Mechanisms mediating regional sympathoactivatory responses to stimulation of NTS A₁ adenosine receptors

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Scislo, Tadeusz J., and Donal S. O’Leary. Mechanisms mediating regional sympathoactivatory responses to stimulation of NTS A₁ adenosine receptors. Am J Physiol Heart Circ Physiol 283: H1588–H1599, 2002; 10.1152/ajpheart.00897.2001.—Selective activation of adenosine A₁ and A₂a receptors in the subpostremal nucleus tractus solitarius (NTS) increases and decreases mean arterial pressure (MAP), respectively, and decreases heart rate (HR). We have previously shown that the decreases in MAP evoked by NTS A₂a receptor stimulation were accompanied with differential sympathetic responses in renal (RSNA), lumbar (LSNA), and preganglionic adrenal sympathetic nerve activity (pre-ASNA). Therefore, now we investigated whether stimulation of NTS A₁ receptors via unilateral microinjection of N°-cyclopentyladenosine (CPA) elicits differential activation of the same sympathetic outputs in α-chloralose-urethane-anesthetized male Sprague-Dawley rats. CPA (0.33–330.0 pmol in 50 nl) evoked dose-dependent increases in MAP, variable decreases in HR, and differential increases in all recorded sympathetic outputs: ↑ pre-ASNA >> ↑ RSNA ≥ ↑ LSNA. Sinoaortic denervation + vagotomy abolished the MAP and LSNA responses, reversed the normal increases in RSNA into decreases, and significantly attenuated increases in pre-ASNA. NTS ionotropic glutamatergic receptor blockade with kynurenate sodium (4.4 nmol/100 nl) reversed the responses in MAP, LSNA, and RSNA and attenuated the responses in pre-ASNA. We conclude that afferent inputs and intact glutamatergic transmission in the NTS are necessary to mediate the pressor and differential sympathoactivatory responses to stimulation of NTS A₁ receptors.

nucleus of the solitary tract; purinergic receptors; adrenal sympathetic nerve; renal sympathetic nerve

THE NUCLEUS TRACTUS SOLITARIUS (NTS) is the primary integrative center for cardiovascular control and other autonomic functions and contains a great variety of neurotransmitters/neuromodulators (10, 15, 33). Recent studies from our laboratory and by others showed that among the numerous neuroactive substances, adenosine modulates cardiovascular control at the level of the NTS (3, 17, 18, 25–28, 32). Interestingly, NTS contains the highest number of adenosine uptake sites in the central nervous system, and this fact strongly suggests an important physiological role of adenosine in NTS mechanisms (5). A natural source of adenosine in the NTS and other cardiovascular centers is ATP, operating as a neurotransmitter/neuromodulator in these structures, which is rapidly catabolized by ectonucleotidases upon synaptic release (21, 31). Adenosine is also naturally released into the central nervous system, including the NTS, as a result of breakdown of intracellular ATP during hypoxia, ischemia, and severe hemorrhage (20, 34, 37). Adenosine may either inhibit or facilitate neurotransmitter release acting via presynaptic A₁ or A₂a receptors, respectively (21, 29). We have shown that selective stimulation of adenosine A₂a receptors located in the subpostremal NTS, in addition to previously reported decreases in mean arterial pressure (MAP) and heart rate (HR) (1, 3), exerts qualitatively different effects on regional sympathetic outputs (25–28). Stimulation of NTS A₂a receptors decreases renal (RSNA) and postganglionic adrenal sympathetic nerve activity (post-ASNA), whereas it increases preganglionic adrenal sympathetic nerve activity (pre-ASNA) and does not markedly change efferent lumbar sympathetic nerve activity (LSNA) (25–28).

Previous studies have shown that stimulation of NTS A₁ receptors evokes variable hemodynamic effects: it usually increases MAP in a dose-dependent manner, whereas HR slightly decreases (1); however, the depressor effects also have been reported (36). There is also evidence that adenosine A₁ receptors located in the NTS and rostral ventrolateral medulla play a role in hypothalamic defense response facilitating the pressor component (30, 31).

The pressor responses to stimulation of NTS A₁ receptors are likely mediated via increases in efferent sympathetic vasoconstrictor activity; however, this hypothesis has not been tested. Because we observed that stimulation of subpostremal NTS adenosine A₂a receptors evoked a highly diverse pattern of regional sympathoactivatory responses, we felt it was likely that the same neuromodulator operating in the same site of the NTS via its A₁ receptor subtype also elicits differential regional sympathoactivatory responses. Therefore, in the present study, we evaluated the effect of stimulation of NTS A₁ receptors on RSNA, pre-ASNA, and LSNA, i.e., the same sympathetic outputs that were modulated in
a qualitatively different manner by stimulation of NTS A2 receptors in our previous studies (25–28).

The pressor and presumably sympahtoactivatory responses may be a result of A1 receptor-mediated inhibition of glutamate release from arterial and cardio-pulmonary baroreceptor afferents terminating in the NTS. Therefore, in the present study, we also tested the hypothesis that intact afferent inputs to the NTS and intact glutamatergic transmission in the NTS are necessary to mediate pressor and sympathoactivatory responses evoked by stimulation of adenosine A1 receptors. Our results showed that the pressor and differential sympahtoactivatory responses evoked by stimulation of NTS adenosine A1 receptors are mediated mostly via modulation of afferent glutamatergic transmission to the NTS.

MATERIALS AND METHODS

All protocols and surgical procedures employed in this study were reviewed and approved by the institutional Animal Care and Use Committee and were performed in accordance with the Guiding Principles in the Care and Use of Laboratory Animals endorsed by the American Physiological Society and published by the National Institutes of Health.

Design. The effects of activation of A1 adenosine receptors in the subpulmonary region of the NTS on regional sympathetic nerve activity directed to the kidney, adrenal medulla, and hindquarters vasculature were investigated in three groups of rats: 1) intact (INT), 2) sinoaortic denervated plus vagotomized (SAD+VX), and 3) after ionotropic glutamatergic blockade of NTS neurons with kynurenate sodium (KYN). MAP and HR responses were also recorded. In 58 intact animals, NTS A1 adenosine receptors were activated via microinjections of the selective agonist N⁶-cyclopentyl-adenosine (CPA, RBI) in four gradual concentrations (from 0.33 to 33.0 pmol in a 50-nl volume) to evaluate dose-response functions for each analyzed sympathetic output. The maximal responses evoked by the greatest dose of CPA were compared between INT (n = 26) versus SAD+VX (n = 11) and KYN (n = 9) animals. In addition, in two SAD+VX animals, four responses of post-ASNA were recorded. The effect of microinjection of artificial cerebrospinal fluid (ACF) in a volume equal to that of KYN (100 nl) on responses to the greatest dose of CPA was evaluated in 11 animals. Time control for each recorded variable following the ionotropic glutamatergic blockade was accomplished in additional eight animals.

Instrumentation and measurements. All the procedures were described in detail previously (3, 22–27). Briefly, male Sprague-Dawley rats (350–400 g) (Charles River) were anesthetized with a mixture of α-chloralose (80 mg/kg) and urethane (500 mg/kg ip), tracheotomized, connected to a small animal respirator (SAR-830, CWE; Ardmore, PA), and artificially ventilated with a 40% oxygen-60% nitrogen mixture. Arterial blood gases were tested occasionally (Radiometer, ABL500, OSM3), and ventilation was adjusted to maintain PO2, PCO2, and pH within normal ranges. Average values measured at the end of each experiment were PO2 = 132.8 ± 3.0 mmHg, PCO2 = 40.8 ± 0.6 mmHg, and pH = 7.390 ± 0.004 (n = 97). The right femoral artery and vein were catherzied to monitor arterial blood pressure and infuse drugs. SAD and vagotomy were accomplished at the beginning of surgery, as described before (24). The completeness of the denervation procedure was tested ~3 h latter, just before the protocol was started. The procedure was considered complete if intravenous phenylephrine-induced increase in MAP > 30 mmHg did not change HR and decreased sympahtoactivatory activity <10%.

In each experiment, simultaneous recordings from two sympathetic outputs (RSNA + pre-ASNA or RSNA + LSNA) were performed. The adrenal and renal nerves were exposed retroperitoneally, whereas the lumbar sympathetic trunk and adrenal nerve were exposed through midabdominal incision, and neural recordings were accomplished as described previously (22–27). Neural signals were initially amplified (>2,000–20,000) with bandwidth set at 100–1,000 Hz, digitized, rectified, and averaged in 1-s intervals. Background noise was determined 30–60 min after the animal was euthanized. Basal nerve activity was normalized to 100%.

The ratio between preganglionic versus total nerve activity was initially tested with an intravenous bolus injection of the short-lasting (1–2 min) ganglionic blocker trimethaphan (Arfonad, 2 mg/kg; Rohe Pharmaceuticals) and reevaluated at the end of each experiment with hexamethonium (20 mg/kg iv). RSNA was almost completely postganglionic: only 2.5 ± 1.4% (n = 97) of the activity persisted after the ganglionic blockade. LSNA was mostly postganglionic: 33.9 ± 2.6% (n = 42) of the activity persisted after the ganglionic blockade. The adrenal nerve consists of several separate bundles containing both pre- and postganglionic fibers with a very different ratio for each bundle. Therefore, with the use of criteria established in our previous studies, pre-ASNA was considered as predominantly preganglionic if the activity remaining after ganglionic blockade at the end of each experiment was >75% (26, 27). Average pre-ASNA after ganglionic blockade was 102.7 ± 2.5% (n = 55). In two other SAD+VX animals, post-ASNA was recorded, i.e., 48% and 33% of the neural activity remained after the ganglionic blockade.

The arterial pressure and neural signals were digitized and recorded with Hemodynamic and Neural Data Analyzer (Biotech Products; Greenwood, IN), averaged over 1-s intervals, and stored on hard disk for subsequent analysis.

Microinjections into the NTS. Unilateral microinjections of CPA in four different concentrations (0.33, 3.3, 33, and 330 pmol in 50 nl of ACF) were made with multibarrel, glass micropipettes into the medial region of the caudal subpulmonary NTS as described previously (3, 22, 25–27). In several previous studies we have shown that microinjections of the same amount of vehicle (ACF) into the same site of the NTS did not markedly affect MAP, HR, RSNA, LSNA, and pre-ASNA. The changes in all these variables were either not different from zero or smaller than natural, random fluctuations of these variables over the time of measurements (3, 22, 25–27). All microinjection sites were verified histologically as described previously (3, 22, 25–27) and are presented in Fig. 1.

The doses of CPA used in the present study were similar to those used by Barraco and co-workers (1) who distinguished between hemodynamic effects evoked by selective stimulation of A1 versus A2 adenosine receptor agonist. CPA (5 nmol/kg), were completely blocked by pretreatment with the selective A1 adenosine receptor antagonist DPCPX (1). In the present study, we used four doses of CPA in 10-fold increments from the approximate threshold hypertensive dose (0.33 pmol in 50 nl) to the approximate saturation dose (330 pmol in 50 nl). To avoid the effect of desensitization of purinoceptors, in all experiments only one dose of the agonist was microinjected into the left and/or right side of the NTS. If the agonist was injected bilaterally,
at least a 90-min interval between the injections was allowed. CPA was dissolved in ACF and the pH adjusted to 7.2.

Ionotropic glutamatergic blockade. Ionotropic glutamatergic blockade of NTS neurons was performed as described previously (27). Briefly, the blockade was accomplished with unilateral microinjection of KYN (4.4 nmol in 100 nl) into the subpostremal NTS. Previously, we have shown that this dose of KYN, when applied bilaterally, severely and reversibly impaired the arterial baroreflex control of HR and efferent sympathetic nerve activity for over 20 min (27). Therefore, the effective glutamatergic blockade lasted longer than the total responses analyzed in the current study, i.e., 10 min of the response to CPA starting 5 min after microinjection of KYN.

Unilateral microinjection of KYN resulted in a slow ramp-like increase in MAP and neural activity and stabilization of these variables 5 min after the microinjection (Table 1). Stimulation of A1 receptors was performed during this stable period. However, after several minutes of relatively stable response to KYN, the hemodynamic and neural variables start to return very slowly toward their resting values, and the long-lasting responses to stimulation of A1 receptors were superimposed on these spontaneous changes in all recorded variables.

<table>
<thead>
<tr>
<th>After KYN (4.4 nmol/100 nl)</th>
<th>n</th>
<th>After ACF (100 nl)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, Δ%</td>
<td>6.9 ± 1.7††</td>
<td>28</td>
<td>-4.6 ± 2.3</td>
</tr>
<tr>
<td>HR, Δbeats/min</td>
<td>-12.2 ± 2.5†</td>
<td>28</td>
<td>-14.5 ± 3.2†</td>
</tr>
<tr>
<td>LSNA, Δ%</td>
<td>6.1 ± 1.7††</td>
<td>12</td>
<td>-2.3 ± 3.3</td>
</tr>
<tr>
<td>RSNA, Δ%</td>
<td>7.9 ± 2.0††</td>
<td>28</td>
<td>-2.8 ± 3.6</td>
</tr>
<tr>
<td>Pre-ASNA, Δ%</td>
<td>23.4 ± 6.1††</td>
<td>16</td>
<td>4.4 ± 3.8</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, number of animals. KYN, kynurenate sodium; ACF, artificial cerebrospinal fluid; MAP, mean arterial pressure; HR, heart rate; LSNA, RSNA, and pre-ASNA, lumbar, renal, and preganglionic adrenal sympathetic nerve activity, respectively; Δ, change in. Measurements were made during a stable period of the response, approximately 5 min after microinjection of KYN. Note that pre-ASNA increased several times more than RSNA and LSNA after the blockade of NTS glutamatergic receptors. *P < 0.05 vs. ACF; †P < 0.05 vs. zero.
variables. Therefore, in a separate group of eight animals, the time course of the spontaneous changes following the microinjection of KYN was evaluated for all recorded variables (Table 2), and these spontaneous changes were subtracted from the responses to stimulation of the purinergic receptors after KYN. The short-lasting and relatively small responses to microinjection of vehicle (ACF, 100 nl) (Table 1), similar to random fluctuations in all recorded variables and not significantly different from zero (except that for HR response), were not subtracted.

Data analysis. Hemodynamic and sympathetic nerve responses were analyzed over a 10-min period following the microinjections and quantified in two ways as described previously (3, 22, 25–27): 1) the maximal percent changes from a 60-s baseline control period that was taken immediately before microinjection, and 2) integration of the percent changes from the control values. The integral reflects a predominant trend of the response despite transient, sometimes large, bidirectional fluctuations in each variable. Because neural and hemodynamic effects evoked by stimulation of NTS A\(_1\) receptors were variable, often biphasic, or even polyphasic, we used the integral values for the comparisons between the experimental groups. The HR responses, calculated from pulse intervals, were expressed in absolute values (beats/min). Neural recordings were additionally filtered using a running average in 10-s intervals to minimize the effect of random spikes on maximal response values. Because there were no significant differences between the responses in RSNA recorded simultaneously with pre-ASNA versus those recorded simultaneously with LSNA, these data were combined for further calculations. One-way ANOVA for independent measures was used to compare MAP and HR responses versus different doses of CPA. A two-way ANOVA for independent measures was used to compare the responses of three sympathetic outputs (RSNA, pre-ASNA, and LSNA) versus four doses of CPA and to compare the responses evoked by the greatest dose of CPA in three sympathetic outputs versus experimental groups (INT vs. SAD+VX vs. KYN+CPA, KYN+CPA vs. ACF+CPA, and INT vs. ACF+CPA). Differences observed were further evaluated by t-test with Bonferroni adjustment for independent measures. The changes in all recorded variables were also compared with zero by means of SYSTAT univariate P-test. An alpha level of \(P < 0.05\) was used to determine statistical significance.

**RESULTS**

The resting MAP and HR measured in intact animals before each microinjection of drugs (128 microinjections in 86 animals) were 85.0 ± 0.9 mmHg and 358 ± 3 beats/min, respectively. The hemodynamic and neural responses evoked by microinjections of CPA into the subpostremal NTS were variable. In most cases microinjection of CPA produced gradually developing and long-lasting increases in MAP, LSNA, RSNA, and pre-ASNA and simultaneous decreases in HR as illustrated in Fig. 2. This was the most typical pattern of the response for the greatest dose of CPA (330 pmol); 24 of 26 microinjections resulted in overall pressor response (Fig. 1, Table 3). With smaller doses of CPA (0.33–33 pmol), biphasic or even depressor and/or sympathoinhibitory effects were occasionally observed. However, pressor responses prevailed with a ratio of ~2:1 as summarized in Table 3. There was no correlation between specific sites of the microinjections and pressor or depressor responses (Fig. 1). Maximal pressor responses developed slowly, i.e., 2.8 ± 0.5, 6.9 ± 0.7, 5.3 ± 0.7, and 6.3 ± 0.4 min after the injections of 0.33, 3.3, 33, and 330 pmol of CPA into the NTS, respectively. Interestingly, despite variable responses in most analyzed parameters, pre-ASNA always increased in response to all doses of CPA (Table 3).

**Dose-response functions.** Averaged dose-response curves for all integral responses are presented in Fig. 3. Significant dose-dependent increases were observed in MAP and all neural outputs. The same tendency was seen for the maximal values averaged for the experiments where pressor responses prevailed (Table 4). One-way ANOVA for doses of CPA versus integral responses in MAP showed a highly significant dose effect for the increases in MAP (\(P < 0.0001\)). Also, two-way ANOVA for doses of CPA versus integral responses in three neural outputs showed a highly significant dose and neural output effects (\(P < 0.0001\)); however, there was no significant dose versus neural output interaction (\(P = 0.313\)). This indicates that there were significant differences in the magnitude of the sympathoactivation between the nerves; however, there were no significant differences between the slopes of the dose-response curves. Pre-ASNA increased markedly more than RSNA and LSNA (\(P < 0.05\) for all doses of the agonist). Finally, the significant decreases in HR were not dose related (\(P = 0.773\)).

**Sinoaortic denervation plus vagotomy.** The effects of bilateral SAD and subsequent vagotomy on resting MAP and HR were very similar to those observed in our previous study (26). SAD+VX resulted in a marked, sustained increase in HR and a transient elevation in MAP. HR increased in SAD+VX animals (\(n = 11\)) just before the microinjections of the drug (3–10 h after the denervation) remained elevated compared with HR measured in intact animals (443 ± 5 vs. 358 ± 3 beats/min, respectively, \(P < 0.0001\)). In contrast, MAP returned gradually toward resting values during the first 30–60 min after the denervation, and no differences between SAD+VX versus intact animals were observed 3–10 h later during the experiments (85.0 ± 0.9 vs. 82.7 ± 2.2 mmHg, respectively, \(P = 0.495\)).

SAD+VX abolished the increases in MAP and LSNA and reversed the increases in RSNA into significant

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**Table 2. Changes in resting values of MAP, HR, LSNA, RSNA, and pre-ASNA from levels that stabilized 5 min after unilateral blockade of glutamatergic receptors in the subpostremal NTS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Average Change at 15 vs. 5 min After KYN</th>
<th>Integral for 10 min Starting 5 min After KYN</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, Δ%</td>
<td>15</td>
<td>6.0 ± 1.44*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>15</td>
<td>6.7 ± 1.5*</td>
</tr>
<tr>
<td>LSNA, Δ%</td>
<td>6</td>
<td>0.1 ± 1.7</td>
</tr>
<tr>
<td>RSNA, Δ%</td>
<td>15</td>
<td>4.3 ± 1.7*</td>
</tr>
<tr>
<td>Pre-ASNA, Δ%</td>
<td>9</td>
<td>4.5 ± 1.7*</td>
</tr>
</tbody>
</table>

Data are means ± SE after microinjection of KYN; \(n\), number of animals. *\(P < 0.05\) vs. zero.

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Fig. 2. Mean arterial pressure (MAP), heart rate (HR), lumbar (LSNA), renal (RSNA), and predominantly preganglionic adrenal sympathetic nerve activity (pre-ASNA) responses to microinjection of selective A1 receptor agonist CPA (330 pmol in 50 nl) into the subpostremal NTS in an intact (left) and SAD+VX animal (right). In both panels MAP, HR, LSNA, and RSNA were recorded in single animals, whereas pre-ASNA recordings from the other experiments were added to the graphs for comparison. SAD+VX abolished pressor and sympathoactivatory responses in LSNA and RSNA; however, sympathoactivation in pre-ASNA partially persisted. bpm, Beats per minute.

Table 3. Number of individual experiments in intact animals where overall increments or decrements were observed for each recorded parameter based on its integral values

<table>
<thead>
<tr>
<th>CPA pmol</th>
<th>MAP</th>
<th>HR</th>
<th>LSNA</th>
<th>RSNA</th>
<th>Pre-ASNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incr</td>
<td>Decr</td>
<td>Incr</td>
<td>Decr</td>
<td>Incr</td>
</tr>
<tr>
<td>330.0</td>
<td>24 †</td>
<td>2</td>
<td>17 †</td>
<td>14 †</td>
<td>2</td>
</tr>
<tr>
<td>33.0</td>
<td>16 †</td>
<td>7</td>
<td>19 †</td>
<td>6 †</td>
<td>3</td>
</tr>
<tr>
<td>3.3</td>
<td>14 †</td>
<td>5</td>
<td>14 †</td>
<td>5 †</td>
<td>2</td>
</tr>
<tr>
<td>0.33</td>
<td>13 †</td>
<td>7</td>
<td>15 †</td>
<td>7 †</td>
<td>3</td>
</tr>
</tbody>
</table>

Note that pre-ASNA increased in all experiments. Increments (Incr) (†) prevailed in MAP, LSNA, and RSNA, whereas decrements (Decr) (†) prevailed in HR. With the largest dose of A1 receptor agonist, more consistent increases in MAP and neural responses were observed than for lower doses of the agonist.
decreases. This is illustrated by the example of recordings (Fig. 2) and averaged integral values presented in Fig. 4. After SAD+VX, the responses in MAP and LSNA were not different from zero \( (P > 0.05) \). In contrast, pre-ASNA continued to increase under SAD+VX condition, although these increments were markedly attenuated compared with that observed in intact animals \( (P = 0.0013) \). Interestingly, post-ASNA significantly increased in response to stimulation of NTS A1 receptor (integral values: -36.4 \pm 5.5, n = 4, vs.

![Graphs](image)

Fig. 4. Comparison of neural and blood pressure responses evoked by the maximal dose of CPA (330 pmol in 50 nl) microinjected into the subpostremal NTS in intact animals versus animals subjected to bilateral sinoaortic denervation plus vagotomy (SAD+VX) and unilateral ionotropic glutamatergic blockade (KYN). Data are means \( \pm \) SE; numbers of analyzed MAP and RSNA responses were 26, 19 and 13 for intact, SAD+VX, and KYN animals, respectively. Numbers of analyzed pre-ASNA responses were 12, 10, and 7 for intact, SAD+VX, and KYN animals, respectively. *Different vs. intact \( (P > 0.05) \); the ends of horizontal bars join significantly different neural responses \( (P > 0.05) \). SAD+VX abolished and KYN reversed the pressor and sympathoactivatory responses in LSNA and RSNA. Interestingly, following both SAD+VX and KYN, pre-ASNA continued to increase in response to stimulation of NTS A1 receptors, although the increments were significantly attenuated compared with that observed in intact animals \( (P = 0.0013) \) and \( P = 0.0043 \) for SAD+VX and KYN, respectively.

![Table](image)

Table 4. Maximal responses in MAP, HR, LSNA, RSNA, and pre-ASNA to different doses of CPA recorded in intact animals where pressor responses prevailed

<table>
<thead>
<tr>
<th>CPA, pmol</th>
<th>MAP, ΔmmHg</th>
<th>HR, Δbeats/min</th>
<th>LSNA, Δ%</th>
<th>RSNA, Δ%</th>
<th>Pre-ASNA, Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>330.0</td>
<td>31.6 \pm 3.2 (24)</td>
<td>-21.8 \pm 4.2 (24)</td>
<td>20.9 \pm 2.9 (12)</td>
<td>35.7 \pm 4.7 (24)</td>
<td>50.0 \pm 8.1 (12)</td>
</tr>
<tr>
<td>33.0</td>
<td>17.7 \pm 1.7 (16)</td>
<td>-21.5 \pm 5.4 (16)</td>
<td>9.8 \pm 1.6 (7)</td>
<td>18.5 \pm 2.1 (16)</td>
<td>35.8 \pm 4.5 (9)</td>
</tr>
<tr>
<td>3.3</td>
<td>17.0 \pm 1.4 (14)</td>
<td>-11.3 \pm 2.3 (14)</td>
<td>15.8 \pm 2.5 (5)</td>
<td>24.5 \pm 2.6 (14)</td>
<td>28.7 \pm 4.5 (9)</td>
</tr>
<tr>
<td>0.33</td>
<td>11.9 \pm 1.1 (13)</td>
<td>-22.4 \pm 3.6 (13)</td>
<td>10.2 \pm 1.1 (7)</td>
<td>12.8 \pm 1.0 (13)</td>
<td>19.9 \pm 3.6 (6)</td>
</tr>
</tbody>
</table>

Data are means \( \pm \) SE; number of responses showed in parentheses. *Different vs. 330.0 pmol, \( P < 0.05 \); †different vs. 0.33 pmol, \( P < 0.05 \).
92.1 \pm 20.5, n = 8, for post-ASNA vs. pre-ASNA, respectively, P = 0.0013), indicating that the activation of pre-ASNA under these conditions was very selective.

SAD+VX did not alter the relatively small, dose-independent HR decreases evoked by microinjection of CPA into the NTS (maximal decreases, \(-23.2 \pm 4.1\) vs. \(-19.9 \pm 2.7\) beats/min; integral decreases, \(-85.2 \pm 35.7\) vs. \(-98.3 \pm 19.9\) beats\(\cdot\)min\(^{-1}\)\(\cdot\)s, for intact vs. SAD+VX animals, respectively, P < 0.05).

**Ionotropic glutamatergic blockade.** Figure 5 presents an example of the responses to stimulation of NTS A\(_1\) receptors following pretreatment with KYN (right) or equivalent volume of vehicle (ACF) (left). Unilateral blockade of ionotropic glutamatergic receptors in the subpostremal NTS increased MAP, decreased HR, and markedly increased pre-ASNA compared with a smaller increases in RSNA and LSNA (Table 1, Fig. 5); these results were similar to those observed in our previous study (27). Microinjection of the same volume of ACF did not affect MAP and neural outputs; very small changes in these variables were not significantly different from zero (Table 1 and Fig. 5). Pretreatment with ACF did not significantly change hemodynamic and neural responses to stimulation of NTS A\(_1\) receptors compared with the effects evoked by microinjection of CPA alone (Table 5, P > 0.05 for all comparisons).

Ionotropic glutamatergic blockade reversed integral pressor and sympathoactivatory responses in LSNA and RSNA into depressor and sympathoinhibitory responses (Fig. 4 and Table 5). In contrast, pre-ASNA

![Fig. 5. Responses to stimulation of NTS A\(_1\) receptors after pretreatment with ACF (100 nl, left) and after blockade of ionotropic glutamatergic receptors with KYN (4.4 nmol in 100 nl, right) in the same site of the NTS. Recordings were made in two different animals; pre-ASNA recordings for ACF+CPA and LSNA recordings for KYN+CPA from different experiments were added for comparison. Blockade reversed increases in MAP, LSNA, and RSNA into decreases and attenuated the sympahtoactivation in pre-ASNA.](http://ajpheart.physiology.org/)
continued to increase following the blockade as it did in control and following SAD+VX (Fig. 4), although the increases were attenuated compared with those observed in intact animals ($P = 0.0043$). The differences between the responses of three neural outputs to stimulation of NTS A1 receptors following KYN were statistically significant (Fig. 4).

The bradycardia evoked by A1 receptor stimulation tended to be greater following the glutamatergic blockade in comparison with that observed following pretreatment with ACF or microinjection of CPA alone; however, these differences did not reach statistical significance (Fig. 5 and Table 5).

**DISCUSSION**

This is the first study to investigate the effects of selective stimulation of A1 adenosine receptors located in the subpostremal NTS on regional sympathetic outputs. Also, for the first time, the roles of NTS glutamatergic mechanisms and peripheral afferents in neural and hemodynamic responses to stimulation of NTS A1 receptors were evaluated. There are two major new findings of the present study: 1) the stimulation of A1 adenosine receptors located on neurons/neural terminals in the subpostremal NTS evoked differential patterns of regional sympathoactivation ($\uparrow$ pre-ASNA $\gg$ $\uparrow$ RSNA $\geqslant$ $\uparrow$ LSNA); and 2) the responses of different sympathetic outputs to NTS A1 receptor stimulation were differently affected by SAD+VX and by the blockade of ionotropic glutamatergic transmission in the NTS. SAD+VX virtually abolished the increases in MAP and LSNA, reversed increases in RSNA into decreases, and markedly attenuated the increases in pre-ASNA, indicating that the afferent inputs into the NTS are necessary to evoke the pressor and sympathoactivatory responses. Ionotropic glutamatergic blockade reversed responses in MAP, LSNA, and RSNA from increases into significant decreases and attenuated the increases in pre-ASNA, indicating that the pressor and sympathoactivatory responses are mediated by a glutamatergic mechanism. Interestingly, pre-ASNA in contrast to RSNA and LSNA continued to increase in response to NTS A1 receptor stimulation following SAD+VX and KYN. This suggests that NTS A1 receptors trigger sympathoactivation in pre-ASNA via both glutamatergic and nonglutamatergic mechanisms and that the responses of this sympathetic output are not counteracted by inhibitory mechanisms, as it was observed in RSNA and LSNA.

**Variability of the effects evoked by stimulation of NTS A1 receptors.** Stimulation of NTS A1 receptors evoked moderate, slowly developing (several minutes), predominantly pressor, and sympathoexcitatory responses accompanied with variable cardiac slowing responses; however, in several cases, a biphasic, polyphasic or even depressor and sympathoinhibitory responses were occasionally observed, especially at the lower doses of CPA (Table 3). The present results are consistent with those reported previously by Barraco et al. (1) who observed dose-dependent increases in MAP and dose-independent decreases in HR in response to stimulation of A1 receptors in the same site of the NTS by using the same A1 receptor agonist CPA. Interestingly, in both studies, the dose-response curves for increases in MAP were not quite linear showing a slight decline for medium doses of CPA. The present study also showed that the dose-response curves for RSNA and LSNA exhibited similar patterns to that observed in MAP. These “bimodal” dose-response curves for MAP and two neural outputs suggest that stimulation of NTS A1 receptors triggers at least two different mechanisms with different thresholds for A1 receptor stimulation and that these apparently counteracting mechanisms may be a source of observed variability of the results. In contrast, the dose-response curve for pre-ASNA was linear, suggesting more homogenous mechanism of A1 receptor-mediated sympathoactivation in this sympathetic output compared with that observed in RSNA and LSNA.

The present study and previous data reported by Barraco et al. (1) remain in disagreement with the report by White and co-workers (36) who observed marked and rapid (up to 60 s) dose-dependent decreases in MAP in response to the same A1 receptor agonist (CPA) microinjected into the NTS. However, these authors microinjected increasing doses of CPA in proportionally increasing, large volumes (100 nl–10 μl) of vehicle containing 10% of dimethylsulfoxide (36). Therefore, nonspecific, mechanical, and chemical stimulation of the NTS could have been responsible for the fast depressor responses reported by these authors (36).

The responses to stimulation of NTS A1 receptors observed in the present study were much more variable than the responses to stimulation of NTS A2a and P2x
purinergic receptor subtypes observed in our previous studies (3, 22, 25–27). With the assumption that stimulation of A2a and P2x receptors facilitate neurotransmitter release (21, 29), the effects of stimulation of these receptors are relatively independent of ongoing afferent activity terminating in the NTS, because the release of neurotransmitters on stimulation of these purinoreceptors may be evoked in both active and currently nonactive neural terminals. In fact, SAD+VX did not markedly change the responses to stimulation of NTS A2a and P2x receptors (26). In contrast, stimulation of A1 receptors is known to inhibit neurotransmitter release (21, 29); therefore, the responses to stimulation of NTS A1 receptors may depend on the level of ongoing reflex activity at the moment of the stimulation. This may naturally lead to a greater variability of the responses. In support of this concept, SAD+VX abolished pressor and sympathoactivatory responses in RSNA and LSNA and markedly attenuated the responses in ASNA evoked by stimulation of NTS A1 receptors, indicating that the ongoing afferent activity to the NTS is crucial for these responses.

It is also possible that nonselective activation of NTS A2a receptors by the microinjections of A1 receptor agonist (CPA), especially in its greater concentrations, could occur and contribute to the reported variability of the responses. NTS A2a receptors when activated might evoke typical depressor and sympathoinhibitory responses (1, 3, 25, 26), which could counteract pressor and sympathoactivatory responses elicited by primary stimulation of A1 receptors. However, the last possibility was rather unlikely because CPA is 400- to 800-fold more selective for A1 versus A2 receptors (12), and the largest dose of CPA (330 pmol/50 nl) used in the present study evoked the most homogenous pressor and sympathoactivatory responses, whereas more variable effects were observed upon the microinjections of lower doses of CPA (Table 3).

Predominantly pressor or depressor responses to CPA were evoked from similar sites of the caudal subpostremal NTS (Fig. 1), indicating that the type of the response was not related to anatomically specific groups of NTS neurons in this area. Previously, we have shown that microinjections of glutamate (100 pmol in 50 nl) into similar sites of the NTS, under similar experimental conditions, and in the same volume as used for CPA microinjections in the present study, evoked predominantly depressor responses (26). This suggests that variable responses to CPA were not a result of targeting the “depressor” (rostral and subpostremal) versus the “pressor” (caudal) portion of the NTS, which respond reciprocally to much smaller volumes of glutamate (19).

Taken together, the above considerations suggest two major sources of variability of the responses to NTS A1 receptor stimulation observed in the present study, i.e., fluctuations of ongoing reflex and/or descending activity terminating in the NTS and possible simultaneous modulation of different, counteracting NTS mechanisms, which overlap anatomically but are functionally different.  

**Physiological implications.** Pressor and sympathoactivatory responses to NTS A1 receptor stimulation were abolished by SAD+VX and reversed by ionotropic glutamatergic blockade of NTS neurons. This indicates that stimulation of NTS A1 receptors may act via modulation of ongoing afferent activity utilizing glutamatergic neurotransmission in the NTS. With the assumption that A1 adenosine receptors exert inhibitory effects on central neurons via presynaptic inhibition of excitatory neurotransmitter release from neural terminals or postsynaptic activation of various K+ channels (21, 29), it is likely that the pressor and sympathoactivatory responses evoked by NTS A1 receptor stimulation were a result of inhibition of glutamate release from baroreceptor terminals and/or NTS interneurons participating in baroreflex arc. This concept is consistent with our previous observation that unloading of arterial baroreceptors by steady-state decreases in MAP evoked a similar pattern of sympathoactivation to that observed during stimulation of NTS A1 receptors, i.e., the greatest increases in pre-ASNA versus RSNA and LSNA (23). In addition, CPA may facilitate/dissinhibit chemoreflex and descending sympathoactivatory pathways via A1 receptor inhibition of intrinsic inhibitory NTS neurons/neural terminals, as we suggested previously (28). The selective action of CPA on NTS mechanisms may be explained by differential location/expression of A1 adenosine receptors on NTS neurons/neural terminals participating in different afferent and descending pathways (28). This selective action of CPA (inhibiting depressor and facilitating pressor pathways in the NTS) may lead to a greater pressor and sympathoactivatory responses than that observed following KYN, which nonselectively blocked all pressor and depressor, afferent and descending NTS glutamatergic mechanisms. Because SAD+VX virtually abolished the pressor and sympathoactivatory responses to CPA, these responses were mediated mostly via inhibition of depressor and disinhibition of pressor afferents terminating in the NTS.

Interestingly, HR responses to A1 receptor agonist, CPA were not dose related in the present and previous studies (1). In addition, the bradycardia evoked by NTS A1 receptor stimulation did not change following SAD+VX and even tended to increase following the glutamatergic blockade. Therefore, it is likely that this dose-independent bradycardia was a net result of at least two counteracting mechanisms simultaneously triggered by NTS A1 receptor stimulation, for example, facilitation/disinhibition of reflex and descending, non-ionotropic-glutamatergic, cardiac slowing pathways, and counteracting inhibition of tonic baroreflex restraint of HR. Similar mechanisms were proposed to explain the observed bradycardia instead of expected tachycardia following blockade of baroreflex responses via microinjection of KYN into the NTS and ventrolateral medulla (11, 27).

Glutamatergic blockade unmasked the depressor and sympathoinhibitory mechanisms elicited via A1 receptors, which were obscured by the predominantly pressor and sympathoactivatory responses observed in
the intact animals. The physiological role and specific neurotransmitters of these nonionotropic-glutamatergic inhibitory mechanisms remain unknown. Possible contribution of metabotropic glutamatergic receptors and nonglutamatergic neurotransmitters/neuromodulators, which exert depressor effects at the level of the NTS via afferent or descending pathways, for example, substance P, neuropeptide Y, serotonin, or vasopressin, should be considered (15).

The pressor and sympathoactivatory effects evoked by stimulation of NTS A1 receptors in the present study are consistent with the data recently reported by Spyer and colleagues (30) who showed that adenosine A1 receptors facilitate the pressor component of hypothalamic defense response at the level of the NTS and rostral ventrolateral medulla. The hypothalamic defense response increases MAP partially via inhibition of baroreflex neurons by intrinsic GABA-ergic interneurons at the level of the NTS (13). Because stimulation of adenosine A1 receptors usually inhibits neurotransmitter release, it is unlikely that they may facilitate the release of GABA. Instead, they may contribute to the hypothalamic defense response via direct inhibition of the release of glutamate from baroreceptor afferents, which is consistent with our observation that pressor and sympathoactivatory responses to NTS A1 receptor stimulation are mediated via a glutamatergic mechanism. Still it remains unclear whether NTS A1 receptors are involved in a tachycardic component of the hypothalamic defense response, because the stimulation of NTS A1 receptors evokes bradycardia instead of expected tachycardia as shown in the present and previous studies (1).

Adrenal nerve. In contrast to the variable responses observed in MAP, RSNA, and LSNA, the sympathetic activity directed to the adrenal medulla (pre-ASNA) consistently increased in response to stimulation of NTS A1 receptors. The increases in pre-ASNA were linearly dose dependent and significantly greater than those observed in the other sympathetic outputs. We have previously shown that pre-ASNA markedly increased also in response to stimulation of NTS A2a receptors, whereas MAP, HR, and RSNA decreased and LSNA did not change under those experimental conditions (25, 26). Taken together, these observations indicate that despite different, often reciprocal, hemodynamic and neural effects of adenosine A1 versus A2a receptor stimulation in the subpostrenal NTS, the activation of both adenosine receptor subtypes evokes consistent, marked increases in the sympathetic output directed to the adrenal medulla. This suggests a special link between centrally operating adenosine and selective activation of the adrenal medulla. Interestingly, adenosine levels in the central nervous system, including the NTS, increase during hypoxia, ischemia, and severe hemorrhage (20, 34, 37). In these pathophysiological situations, efferent adrenal nerve activity increases and the adrenal medulla is powerfully activated to release catecholamines and restore homeostatic imbalance (4, 35). Therefore, adenosine may be a unique central neuromodulator that “senses” severe deterioration of homeostasis and triggers central mechanisms selectively activating the adrenal medulla.

It is likely that preferential stimulation of the adrenal medulla via activation of NTS A1 receptors may evoke β-adrenergic vasodilation in muscle vascular bed, similar to what we observed previously for activation of NTS A2a receptors (14). This vasodilation may counteract the pressor effects evoked by NTS A1 receptor stimulation and contribute to the observed variability of MAP responses.

What specific nonglutamatergic mechanisms may be triggered in the NTS to selectively increase pre-ASNA remains unknown. Among several possible neurotransmitters/neuromodulators, corticotropine releasing factor (CRF) and histamine should be considered. In addition to the activation of the hypothalamo-pituitary-adrenal axis, both of these neurotransmitters/neuromodulators are involved in central cardiovascular responses to stress, and both operate via respective receptors at the level of the NTS (7, 16). Finally, centrally administered CRF antagonist (α-helical CRF 9–41) attenuates hemorrhage- and hypoglycemia-induced increases in blood epinephrine but not norepinephrine levels, indicating that naturally released CRF may selectively activate sympathetic output to the adrenal medulla (6).

Limitations of the method. The limitations and advantages of the methods used in the present study for the evaluation of hemodynamic and regional sympathetic responses to selective stimulation of A1 purinergic receptor subtype in the NTS in intact versus SAD+VX and KYN animals were discussed in detail in our previous studies where the responses to stimulation of A2a and P2x purinoceptors were compared under similar experimental conditions (27, 28). Briefly, anesthesia used in the present and previous studies could affect the responses, especially their HR component; however, only in anesthetized animals are direct comparisons of simultaneous recordings from regional sympathetic outputs currently possible, and these comparisons are more reliable than those recorded separately in different animals (23). Anesthesia may also attenuate NTS chemoresponse mechanisms as suggested by comparison of the predominantly pressor, chemoreceptor-like responses evoked by stimulation of NTS glutamatergic receptors in conscious animals versus the predominantly depressor, baroreflex-like responses observed in anesthetized animals (9, 26). Therefore, the results of the present study may reflect mostly the interactions between adenosine A1 and glutamatergic neurotransmission/neuromodulation in NTS baroreflex mechanisms. Nonuniform changes in all recorded variables following the glutamatergic blockade of the NTS neurons (Table 1) slightly complicated comparisons of the responses to A1 receptor stimulation in intact versus blockade conditions. However, these changes were negligible considering that KYN qualitatively changed the responses from increases in MAP, RSNA, and LSNA to significant decreases. Only the relatively large increase in resting pre-ASNA following KYN (Table 1) could contribute to the observed atte-
ation of further increases in pre-ASNA in response to stimulation of NTS A1 receptors after KYN.

One may argue that the differences in regional sympathoactivation evoked by the stimulation of NTS A1 receptors (↑ pre-ASNA ≥ ↑ RSNA ≥ ↑ LSNA) may be a result of different ratio of pre- versus postganglionic fibers in each nerve. However, we did not find a significant correlation between the percentage of preganglionic sympathetic activity versus the magnitude of integral responses to A1 receptor stimulation within each sympathetic output (correlation coefficients < 0.2). In addition, under conditions of SAD+VX and glutamatergic blockade, the responses in pre-ASNA were qualitatively different, i.e., in the opposite direction to those in RSNA and LSNA. Although quantitative differences between the responses of pre- versus postganglionic fibers may occur, it is unlikely that these responses may differ qualitatively. Therefore, in our opinion, the opposite responses of pre-ASNA versus RSNA and LSNA to NTS A1 receptor stimulation following SAD+VX and KYN likely reflect differences in location/ expression of A1 receptors on NTS neurons targeting different sympathetic outputs.

Although we microinjected drugs into the medial subpostremal NTS, where mostly arterial baro- and chemoreflex mechanisms are integrated (8), it is possible that other reflex mechanisms operating in this and neighboring parts of the NTS were also affected. For example, stimulation of NTS A1 receptors increases tidal volume, and thus may indirectly affect cardiovascular responses (1). Using artificial ventilation, we eliminated most of the possible peripheral interactions between respiratory and cardiovascular system; however, central interactions could still occur. The method of stimulation of NTS purinoreceptor subtypes used in the present and our previous studies was chemically selective, but, by the design, anatomically nonselective (28). Therefore, based on the patterns of hemodynamic and neural responses, we may only suggest, but cannot prove, what specific NTS mechanisms were affected by stimulation of certain purinergic receptor subtype. The present data indicate that A1 receptors differentially modulate reflex and descending control of regional sympathetic outputs, affecting both glutamatergic and nonglutamatergic mechanisms, integrated in the subpostremal NTS. This strongly suggests that A1 purinoreceptors, similarly as A2a and F2x purinoreceptors, are differentially located/expressed on NTS neurons/neural terminals targeting different sympathetic outputs. Further studies using precisely identified NTS neurons involved in transmission/integration of specific afferent modalities are necessary to further elucidate the role of adenosine receptor subtypes in various reflex mechanisms integrated in the NTS.

**Summary and conclusions.** Intact glutamatergic transmission and afferent inputs to the NTS are necessary to mediate the pressor and differential sympathoactivatory effects evoked by stimulation of NTS A1 adenosine receptors. This suggests that adenosine A1 receptors may inhibit glutamate release from baroreceptor afferents terminating in the NTS and attenuate baroreflex restraint of efferent sympathetic activity and arterial pressure. The activation of pre-ASNA is partially independent of ionotropic glutamatergic mechanisms and afferent inputs to the NTS; therefore, it may be a result of facilitation/disinhibition of descending, central inputs to the NTS, which may selectively control pre-ASNA via a nonglutamatergic mechanism. Ionotropic glutamatergic blockade unmasked the depressor and sympathoinhibitory effects elicited by NTS A1 receptor stimulation. These effects were mediated by nonglutamatergic mechanisms. Differential effects of sinoaortic denervation plus vagotomy and ionotropic glutamatergic blockade on regional sympathetic responses to NTS A1 receptor stimulation supports the hypothesis that purinergic receptor subtypes are differentially located and expressed on NTS neurons/neural terminals controlling different sympathetic outputs.

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