergic synapses and AVP receptors in the NTS are involved in the potentiation of tachycardiac response to exercise. The vasopressinergic mechanism operates in both S and T rats, but training alters the sensitization of AVP receptors by AVP.

V1 receptors; brain stem; peptides; blood pressure; training; rats

The nucleus tractus solitarii (NTS) in the dorsal brain stem is an important relay station in central cardiovascular control. It receives afferents from peripheral viscero-sensory fibers, from other bulbar and spinal areas, and from several autonomic and endocrine suprabulbar modulatory centers involved in cardiovascular regulation (28), providing anatomic support for a complex control system operating at the NTS level. A wide range of neurotransmitters/neuromodulators was localized in the NTS (27, 41). Immunohistochemical techniques have revealed that arginine vasopressin (AVP) is present in suprabulbar pathways to the NTS (4, 37, 39). Evidence suggests that AVP may have tonic modulatory actions on neural pathways in the NTS. 1) All cardiovascular information from periphery to paraventricular nucleus (PVN), as well as to other integrative forebrain centers, is conveyed via the NTS, which also receives direct projections from the PVN (10, 36); 2) the PVN of the hypothalamus is the only source of AVP projections to the NTS (26, 37, 39); 3) in the NTS, AVP is contained only in fibers (44); 4) these fibers make axosomatic and axodendritic connections with the NTS neurons (44), an arrangement characteristic of a modulatory circuitry; and 5) AVP and its receptors are present in the NTS (8, 29, 40, 42, 44).

In a previous functional study, we showed that AVP administration into the NTS of conscious rats produced a smaller bradycardic response during transient pressure increases without changing the baroreceptor reflex sensitivity (19). Interestingly, with small pressure increases (range of 5–15 mmHg, similar to that observed during dynamic exercise), the AVP treatment abolished the bradycardic response, whereas the blockade of endogenous NTS V1 receptors caused a larger pressure-induced reflex bradycardia (18, 19). These data suggested a possible involvement of vasopressinergic synapses in the NTS in the modulation of heart rate (HR) response during dynamic exercise.

It is accepted that circulatory control during exercise is governed by two main neural mechanisms: a central command, serving as a feed-forward control to set the basic pattern of effector activity, and a feedback control, driven by the mechano- and chemoreceptors from the cardiovascular areas and active muscles (21, 34, 35). However, little is known about the way these mechanisms interact to regulate HR and blood pressure during exercise. Most of the studies on cardiovascular regulation during acute and chronic exercise make reference only to peripheral adaptive responses (16, 21, 34) and not to central nervous system (CNS) effects and/or modulatory mechanisms. An attractive hypothesis is that vasopressinergic synapses at the NTS serve as a link between the feed-forward and the feedback controllers of circulation during exercise.

The objectives of the present study were 1) to determine the effects of exogenous AVP administered in the NTS on the blood pressure and HR responses to treadmill exercise, and 2) to investigate a possible endogenous role of this peptide by blockade of its action during rest.
and exercise, 3) to determine the effect of the exercise paradigm on CNS and plasma AVP levels, and 4) to compare those responses in sedentary (S) and trained (T) rats. The experimental protocol involves a technique developed in our laboratory (19) in which drugs are microinjected into the dorsal brain stem of conscious, freely moving rats. This allows for the evaluation of CNS actions on cardiovascular responses in T and S rats during treadmill exercise.

MATERIALS AND METHODS

Young male Wistar-Kyoto (WKY) rats, weighing 170–200 g at the beginning of the experiments, were housed in Plexiglas cages (4 per cage) and kept in a 12:12-h light-dark cycle with standard laboratory chow and water provided ad libitum. Body weight was measured weekly. All surgical procedures and protocols used were in accordance with Institutional Guidelines for Animal Experimentation.

The rats were initially selected by their ability to walk on a treadmill (4–5 sessions at 0.4 km/h, 0% grade, 10 min/day) and then randomly assigned to T or S groups. T rats followed a progressive training protocol in which they ran on a motor treadmill 5 days/wk for 13 wk. The training protocol was similar to that described by Negrao et al., (25) with some adaptations. It progressed from 0.5 km/h, 0% grade, 20 min/day, at the beginning, reaching 60 min/day on week 2, 15% grade on week 9, and 1.6 km/h on week 10. These parameters were maintained up to week 13. S rats were handled every day and submitted once a week to short 5- to 10-min periods of exercise on the treadmill at 0.4–0.8 km/h, with no grade. This allowed the control animals to become fully habituated to the experimental procedures.

At the end of the 13-wk training period, T and S rats were 5 mo old and weighed 450 ± 8 and 476 ± 5 g, respectively. The rats were submitted to chronic cannulation of the brain stem, according to a technique developed in our laboratory (19). Briefly, the rats were anesthetized with Nembutal (40 mg/kg ip) and placed in a stereotaxic apparatus (Kopf, Tujunga, CA), with their heads in a horizontal position. The skull was widely exposed, one screw was fixed at the parietal bone, and with their heads in a horizontal position. The skull was widely exposed, one screw was fixed at the parietal bone, and the remaining skull parameters were maintained up to week 13. S rats were handled every day and submitted once a week to short 5- to 10-min periods of exercise on the treadmill at 0.4–0.8 km/h, with no grade. This allowed the control animals to become fully habituated to the experimental procedures.

To determine the effect of exercise on CNS and plasma AVP levels, another group of WKY rats of similar age and body weight was used. The rats were randomly assigned to T or S groups. After 13 wk, one-half of S and one-half of T rats were killed by decapitation at rest, whereas the remaining rats were killed immediately after the acute exercise protocol. Mixed blood samples were collected in heparinized tubes on ice (15°C) and centrifuged to separate the plasma, which was stored at −80°C. The brains and posterior pituitary PP were rapidly removed, immediately frozen in liquid nitrogen, and stored at −80°C. The PVN, supraoptic nucleus (SON), median eminence (ME), dorsal and ventral brain stem (DBS and VBS, respectively), and spinal cord (SC) regions were microdissected from frozen brain sections. The tissue samples were weighed and sonicated in methanol-acetic acid (98%-0.02 N), and peptide content was measured in the supernatant. Plasma samples were extracted with the use of acetone precipitation and petroleum ether extraction. Plasma and tissue extracts were measured for AVP by a sensitive and specific radioimmunassay (33). The rabbit antiserum is specific for the amidated peptide, with negligible cross-reactivity with oxytocin or other related peptides. The peptide standard and [125I]-labeled AVP were purchased from commercial sources (Peninsula Labs, Belmont, CA, and DuPont, Boston, MA, respectively).

All data are presented as means ± SE. Data are reported as changes of MAP and HR from rest values for S and T rats during exercise and recovery. A three-way analysis of variance (treatment × exercise intensity × time) with repeated measures on the third factor (BMDP, Statistical Software) was used to compare HR and MAP changes in S and T groups (time course effects). Maximal HR and MAP responses of S and T groups at each exercise load were also compared by two-way interaction. The maximal responses were compared by one-way ANOVA, and post hoc tests were performed using the Student–Newman–Keuls test.
three-way analysis of variance using group \times treatment \times exercise intensity as a factor design. Differences of plasma and CNS AVP levels between groups (S and T) and conditions (rest and exercise) were evaluated by two-way analysis of variance. Tukey's test was used as a post hoc test. The level of significance was $P < 0.05$.

**RESULTS**

Adequacy of the Exercise Protocol

The first goal was to establish an adequate exercise protocol for the study of peptidergic modulation of exercise-induced cardiovascular changes. S male rats ($n = 10$) with chronic NTS cannulas were used in this study. The selected protocol consisted of three to four exercise levels, 0% grade at 0.4, 0.8, 1.1, and 1.4 km/h for 2 min each, with the Veh and/or peptide injected into the NTS. S group was exercised up to 1.1 km/h, and T group was exercised up to 1.4 km/h. We determined that running tests performed with or without Veh administration into the NTS showed similar pressor and tachycardic responses. After Veh treatment, (Veh 1 = 0.2 µl, Fig. 1) the exercise caused gradual, well-defined increases in HR according to the exercise level (mild, moderate, or heavy), with small and maintained increases in MAP (range of 10–20 mmHg). After exercise, the recovery of MAP and HR values to baseline levels was gradual, being completed after 5–10 min. The exercise response was reproducible, with a similar pattern observed after the second Veh injection into the NTS (Veh 2, Fig. 1).

Effects of AVP and $V_1$ Blocker at the NTS on Exercise Tachycardia

S rats. The baseline values of MAP and HR of S rats after the different treatments are shown in Table 1 ($n = 12$). Administration of Veh, AVP, or AVPant into the NTS did not change baseline MAP. Basal HR was decreased after AVP but unchanged after other treatments.

The control MAP response to exercise (after Veh injection into the NTS, Fig. 2) was characterized by an initial increase (with no apparent relationship with the exercise intensity) that was sustained throughout the exercise. The recovery to baseline levels was reached 4–5 min after the exercise had stopped. Neither basal MAP nor MAP response during exercise or recovery is altered by AVP treatment. The HR response was increased gradually in relation to the exercise load: $+50 \pm 9$, $+89 \pm 12$, and $+127 \pm 13$ beats/min at the end of each level, with a more rapid increase observed in the first 5–15 s. The recovery to basal HR values was initially rapid (almost 50% of the response being reversed at 30 s), with the return to the baseline levels attained 5–10 min after the exercise had stopped (Fig. 2 and Table 2).

The administration of 20 pmol of exogenous AVP into the NTS significantly increased the HR response to

![Fig. 1. Values of heart rate (HR, A) and mean arterial pressure (MAP, B) of 10 sedentary (S) rats during rest, exercise at 3 different levels, and recovery. Values were obtained after 2 injections of vehicle (Veh, 0.2 µl each) 60–120 min apart into the nucleus tractus solitarii (NTS). $^\circ$, $^\dagger$, $^\ddagger$; $P < 0.05$ vs. rest, 5 s in each exercise level, and other exercise levels, respectively. b/min, Beats/min.](http://ajpregu.physiology.org/)
exercise: +82 ± 13, +125 ± 10, and +145 ± 10 beats/min over control value for each intensity, which corresponds to elevations over Veh response of 63, 40, and 14% (Fig. 3). Evaluation of HR changes during exercise at the steady-state only showed significant potentiation of the tachycardia at 0.4 and 0.8 km/h (Fig. 3). The effect of AVP administration was long lasting,

Table 1. Baseline values of MAP and HR of S and T rats submitted to treatment with Veh, AVP, or AVP<sub>ant</sub> into the NTS

<table>
<thead>
<tr>
<th>Groups and Treatments</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh 1</td>
<td>12</td>
<td>112 ± 4</td>
<td>337 ± 10</td>
</tr>
<tr>
<td>AVP</td>
<td>12</td>
<td>112 ± 5</td>
<td>311 ± 9&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Veh 2</td>
<td>9</td>
<td>112 ± 2</td>
<td>332 ± 7</td>
</tr>
<tr>
<td>AVP&lt;sub&gt;ant&lt;/sub&gt;</td>
<td>9</td>
<td>116 ± 3</td>
<td>336 ± 7</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh 1</td>
<td>10</td>
<td>116 ± 3</td>
<td>320 ± 5*</td>
</tr>
<tr>
<td>AVP</td>
<td>10</td>
<td>117 ± 3</td>
<td>304 ± 4*&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Veh 2</td>
<td>7</td>
<td>113 ± 3</td>
<td>310 ± 10*</td>
</tr>
<tr>
<td>AVP&lt;sub&gt;ant&lt;/sub&gt;</td>
<td>7</td>
<td>110 ± 3</td>
<td>321 ± 12*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; S, sedentary; T, trained; Veh, vehicle; AVP, vasopressin; AVP<sub>ant</sub>, vasopressin antagonist; NTS, nucleus tractus solitarii; NS, not significant. *Significant vs. S, †significant vs. Veh 1; <i>P</i> < 0.05.

Table 2. Statistical summary of comparisons of MAP and HR responses during different intensities of exercise in S and T rats treated with Veh, AVP, and AVP<sub>ant</sub> in the NTS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exercise Intensity</th>
<th>Time</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>ΔMAP</td>
<td>NS</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.05</td>
</tr>
<tr>
<td>ΔMAP</td>
<td>NS</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>ΔHR</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.01</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>ΔHR</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.001</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>ΔMAP</td>
<td>NS</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.001</td>
</tr>
<tr>
<td>ΔMAP</td>
<td>NS</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>ΔHR</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.001</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>ΔHR</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.001</td>
<td>&lt;i&gt;P&lt;/i&gt; &lt; 0.001</td>
<td></td>
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</tbody>
</table>

For significant interactions of main effects, see text. *<i>P</i> value for main effect shown only if not confounded by the interaction. Δ, Change.

Fig. 2. Changes (delta) from baseline HR (A) and MAP (B) during 3 exercise intensities and recovery in 12 S rats microinjected with Veh 1 and vasopressin (AVP) into the NTS. Significance values are in Table 2.
with evidence for an altered HR response to exercise 1–2 days after the injection. Nevertheless, administration of V₁ antagonist reduced the maximal HR response by 30, 24, and 15% at 0.4, 0.8, and 1.1 km/h, respectively (Fig. 4), without any changes in MAP response. Notice that, after Veh 2, the baseline HR was similar to that observed after Veh 1 and that AVP₃ antagonist administration in the NTS did not affect this value (Table 1).

**T rats.** Baseline MAP in T rats was not different from that in S rats, but HR was significantly lower (Table 1). Similar to results in S rats, the NTS administration of AVP caused a significant decrease in basal HR. Exercise-induced MAP changes in T rats exhibited the same time course observed in S rats. Neither AVP (Fig. 5) nor AVP₃ antagonist (AVP₃ ant) (Fig. 6) altered MAP during exercise or during recovery. On the other hand, AVP administered into the NTS of T rats potentiated the HR response to exercise (Fig. 5), whereas exercise-induced tachycardia was blunted by blockade of V₁ receptors (Fig. 6). This was observed throughout the exercise period, with maximal differences observed at moderate exercise intensity. Again, no change was observed in the pressor response. To further challenge the T group, the exercise intensity was increased to 1.6 km/h in some rats (not shown). This did not further increase the MAP and HR responses already observed at 1.4 km/h.

AVP injection into the NTS caused marked increases in the HR response at the steady state: +59, +50, +18, and +10%; in contrast, AVP₃ antagonist significantly reduced the exercise tachycardia by −28, −30, −19, and −12%, for 0.4, 0.8, 1.1, and 1.4 km/h, respectively (Fig. 7). There was also an interaction among treatments × exercise intensity × time (Table 2). The tachycardia of exercise was significantly potentiated by AVP and significantly blunted by AVP₃ antagonist, and the HR response to Veh 1 was also different from Veh 2. The tachycardiac response after Veh 2 administration was almost identical to that observed immediately after AVP administration into the NTS performed 1–2 days earlier, indicating a pronounced long-lasting effect of AVP that was significantly blunted by the administration of V₁ blocker, bringing it back to initial control values.

The recovery of HR response was affected by the treatment administered into the NTS (Figs. 5 and 6); it was delayed by AVP and facilitated by AVP₃ antagonist (Table 2). There was also an interaction between time and treatment in such a way that 50% and complete recovery occurred earlier after AVP₃ antagonist (30 s and 5–10 min, respectively) and later after AVP treatment (1–2 min and >10 min, respectively).

**Comparison of Vasopressinergic Effects: T × S Rats**

To understand the effects of facilitation/inhibition of vasopressinergic synapses at the NTS on the tachycardia of exercise and the effects caused by training, we compared the maximal HR responses at three different exercise intensities in T and S rats.
exercise intensities in T and S rats treated with Veh, AVP, and AVP_

ant. Table 3 shows that treatment as well as exercise load changed the maximal HR response to exercise and that there was a significant difference in the exercise tachycardia between T and S rats. The comparison of the maximal HR effects (that is, the differential effect of both agonist and antagonist, Fig. 8) shows clearly that the potentiation of exercise tachycar-

![Fig. 5. Changes from baseline HR (A) and MAP (B) during 4 exercise levels and recovery in 10 trained (T) rats microinjected with Veh 1 and AVP into the NTS. Significance values are in Table 2.](image1)

![Fig. 6. Changes from baseline HR (A) and MAP (B) during 4 exercise levels and recovery in 7 T rats microinjected with Veh 2 and AVP_
ant into the NTS. Significance values are in Table 2.](image2)
dia by AVP was observed in both groups and was of similar magnitude. On the other hand, the blunting of tachycardic response by endogenous blockade of V₁ receptors at the NTS was significantly larger in T than S rats (Fig. 8, Table 3). In addition, there was another treatment effect: in T rats, the HR response to exercise after Veh 2 (control response obtained 1–2 days after AVP treatment) was not significantly different from that observed shortly after the single administration of AVP into the NTS (Table 3), showing that the potentiation by AVP was larger and longer in T rats. Maximal MAP responses were not significantly different between groups and not affected by any treatment (Table 3).

Injection Sites

Microscopic examination of the brain stem in S and T rats revealed that, in both, the microinjections were directed mainly to the commissural NTS (Fig. 9). The dye injected apparently followed the structure of the NTS by spreading predominantly in a rostrocaudal direction that extended 517 ± 47 µm in S and 511 ± 41 µm in T groups, with the mean point of injection located, on average, at +155 ± 67 and +158 ± 77 µm rostral to the calamus scriptorius in S and T rats, respectively. As illustrated in Fig. 9, the injection sites were centered in the lateral commissural NTS, but the medial portion of commissural NTS was also microinjected. Additionally, dye stained a portion of the dorsal motor nucleus of the vagus (DMV) in five S and five T rats, but in these rats the tachycardic response after AVP treatment was similar to that observed in rats in Fig. 8.

Table 3. Maximal responses of ΔMAP and ΔHR at different exercise intensities in S and T rats treated with Veh, AVP, and AVPant in the NTS

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>0.4 km/h</th>
<th>0.8 km/h</th>
<th>1.1 km/h</th>
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<tr>
<td></td>
<td>ΔMAP, mmHg</td>
<td>ΔHR, beats/min</td>
<td>ΔMAP, mmHg</td>
</tr>
<tr>
<td>S Veh 1</td>
<td>7 ± 3</td>
<td>50 ± 9</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>AVP 10 ± 2</td>
<td>13 ± 3</td>
<td>130 ± 10</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Veh 2 12 ± 2</td>
<td>16 ± 2</td>
<td>88 ± 11</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>AVPant 10 ± 2</td>
<td>14 ± 3</td>
<td>67 ± 9</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>T Veh 1</td>
<td>14 ± 2</td>
<td>60 ± 9</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>AVP 14 ± 2</td>
<td>21 ± 2</td>
<td>128 ± 10</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Veh 2 15 ± 3</td>
<td>17 ± 4</td>
<td>114 ± 11</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>AVPant 17 ± 4</td>
<td>20 ± 4</td>
<td>81 ± 14</td>
<td>24 ± 5</td>
</tr>
</tbody>
</table>

Fig. 7. HR responses at steady-state exercise during different workloads after AVP and AVPant or Veh 1 (A) or Veh 2 (B) treatments into the NTS of T rats. Treatments were made 20–30 min before acute exercise; agonist and antagonist treatments were made 1–2 days apart. *Significant (P < 0.05) vs. Veh 1 or Veh 2.

Table 3. Values are means ± SE.

Fig. 8. Comparison of maximal HR effects of AVP (A) and AVPant (B) treatments in T and S groups. Bars represent differential effect of agonist (AVP – Veh 1) and antagonist (AVPant – Veh 2) administration into the NTS. *Significant (P < 0.05) vs. S group.
which only the NTS was injected. A small portion of the area postrema was also stained in one T and two S rats.

**Plasma and Brain AVP Levels**

Measurement of regional brain AVP levels (nuclear and terminal projection areas) showed that there were no significant differences between S and T rats under resting conditions (Table 4), although there was a tendency for reduced AVP levels in the DBS of T rats (−86%). After training, basal DBS content was so low (0.02 ± 0.01 pg/mg) that the increment of 0.85 pg/mg observed immediately after acute exercise represented a dramatic increase. AVP content in VBS was also significantly increased (6-fold) after acute exercise in T rats (Table 4). These changes were specific for the brain stem because there were no significant alterations in the AVP concentrations in the PVN, SON, ME, or PP. Plasma AVP levels were not different between the groups nor was there any effect of exercise.

**DISCUSSION**

In the present study, we described and analyzed for the first time the modulatory effect of the NTS vasopressinergic system on the tachycardia of exercise. Several new observations were made. 1) Exogenous AVP administered into the NTS (mimicking increased release in this area) potentiates the HR response during acute exercise; 2) the AVP effect is long lasting and is observed in both S and T rats; 3) endogenous blockade of AVP at the NTS significantly blunts the HR response during exercise, the effect being larger in T rats; and 4) exercise produces an increase in brain stem

**Table 4. Vasopressin content at rest and immediately after acute exercise in S and T rats**

<table>
<thead>
<tr>
<th></th>
<th>Rest (n = 11)</th>
<th>Acute exercise (n = 8)</th>
<th>Rest (n = 7)</th>
<th>Acute exercise (n = 7)</th>
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<tr>
<td>PVN</td>
<td>15.44 ± 5.36</td>
<td>23.53 ± 8.81</td>
<td>25.58 ± 15.62</td>
<td>14.77 ± 6.87</td>
</tr>
<tr>
<td>SON</td>
<td>68.48 ± 12.48</td>
<td>51.80 ± 14.20</td>
<td>60.15 ± 24.69</td>
<td>42.01 ± 12.01</td>
</tr>
<tr>
<td>PP, ng</td>
<td>35.32 ± 7.04</td>
<td>32.19 ± 8.86</td>
<td>52.43 ± 8.99</td>
<td>43.93 ± 9.05</td>
</tr>
<tr>
<td>ME</td>
<td>438.49 ± 135.74</td>
<td>288.30 ± 54.21</td>
<td>345.95 ± 79.75</td>
<td>112.57 ± 34.39</td>
</tr>
<tr>
<td>DBS</td>
<td>0.14 ± 0.12</td>
<td>0.17 ± 0.10</td>
<td>0.02 ± 0.01</td>
<td>0.87 ± 0.32†</td>
</tr>
<tr>
<td>VBS</td>
<td>0.05 ± 0.02</td>
<td>0.16 ± 0.05</td>
<td>0.06 ± 0.04</td>
<td>0.33 ± 0.10†</td>
</tr>
<tr>
<td>SC</td>
<td>0.12 ± 0.05</td>
<td>0.06 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.31 ± 0.15</td>
</tr>
<tr>
<td>Plasma, pg/ml</td>
<td>0.52 ± 0.15</td>
<td>0.12 ± 0.04</td>
<td>0.52 ± 0.27</td>
<td>0.85 ± 0.36</td>
</tr>
</tbody>
</table>

Values are means ± SE in pg/mg, unless otherwise indicated; n = no. of rats. PVN, paraventricular nucleus; SON, supraoptic nucleus; PP, posterior pituitary; ME, median eminence; DBS, dorsal brain stem; VBS, ventral brain stem; SC, spinal cord (near C1 level). *Significant vs. rest, †significant vs. S; P < 0.05.
AVP content, with the greatest change noted in the dorsal brain stem of T rats. The data provide evidence for a role of NTS vasopressin V1 receptors in the genesis of exercise-induced tachycardia.

It is well known that dynamic exercise produces instantaneous but maintained cardiovascular responses, including increases in HR and cardiac output, associated with decreased venous capacitance and redistribution of blood to different beds (regional vasoconstriction or vasodilatation) via neural, hormonal, and local mechanisms (11, 16, 21, 35, 43, 45). According to current theory, the circulatory control during exercise is governed by the central command and feedback control mechanisms. The central command (a feedforward control) sets the basic pattern of effector activity, which is modulated by the arterial baroreceptors and other mechano- and chemoreceptors from cardiovascular areas and active muscles (21, 34, 35). The feedback control systems are centered in the brain stem (NTS, DMV, nucleus ambiguous, rostral and caudal ventrolateral and ventromedial medullas) and are interconnected with higher modulatory centers (18, 28) in such a way that these mechanisms are complementary, exerting their effects on heart and musculature by autonomic effector mechanisms. Although the interactions (afferent/efferent pathways and transmitters involved) are not known, there are data to support a role for brain stem AVP as a neurochemical mediator. First, immunohistochemical studies (4, 26, 37, 39, 44) have shown that vasopressinergic pathways are not restricted to the hypotalamohypophysial system but are widespread throughout the brain, with projections to the limbic system, brain stem, and SC. In the brain stem, immunoreactive AVP fibers are concentrated in the NTS and ventrolateral medulla (4, 26, 37, 39, 44). AVP receptors are also present in this region (29, 40, 42), and there is evidence for local peptide release (13). The anatomic evidence is supported by physiological data that show that brain stem AVP alters blood pressure, HR, and sympathetic activity (15, 18, 19, 41).

The present results support and extend previous findings; they suggest that NTS vasopressin systems are involved in the cardiac response to exercise. Using direct injection of the peptide and its antagonist into the NTS region in conscious rats, we demonstrate potentiation and blunting, respectively, of exercise-induced tachycardia. Furthermore, the measurement of AVP content in this and other brain regions shows that there are anatomically specific changes in content that are related to physiological status. Exercise in the T group led to a marked increase in AVP content in the dorsal (NTS region) and ventral brain stem regions, with no changes detected in the biosynthetic areas or the PVN, SON, or PP. Furthermore, exercise had no effect on plasma AVP, emphasizing the differential changes in central and peripheral AVP systems. It appears that exercise has a greater effect on the paravascular AVP pathways from PVN to brain stem. Interactions between baroreceptor function and peptide content were documented in a previous study (1) in which afferent input to the NTS region was interrupted via surgical denervation. Sinoaortic denervation produced differential changes in hypothalamic and brain stem AVP levels, with an increase in the brain stem and a decrease in the SON and PVN. The key question is whether alterations in peptide content are associated with changes in local secretion. Certainly, the peptide content data are consistent with the cardiovascular results. AVP content was increased significantly in DBS (and VBS) of T rats immediately after acute exercise, and endogenous blockade of AVP caused a significantly larger decrease in exercise tachycardia of T rats (Fig. 8). It should be noted that even the equal HR response of T and S rats after exogenous AVP into the NTS indicates a larger effect in the T group in which the smaller tachycardia at similar workload, an important adaptative effect of training (38), was abolished by AVP treatment.

Histological evaluation of the brain stem of rats submitted to physiological experiments showed that changes in tachycardiac response were obtained only when dorsal injection sites included the NTS. The microdissected DBS region used for the peptide measurements would include the DMV and parts of hypoglossal, gracilis, and cuneatus nucleus, as well as the NTS. Whether the AVP changes are specific for the NTS is not known, although immunohistochemical studies indicate a concentration of fibers within this central area (37, 39, 41, 44). We propose that the modulation of exercise-induced tachycardia by AVP is mediated through synapses in the NTS. This is supported by both the similar HR changes when AVP or its blockade are restricted to the NTS or included in part of the DMV and the time course of the events, because both the AVP potentiation and antagonist-induced attenuation of the HR response appeared 30–60 s after the initiation of exercise, when HR is mainly driven by the sympathetic tone (9, 17, 24). If the effect were caused by a modulation of DMV (also injected in some animals) where some cell bodies of parasympathetic preganglionic neurons are located (most of them arise from the nucleus ambiguous), a larger change would be expected in the first seconds of exercise, when HR increase is mainly due to vagal withdrawal (9, 17, 24, 34). Although we cannot rule out a possible direct effect on DMV, changes in vagal tone are not alike (above evidences), whereas changes in sympathetic tone appear to be the primary mechanism for AVP-induced HR changes during exercise. The central command could regulate sympathetic tone by a combined action on dorsal (afferent input) and ventral (efferent output) brain stem areas. Exogenous AVP into the NTS also decreased baseline HR of S and T rats, an effect that could have been related to the peptide dose. Possible contributions of vagal and/or sympathetic tone to this effect remain to be determined.

It has been proposed that during exercise the central command exerts its effects on cardiovascular parameters by changing or resetting the operating point of the arterial baroreflex, the main feedback controller of the circulation (11). The results of the present experiments suggest that AVP released at the NTS could be one of
the links between the feed-forward and feedback control of circulation during exercise. In a previous study in conscious, chronically cannulated rats (19) we showed that administration of a low dose of AVP into the NTS changed the set point of the arterial baroreceptor reflex by displacing the HR response to higher HR values during transient changes in pressure without changing baroreflex sensitivity. We also showed (19) that endogenous blockade of V₁ receptors at this level completely abolished the sensitivity of the bradycardic response, indicating that the long-descending vasopressinergic projections to the NTS are important for the tonic maintenance of baroreflex sensitivity. In addition, the present results show that AVP acting on V₁ receptors in the NTS contributes to the genesis of exercise-induced tachycardia. It has been shown that baroreflex sensitivity is maintained during exercise in the presence of tachycardia because the reflex is reset to higher blood pressure (7, 35).

As demonstrated by the present data, the modulation of AVP by AVP is mediated through V₁ receptors and is specific for the tachycardic response during exercise, because AVP receptor blockade did not control change levels of HR and MAP (observed during the rest period) nor did it change the pressor response to exercise.

An interesting aspect of the results was the long-lasting effect of AVP on the NTS. Alterations in the maximal HR response during exercise were still observed 1–2 days after peptide administration in S rats (Table 3). In T rats, the potentiation was similar both immediately after the treatment with AVP and 2 days later, showing that 3 mo of training facilitated even more the potentiation of the tachycardic response by AVP. There is evidence that central AVP sensitizes the organism to further stimulation (3, 15, 30). For example, preexposure to a single central AVP injection increases the likelihood of motor disturbances and convulsion (3, 30) and sensitizes pressor effects and efferent sympathetic neural responses (15). As in the present study, motor responses and pressor and NTS sensitization were observed with similar doses (in the ng range), were mediated by V₁ receptors, and lasted from hours to days (30). Lebrun et al. (14) and Poulin and Pittman (30) provided further evidence for V₁-mediated sensitization, because injection of AVP stimulated phosphoinositol (IP₃) metabolism (increased IP₃ release) in septal slices prepared from rats centrally pretreated with AVP. Complementary to these observations, Burnard et al. (5) showed that hemorrhage and hypertonic saline, which are known to stimulate the central release of AVP, mimic the effects of exogenous peptide administration; the motor disturbances produced by acute intracerebroventricular administration of AVP were increased after these treatments. Accordingly, the exercise test causing endogenous release of AVP and performed 1–2 days after exogenous AVP administration in the NTS propitiates the conditions for the sensitization phenomenon. In addition, our results showing larger effects of AVP in T rats indicate that repeated AVP release by daily exercise caused a long-lasting potentiation of AVP-induced sensitization.

It was demonstrated that enhancement of IP₃ release after subsequent AVP administrations occurred without changes in affinity and density of AVP binding sites (30), indicating a postreceptor mechanism. The presence of changes in postreceptor mechanisms does not exclude the possibility that they may be due to a change in V₁ receptor number. Research on the effects of AVP or AVP ant treatment also produced alterations in the recovery of HR response after exercise. This was most prominent in the T rats, in which the effects of AVP during exercise were greatest. Therefore, in the absence of effects of AVP at the NTS, not only was the tachycardic response to exercise smaller, but the recovery, reflecting the gradual decrease of sympathetic and the gradual return of vagal activity (2), was faster. In keeping with the present observations, we hypothesize that, apart from the well-known peripheral cardiovascular adaptations (6, 23, 38), training also alters the mechanisms involved in the integration of the central command with the brain stem feedback control systems. Our results support the hypothesis that the central vasopressinergic system may be one of the mechanisms involved.

We should clarify that the vasopressinergic system at the NTS may not be the only central mechanism involved in the genesis of exercise tachycardia (and/or central adaptations to training) because AVP ant in this area did not block the HR response but only caused a partial blunting. Other peptidergic systems may be involved in the genesis of exercise tachycardia. Kregel et al. (12) showed that corticotropin-releasing factor is involved in the genesis of exercise tachycardia and may not be the only central mechanism involved in the potentiation of exercise tachycardia. As demonstrated by the present data, the modulation of AVP by AVP ant treatment also produced alterations in the recovery of HR response after exercise. This was most prominent in the T rats, in which the effects of AVP during exercise were greatest. Therefore, in the absence of effects of AVP at the NTS, not only was the tachycardic response to exercise smaller, but the recovery, reflecting the gradual decrease of sympathetic and the gradual return of vagal activity (2), was faster. In keeping with the present observations, we hypothesize that, apart from the well-known peripheral cardiovascular adaptations (6, 23, 38), training also alters the mechanisms involved in the integration of the central command with the brain stem feedback control systems. Our results support the hypothesis that the central vasopressinergic system may be one of the mechanisms involved.

In conclusion, vasopressinergic projections to the NTS (probably arising from the parvicellular PVN) are activated during dynamic exercise and could act as a link between the feed-forward and the feedback controllers of circulation. The vasopressin synapses seem to be involved in setting the operating point of the arterial baroreflexes to permit the appearance of tachycardia...
even when the MAP is elevated. This alteration in blood pressure-HR interactions is likely mediated by V1 receptors and is changed under conditions of exercise training.

Perspectives

The present study, which uses a technique developed by us to chronically administer drugs into the dorsal brain stem areas of freely moving rats, uniquely documents the effect of vasopressinergic input to the NTS on exercise-induced changes in cardiovascular function. It is also the first to document the marked increase of endogenous AVP content in dorsal (and ventral) brain stem areas after acute exercise. Both observations suggest that vasopressinergic projections to the NTS are excited during dynamic exercises, determining a higher tachycardic response for the same blood pressure increase. By comparing the exercise-induced changes in brain stem AVP content with HR response after NTS AVP administration or its endogenous blockade in S and T exercising rats, this study bridges the disciplines of exercise physiology and neural control of circulation with biochemical measurements of peptide content in different areas of the brain. It highlights the physiological significance of vasopressinergic synapses in the modulation of baroreceptor reflex control of HR during exercise and, more importantly, opens a new field for the study, during specific behaviors, of the modulatory effects on autonomic functions by different brain stem areas.

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Address for reprint requests: L. C. Michelini, Dept. Fisiologia e Biofisica, ICB-USP, Av. Prof. Lineu Prestes, 1524, 05508–900, Sao Paulo, SP, Brazil.

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