Differential control of renal vs. adrenal sympathetic nerve activity by NTS A2a and P2x purinoceptors

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Scislo, Tadeusz J., and Donal S. O’Leary. Differential control of renal vs. adrenal sympathetic nerve activity by NTS A2a and P2x purinoceptors. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H2130–H2139, 1998.—Activation of aden- osine A2a and ATP P2x purinoceptors in the subpostremal nucleus tractus solitarii (NTS) via microinjection of the selective agonists CGS-21680 and α,β-methylene ATP (α,β- MeATP), respectively, elicits large dose-dependent decreases in arterial pressure and heart rate, differential regional vasodilation, and differential inhibition of regional sympathetic outputs. With marked hypotensive hemorrhage, preganglionic adrenal sympathetic nerve activity (pre-ASNA) increases, whereas renal (RSNA) and postganglionic adrenal sympathetic nerve activity (post-ASNA) decrease. In this setting, adenosine levels in the brain stem increase. Therefore, we investigated whether stimulation of specific purinoceptors in the NTS may evoke differential sympathetic responses. RSNA was recorded simultaneously with pre-ASNA or post-ASNA in chloralose-urethan-anesthetized male Sprague-Dawley rats. CGS-21680 (2 and 20 pmol in 50 nl) inhibited RSNA and post-ASNA, whereas pre-ASNA increased markedly. α,β-MeATP (25 and 100 pmol in 50 nl) inhibited all sympathetic outputs. Sinoaortic denervation plus vagotomy markedly prolonged the responses to P2x-purinoceptor stimulation. Glutamate (100 pmol in 50 nl) caused differential inhibition of all sympathetic outputs similar to that evoked by α,β-MeATP. We conclude that NTS A2a-purinoceptor activation evokes differential sympathetic responses similar to those observed during hemorrhage, whereas P2x-purinoceptor and glutamate-receptor activation evokes differential inhibition of sympathetic outputs similar to arterial baroreflex responses.

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nts in ASNA and a simultaneous decrease in RSNA (16, 32). To test this hypothesis, we compared ASNA and RSNA responses to selective stimulation of A2a purinoceptors in the subpostremal NTS. To evaluate whether the pattern of sympathetic responses (ASNA vs. RSNA) is specific to stimulation of A2a purinoceptors, we compared these responses with those evoked by selective stimulation of P2x purinoceptors and nonselective stimulation of glutamate receptors in the same site of the NTS. To distinguish between primary responses to stimulation of the purinoceptor subtypes in the NTS and reflex compensation to these responses via arterial and cardiopulmonary afferents, the effects evoked by microinjections of selective A2a- and P2x-purinoceptor agonists (CGS-21680 and α,β-MeATP, respectively) were compared in intact versus sinoaortic denervated and vagotomized (SAD + VX) animals.

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 Animals” endorsed by the American Physiological Society and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Design. The effect of activation of A2a and P2x purinoceptors in the subpostremal region of the NTS on simultaneously recorded sympathetic nerve activity directed to the adrenal gland and the kidney was investigated in 26 intact and 11 SAD + VX male Sprague-Dawley rats (350–400 g) (Charles River Laboratories, Wilmington, MA). MAP and HR responses were also recorded. Activation of purinoceptors was accomplished via microinjections of the selective A2a- and P2x-purinoceptor agonists CGS-21680 and α,β-MeATP, respectively. In some animals the effects of microinjection of vehicle [artificial cerebrospinal fluid (ACF)] (5, 28, 31) on cardiovascular and neural parameters were observed over the average time of the response to the maximal dose of CGS-21680 or α,β-MeATP, respectively. In an additional five animals with intact baroreceptors and vagal afferents, nonselective glutamatergic receptors were stimulated via microinjections of sodium glutamate into various sites of subpostremal and immediately adjacent commissural NTS. This was performed to compare responses evoked by selective activation of purinoceptor subtypes with a standard “nonspecific” stimulation of NTS neurons in this area.

Instrumentation and measurements. All the procedures were described in detail previously (2, 5, 14, 28, 29, 31). Briefly, rats were anesthetized with a mixture of α-chloralose (80 mg/kg) and urethane (500 mg/kg ip) and tracheotomized. Intact animals breathed spontaneously, similar to our previous studies (28, 31). SAD + VX animals were connected to a small animal respirator (SAR-830, CWE, Ardmore, PA) to compensate for the changes in respiratory pattern. All animals breathed oxygen-enriched air. Arterial blood gases were tested occasionally (ABL500, OSM 3; Radiometer), and ventilation was adjusted to maintain PaO2, PaCO2, and pH within normal ranges. The right femoral artery and vein were catheterized to monitor arterial blood pressure and infusion drugs. SAD + VX was accomplished and its completeness tested as described previously (29).

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 a hard disk for subsequent analysis.

Microinjections into the NTS. Unilateral microinjections of CGS-21680, α,β-MeATP, glutamate, or vehicle (ACF) were made with multibarrel glass micropipettes into the medial region of the caudal subpostremal NTS as described previously (2, 5, 28, 31). Some microinjections of glutamate were made into the adjacent portions of the NTS, i.e., commissural and rostral subpostremal NTS. Nonselective activation of glutamatergic receptors allowed evaluation of whether the relative ratio between regional neural responses (ASNA vs. RSNA) to a nonspecific stimulus (glutamate) depends on the precise anatomic location of the stimulus in this area of the NTS. All microinjection sites were verified histologically as described previously (28, 30, 31) and presented schematically in Fig. 1.

The doses of CGS-21680 and α,β-MeATP were the same as those used in our previous studies (5, 28, 31): 1) the approximate threshold hypotensive dose (2 and 25 pmol for CGS-21680 and α,β-MeATP, respectively), and 2) the maximally effective hypotensive dose (20 and 100 pmol for CGS-21680 and α,β-MeATP, respectively) (5, 28, 31). We have previously shown that the effects elicited with the high doses of both purinoceptor agonists were completely and selectively blocked by microinjection of the selective A2a-purinoceptor antagonist CGS-15943A (2) or P2x-purinoceptor antagonist suramin, respectively (14, 28). To avoid the effect of desensitization of purinoceptors, in all experiments only one dose of purinoceptor agonist was microinjected into the left and/or right side of the NTS. If purinergic agonists were injected bilaterally, at the end of each experiment with washout with hexamethonium (20 mg/kg iv), RSNA was almost completely postganglionic; only 4.3 ± 1.1% (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 (8, 9). Therefore, ASNA was considered predominantly postganglionic if the activity remaining after ganglionic blockade at the end of each experiment was >75% or predominantly preganglionic if the remaining activity was <50%. These criteria were set because, in some experiments, ASNA slightly increased in response to arfonad at the beginning of surgery and then decreased slightly in response to hexamethonium after 6–10 h of the experiment. Data from animals that exhibited ASNA between 50 and 75% after ganglionic blockade were excluded from further calculations. Average ASNA after ganglionic blockade was 121.4 ± 5.1 (n = 36) and 38.8 ± 2.4% (n = 6) of control level immediately after microinjection of hexamethonium for “preganglionic” (pre-ASNA) and “postganglionic” ASNA (post-ASNA), respectively. Pre-ASNA increased over 100%, likely because of an arterial baroreflex response caused by the decrease in MAP after ganglionic blockade.

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formed. All the drugs were dissolved in ACF and the pH adjusted to 7.2. Microinjections of ACF (50 nl) served as a vehicle control.

Data analysis. Hemodynamic and sympathetic nerve responses were quantified in two ways: 1) the maximal percent difference from a 30-s basal control period taken immediately before microinjection, and 2) integration of the percent changes from control over the period of the change in MAP. 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 maximum response values. Because the responses to α,β-MeATP frequently exhibited a biphasic pattern, i.e., fast recovery to ~80% of the depressor response followed by variable residual depression, only the fast part of the response (to 80% of depression) was analyzed, similar to our previous study (28). However, time to recovery was measured for both fast and slow components of the response. Maximal and integral responses to control microinjections of ACF were measured over the time to recovery of MAP after the high dose of CGS-21680 and/or α,β-MeATP, respectively. One-way ANOVA for independent measures was used to compare MAP and HR responses to ACF versus different doses of CGS-21680 and α,β-MeATP. A two-way ANOVA for independent measures was used to compare neural responses versus doses of the drug and ASNA versus RSNA. Differences observed were further evaluated by the test of simple effect. An α-level of P < 0.05 was used to determine statistical significance.

RESULTS

The resting values for MAP and HR measured in intact animals (n = 31) before microinjection of drugs or vehicle into the subpostremal NTS were 81.8 ± 1.3 mmHg and 367 ± 4 beats/min. The effects of microinjections of CGS-21680 and α,β-MeATP at the high doses (20 and 100 pmol/rat, respectively) on MAP, HR, and simultaneously recorded pre-ASNA and RSNA are presented in Fig. 2. Microinjection of CGS-21680 produced gradually developing and long-lasting reductions in MAP, HR, and RSNA; however, pre-ASNA increased significantly. In contrast to the directionally opposite responses of RSNA and pre-ASNA to CGS-21680, α,β-MeATP evoked decreases in both sympathetic outputs. The responses to α,β-MeATP started abruptly and usually showed a biphasic pattern of recovery (fast and slow components), especially for the high dose of the drug (Fig. 2). Fast recovery, to ~80% of the depressor response, was followed by a weak residual depression,
cardiac slowing, and sympathoinhibition, lasting several times longer than the fast component of the response (Fig. 2 and Table 1). Both drugs demonstrated a dose dependency of the duration of the depressor responses (Table 1).

A2a-purinoceptor stimulation. The average neural and hemodynamic responses to ACF (50 nl) and both doses of CGS-21680 (2 and 20 pmol in 50 nl) are shown in Fig. 3. Microinjections of CGS-21680 evoked substantial dose-dependent increases in pre-ASNA, whereas RSNA and hemodynamic parameters markedly decreased in a dose-dependent manner. The differences between neural responses were highly significant for both methods of evaluation of the effect (i.e., maximum and integral responses, P < 0.0001). HR and RSNA responses were similar in magnitude and time course to those observed in previous studies from our laboratory (5, 31). However, the decrease in MAP was ~30–50% smaller compared with that previously observed for both doses of the drug in terms of both the maximal and integral responses (5, 31; see DISCUSSION). Microinjections of the same volume of ACF did not evoke marked integral responses measured over the average time to recovery of MAP for the high dose of CGS-21680 (Fig. 3). The integral RSNA, pre-ASNA, and HR responses to ACF were not different from zero (P > 0.05), and the integral response of MAP did not decrease but rather tended to increase slightly (P = 0.034). Because hemodynamic and neural parameters fluctuate spontaneously, some decreases and equivalent increases in all parameters were observed over the relatively long time period of measurement (almost a half hour); however, these minor spontaneous fluctuations were significantly smaller than the responses to even the low dose of CGS-21680 (P < 0.05) (Fig. 3). Note that pre-ASNA responses to CGS-21680 were compared with the maximal, spontaneous increases in the activity recorded after microinjections of ACF, whereas responses in RSNA and hemodynamic parameters (decreases) were compared with maximal spontaneous decreases in these parameters. The magnitude of spontaneous increases was not different from the magnitude of spontaneous decreases for all recorded parameters (P > 0.05; the remaining data for all these comparisons are not presented).

Table 1. Time to recovery of mean arterial blood pressure after microinjections of α,β-MeATP and CGS-21680 into the NTS in intact and sinoaortic baroreceptor-denervated plus vagotomized animals

<table>
<thead>
<tr>
<th>Drug</th>
<th>Intact</th>
<th>SAD + VX</th>
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<tbody>
<tr>
<td>α,β-MeATP</td>
<td>Low dose</td>
<td>High dose</td>
</tr>
<tr>
<td>Fast response</td>
<td>0.44 ± 0.08* (9)</td>
<td>4.29 ± 0.76 (8)</td>
</tr>
<tr>
<td>Slow response</td>
<td>8.39 ± 1.41* (9)</td>
<td>21.00 ± 1.61 (8)</td>
</tr>
<tr>
<td>CGS-21680</td>
<td>20.48 ± 1.03* (8)</td>
<td>28.19 ± 1.46 (9)</td>
</tr>
</tbody>
</table>

Data are means ± SE. Numbers in parentheses are no. of animals receiving α,β-methylene ATP (α,β-MeATP; 25 or 100 pmol/50 nl) or CGS-21680 (2 or 20 pmol/50 nl). Time of responses was dose dependent. Sinoaortic denervation plus vagotomy (SAD + VX) markedly prolonged fast component of response to α,β-MeATP and slightly increased time of response to CGS-21680, likely because responses were not reflexly buffered after SAD + VX. NTS, nucleus tractus solitarii. *P < 0.05 vs. intact (high dose).

P2x-purinoceptor stimulation. Averaged fast components of hemodynamic and neural responses to both...
doses of α,β-MeATP compared with respective vehicle control (ACF) are presented in Fig. 4. Microinjection of α,β-MeATP produced powerful, dose-related reductions in the activity of both sympathetic outputs and decreases in MAP and HR. The decreases in MAP, HR, and RSNA were similar in magnitude and time course to those observed in our previous studies (5, 28). The responses evoked by the high dose of α,β-MeATP were significantly greater than those caused by the low dose for both maximal and integral changes (P < 0.05 for all comparisons). However, dose-related differences were much more pronounced for integral changes, especially for the neural activity, which was abruptly inhibited, sometimes to virtually zero levels for a very short time (a few seconds) just after the microinjection. Large dose-related differences for integral responses and small dose-related differences for maximum changes reflect the observation that higher doses of α,β-MeATP evoked much longer-lasting inhibitory effects compared with those evoked with low doses (Table 1; compare also Fig. 2, right, and Fig. 5, left). The fast responses to α,β-MeATP were several times shorter than those evoked by CGS-21680, especially for the small, near-threshold doses of both drugs (Table 1).

RSNA was inhibited to a slightly greater extent than pre-ASNA for both high and low doses of α,β-MeATP and for both methods of evaluation of the effects (P < 0.05).

Microinjection of vehicle (ACF) into the same site of the NTS had no marked effect on any of the parameters measured over the average time of fast depressor responses to the higher dose of α,β-MeATP (Fig. 4). Sinoaortic denervation and vagotomy. Bilateral sinoaortic denervation and subsequent vagotomy resulted in a marked, sustained increase in HR and transient elevation in MAP. HR measured in SAD + VX animals (n = 11) just before the microinjections of the drugs (3–10 h after denervation) remained elevated compared with HR measured in intact animals (441 ± 6 vs. 367 ± 4 beats/min, respectively; P < 0.0001). In con-
Table 2. Effect of SAD + VX on hemodynamic and neural responses to microinjections of α,β-MeATP and CGS-21680 into the subpostremal NTS

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Maximum Responses</th>
<th>Integral Responses</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>%ΔMAP</td>
<td>%ΔHR</td>
</tr>
<tr>
<td>αβ-MeATP (100 pmol/50 nl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>52.3±3.3</td>
<td>57.8±12.3</td>
</tr>
<tr>
<td>SAD + VX</td>
<td>49.5±2.4</td>
<td>64.0±11.0</td>
</tr>
<tr>
<td>CGS-21680 (20 pmol/50 nl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>26.8±1.5</td>
<td>58.7±11.5</td>
</tr>
<tr>
<td>SAD + VX</td>
<td>22.3±3.8</td>
<td>43.4±10.9</td>
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</table>

Data are means ± SE. Maximum (%Δ) and integral (Δ%) responses include mean arterial blood pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), and preganglionic adrenal sympathetic nerve activity (pre-ASNA). Integral responses to αβ-MeATP (fast components) were markedly greater in SAD + VX animals compared with those recorded in intact animals, mostly because of markedly longer times of responses. Opposite neural responses to stimulation of A2a purinoceptors, i.e., marked decrease in RSNA and marked increase in pre-ASNA, were not affected by SAD + VX. *P < 0.05 vs. intact; †P < 0.05 vs. RSNA.

In contrast, MAP returned gradually toward resting values during the 30–60 min after denervation, and no differences between SAD + VX vs. intact animals were observed throughout the time course of experiments (82.9 ± 1.3 vs. 81.8 ± 1.3 mmHg, respectively; P = 0.681).

The comparison of average maximum and integral responses evoked by microinjections of the high doses of CGS-21680 and αβ-MeATP in intact and SAD + VX animals is presented in Table 2. The opposite regional neural responses to stimulation of A2a purinoceptors, i.e., a decrease in RSNA and an increase in pre-ASNA, were not affected by SAD + VX. Also, the magnitude of hemodynamic responses to CGS-21680 was not markedly changed by SAD + VX, although the maximum responses tended to decrease (Table 2). However, SAD + VX slightly increased the time to recovery of MAP (Table 1).

SAD + VX significantly increased the fast component of integral neural and MAP responses to stimulation of P2x purinoceptors, reflecting the much longer-lasting inhibitory effects of αβ-MeATP in SAD + VX versus intact animals (Tables 1 and 2). However, HR responses were not significantly altered with SAD + VX. A slight tendency to decrease maximum HR responses and to increase integral HR responses in SAD + VX versus intact animals did not reach statistical significance (P = 0.429 and P = 0.0968, respectively).

Glutamatergic microinjections. Similarities between neural and hemodynamic responses evoked by the low dose of αβ-MeATP (25 pmol in 50 nl) and a moderate dose of glutamate (100 pmol in 50 nl) are shown in Fig 5 (left). The microinjection of glutamate elicited a short-lasting decrease in MAP, HR, RSNA, and ASNA. The time course of these responses was very similar to that of the fast component of the responses evoked by a low dose of glutamate.
alpha,beta-MeATP. Both drugs inhibited pre-ASNA to a slightly lesser extent than RSNA. The ratio between maximal neural responses (Δ%pre-ASNA/Δ%RSNA) was virtually the same for both agonists microinjected into the same area of the caudal subpostremal NTS [0.668 ± 0.055 vs. 0.641 ± 0.063 for alpha,beta-MeATP (n = 9) and glutamate (n = 7), respectively; P = 0.750].

Averaged responses to glutamate microinjected into the caudal subpostremal NTS and immediately adjacent areas of the NTS, i.e., rostral subpostremal and comissural, are presented as bar graphs in Fig. 5 (see also microinjection sites in Fig. 1). Neural and MAP responses did not differ between specific microinjection sites; however, HR responses tended to increase toward the rostral subpostremal NTS (P = 0.039). The ratio between pre-ASNA and RSNA responses was similar regardless of the specific location of the microinjections (0.685 ± 0.063, 0.641 ± 0.063, and 0.668 ± 0.55 for rostral subpostremal, caudal subpostremal, and comissural NTS, respectively; P > 0.05 for each comparison).

Post-ASNA responses. In contrast to pre-ASNA, post-ASNA responded similarly to RSNA. Stimulation of A2a purinoceptors evoked a decrease in predominantly post-ASNA in contrast to the increase observed in predominantly pre-ASNA (compare Figs. 3 and 6). The inhibition of post-ASNA was smaller than that for RSNA; however, predominantly post-ASNA exhibited almost 40% of preganglionic activity, which presumably increased in response to stimulation of A2a purinoceptors, whereas RSNA was ~96% postganglionic.

The high dose of alpha,beta-MeATP evoked virtually the same inhibition of post-ASNA and RSNA in terms of both maximal and integral responses (P > 0.05 for both comparisons). Also, the responses to glutamate were very similar for both post-ASNA and RSNA. Integral neural responses to glutamate were virtually the same between post-ASNA and RSNA, although a very small (3.2%) "significant" (P = 0.047) difference for the maximum responses was observed. The ratio between post-ASNA and RSNA responses to glutamate (Δ%post-ASNA/Δ%RSNA) was significantly closer to 1 than that calculated for all (n = 17) pre-ASNA versus RSNA responses (0.953 ± 0.016 vs. 0.676 ± 0.036, respectively, P = 0.006).

**DISCUSSION**

This is the first study to investigate the effects of selective stimulation of A2a and P2x purinoceptors located in subpostremal NTS on preganglionic sympathetic nerve activity directed to the adrenal gland. Also, for the first time, hemodynamic and neural responses to stimulation of purinoceptor subtypes in the NTS were compared in intact versus SAD + VX animals. The major new finding of the present study is that pre-ASNA increased in response to stimulation of NTS A2a purinoceptors and decreased in response to stimulation of P2x and glutamate receptors, whereas simultaneously recorded RSNA and post-ASNA decreased in response to stimulation of all of these receptor subtypes. These differential neural responses were dose dependent and were accompanied by respective decreases in MAP and HR, as observed previously (5, 28, 31). In addition, SAD + VX markedly prolonged the responses to activation of P2x purinoceptors and slightly extended the responses to activation of A2a purinoceptor stimulation without marked changes in their initial magnitudes. SAD + VX experiments demonstrate that the increase in pre-ASNA after stimulation of A2a purinoceptors was not a baroreflex response to the concomitant decrease in MAP but a primary response to central stimulation of these receptors.

Differential sympathetic control via NTS neurons. Stimulation of P2x purinoceptors in the subpostremal NTS inhibited pre-ASNA to a lesser extent than RSNA; however, there was no difference between the inhibition of post-ASNA and RSNA. More dramatic differences among these regional sympathetic outputs were observed in response to A2a-purinoceptor stimulation: pre-ASNA increased, whereas post-ASNA decreased and RSNA decreased to an even greater extent. In addition, our previous studies showed that stimulation of P2x purinoceptors inhibited RSNA to a lesser extent than LSNA (directed mostly to the hindlimb), whereas stimulation of A2a purinoceptors, which inhibited RSNA to an extent similar to that in the present study, caused no significant change in LSNA (28, 31). The ratio between regional sympathetic responses to selective stimulation of A2a and P2x purinoceptors was different...
and characteristic for each pair of sympathetic outputs (Δ%pre-ASNA/Δ%RSNA, Δ%post-ASNA/Δ%RSNA, and Δ%SNA/Δ%RSNA) and each purinoceptor subtype (A2a and P2x). These differential sympathetic responses to stimulation of different purinoceptor subtypes were evoked from the same anatomic site of the NTS. It is worthwhile to note that the relative ratio between inhibitory neural responses to nonselective glutamatergic stimulation (Δ%pre-ASNA/Δ%RSNA) remained virtually the same for all the microinjection sites located in various parts of subpostremal NTS and the immediately adjacent comissural NTS. This indicates that the differential neural response patterns were not related to a very discrete anatomic location of neurons targeting different sympathetic output. Instead, it is likely that NTS neurons finally targeting sympathetic output directed to different organs exhibited differential expression of A2a versus P2x and glutamate receptor subtypes. In support of this concept, a recent study confirmed that relative expression of various types of K⁺ channels was different for subpostremal and comissural NTS neurons that are involved in arterial baroreflex versus cardiopulmonary reflex control of the circulation (7). Taken together, the results of the present and our previous studies support the hypothesis that different neurotransmitters/neuromodulators operating in the same site of the NTS may be related to specific functional subsystems differentially controlling regional sympathetic outputs.

Physiological implications. Stimulation of A2a purinoceptors in subpostremal NTS created a very similar pattern of hemodynamic and differential sympathetic neural responses to that observed during the latter hypotensive stage of severe hemorrhage, i.e., hypotension accompanied with a large and long-lasting decrease in HR and RSNA and a contrasting, marked increase in pre-ASNA (8, 35, 39). Adenosine naturally accumulates in the brain stem, including the NTS, during severe hemorrhage, hypoxia, or cerebral ischemia (38, 40). This coincidence strongly suggests that adenosine naturally released in the NTS during hemorrhage may participate via its A2a-purinoceptor action in creating specific regional responses that occur during hemorrhagic shock.

Interestingly, the decreases in MAP in response to stimulation of NTS adenosine receptors recorded in the present study were ~30−50% smaller than those observed previously, whereas the decreases in HR and RSNA were virtually the same (5, 31). One major difference among the experimental preparations was that in the present study the left adrenal nerve was cut for recording ASNA, whereas in previous studies sympathetic innervation of both adrenal glands remained intact. Considering that stimulation of A2a purinoceptors in the subpostremal NTS evoked large, long-lasting increases in pre-ASNA directed predominantly to the adrenal medulla, it is likely that release of epinephrine is increased in this setting. In the rat, stimulation of pre-ASNA results in a 4:1 increase in the release of epinephrine versus norepinephrine as measured in the adrenal vein (20). Epinephrine operating via vascular β-receptors located preferentially in the muscle vascular bed (37) may contribute to the depressor response evoked by stimulation of A2a purinoceptors in the NTS via preferential vasodilation of skeletal muscle. We previously observed that stimulation of NTS A2a purinoceptors evoked marked, preferential vasodilation of the hindlimb versus the renal and mesenteric vascular beds (5). In the present study, because one adrenal gland was denervated, less epinephrine would be released, thereby limiting peripheral vasodilation and hypotension via this mechanism. In support of this concept, we recently reported that the hypotensive and hindlimb vasodilator responses to stimulation of NTS A2a purinoceptors were abolished by systemic β-adrenergic blockade (19).

Stimulation of P2x purinoceptors exerted MAP and HR depression and sympathoinhibition of a very rapid onset and biphasic pattern of recovery; the first ~80% of the response recovered relatively fast (in 28 s for the low dose of α,β-MeATP), whereas weak residual depression lasted several times longer (Table 1). This biphasic time course of the responses suggests that two different mechanisms were triggered by stimulation of P2x purinoceptors in the NTS; presumably, neuromediator-like action was followed by neuromodulator-like action. Interestingly, the fast response elicited by the low, near-threshold dose of α,β-MeATP was similar to that evoked by microinjection of glutamate and mimicked baroreflex response (Fig. 5). The ratio between these neuromodulatory responses of pre-ASNA and RSNA was virtually the same for α,β-MeATP and glutamate. In addition, blockade of P2x purinoceptors with suramin microinjected into the same site of the NTS markedly impaired HR baroreflex responses (30). Previous studies indicate that ATP operating via P2x purinoceptors may serve as a fast neurotransmitter between central neurons (12, 13, 17) and that ATP is neurally released into the NTS (33). Therefore, it is possible that ATP may act as a fast neurotransmitter between NTS interneurons possibly linked in chain with glutamatergic neurons operating in the baroreflex arc. Alternatively, stimulation of P2x purinoceptors may trigger the release of glutamate in the baroreflex arc at the level of the NTS, as was postulated for hippocampal structures (17). The secondary weak, slow, and long-lasting response may be mediated via triggering of the release of one of numerous neuromodulators operating in the NTS (21). This interesting possibility awaits further, detailed investigations.

In summary, different neurotransmitters/neuromodulators microinjected into the same site of the NTS evoked different patterns of regional sympathetic responses. Differential neural response to stimulation of A2a purinoceptors resembled differential neural response to severe hemorrhage, whereas the rapid onset and fast initial recovery of the responses to stimulation of P2x purinoceptors were similar to typical responses evoked with stimulation of glutamate receptors and resembled rapid baroreflex responses. The ratio between neural responses to selective stimulation of A2a and P2x purinoceptors was characteristic for each pair.
of sympathetic outputs and each purinoceptor subtype. We believe that a comparison of specific patterns of regional peripheral responses to administration of specific neurotransmitters/neuromodulators into discrete groups of central neurons performed in vivo in a whole animal may help explain the physiological role and significance of molecular mechanisms described at the cellular level in vitro.

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