Stimulation of NTS A<sub>1</sub> adenosine receptors differentially resets baroreflex control of regional sympathetic outputs

Tadeusz J. Scislo,1 Tomoko K. Ichinose,1,2 and Donal S. O’Leary1

1Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan; and 2Osaka International University, Osaka, Japan

Submitted 21 September 2007; accepted in final form 31 October 2007

Scislo TJ, Ichinose TK, O’Leary DS. Stimulation of NTS A<sub>1</sub> adenosine receptors differentially resets baroreflex control of regional sympathetic outputs. Am J Physiol Heart Circ Physiol 294: H172–H182, 2008. First published November 2, 2007; doi:10.1152/ajpheart.01099.2007.— Previously we showed that presor and differential regional sympathoexcitatory responses (adrenal &gt; renal &gt; lumbar) evoked by stimulation of NTS A<sub>1</sub> adenosine receptors located in the nucleus of the solitary tract (NTS) were attenuated/abolished by baroreceptor denervation or blockade of glutamatergic transmission in the NTS, suggesting A<sub>1</sub> receptor-elicited inhibition of glutamatergic transmission in baroreflex pathways. Therefore we tested the hypothesis that stimulation of NTS A<sub>1</sub> adenosine receptors differentially inhibits/resets baroreflex responses of preganglionic adrenal (pre-ASNA), renal (RSNA), and lumbar (LSNA) sympathetic nerve activity. In urethane-chloralose-anesthetized male Sprague-Dawley rats (n = 65) we compared baroreflex-response curves (iv nitropusside and phenylephrine) evoked before and after bilateral microinjections into the NTS of A<sub>1</sub> adenosine receptor agonist (N<sup>6</sup>-cyclopentyladenosine, CPA; 0.033–30 pmol/50 nl). CPA evoked typical dose-dependent pressor and differential sympahtoexcitatory responses and similarly shifted baroreflex curves for pre-ASNA, RSNA, and LSNA toward higher mean arterial pressure (MAP) in a dose-dependent manner; the maximal shifts were 52.6 ± 2.8, 48.0 ± 3.6, and 56.8 ± 6.7 mmHg for pre-ASNA, RSNA, and LSNA, respectively. These shifts were not a result of simple baroreceptor resetting because they were two to three times greater than respective increases in baseline MAP evoked by CPA. Baroreflex curves for pre-ASNA were additionally shifted upward: the maximal increases of preganglionic adrenal sympathetic nerve activity (pre-ASNA) were 2.3% vs. 2.8, 48.0% vs. 48.0% and 56.8% vs. 56.8%, respectively. Maximal gain (%/mmHg) measured before vs. after CPA increased for pre-ASNA (3.0 ± 0.6 vs. 4.9 ± 1.3), decreased for RSNA (4.1 ± 0.6 vs. 2.3 ± 0.3), and remained unaltered for LSNA (2.1 ± 0.2 vs. 2.0 ± 0.1). Vehicle control did not alter the baroreflex curves. We conclude that the activation of NTS A<sub>1</sub> adenosine receptors differentially inhibits/resets baroreflex control of regional sympathetic outputs.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MAP under similar experimental conditions (23, 29). The pressor and sympathoactivatory responses to stimulation of NTS A<sub>1</sub> adenosine receptors were abolished/attenuated after bilateral sinoaortic denervation and vagotomy as well as after the blockade of ionotropic glutamatergic transmission in the NTS (28). In addition, our very recent study (13, 14) suggested that stimulation of NTS A<sub>1</sub> adenosine receptors may facilitate glumatate release from baroreceptor terminals into the circulation, the effect consistent with the inhibition of tonic baroreflex restraint of vasopressin release. Together the above data imply that activation of A<sub>1</sub> adenosine receptors in the subpostremal NTS may inhibit glumatate release from baroreceptor terminals and/or interneurons. However, to our knowledge, the direct effects of activation of NTS A<sub>1</sub> adenosine receptors on baroreflex control of the cardiovascular system have never been studied. Therefore, in the present study we compared sigmoidal baroreflex stimulus-response curves for regional sympathetic nerve activity (SNA) and HR before and after graded, selective activation of A<sub>1</sub> adenosine receptors located in the subpostremal NTS. Since stimulation of NTS A<sub>1</sub> adenosine receptors preferentially increases pre-ASNA vs. RSNA and LNSA (28), and baroreflex functions for these sympathetic outputs have differential characteristics (23), we expected that the stimulation of NTS A<sub>1</sub> adenosine receptors would differentially affect baroreflex control of these regional sympathetic outputs.

**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 Guide for 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 A<sub>1</sub> adenosine receptors located in the NTS on sigmoidal baroreflex-response curves constructed for regional SNA (pre-ASNA, RSNA, and LNSA) and HR were studied in 50 male Sprague-Dawley rats. A<sub>1</sub> adenosine receptors were activated via bilateral microinjections into the subpostremal NTS of different doses of the selective agonist N<sup>6</sup>-cyclopentyladenosine (CPA; 0.033–330 pmol/50 nl). In an additional 15 animals, the effects of bilateral microinjections of vehicle [50 nl of artificial cerebrospinal fluid (ACF)] on the baroreflex functions were evaluated.

**Instrumentation and measurements.** All procedures were described in detail previously (3, 25–28, 30–32). 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 PO<sub>2</sub>, PCO<sub>2</sub>, and pH within normal ranges. Average values measured at the end of each experiment were PO<sub>2</sub> = 161.4 ± 3.4, PCO<sub>2</sub> = 39.3 ± 0.5, and pH = 7.403 ± 0.011 (n = 65). The left femoral artery and vein were catheterized to monitor arterial blood pressure and infuse supplemental doses of anesthesia (12–21 mg kg<sup>−1</sup> h<sup>−1</sup> α-chloralose and 76–133 mg kg<sup>−1</sup> h<sup>−1</sup> urethane dissolved in 2.4–4.2 ml kg<sup>−1</sup> h<sup>−1</sup> saline), respectively. Two additional catheters were placed into the right femoral vein to infuse sodium nitroprusside and phenylephrine, respectively.

In each experiment simultaneous recordings from two sympathetic outputs (RSNA + pre-ASNA or RSNA + LNSA) were performed. The adrenal and renal nerves were exposed retroperitoneally, the lumbar sympathetic trunk and renal nerve were exposed through a midabdominal incision, and neural recordings were accomplished as described previously (23, 25–28, 30–32). 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 and total nerve activity was initially tested with an intravenous bolus injection of the short-lasting (1–2 min) ganglionic blocker Arfonad (trimethaphan, 2 mg/kg; Hoffmann-La Roche) and reevaluated at the end of each experiment with hexamethionium (20 mg/kg iv). RSNA was almost completely postganglionic; only 3.4 ± 0.5% (n = 65) of the activity persisted after the ganglionic blockade. LNSA was mostly pre- and ganglionic; 27.0 ± 3.4% (n = 23) of the activity persisted after the ganglionic blockade. The adrenal nerve consists of several separate bundles containing both preganglionic and postganglionic fibers.

**Fig. 1.** Microinjection sites in subpostremal nucleus of the solitary tract (NTS) for all experiments. Schematic diagrams show transverse sections of medulla oblongata from a rat brain. AP, area postrema; c, central canal; 10, dorsal motor nucleus of vagus nerve; 12, nucleus of hypoglossal nerve; Gr, gracile nucleus; Cu, cuneate nucleus. Scale is shown at bottom. Numbers at left denote the rostrocaudal position in millimeters of the section relative to the obex according to the atlas of the rat subpostremal NTS by Barraco et al. (2). Microinjection sites for different doses of A<sub>1</sub> adenosine receptor agonist [N<sup>6</sup>-cyclopentyladenosine (CPA)] and volume control [50 nl of artificial cerebrospinal fluid (ACF)] were marked with fluorescent dye. A: •, 0.053 pmol CPA; ◦, 0.33 pmol CPA; ▲, volume control. B: •, 3.3 pmol CPA; ◦, 330 pmol CPA.
and postganglionic fibers with a very different ratio for each bundle. Therefore, with criteria established in our previous studies (26–28, 30–32), pre-ASNA was considered as predominantly preganglionic if the activity remaining after ganglionic blockade at the end of each experiment was $<75\%$. Average pre-ASNA measured after ganglionic blockade was 116.7$\pm$4.2% ($n$ = 42). Pre-ASNA increased over 100% likely because of an arterial baroreflex response caused by the decrease in MAP after ganglionic blockade. The arterial pressure and neural signals were digitized and recorded with a 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.** Bilateral microinjections of four different doses of CPA (0.033, 0.33, 3.3, and 330 pmol diluted in 50 nl of ACF) were made with multibarrel glass micropipettes into the medial region of the caudal subpostremal NTS as described previously (3, 25–28, 30–32). In a separate group of animals microinjections of the same amount of vehicle (50 nl of ACF) into the same site of the NTS were performed. All microinjection sites were verified histologically as described previously (3, 25–28, 30–32) and are presented in Fig. 1.

**Fig. 2.** Example of baroreflex responses of renal (RSNA) and preganglionic adrenal (pre-ASNA) sympathetic nerve activity evoked by gradual decreases and increases in mean arterial pressure (MAP) (**A**) and schematic diagram of the experimental protocol (**B**). MAP was decreased and increased via intravenous infusion of sodium nitroprusside (NP) and phenylephrine (PE), respectively. Baroreflex curves were performed under control conditions, after bilateral microinjections into the NTS of selective $A_1$ adenosine receptor agonist CPA or ACF (volume control), and $\sim70$ min after microinjections of the agonist (recovery). HR, heart rate; bpm, beats per minute.

**Fig. 3.** Example of baroreflex responses of RSNA and pre-ASNA obtained in 1 animal under control conditions (**top**), after bilateral microinjections into the NTS of a maximal dose (330 pmol) of selective $A_1$ adenosine receptor agonist CPA (**middle**), and $\sim70$ min after microinjections (recovery, **bottom**). Vertical dotted, solid, and dashed lines indicate resting MAP and the midpoint of baroreflex response curves (BP50) for RSNA and pre-ASNA, respectively. Note that baroreflex curve for pre-ASNA was markedly shifted upward and both baroreflex curves were shifted toward higher MAP after maximal stimulation of NTS $A_1$ adenosine receptors. Also note that the rightward shifts in BP50 were much greater than the shifts in baseline MAP. The responses partially recovered $\sim70$ min after the microinjections.
The doses of CPA used in the present study were similar to those used in our previous study (28) and those used by Barraco and coworkers (2), who for the first time characterized reciprocal hemodynamic responses evoked by selective stimulation of A₃ and A₂a adenosine receptor subtypes located in the subposteminal NTS. Those authors also showed that the effects elicited with the highest dose of a selective A₁ receptor agonist, CPA (5 nmol/kg), were completely blocked by pretreatment with the selective A₁ adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (2). In the present study initially we assessed the effects on baroreflex control of RSNA, pre-ASNA, and LSNA evoked by bilateral microinjections of two doses of CPA, the approximate threshold hypertensive dose (3.3 pmol in 50 nl) and the approximate saturation dose (330 pmol in 50 nl), based on the effects of unilateral microinjections performed in previous studies (2, 28). However, the responses of pre-ASNA evoked by bilateral microinjections of A₁ adenosine receptor agonist were relatively large for both doses of the agonist. Therefore, in an additional group of animals we evaluated the effects of 10- and 100-fold lower doses of the agonist (0.33 and 0.033 pmol) on baroreflex control of pre-ASNA and RSNA. LSNA responses were not evaluated in this group. To avoid the effect of desensitization of purinoceptors, in all experiments only one dose of the agonist was microinjected into each side of the NTS in ~2- to 3-min intervals. CPA was dissolved in ACF, and the pH was adjusted to 7.2. Baroreflex functions. The sigmoidal baroreflex-response curves were constructed similarly as described in our previous paper (23) with modifications reflecting the relatively short period (~10–12 min) of maximal plateau of hemodynamic and sympathetic responses to the selective activation of NTS A₁ adenosine receptors. Briefly, MAP was decreased by graded intravenous infusion of sodium nitroprusside (200 mg/ml, 4–6 steps, 0.25–8 ml/h) until maximal steady-state decreases in SNA and HR were observed for at least 1 min. MAP was allowed to return to the resting values and then increased via graded intravenous infusion of phenylephrine (200 mg/ml, 4–6 steps, 0.5–8 ml/h) until the steady-state maximal decreases in SNA and HR were observed. An example of cardiac and sympathetic responses to experimental alterations of MAP evoked under control conditions is presented in Fig. 2A. The values between the upper and lower plateaus of the responses were used to construct baroreflex-response curves for each variable (RSNA, pre-ASNA, LSNA, and HR). This method allowed us to generate whole sigmoidal baroreflex-response curves (slow ramp changes) over ~6–10 min, a period short enough to match the plateau of maximal responses to stimulation of NTS A₁ adenosine receptors. The timeline of the protocol is presented in Fig. 2B.

Data analysis. The baroreflex-response curves (for RSNA, pre-ASNA, LSNA, and HR) generated under control conditions were compared with the functions generated after bilateral stimulation of NTS A₁ adenosine receptors with four doses of the selective agonist (CPA; 0.033, 0.33, 3.3, and 330 pmol) and after bilateral microinjections of ACF into the NTS (volume control). Control baroreflex response functions obtained before microinjections of CPA were also compared with the functions generated ~70 min after the microinjections (recovery). Sigmoidal logistic baroreflex curves were approximated to experimental data points (averaged in 1-s intervals) according to the model described by Kent et al. (9) with SigmaPlot for Windows 9.0 and the formula: \[ SNA (HR) = \left( P_1 - P_2 \right) / \left( 1 + \exp\left[ P_3 \ast (MAP - P_4) \right] \right) + P_5, \]
where \( P_1 \) is the upper plateau of the curve, \( P_3 \) is the lower plateau, \( P_5 \) is a coefficient describing the distribution of gain along the curve, and \( P_4 \) is MAP in the midpoint of the curve (BP₅₀). Maximum gain (Gₐₕ₅) was calculated according to the formula \( G_{max} = P_5(P_1 - P_2)/4 \). The range of baroreflex control was measured as a difference between upper and lower plateaus (range \( = P_1 - P_2 \)). An example of sigmoidal baroreflex-response functions obtained in one animal under control conditions, after microinjections of CPA into the NTS, and ~70 min after the microinjections is presented in Fig. 3. The baroreflex-response curves were calculated for each animal under each experimental conditions and then averaged across animals.

The analysis of variance and significance of differences between mean values were calculated with the statistical package SYSTAT 11.0 for Windows. Two-way ANOVA for repeated measures was used to compare the parameters of the baroreflex curves across the experimental conditions and across the doses of A₁ adenosine agonist. Two-way ANOVA for independent measures was used to analyze the responses evoked by four doses of CPA in three sympathetic nerves; similarly, the shifts in BP₅₀ were calculated for the three sympathetic outputs and four doses of CPA. If significant interactions were found (experimental conditions \( \times \) doses, or nerves \( \times \) doses), the differences between the means were calculated with a modified Bonferroni t-test. One-way ANOVA for independent measures followed by modified Bonferroni t-test was used for comparison of HR and MAP responses to microinjections of four doses of CPA and for shifts in BP₅₀ for HR baroreflex curves across the doses of CPA. The responses evoked by bilateral microinjections of different doses of CPA and respective volume of vehicle (ACF) were calculated as a Δ% between resting values averaged during the last 30 s preceding the microinjections and the last 30 s of the 5-min period of the response to CPA or ACF measured before generating the second baroreflex curve (see schematic diagram of the experimental protocol, Fig. 2B). The differences between the responses to ACF vs. CPA were evaluated with a t-test for independent measures. The responses observed in all recorded variables were also compared with zero by means of the SYSTAT univariate F-test. An α-level of \( P < 0.05 \) was used to determine statistical significance.

RESULTS

Basal MAP and HR measured before first, control baroreflex response curves in each animal (\( n = 65 \)) were 90.2 ± 1.2 mmHg and 366.8 ± 5.3 beats/min, respectively. Approximately 45 min after the control baroreflex curves, the basal parameters (measured just before microinjections into the NTS)

Table 1. Changes in resting values of MAP, HR, RSNA, pre-ASNA, and LSNA after bilateral microinjections of selective NTS A₁ adenosine receptor agonist (CPA) and volume control (ACF)

<table>
<thead>
<tr>
<th></th>
<th>CPA, pmol/50 nl</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACF, 50 nl</td>
<td>0.033</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>-3.5 ± 1.2 (15)</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>-21.6 ± 4.4 (15)</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>-9.0 ± 2.9 (15)</td>
</tr>
<tr>
<td>pre-ASNA, %</td>
<td>10.1 ± 2.3 (8)</td>
</tr>
<tr>
<td>LSNA, %</td>
<td>3.2 ± 5.7 (7)</td>
</tr>
</tbody>
</table>

Data are means ± SE for no. of animals in parentheses. NTS, nucleus of the solitary tract; ACF, artificial cerebrospinal fluid; CPA, N°-cyclopentyladenosine; MAP, mean arterial pressure; HR, heart rate; RSNA, pre-ASNA, LSNA, renal, preganglionic adrenal, and lumbar sympathetic nerve activity, respectively. **P < 0.05 vs. ACF; #P < 0.05 vs. RSNA; †P < 0.05 vs. LSNA.

AJP-Heart Circ Physiol • VOL 294 • JANUARY 2008 • www.ajpheart.org
of CPA or ACF, preceding second baroreflex curves) returned to normal levels: 89.7 ± 1.2 mmHg and 369.4 ± 4.8 beats/min for MAP and HR, respectively. Also, in those animals in which the third baroreflex curve was performed ~70 min after the microinjections of CPA (recovery, n = 50), basal MAP (91.4 ± 1.6 mmHg) and HR (372.0 ± 5.8 beats/min) were similar to those measured at the beginning of the experiment. There were no significant differences between basal levels of MAP and HR measured during the three stages of the experiments: control, stimulation of NTS A₁ adenosine receptors, and recovery (P > 0.05 for all comparisons).

Effects of stimulation of NTS adenosine A₁ receptors on baseline variables. The effects of bilateral microinjections into the NTS of different doses of A₁ adenosine receptor agonist (CPA) and vehicle (ACF) on resting hemodynamic and neural variables are presented in Table 1. The responses were similar although larger than those observed in our previous studies (13, 25, 26, 28, 30, 32), in which unilateral microinjections of the agonist and/or vehicle were performed. Bilateral stimulation of NTS A₁ adenosine receptors evoked dose-dependent increases in MAP (dose effect, P = 0.001), moderate, dose-independent decreases in HR (dose effect, P = 0.568), and preferential

Fig. 4. Averaged baroreflex-response curves for RSNA, pre-ASNA, and lumbar sympathetic nerve activity (LSNA) under control conditions (left) and after bilateral microinjections of 50 nl of ACF (vehicle) and different doses of A₁ adenosine receptor agonist CPA (0.033, 0.33, 3.3, and 330 pmol in 50 nl of ACF) (right). Vertical dotted, solid, dashed, and dash-dotted lines indicate resting MAP and BP50 for RSNA, pre-ASNA, and LSNA, respectively. Note that stimulation of NTS A₁ adenosine receptors shifted upward baroreflex curves for pre-ASNA vs. those for RSNA and LSNA. The dose-dependent rightward shifts of the baroreflex curves toward higher MAP were similar for all nerves and much greater than the increases in baseline MAP.
activation of pre-ASNA vs. RSNA and LSNA. Two-way ANOVA showed that neural responses were significantly different from each other (nerve effect, $P < 0.001$) and overall dose dependent (dose effect, $P = 0.016$). Different sympathetic outputs responded in a significantly different, nonlinear manner to the increasing doses of CPA (nerve x dose interaction, $P = 0.019$). The increases in pre-ASNA were severalfold greater than the increases in RSNA and LSNA for all doses of CPA. Interestingly, even the smallest dose of CPA (0.033 pmol) increased pre-ASNA by $>40\%$ ($P = 0.004$ vs. ACF), whereas it did not affect RSNA, MAP, or HR ($P > 0.05$ vs. ACF).

Effects of stimulation of NTS $A_1$ adenosine receptors on baroreflex control of SNA. Sigmoidal baroreflex-response curves for RSNA, pre-ASNA, and LSNA obtained under control conditions (Fig. 4, left) were similar to those observed in our previous study (23). The averaged parameters of control baroreflex functions combined for all animals ($n = 65$) are presented in Table 2. Activation of arterial baroreceptors inhibited RSNA to a greater extent than pre-ASNA and LSNA (lower plateaus), whereas unloading of baroreceptors increased pre-ASNA to a higher level (upper plateau) than that for RSNA and LSNA. The range of baroreflex control was greater for pre-ASNA compared with both other nerves (Table 2). The maximal gain of baroreflex functions ($G_{\text{max}}$) was significantly greater for RSNA than for LSNA (Table 2). The midpoint of baroreflex functions ($BP_{50}$) was similar for all analyzed sympathetic outputs and was located close to the resting MAP (Fig. 4, left; Table 2).

Stimulation of NTS $A_1$ adenosine receptors shifted baroreflex-response curves toward higher MAP in a dose-dependent manner (dose effect, $P < 0.001$) (Fig. 4, right; Fig. 5; Table 3, $BP_{50}$). There were no differences between the shifts for different sympathetic outputs (nerve effect, $P = 0.184$; nerve x dose interaction, $P = 0.624$) despite highly significant differences between activation of different sympathetic outputs evoked by microinjections of CPA into the NTS (nerve effect, $P < 0.001$) (Table 1). The shifts were two to three times greater than the respective increases of resting MAP evoked by activation of NTS $A_1$ adenosine receptors (Fig. 5), indicating that the shifts were not a result of simple baroreceptor resetting caused by elevation of MAP. Importantly, higher doses of CPA (3.3 and 330 pmol) abolished the increases in SNA that normally occurred during unloading of arterial baroreceptors under con-

\begin{table}
\centering
\caption{Parameters of control sigmoidal baroreflex-response curves combined for all experiments}
\begin{tabular}{lcccccc}
\hline
 & $n$ & $BP_{50}$, mmHg & Range, % & Upper Plateau, % & Lower Plateau, % & $G_{\text{max}}$, %/mmHg \\
\hline
RSNA & 65 & $98.8 \pm 1.4$ & $106.3 \pm 3.1$ & $121.8 \pm 2.9$ & $15.4 \pm 1.1$ & $2.9 \pm 0.3$

Pre-ASNA & 42 & $95.6 \pm 1.6$ & $132.0 \pm 6.3^*$ & $162.6 \pm 6.2^*$ & $30.6 \pm 2.2^*$ & $2.5 \pm 0.2$

LSNA & 23 & $97.2 \pm 2.6$ & $90.7 \pm 6.1^#$ & $134.9 \pm 5.0^*$ & $44.2 \pm 3.6^*$ & $1.8 \pm 0.1^*$
\hline
\end{tabular}
\end{table}

Data are means $\pm$ SE. $BP_{50}$, MAP at midpoint of baroreflex curve. $G_{\text{max}}$, maximum gain of sigmoidal baroreflex curve. $^*P < 0.05$ vs. RSNA; $^#P < 0.05$ pre-ASNA vs. LSNA.

![Fig. 5. Shifts of baroreflex response curves ($BP_{50}$) toward higher MAP for RSNA, pre-ASNA, and LSNA (A) compared with corresponding changes in resting MAP (B) evoked by bilateral microinjections into the NTS of vehicle (ACF) and different doses of $A_1$ adenosine receptor agonist CPA (0.033, 0.33, 3.3, and 330 pmol). Horizontal lines connect pairs of columns that are significantly different from each other ($P < 0.05$). $^*P < 0.05$ vs. ACF. Note that the shifts of the baroreflex curves were 2–3 times greater than the increases of resting MAP evoked by stimulation of NTS $A_1$ adenosine receptors.](http://ajpheart.physiology.org/)
Table 3. Averaged parameters of sigmoidal baroreflex functions for RSNA, pre-ASNA, and LSNA measured before (Control) and after bilateral microinjections of selective A1 adenosine receptor agonist CPA into NTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CPA 0.033</th>
<th>CPA 0.33</th>
<th>CPA 3.30</th>
<th>CPA 33.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Plateau %</td>
<td>12.5±2.5</td>
<td>15.9±2.5</td>
<td>16.9±2.5</td>
<td>16.7±2.5</td>
<td>16.7±2.5</td>
</tr>
<tr>
<td>Lower Plateau %</td>
<td>2.2±0.2</td>
<td>2.2±0.2</td>
<td>2.2±0.2</td>
<td>2.2±0.2</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>Gmax, %/mmHg</td>
<td>4.5±1.1</td>
<td>7.0±1.1</td>
<td>7.0±1.1</td>
<td>7.0±1.1</td>
<td>7.0±1.1</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.6±0.1</td>
<td>0.6±0.1</td>
<td>0.6±0.1</td>
<td>0.6±0.1</td>
<td>0.6±0.1</td>
</tr>
</tbody>
</table>

Data are means ± SE for CPA doses expressed in pmol/50 nl. Range and upper and lower plateaus are expressed as % of resting neural activity normalized to 100% for each nerve. *P < 0.05 vs. Control.
of vehicle (ACF) did not affect any of the parameters of HR baroreflex curves (Fig. 6).

**DISCUSSION**

The present study supports the hypothesis emerging from our previous data, which suggested that activation of A1 adenosine receptors located in the NTS may impair baroreflex inhibition of regional SNA (28). There are three major findings of the present study.

1) Stimulation of NTS A1 adenosine receptors similarly shifted baroreflex functions for RSNA, pre-ASNA and LSNA rightward, toward higher arterial pressure, despite markedly different responses of these three sympathetic outputs to microinjections of A1 adenosine receptor agonist. The shifts of baroreflex functions toward higher MAP were independent of potential resetting of arterial baroreceptors, because the shifts were two to three times greater than the respective increases in baseline MAP caused by the stimulation.

2) Higher doses of CPA (3.3 and 330 pmol) abolished increases of neural activity that were normally observed in all sympathetic outputs during unloading of arterial baroreceptors with decreases in MAP.

3) The stimulation of NTS A1 adenosine receptors differentially affected specific parameters of sigmoidal baroreflex-response curves for each sympathetic nerve. Similar and differential A1 adenosine receptor effects on regional baroreflex functions. A1 adenosine-receptor-mediated modulation of baroreflex functions had similar as well as differential effects on regional sympathetic outputs as shown schematically in Table 5. The similar, regionally uniform, and dose-dependent alterations of baroreflex functions were as follows.

1) The threshold for baroreflex sympathoinhibition increased as shown by the uniform increases in BP50 toward higher MAP; i.e., a greater afferent stimulus (increase in MAP) was needed to overcome A1 adenosine receptor-mediated inhibition of NTS baroreflex mechanisms and trigger baroreflex responses.

2) Resting tonic baroreflex activity was inhibited, because increasing stimulation of NTS A1 adenosine receptors gradually attenuated/abolished normal sympathoexcitatory responses to unloading of arterial baroreceptors.

3) Maximal baroreflex inhibition of the regional sympathetic outputs was impaired, i.e., the lower plateaus of baroreflex functions were significantly elevated for RSNA and pre-ASNA (P < 0.05) and

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BP50, mmHg</th>
<th>Range, %</th>
<th>Upper Plateau, %</th>
<th>Lower Plateau, %</th>
<th>Gmax, %/mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>ACF</td>
<td>Control</td>
<td>ACF</td>
<td>Control</td>
</tr>
<tr>
<td>RSNA</td>
<td>15</td>
<td>96.9±2.3</td>
<td>96.9±2.3</td>
<td>112.1±9.6</td>
<td>107.0±8.2</td>
<td>131.6±9.5</td>
</tr>
<tr>
<td>pre-ASNA</td>
<td>8</td>
<td>94.9±3.5</td>
<td>95.4±4.7</td>
<td>115.5±5.2</td>
<td>125.5±7.7</td>
<td>149.8±6.3</td>
</tr>
<tr>
<td>LSNA</td>
<td>7</td>
<td>101.3±2.8</td>
<td>103.5±2.8</td>
<td>65.6±9.5</td>
<td>76.5±4.4</td>
<td>123.8±8.1</td>
</tr>
</tbody>
</table>

Data are means ± SE. Range and upper and lower plateaus are expressed as % of resting neural activity normalized to 100% for each nerve. There were no significant differences between Control and ACF for all comparisons.
tended to be elevated for LSNA ($P = 0.107$). Together these observations are consistent with regionally uniform A1 adenosine-receptor-mediated inhibition of baroreflex pathways at the level of the NTS. These data suggest that A1 adenosine receptors may be similarly located on baroreflex terminals/interneurons targeting different specific sympathetic outputs. The inhibition may occur via presynaptic inhibition of neurotransmitter release and/or via postsynaptic inhibition of interneurons participating in the baroreflex arch, because both pre- and postsynaptic locations of A1 adenosine receptors in the NTS have been found (11, 20).

Small quantitative differences between expression of A1 adenosine receptors on NTS baroreflex neurons/terminals targeting each sympathetic output may exist, as suggested by the differential effects of activation of these receptors on specific parameters of regional baroreflex functions as shown in Table 5. For example, the range and maximal gain of baroreflex control of RSNA decreased, which is consistent with the impairment/inhibition of baroreflex mechanisms controlling this sympathetic output. The range also decreased for LSNA; however, $G_{\text{max}}$ and the lower plateau remained unaltered. This may suggest that the impairment of baroreflex control was slightly smaller for LSNA than for RSNA. In contrast to RSNA and LSNA, the range of baroreflex control of pre-ASNA remained unaltered, as a result of similar increases in upper and lower plateaus suggesting that a nonbaroreflex mechanism was responsible for the upward shift of pre-ASNA baroreflex function.

Baroreflex and nonbaroreflex responses to stimulation of NTS A1 adenosine receptors. Stimulation of NTS A1 adenosine receptors similarly reset regional sympathetic baroreflex functions toward higher MAP and similarly abolished regional responses to unloading of arterial baroreceptors. Therefore the inhibition of NTS baroreflex mechanisms similarly contributed to the increases (disinhibition) of baseline pre-ASNA, RSNA, and LSNA caused by microinjections of A1 adenosine receptor agonist into the NTS. In contrast, the preferential increases in pre-ASNA were only partially mediated via the baroreflex disinhibition, because these responses partially persisted after bilateral sinoaortic denervation plus vagotomy as well as blockade of ionotropic glutamatergic transmission in the NTS (28). The nonbaroreflex component of activation of pre-ASNA was most evident after microinjections of the lowest dose of the agonist (CPA, 0.033 pmol) into the NTS. This dose of the agonist evoked an $\sim$40% increase in baseline pre-ASNA and respective upward shift of the baroreflex curve (Fig. 4; Tables 1 and 3); however, no significant resetting of the baroreflex function toward higher MAP was observed. Clearly, the impairment of baroreflex transmission required bigger doses of the agonist, whereas the nonbaroreflex component of adrenal sympathoactivation occurred with much lower doses of the agonist, probably even severalfold lower than those used in the present study. This suggests that the threshold for activation of A1 adenosine receptors operating in nonbaroreflex mechanisms responsible for the increases in pre-ASNA is much lower than the threshold for baroreflex disinhibition of this sympathetic output. Consequently, differential expression/distribution of A1 adenosine receptors on NTS neurons/neural terminals participating in these two mechanisms is likely.

The specific mechanism(s) responsible for the selective nonbaroreflex activation/disinhibition of pre-ASNA via A1 adenosine receptors located in the NTS remains unknown. Among many possibilities at least three potential NTS mechanisms increasing pre-ASNA should be considered: 1) disinhibition/facilitation of chemoreflex pathway controlling pre-ASNA; 2) disinhibition/facilitation of descending hypothalamic pathways that may activate NTS glutamatergic neurons projecting directly to those neurons of the rostral ventrolateral medulla (RVLM) that control the adrenal medulla; 3) disinhibition/facilitation of ascending projections from the NTS to hypothalamic nuclei [most likely paraventricular (PVN) and dorsomedial nuclei] that control the adrenal medulla via RVLM or via direct projections from PVN to the respective spinal sympathetic nuclei (7). In these nonbaroreflex mechanisms A1 adenosine receptors most likely modulate release of nonglutamatergic neurotransmitters, because the blockade of ionotropic glutamatergic transmission in the NTS did not abolish A1 receptor-mediated increases in pre-ASNA. For example, A1 adenosine receptors may disinhibit release of vasopressin and/or nitric oxide, because these neurotransmitters operate in reciprocal NTS-PVN connections (12, 22). Among these three potential mechanisms that may be responsible for nonbaroreflex increases in pre-ASNA the facilitation/disinhibition of chemoreflex pathways seems less likely than the facilitation of reciprocal NTS-hypothalamic connections because it has been shown that the blockade of NTS A1 adenosine receptors did not alter MAP and HR responses to stimulation of arterial chemoreceptors in conscious rats (8). The specific NTS mechanism(s) via which A1 adenosine receptors selectively activate the adrenal medulla requires further investigation.

Limitations of the method. The present study was performed in anesthetized animals; therefore baroreflex mechanisms were likely inhibited, especially the responses to unloading of arterial baroreceptors. However, general differences between baroreflex characteristics of regional sympathetic outputs are qualitatively similar for conscious and anesthetized animals as we reviewed previously (23). In the present study, we did not wait until all variables fully recovered after generating the depressor part of the baroreflex curves. Instead, we initiated the pressor part of the curve immediately after MAP returned to control values (Fig. 2A). Nevertheless, the modified baroreflex curves obtained under control conditions in the present study

---

**Table 5. Comparison of changes in regional sympathetic baroreflex functions evoked by selective stimulation of NTS A1 adenosine receptors with different doses of selective agonist CPA (0.033–330 pmol)**

<table>
<thead>
<tr>
<th>Parameters of Sigmoidal Baroreflex Function</th>
<th>Sympathetic Nerve Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP$_{SO}$</td>
<td>Adrenal</td>
</tr>
<tr>
<td>Upper plateau</td>
<td>*</td>
</tr>
<tr>
<td>Lower plateau</td>
<td>*</td>
</tr>
<tr>
<td>Range</td>
<td>*</td>
</tr>
<tr>
<td>$G_{\text{max}}$</td>
<td>*</td>
</tr>
</tbody>
</table>

Significant increases and decreases of parameters are marked with up and down arrows, respectively; —, no change. *Effect was dose dependent (2-way ANOVA showed significant dose vs. experimental condition interaction).
revealed differences between regional sympathetic outputs similar to those observed in our previous study in which steady-state alterations of MAP were used (Table 2; Ref. 23).

Baroreflex control of HR requires steady-state alterations in MAP (at least 30–60 s) to fully express the sympathetic component of the responses. Therefore in the present study mostly the parasympathetic component of baroreflex control of HR has likely been assessed. This may explain the smaller resetting of HR baroreflex-response curves toward higher pressures compared with the resetting of sympathetic baroreflex-response curves. Interestingly, although baroreflex control of HR was shifted toward higher MAP after stimulation of NTS A₁ adenosine receptors, the microinjections of all doses of CPA as well as ACF evoked moderate, dose-independent cardiac slowing similar to that observed in our previous studies (13, 25, 26, 28, 30, 32) and by others (2). These responses may reflect mechanical stimulation of the NTS especially given that the micropipettes were inserted in close proximity of the dorsal vagal nucleus, which contributes to vagal cardiac slowing in rats (18).

We were unable to perform selective blockade of NTS A₁ adenosine receptors, which might reveal potential tonic action of these receptors on cardiovascular control suggested by our previous study (31). Unfortunately, to our knowledge, there are no water-soluble selective A₁ adenosine receptor antagonists that may be microinjected into the NTS. Our attempts to use DPCPX dissolved in 2.5% DMSO, as done previously in conscious rats (8), were unsuccessful because this solution/suspension clogged our standard micropipettes with an internal diameter of ~10–15 μm per barrel.

Conclusion. Activation of A₁ adenosine receptors in the NTS differentially increases regional SNA, whereas it similarly resets baroreflex control of pre-ASNA, RSNA, and LSNA toward higher arterial pressure and impairs sympathoactivatory responses to unloading of arterial baroreceptors. These observations are consistent with regionally uniform A₁ adenosine receptor-mediated inhibition/resetting of baroreflex pathways at the level of the NTS. The increases in baseline RSNA and LSNA following microinjections of selective A₁ adenosine agonist into the NTS may be explained by inhibition of neurotransmitter release in baroreflex pathways controlling these two sympathetic outputs. However, the preferential increases in sympathetic activity directed to the adrenal medulla (pre-ASNA) only partially depend on the impairment of baroreflex restraint of this sympathetic output. The nonbaroreflex component of activation of pre-ASNA depends most likely on activation (disinhibition) of descending pathways that selectively activate pre-ASNA via direct NTS-RVL connections. The last mechanism may be responsible for the upward shift of pre-ASNA baroreflex functions. Together these data support the hypothesis that A₁ adenosine receptors are differentially expressed on NTS neurons/nerve terminals controlling different sympathetic outputs.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the generous gift of Arfonad by Hoffmann-La Roche Ltd., Basel, Switzerland. We also gratefully acknowledge the technical assistance of J. McClure. T. Ichinose is a participant in a research fellowship of the Japan Society for the Promotion of Science for Young Scientists.


