Differential role of nitric oxide in regional sympathetic responses to stimulation of NTS A2a adenosine receptors

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Scislo, Tadeusz J., Nobusuke Tan, and Donal S. O’Leary. Differential role of nitric oxide in regional sympathetic responses to stimulation of NTS A2a adenosine receptors. Am J Physiol Heart Circ Physiol 288: H638–H649, 2005. First published September 30, 2004; doi:10.1152/ajpheart.00857.2004.—Our previous studies showed that preganglionic adrenal (pre-ASNA), renal (RSNA), lumbar, and postganglionic adrenal sympathetic nerve activities (post-ASNA) are inhibited after stimulation of arterial baroreceptors, nucleus of the solitary tract (NTS), and glutamateergic and P2X receptors and are activated after stimulation of adenosine A1 receptors. However, stimulation of adenosine A2a receptors inhibited RSNA and post-ASNA, whereas it activated pre-ASNA. Because the effects evoked by NTS A2a receptors may be mediated via activation of nitric oxide (NO) mechanisms in NTS neurons, we tested the hypothesis that NO synthase (NOS) inhibitors would attenuate regional sympathetic responses to NTS A2a receptor stimulation, whereas NO donors would evoke contrasting responses from pre-ASNA versus RSNA and post-ASNA. Therefore, in chloralose/urethane-anesthetized rats, we compared hemodynamic and regional sympathetic responses to microinjections of selective A2a receptor agonist (CGS-21680, 20 pmol/50 nl) after pretreatment with NOS inhibitors Nω-nitro-l-arginine methyl ester (10 nmol/100 nl) and 1-[(2-trifluoromethyl)phenyl]imidazole (100 pmol/100 nl) versus pretreatment with vehicle (100 nl). In addition, responses to microinjections into the NTS of different NO donors [40 and 400 pmol/50 nl sodium nitroprusside (SNP); 0.5 and 5 mmol/50 nl 3,3-bis(aminomethyl)-1-hydroxy-2-oxo-1-triazene (DETA NONOate, also known as NOC-18), and 2 mmol/50 nl 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine (PAPA NONOate, also known as NOC-15), the NO precursor l-arginine (10–50 mmol/50 nl), and sodium glutamate (500 pmol/50 nl) were evaluated. SNP, DETA NONOate, and PAPA NONOate activated pre-ASNA and inhibited RSNA and post-ASNA, whereas l-arginine and glutamate microinjected into the same site of the NTS inhibited all these sympathetic outputs. Decreases in heart rate and depressor or sympathoactivatory responses accompanied the neural responses. Pretreatment with NOS inhibitors reversed the normal depressor and sympathoactivatory responses to stimulation of NTS A2a receptors into pressor and sympathoactivatory responses and attenuated the heart rate decreases; however, it did not change the increases in pre-ASNA. We conclude that NTS NO mechanisms differentially affect regional sympathetic outputs and differentially contribute to the pattern of regional sympathetic responses evoked by stimulation of NTS A2a receptors.

nitric oxide donors and inhibitors; adrenal sympathetic nerve; renal sympathetic nerve

Recent studies from our laboratory and by others (5, 30, 35, 44) have shown that adenosine, among the numerous neuroactive substances operating in the nucleus of the solitary tract (NTS), plays an important role as a neuromodulator of cardiovascular function. Adenosine, when microinjected into the NTS, evokes depressor and cardiac slowing responses, which are effects similar to those evoked by activation of the arterial baroreflex that is initially integrated at the level of the NTS. Hypotensive action of adenosine is mediated via A2a receptors (3). A2a receptors may facilitate the release of glutamate from neural terminals in central structures including the NTS (10, 30), and A2a receptors are present on presynaptic vagal terminals in the NTS (10). In addition, nonsympathetic blockade of adenosine receptors attenuates heart rate (HR) baroreflex responses to increases in MAP (31), and selective blockade of A2a receptors in the NTS attenuates MAP and HR responses evoked by electrical stimulation of the aortic nerve (45). Therefore, on the basis of these general hemodynamic observations (MAP and HR), it has been postulated that the hypotensive action of adenosine in the NTS is mediated mostly via stimulation of presynaptic A2a receptors and the release of glutamate from afferent terminals and/or from intrinsic NTS interneurons mediating baroreflex transmission. However, a line of evidence from our laboratory showed that differential patterns of regional sympathetic responses to selective stimulation of NTS A2a receptors are different from that evoked by activation of arterial baroreceptors (34–37). Specifically, although depressor and bradycardic responses occur and stimulation of NTS A2a receptors inhibited renal sympathetic nerve activity (RSNA) and postganglionic adrenal sympathetic nerve activity (post-ASNA) consistent with baroreflex responses, activation of these receptors did not change lumbar sympathetic nerve activity and markedly increased preganglionic adrenal sympathetic nerve activity (pre-ASNA), effects inconsistent or opposite to those evoked by activation of arterial baroreceptors (34). In addition, sinoaortic denervation plus vagotomy or blockade of ionotropic glutamatergic transmission in the NTS did not markedly change the pattern of neural responses to stimulation of NTS A2a receptors (38). These observations suggested that sympathetic responses to stimulation of A2a receptors in the NTS are at least not entirely mediated via facilitation of glutamate release from baroreflex terminals, as suggested previously (10, 31, 45), and that some unknown nonglutamatergic mechanism may contribute to these responses.

A2a adenosine receptor-induced release of nitric oxide (NO) from neural terminals and/or vascular endothelium should be considered among several possible factors contributing to the differential pattern of regional sympathetic responses evoked by selective stimulation of A2a receptors in the NTS. In support of these observations, increased NO synthesis by iNOS or eNOS may be triggered by activation of A2a receptors in the NTS, as suggested previously (34, 35, 44). Therefore, different neuronal and vascular NO mechanisms differentially contribute to the pattern of regional sympathetic responses evoked by stimulation of A2a receptors in the NTS.
of this concept, stimulation of adenosine $A_{2a}$ receptors activates adenylcyclase and increases intracellular $Ca^{2+}$ levels that may activate NO synthase (NOS) and, consequently, increase NO production (7, 22, 33). Adenosine $A_{2a}$ receptors may also stimulate NO production via phosphorylation of p42/p44 nitrogen-activated protein kinase as recently reported (49). NO containing nerve terminals of vagal and nonvagal origin are abundant in the NTS (25). Microinjections of NO donors into the NTS increase the activity of NTS neurons (13, 42) and evoke differential responses in regional vascular beds (19). Therefore, the diverse pattern of regional sympathetic responses to activation of NTS $A_{2a}$ receptors observed in our previous studies (35–38) could have been a result of triggering the release of NO from baroreceptor and nonbaroreceptor terminals that may differentially affect NTS neurons/neural terminals controlling different sympathetic outputs. This concept is consistent with a previous report (27) that depressor and cardiac slowing responses to microinjections of adenosine into the NTS were attenuated after pretreatment with the nonselective NOS inhibitors $N^G$-monomethyl-L-arginine ($L$-NMMA) and $N^w$-nitro-L-arginine methyl ester ($L$-NAME). Therefore, in the present study, we compared regional patterns of sympathetic responses evoked by selective activation of NTS $A_{2a}$ receptors before and after pretreatment with the selective neuronal NOS (nNOS) inhibitor 1-[2-(trifluoromethyl)phenyl]-imidazole (TRIM) and the nonselective NOS inhibitor ($L$-NAME), which potentially affects both neuronal and endothelial NOS (eNOS) (17, 29). Comparison of the effects of TRIM versus $L$-NAME allowed us to assess the potential contribution of nNOS versus eNOS to the responses evoked by stimulation of NTS $A_{2a}$ receptors. To assess whether NO released into the NTS may trigger a differential pattern of regional sympathetic responses consistent with that observed after stimulation of NTS $A_{2a}$ receptors, we compared the patterns evoked by intra-NTS application of the different NO donors: sodium nitroprusside (SNP), 3,3-bis(aminethyl)-1-hydroxy-2-oxo-1-triazene (DETA NONOate, also known as NOC-15), and 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine (PAPA NONOate, also known as NOC-18), and 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine (PAPA NONOate, also known as NOC-15) and the NO precursor $L$-arginine. Our data showed that NO released into the NTS network differentially affects regional sympathetic outputs and differentially contributes to the pattern of regional sympathetic responses evoked by stimulation of NTS $A_{2a}$ adenosine receptors.

**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 published by the National Institutes of Health.

**Design.** The effect of blockade of NOS or the respective volume control by artificial cerebrospinal fluid (ACF) on hemodynamic and regional sympathetic responses to stimulation of $A_{2a}$ adenosine receptors in the subpostemtral NTS was studied in 25 male Sprague Dawley rats (350–400 g) (Charles River Laboratories, Wilmington, MA). In seven animals, the responses to stimulation of NTS $A_{2a}$ receptors with microinjections of the selective agonist CGS-21680 were compared after microinjection of ACF and the selective nNOS antagonist TRIM. In 15 animals, responses to CGS-21680 were compared after ACF and/or $L$-NAME, a nonselective antagonist of both nNOS and eNOS. In three animals, a time control was performed (2 microinjections of ACF + CGS-21680 in 90-min intervals). In an additional 24 animals, the effects of microinjections into the subpostemtral NTS of NO donors, the NO precursor $L$-arginine, and glutamate on hemodynamic and regional sympathetic responses were assessed.

**Instrumentation and measurements.** All procedures have been previously described in detail (4, 36–39). Briefly, rats were initially anesthetized with a mixture of $\alpha$-chloralose (80 mg/kg) and urethane (500 mg/kg ip), tracheotomized, and artificially ventilated with oxygen-enriched air. After the completion of the surgery, a continuous intravenous infusion of $\alpha$-chloralose (8–16 mg kg$^{-1}$ h$^{-1}$) and urethane (50–100 mg kg$^{-1}$ h$^{-1}$) was applied to maintain a stable level of anesthesia during the experimental protocols as has been performed in all of our previous studies (4, 34–40). The level of anesthesia was monitored via corneal and hindlimb withdrawal reflexes and the stability of hemodynamic and neural variables. Rectal body temperature was maintained at 37–38°C by means of a heating pad and heating lamp. Arterial blood gases were tested occasionally (models ABL500 and OSM3; Radiometer), and ventilation was adjusted to maintain $P_{O_2}$, $P_{CO_2}$, and pH within normal ranges. Average values measured at the end of each experiment were: $P_{O_2}$, 120.1 ± 3.3; $P_{CO_2}$, 37.4 ± 0.7; pH, 7.417 ± 0.007 ($n$ = 49). The right femoral artery and vein were catheterized to monitor arterial blood pressure and infuse drugs.

In each experiment, simultaneous recordings from two sympathetic outputs (RSNA + pre-ASNA or RSNA + post-ASNA) were performed. The adrenal and renal nerves were exposed retroperitoneally, and neural recordings were accomplished as previously described (37–39). Neural signals were initially amplified (×2,000–20,000 times) 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. Resting nerve activity before each microinjection was normalized to 100%.

The ratio between preganglionic and total nerve activity was initially tested with bolus intravenous injection of the short-lasting (1–2 min) ganglionic blocker Arfonad (2 mg/kg; Hoffmann La Roche) (37–39) and reevaluated at the end of each experiment with hexamethonium (20 mg/kg iv). RSNA was almost completely postganglionic: only 2.1 ± 0.8% ($n$ = 49) 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 of both types of fibers in each bundle. Therefore, with the use of criteria established in our previous studies, ASNA was considered as predominantly preganglionic or postganglionic if the activity remaining after ganglionic blockade at the end of each experiment was $>$75% or $<$50%, respectively (37–39). Average pre-ASNA and post-ASNA after ganglionic blockade were 109.8 ± 4.9% ($n$ = 39) and 42.53 ± 3.2% ($n$ = 10), respectively. Pre-ASNA increased over 100%, probably due to an arterial baroreflex response caused by the decrease in MAP after the ganglionic blockade.

The arterial pressure and neural signals were digitized and recorded with a hemodynamic and neural data analyzer (Biotech Products, Greenwood, Indiana), averaged over 1-s intervals, and stored on hard disk for subsequent analysis.

**Microinjections into the NTS.** Unilateral microinjections of drugs, vehicle (ACF), and carboxyamine dye (Dy) were made with three-barrel glass micropipettes into the medial region of the caudal subpostemtral NTS as described previously (4, 36–39). Briefly, with the rat skull adjusted to a 45° angle from the horizontal plane of the stereotaxic apparatus and the micropipette barrel held at a 22° angle from the vertical plane, the surface coordinates used for insertion of the micropipette relative to the caudal tip of the area postrema were anteroposterior = −0.1 mm, mediolateral = 0.3 mm, and dorsoventral = 0.35 mm from the dorsal surface of the brainstem. All drugs were dissolved in ACF, and the pH was adjusted to 7.2. No more than one microinjection protocol was performed on one side of the NTS.
All microinjection sites were verified histologically as previously described (4, 36–39).

Our previous studies (4, 36–39) have shown that microinjections of the selective A2a receptor agonist CGS-21680 into the subposteminal NTS evoke dose-related responses in all hemodynamic and neural parameters recorded in the present study. The effects elicited with the highest dose of the agonist (20 pmol) were previously shown to be completely and selectively blocked by microinjection of the A2a purinoreceptor antagonist CGS-15943A (3). Responses to stimulation of mostly presynaptic NTS A2a receptors may be mediated via release of glutamate or other neurotransmitters operating in NTS circuitry, e.g., release of norepinephrine or serotonin on stimulation of NTS A2a receptors (1, 2). Therefore, we used the maximal effective dose of the selective A2a receptor agonist CGS-21680 (20 pmol in 50 nl) to maximally activate all possible mechanisms triggered by A2a receptor stimulation and evaluate the contribution of nitrooxidergic mechanisms to this response.

Experimental protocols. NO of neuronal or vascular origin may affect the function of NTS circuitry (43, 48). Selective and nonselective NOS antagonists were applied to distinguish between the contribution of neuronal and vascular NO to the hemodynamic and neural responses to stimulation of NTS A2a receptors. Selective blockade of nNOS was accomplished with unilateral microinjection of TRIM (20 nmol in 100 nl; Sigma-Aldrich, St. Louis, MO). Nonselective blockade of both nNOS and eNOS was performed with microinjections of L-NAME hydrochloride (Sigma-Aldrich; 20 nmol in 100 nl). The volume of the NOS antagonists was twice as much as the volume of A2a receptor agonists to ensure that the agonist would not penetrate further than the antagonist. Basic protocols were as follows: protocol 1) ACF, 5-min interval, CGS-21680, 90-min interval, TRIM, 5-min interval, CGS-21680 (n = 7); and protocol 2) ACF, 5-min interval, CGS-21680, 90-min interval, L-NAME, 5-min interval, CGS-21680 (n = 8). In each protocol, microinjections of ACF plus CGS-21680 and NOS antagonist plus CGS-21680 were performed on contralateral sides of the NTS in a random manner. This design allowed us to compare the responses to stimulation of A2a receptors under control and blockade condition in the same animal. A time control [ACF (plus CGS-21680), 90-min interval (ACF plus CGS-21680)] was performed in three animals to ensure that two subsequent stimulations of A2a receptors in the 90-min interval evoked comparable responses. In addition to the above longitudinal protocols, in a separate group of animals (n = 7), stimulation of A2a receptors after pretreatment with L-NAME was performed without the preceding volume control and the results were compared with the combined volume controls evoked as a first one in other groups (protocols 1 and 2 and volume controls). This additional control completely excluded the possibility that the differences between responses observed under control and blockade conditions could be an effect of repetitive stimulation of A2a receptors in one animal.

To assess the effect of NO on baseline hemodynamic and neural variables, we used three different NO donors: sodium nitroprusside (SNP; 40 and 400 pmol), DETA NONOate (0.5 and 5 nmol), and PAPA NONOate (2 mmol) and the natural precursor of NO: l-arginine-HCl (10, 20 and 50 nmol). In some experiments, the responses evoked by NO donors and l-arginine were compared with the responses to microinjections of glutamate (500 pmol/50 nl) into the same site of the NTS. All of the above drugs were obtained from Sigma-Aldrich and injected in a 50-nl volume. Different doses of the drugs were microinjected into the same site of the NTS in 30-min intervals in a random order. No more than three microinjections on each side of the NTS were performed.

Data analysis. Hemodynamic and sympathetic nerve responses were quantified in two ways as described previously (4, 36–39): 1) the maximal percent difference from a 30-s basal control period that was taken immediately before microinjection; and 2) percent changes from the control were integrated over the period of 10 min for CGS-21680, 5 min for NO donors and NO precursor l-arginine, and 30 s for glutamate. Different times of integration of the responses evoked by different drugs were used to reflect different maximal times of the responses: 15–30 min for CGS-21680, 5–10 min for NO donors, and 30–40 s for glutamate. Time of integration affects integrated values, and the end of the responses is not clear-cut for most of the drugs used; therefore, we always compared the same time period of the responses evoked by the same drug to decrease variability caused by spontaneous, small, slow, and variable changes in each variable occurring during the experiments. The integral reflects a predominant trend of the response despite transient, sometimes bidirectional, fluctuations in each variable. The maximum values of the responses were measured over the time of integration for each drug. Maximum decreases were calculated for those variables in which the integral response decreased and vice versa. Maximum increases were calculated for those variables in which the integral response increased.

The most prominent, consistent responses to stimulation of NTS A2a receptors occur during the first 10 min after the microinjection of CGS-21680. Therefore, we compared these initial portions of the responses obtained under control conditions (after pretreatment with ACF) with the same portion of the response after pretreatment with NOS antagonists. This approach optimized the discrimination between clearly distinguished control responses and attenuated, biphasic, reversed, or unchanged responses observed in different variables after pretreatment with the NOS antagonists. The effects of L-NAME, TRIM, and ACF on the resting hemodynamic and neural parameters were evaluated ~5 min after the microinjection, during a relatively stable period of the responses; the last 30 s of the responses preceding subsequent stimulation of NTS A2a receptors were averaged. The HR responses, calculated from the pulse intervals, were expressed in absolute values (beats/min). Neural recordings were additionally filtered by using a running average in 10-s intervals to minimize the effect of random spikes on maximum response values. Because there were no significant differences between the responses in RSNA recorded simultaneously with pre-ASNA and those recorded simultaneously with post-ASNA, 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 NO donors and the NO precursor. A two-way ANOVA for independent measures was used to compare the responses of the three sympathetic outputs (RSNA, pre-ASNA, post-ASNA) versus doses of NO donors and the NO precursor. Also a one-way ANOVA for independent measures was used to compare hemodynamic responses and a two-way ANOVA for independent measures to compare neural responses to microinjections of CGS-21680 after pretreatment with ACF and one of NOS antagonists (TRIM or L-NAME). A two-way ANOVA for repeated measures was used to compare the responses of RSNA versus pre-ASNA and RSNA versus post-ASNA, recorded simultaneously. Differences observed were further evaluated by Student’s t-test with Bonferroni adjustment for independent measures. The changes in all recorded variables were also compared with zero by means of SYSTAT univariate F-test. An alpha level of P < 0.05 was used to determine statistical significance.

RESULTS

The resting MAP and HR measured before any microinjection (136 microinjections in 49 animals) were 89.9 ± 0.9 mmHg and 349.6 ± 2.7 beats/min, respectively. Microinjections into the NTS of A2a receptor agonist (CGS-21680) and glutamate evoked characteristic patterns of neural responses similar to those described previously (35–38). Stimulation of NTS A2a receptors inhibited RSNA and post-ASNA and markedly increased pre-ASNA, whereas stimulation of NTS glutamatergic receptors evoked uniform sympathoinhibition. Microinjections of ACF in this and previous studies (38, 39) evoked changes smaller than those randomly occurring in all recorded
parameters during the experiments (Table 1). Responses to microinjections of TRIM and l-NAME were not different than those evoked by ACF with only one exception: that the nonselective NOS inhibitor (l-NAME) caused a small but statistically significant decrease in pre-ASNA compared with the effects of ACF and TRIM ($P = 0.0014$ and $P = 0.0082$, respectively) (Table 1).

**Patterns of neural responses to NO donors.** The differential neural response pattern evoked by microinjections of NO donors that directly release NO into the NTS (SNP and DETA NONOate) (Figs. 1 and 2, Table 2) was similar to that evoked by stimulation of NTS A2a adenosine receptors in this and our previous studies (33–38). Both NO donors as well as the selective A2a receptor agonist CGS-21680 markedly increased pre-ASNA and inhibited RSNA.

The neural responses to both NO donors were dose dependent, because two-way ANOVA showed significant dose versus nerve interactions: $P = 0.021$ and $P = 0.046$ for integral responses to SNP and DETA NONOate, respectively. A similar differential pattern of neural and hemodynamic responses (an increase in pre-ASNA and a decrease in RSNA and post-ASNA, MAP, and HR) was observed also after microinjections of another NO donor, PAPA NONOate (2 nmol/50 nl), in four additional experiments (Table 3). Hemodynamic integral responses to microinjections of NO donors were generally similar to those observed after activation of NTS A2a receptors, although prevailing decreases in MAP and HR frequently coexisted with biphasic or even pressor responses. The variability of hemodynamic responses to NO donors was probably the reason for lack of dose-response effects in MAP and HR (Fig. 2).

Interestingly, microinjections of l-arginine, a precursor of NO, evoked a pattern of neural responses different than that evoked by microinjections of NO donors (and stimulation of A2a receptors) but similar to that evoked by microinjections of glutamate (Figs. 3 and 4 and Table 4). l-Arginine evoked uniform, dose-dependent inhibition of all analyzed sympathetic outputs and decreases in MAP. Two-way ANOVA showed highly significant dose effect ($P < 0.001$), insignificant nerve effect ($P = 0.362$), and dose versus nerve interaction ($P = 0.217$), indicating that all analyzed nerves responded in a dose-dependent manner to l-arginine with similar response patterns. However, HR responses to l-arginine were usually biphasic; an initial moderate decrease in HR was followed with a longer-lasting increase (Fig. 3). Therefore, integral HR responses (first 5 min of response) were not different from zero ($P > 0.05$) with an overall tendency toward tachycardia (Fig. 4).

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**Table 1. Changes in resting values of MAP, HR, RSNA and pre-ASNA in response to microinjections into the NTS of ACF, l-NAME and TRIM.**

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>MAP, %Δ</th>
<th>HR, beats/min</th>
<th>RSNA, %Δ</th>
<th>Pre-ASNA, %Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACF</td>
<td>21</td>
<td>$-1.8\pm1.4$</td>
<td>$-8.0\pm2.9^{\dagger}$</td>
<td>$-3.7\pm2.6$</td>
<td>$4.0\pm1.9$</td>
</tr>
<tr>
<td>l-NAME</td>
<td>15</td>
<td>$-0.3\pm1.5$</td>
<td>$-4.1\pm3.4$</td>
<td>$-6.7\pm2.5^{\dagger}$</td>
<td>$-7.6\pm2.7^{##}$</td>
</tr>
<tr>
<td>TRIM</td>
<td>7</td>
<td>$-1.2\pm0.7$</td>
<td>$-0.4\pm2.6$</td>
<td>$-3.0\pm1.5$</td>
<td>$7.0\pm4.1$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, number of rats; HR, heart rate; RSNA, renal sympathetic nerve activity; pre-ASNA, preganglionic adrenal sympathetic nerve activity; NTS, nucleus of the solitary tract; ACF, artificial cerebrospinal fluid; l-NAME, N*-nitro-l-arginine methyl ester; TRIM, 1-[2-(trifluoromethyl)phenyl]imidazole. *$P < 0.05$ vs. ACF; #$P < 0.05$ vs. TRIM; †$P < $ vs. zero.

Fig. 1. Mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), and predominantly preganglionic adrenal sympathetic nerve activity (pre-ASNA) responses to microinjection of nitric oxide (NO) donors into the subpostremal nucleus of the solitary tract (NTS). Left: sodium nitroprusside (SNP; 400 pmol in 50 nl). Right: 3,3-bis(aminooethyl)-1-hydroxy-2-oxo-1-triazene (DETA NONOate, also known as NOC-18; 5 nmol in 50 nl). Both NO donors inhibited RSNA and markedly increased pre-ASNA.

4). The maximal initial decreases in HR in response to l-arginine and the maximal responses in other variables, consistent with their integral responses (Fig. 4), are presented in Table 4.

**Effects of NOS antagonists.** Both NOS antagonists (TRIM and l-NAME) similarly altered hemodynamic and sympathoinhibitory responses to selective stimulation of NTS A2a receptors. TRIM, a selective nNOS inhibitor, reversed the normal depressor responses into pressor ones and significantly attenuated A2a-receptor-elicited decreases in HR and RSNA (Fig. 5 and Table 5). l-NAME, a nonselective NOS antagonist, had an even greater effect. It reversed normal A2a-receptor-elicited decreases in all recorded parameters (MAP, HR, and RSNA) (Fig. 6 and Table 5), although the integral responses in HR and RSNA after pretreatment with l-NAME and TRIM were not different from zero (Fig. 7). However, both TRIM and l-NAME did not significantly affect A2a-receptor-elicited decreases in pre-ASNA (Figs. 5–7 and Table 5). In one experiment, the responses of postganglionic ASNA were recorded, and this sympathetic output responded similarly to RSNA. Stimulation of NTS A2a receptors inhibited post-ASNA under
control conditions (−22.6% and −133.7% for maximal and integral values, respectively) and activated it after pretreatment with L-NAME (−22.0% and −117.5% for maximal and integral values, respectively).

Time control performed on three additional animals showed that two consecutive stimulations of NTS A2a receptors performed in 90-min intervals, each after pretreatment with 100 nl of ACF, resulted in similar maximum decreases in MAP (−26.3 ± 12.7% vs. −31.4 ± 4.0%), HR (−39.1 ± 10.2 beats/min vs. −29.0 ± 12.1 beats/min), and RSNA (−46.5 ± 18.5% vs. −48.5 ± 5.1%) and increases in pre-ASNA (34.0 ± 6.0 vs. 29.7 ± 4.6). The corresponding integral responses in all recorded variables also did not change with time. Time control indicated that the reactivity of NTS A2a receptors was unchanged during the longitudinal experiments. The trend observed in longitudinally designed experiments was additionally confirmed by comparison between two groups of animals (control vs. NOS blockade) in which only the effects of one (first) stimulation of NTS A2a receptors after pretreatment with ACF (volume control) or L-NAME were considered (Table 6).

**DISCUSSION**

This is the first study to investigate nitroxidergic mechanisms involved in the hemodynamic and regional sympathetic responses evoked by selective stimulation of A2a-adenosine receptors located in NTS circuitry. Also, for the first time, the effects of microinjections into the NTS of different NO donors on different sympathetic outputs were evaluated. There are two major new findings of the present study. First, blockade of NOS in the NTS abolished or even reversed depressor, cardiac slowing, and regionally selective sympathoinhibitory re-

![Fig. 2. Integral responses of MAP, HR, RSNA, pre-ASNA, and postganglionic ASNA (post-ASNA) evoked by microinjections into the subpostremal NTS of two doses of SNP (40 pmol, n = 10, 6, and 4 for RSNA, pre-ASNA and post-ASNA, respectively; 400 pmol, n = 9, 5, and 4 for RSNA, pre-ASNA, and post-ASNA, respectively) and DETA-NONOate, known also as NOC-18 (0.5 nmol, n = 8 and 5 nmol, n = 11). Data are means ± SE. *Different vs. the lower dose (P < 0.05); #different vs. pre-ASNA (P < 0.05). Both NO donors inhibited RSNA and activated pre-ASNA.](image)

**Table 2. Maximal hemodynamic and neural responses evoked by NO donors**

<table>
<thead>
<tr>
<th>Maximal Responses</th>
<th>MAP, Δ%</th>
<th>HR, Δbeats/min</th>
<th>RSNA, Δ%</th>
<th>Pre-ASNA, Δ%</th>
<th>Post-ASNA, Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP (40 pmol)</td>
<td>−13.5±1.9</td>
<td>−20.4±4.7</td>
<td>−18.8±2.2#</td>
<td>24.5±4.6</td>
<td>−13.4±2.1#</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>SNP (400 pmol)</td>
<td>−17.3±3.3</td>
<td>−30.1±9.0</td>
<td>−29.1±4.7#</td>
<td>35.3±7.0</td>
<td>−17.6±2.5#</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>DETA NONOate (0.5 nmol)</td>
<td>−9.2±0.2</td>
<td>−18.0±2.5</td>
<td>−14.8±3.1#</td>
<td>25.0±6.4</td>
<td>Not observed</td>
</tr>
<tr>
<td>n = 8</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>DETA NONOate (5 nmol)</td>
<td>−9.9±2.2</td>
<td>−23.5±2.2</td>
<td>−16.7±2.7#</td>
<td>31.5±5.9</td>
<td>Not observed</td>
</tr>
<tr>
<td>n = 11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. NO, nitric oxide; post-ASNA, postganglionic ASNA; DETA NONOate, 3,3-bis(aminoethyl)-1-hydroxy-2-oxo-1-triazene *P < 0.05 vs. lower dose; #P < 0.05 vs. pre-ASNA. Note that pre-ASNA increased, whereas RSNA and post-ASNA decreased, in response to NO donors. There were no significant differences between responses in RSNA and post-ASNA (P > 0.05).
responses evoked by selective stimulation of NTS A2α receptors. Second, nitroxidergic mechanisms did not contribute to A2α-receptor-elicited increases in sympathetic output directed to the adrenal medulla, because both TRIM and L-NAME did not affect the increases in pre-ASNA. In addition, microinjections of NO donors (SNP, DETA NONOate, and PAPA NONOate), selectively increased pre-ASNA and decreased RSNA, post-ASNA, MAP, and HR. Consistently, L-NAME selectively decreased resting pre-ASNA without affecting other analyzed variables. Taken together the above observations suggest that nitroxidergic mechanisms operating in the NTS differentially contribute to the control of sympathetic output to the adrenal medulla versus other sympathetic outputs (RSNA and post-ASNA).

Responses to NO donors. Our results are consistent with previous reports (21, 23, 24, 46) showing that microinjections of NO donors, nitrosothiols, and L-arginine into the NTS evoked decreases in MAP, HR, and RSNA. However, in the present study, pre-ASNA, directed to the adrenal medulla, increased after microinjections of NO donors and decreased after microinjections of the NO precursor L-arginine. Reasons for opposite effects of NO donors versus L-arginine on pre-ASNA remain unclear. The major functional difference between these substances is that NO donors act nonselectively when releasing NO into the NTS around the microinjection site, whereas L-arginine may release NO more selectively, i.e., in the proximity of those NTS neurons/neural terminals that contain nNOS. As we have shown previously (34, 37), all analyzed sympathetic outputs are uniformly inhibited by activation of baroreceptors or intra-NTS microinjections of glutamate, a primary neurotransmitter in the baroreflex pathway (12). Therefore, it is likely that after administration of L-arginine into the NTS, NO was released primarily in the proximity of baroreflex neurons that exhibit dense colocalization of nNOS and baroreflex induced c-fos (11). That mechanism may be responsible for the similarity of neural response patterns evoked by L-arginine and glutamate in the present study (Figs. 3 and 4). The above observations are consistent with colocalization of glutamatergic transporters and nNOS in NTS neurons (26), mutual interactions between NTS glutamatergic and nitroxidergic mechanisms mediating depressor responses (24), and attenuation of baroreflex responses after inhibition of nNOS in the NTS (43). In contrast, NO donors release NO in a nonselective manner that may mimic nonselective release of NO from the NTS vasculature. Consistent with this concept is the observation that the nonselective antagonist of both nNOS and eNOS, L-NAME, significantly decreased resting pre-ASNA, whereas the selective nNOS inhibitor, TRIM, did not affect pre-ASNA; the responses to TRIM were not different than those to ACF (Table 1). Because L-NAME (but not TRIM) decreased baseline pre-ASNA and NO donors (but not the NO precursor, L-arginine) increased pre-ASNA, these combined results suggest that NO of endothelial (vascular) origin may penetrate into NTS neurons/neural terminals and exert tonic activation of sympathetic activity directed to the adrenal medulla. The mechanism of this activation remains unknown and may involve modulation of reflex and/or descending inputs to the NTS.

The present study, DETA NONOate (NOC-18) evoked small, variable, or biphasic responses in MAP, HR, and RSNA (Fig. 1) with an overall tendency toward decreases in MAP, HR, and RSNA as shown by values integrated over the first 5 min of the response (Fig. 2). These observations remain in

Table 3. Hemodynamic and neural responses to microinjections of PAPA NONOate (2 nmol/50 nl) into the NTS

<table>
<thead>
<tr>
<th>Response Type</th>
<th>MAP, Δ%</th>
<th>HR, Δbeats/min</th>
<th>RSNA, Δ%</th>
<th>Pre-ASNA, Δ%</th>
<th>Post-ASNA, Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-NAME</td>
<td>−21.2±4.6*</td>
<td>−37.9±8.8*</td>
<td>93.4±41.6</td>
<td>−19.0±1.6*</td>
<td></td>
</tr>
<tr>
<td>Post-ACF</td>
<td>−19.2±4.2*</td>
<td>−8.8*</td>
<td>4.6*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ACF</td>
<td>−15.4±1.5</td>
<td>−13.4±2.0</td>
<td>−21.2±4.6*</td>
<td>39.8±12.2</td>
<td>−19.2±4.2*</td>
</tr>
<tr>
<td>Pre-ASNA</td>
<td>−17.5±3.9</td>
<td>−11.3±7.0</td>
<td>−37.9±8.8*</td>
<td>93.4±41.6</td>
<td>−19.0±1.6*</td>
</tr>
<tr>
<td>Post-ASNA</td>
<td>−8.8*</td>
<td>4.6*</td>
<td>93.4±41.6</td>
<td>−19.0±1.6*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats; PAPA NONOate, 3-(2-hydroxy-2-nitroso-1-propylhydrozino)1-propanamine. *P < 0.05 different vs. pre-ASNA. RSNA was not significantly different from post-ASNA (P > 0.05).
contrast to the report of Matsumura et al. (28) who observed increases in MAP, HR, and RSNA upon microinjections of DETA NONOate into the NTS. The reasons for these discrepancies may include a different strain of rats (Wistar), different anesthesia (urethane only), more rostral (400–500 μm) microinjections, and higher maximal doses of the drug (10 nmol) used by these authors (28). Interestingly, we noticed that the depressor component of the responses evoked by DETA NONOate diminished with increasing dose of DETA NONOate and were not different from zero at a dose of 5 nmol (Fig. 2). It seems that there is a tendency to increase the pressor component of the response to DETA NONOate with increasing doses of the drug. Also, the depressor responses to microinjections of SNP observed in the present study did not show a dose-response relationship, although the decreases in MAP, HR, and RSNA were more pronounced and consistent than those evoked by DETA NONOate. Combined, these data suggest that nonselective release of NO into the NTS may affect different mechanisms and evoke counteracting regional responses that result in variability of the overall MAP responses.

It is possible that some nonspecific effects could contribute to the marked depressor and uniform sympathoinhibitory responses evoked by L-arginine. For example, Matsumura et al. (28) reported similar depressor and cardiac slowing responses evoked by microinjections of both L-arginine and D-arginine into the NTS although NOS is unable to release NO from D-arginine. In contrast, a subsequent study from Tseng’s laboratory (23) showed that D-arginine evokes virtually no responses on microinjection into the NTS compared with large decreases in MAP and HR evoked by microinjections of L-arginine, suggesting a specific, NO-related, action of L-arginine in the NTS. Among possible reasons for the discrepancy between these studies is that different animal models (Wistar vs. Sprague Dawley rats, respectively) and possibly different pH of the microinjected drugs should be considered. Millimolar solutions of arginine (20 nmol in 50 nl of ACF) are strongly basic (pH 10.8), whereas the solutions of arginine-

![Graph](https://via.placeholder.com/150)

**Table 4. Maximal hemodynamic and neural responses evoked by NO precursor (L-arginine) and sodium glutamate**

<table>
<thead>
<tr>
<th>Maximum Responses</th>
<th>MAP, Δ%</th>
<th>HR, Δbeats/min</th>
<th>RSNA, Δ%</th>
<th>Pre-ASNA, Δ%</th>
<th>Post-ASNA, Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine (10 nmol)</td>
<td>-20.3±3.0</td>
<td>-14.6±2.8</td>
<td>-28.2±5.7</td>
<td>-24.5±5.6</td>
<td>-25.1±6.8</td>
</tr>
<tr>
<td>n=16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arginine (20 nmol)</td>
<td>-28.5±3.7</td>
<td>-20.6±5.2</td>
<td>-44.1±6.5</td>
<td>-40.5±5.6</td>
<td>-59.4±13.9</td>
</tr>
<tr>
<td>n=13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arginine (50 nmol)</td>
<td>-27.9±2.0*</td>
<td>-20.9±4.5</td>
<td>-31.9±3.3</td>
<td>-43.1±1.6*</td>
<td>-37.6±2.2</td>
</tr>
<tr>
<td>n=14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate (0.5 nmol)</td>
<td>-48.3±1.7</td>
<td>-53.5±13.4</td>
<td>-87.7±4.8</td>
<td>-76.9±7.0</td>
<td>-79.7±1.5</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. *P < 0.05, higher doses vs. the lowest dose of L-arginine. Only initial decreases of biphasic HR responses are presented. There were no significant differences between responses in RSNA, pre-ASNA, and post-ASNA evoked by L-arginine and sodium glutamate (P > 0.05).
HCl are acidic (pH < 5.5) according to our measurements. Unfortunately, both studies cited above did not report the pH of microinjected solutions.

Effects of blockade of nNOS and eNOS on responses to NTS A2a receptor stimulation. Selective antagonist of nNOS (TRIM) and the nonselective antagonist of both nNOS and eNOS, L-NAME, abolished or reversed the depressor, cardiac slowing, and sympathoinhibitory responses to stimulation of NTS A2a receptors. Therefore, NTS nitroxidergic mechanisms appear crucial for the decreases in MAP, HR, and RSNA evoked by NTS A2a receptor stimulation. Because the effects of L-NAME were only slightly stronger than those of TRIM, the hemodynamic and sympathoinhibitory responses to stimulation of NTS A2a receptors are likely mediated predominantly by nNOS with only a small, if any, contribution of eNOS.

Such a powerful effect of blockade of nitroxidergic mechanisms in the NTS on depressor and sympathoinhibitory responses to selective stimulation of adenosine A2a receptors was unexpected, taking into consideration that previous report from Tseng’s laboratory (27) showed that even a 10-times greater dose (100 nmol) of l-NAME and l-NMMA than those used in our study attenuated but did not abolish the depressor and cardiac slowing responses to microinjections of adenosine into the NTS. The major difference between the studies is that we used the selective agonist of adenosine A2a receptors, whereas Tseng and coworkers (27) used adenosine that activates both A2a (mostly presynaptic) and A1 (both pre- and postsynaptic) receptors in the NTS. Although selective stimulation of NTS A1 adenosine receptors exerts predominantly pressor responses (3), our most recent study showed that smaller doses of A1 receptor agonist CPA also evoke depressor and sympathoinhibitory responses in ~30% of the cases (39). Furthermore, after blockade of glutamatergic transmission in the NTS stimulation of adenosine A1 receptors evokes only depressor and sympathoinhibitory responses (39). Therefore, it is possible that blockade of NTS nitroxidergic mechanisms may have abolished the pressor component of the response mediated via A1 receptors and uncovered the depressor component of the

Table 5. Maximum responses to microinjections of CGS-21680 (20 pmol/50 nl) following pretreatment with ACF (volume control, 100 nl) and subsequent pretreatment with TRIM (20 nmol/100 nl) or l-NAME (20 nmol/100 nl): longitudinal design

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>MAP, Δ%</th>
<th>HR, Δbeats/min</th>
<th>RSNA, Δ%</th>
<th>Pre-ASNA, Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACFt + CGS</td>
<td>-24.25±3.81</td>
<td>-63.89±12.66</td>
<td>-47.03±7.95</td>
<td>62.59±14.96</td>
</tr>
<tr>
<td>TRIM + CGS</td>
<td>12.47±1.15*</td>
<td>-22.71±7.30*</td>
<td>-16.03±6.92*</td>
<td>44.13±7.92</td>
</tr>
<tr>
<td>ACFt + CGS</td>
<td>-17.52±5.01</td>
<td>-49.03±5.50</td>
<td>-48.21±9.14</td>
<td>46.33±11.73</td>
</tr>
<tr>
<td>l-NAME + CGS</td>
<td>21.34±5.03*</td>
<td>16.48±4.45*</td>
<td>36.21±17.23*</td>
<td>51.29±13.44</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 rats in each group. CGS, CGS-21680; ACFt + CGS, control before pretreatment with TRIM; ACFt + CGS, control before pretreatment with l-NAME. *P < 0.05, TRIM + CGS vs. ACFt + CGS and l-NAME + CGS vs. ACFt + CGS.
response, similar to what we observed after blockade of glutamatergic transmission in the NTS in our previous study (39). The above mechanism may explain why combined stimulation of both receptor subtypes with microinjections of adenosine still evoked depressor responses after blockade of NOS in the NTS (possibly via A₁ receptors) (27), whereas depressor and sympathoinhibitory responses to selective stimulation of NTS A₂a receptors were abolished or even reversed after the blockade in the present study.

A possible explanation for the dramatic changes in responses to selective stimulation of NTS A₂a receptors after the blockade of nitrooxidergic mechanisms in the NTS may be that NO plays the role of a balancing factor between reciprocal effects potentially facilitated via A₂a receptors in the complex NTS

Fig. 6. Responses to stimulation of NTS A₂a receptors with CGS-21680 (20 pmol in 50 nl) after pretreatment with ACF (100 nl) and nonselective blockade of nNOS plus endothelial NOS (eNOS) with L-nitro-l-arginine methyl ester (l-NAME; 20 nmol in 100 nl) in the same animal. The blockade reversed decreases in MAP, HR, and RSNA into increases and did not affect the sympathoactivation in pre-ASNA.

Fig. 7. Comparison of neural and hemodynamic responses to stimulation of NTS A₂a receptors with CGS-21680 (20 pmol in 50 nl) after pretreatment with selective nNOS inhibitor TRIM (n = 7) and nonselective nNOS plus eNOS inhibitor l-NAME (n = 7) and respective volume controls for each drug [control (CTR; n = 7)]. Data are means ± SE. *P > 0.05 vs. CTR; #P > 0.05, RSNA vs. pre-ASNA. Responses to stimulation of NTS A₂a receptors were not significantly different after TRIM vs. l-NAME in all recorded variables.
circuity. For example, because it has been shown that stimulation of NTS A2a adenosine receptors facilitates glutamate release into the NTS (10) and given that glutamate is a primary mediator in both baro- and chemoreflex pathways at the level of the NTS (12), the overall depressor and sympathoinhibitory responses mediated via A2a receptors located in NTS circuitry may be a net effect of both depressor (baroreflex-like) and pressor (chemoreflex-like) components. Blockade of nitroxidergic mechanisms in the NTS may shift the balance toward the pressor component of the responses and consequently reverse the depressor and sympathoinhibitory responses into pressor and sympathoactivatory ones, as observed in the present study.

The increases of pre-ASNA in response to NTS A2a receptor stimulation were not altered by either TRIM or L-NAME, whereas pre-ASNA increased after microinjections of NO donors (but not the NO precursor, L-arginine) into the NTS. This indicates that selective activation of pre-ASNA by nonselective release of NO from donors and stimulation of A2a receptors occurs via two different, independent mechanisms. We (38) have shown previously that the selective increase in pre-ASNA after stimulation of NTS A2a adenosine receptors was not markedly affected by ionotropic glutamatergic blockade, and in the present study, we found that it is also not affected by nitroxidergic blockade. It is possible that the selective increases in pre-ASNA after stimulation of NTS A2a receptors or nonselective diffusion of NO into the NTS may be a result of facilitation of the release of different neurotransmitter(s) in certain afferent or descending terminals activating those NTS neurons that directly stimulate sympathetic premotorneurons in the rostral ventrolateral medulla that activate pre-ASNA.

In support of this hypothesis, stimulation of cardiac receptors via intrapericardial infusions of phenylbiguanide or stimulation of hypothalamic paraventricular nucleus with glutamate increases pre-ASNA and simultaneously decreases RSNA (18, 20), whereas other reflex effects, mediated via glutamate at the level of the NTS, uniformly inhibit (baroreflex) or facilitate (arterial chemoreflex) these sympathetic outputs (6, 15, 34). Our preliminary data (40) showed that vasopressin microinjected into the NTS also selectively increased pre-ASNA, whereas it decreased RSNA and post-ASNA. Therefore, among the many possibilities, A2a receptor-mediated and/or NO-mediated facilitation of vasopressin release from fibers descending from PVN to the NTS (47) should be considered in further studies.

Interestingly, two sympathetic outputs directed to the same organ, the adrenal gland, responded in an opposite manner to stimulation of NTS A2a receptors and NO donors. Preganglionic fibers of ASNA innervate the adrenal medulla, whereas postganglionic fibers of ASNA innervate the adrenal cortex (zona glomerulosa) and vasculature of the adrenal gland (9). Although responses of post-ASNA and pre-ASNA were opposite, they may be functionally complementary. An increase in pre-ASNA stimulates release of catecholamines into the blood stream, whereas a decrease in post-ASNA probably diminishes vasoconstrictor tone and increases blood flow through the adrenal gland. In support of that concept, during severe hemorrhage in rats, post-ASNA decreases, whereas pre-ASNA and adrenal blood flow increase (8, 41).

**Perspectives.** Endothelial NO may selectively, tonically activate pre-ASNA at the level of the NTS, as blockade of eNOS, but not nNOS, selectively decreased resting pre-ASNA and nonselective diffusion of NO from microinjected NO donors, which mimics the diffusion of NO from NTS vasculature, selectively increased pre-ASNA but not other variables. The selective activation of sympathetic activity directed to the adrenal medulla may increase during hypoxia and ischemia, as hypoxia markedly increases eNOS expression in brain vessels (14, 16). Interestingly, activation of both A1 and A2a adenosine receptors in the NTS also preferentially activate pre-ASNA (35–39), and adenosine is released into the central structures, including the NTS during hypoxia and ischemia (32, 50). The mechanisms of activation of pre-ASNA via NO of endothelial origin and adenosine are synergistic although independent of each other, because blockade of nitroxidergic mechanisms in the NTS did not affect increases in pre-ASNA evoked by stimulation of NTS A2a adenosine receptors. Taken together, the above observations suggest that both adenosine and NO of endothelial origin may be mobilized in the NTS during severe homeostatic imbalance and preferentially stimulate the adrenal medulla to release catecholamines and restore homeostasis.

In summary, intact synthesis of NO is necessary to develop depressor and sympathoinhibitory responses evoked by selective activation of adenosine A2a receptors in the NTS. Mainly nNOS contributes to the depressor and sympathoinhibitory responses evoked by stimulation of NTS A2a adenosine receptors as both TRIM and L-NAME abolished or reversed the responses. In contrast, NO of endothelial origin may tonically activate sympathetic output to the adrenal medulla as L-NAME but not TRIM decreased resting pre-ASNA. The increases in pre-ASNA evoked by stimulation of NTS adenosine A2a receptors were unaffected by the blockade of NO synthesis, suggesting that these responses are mediated via nonnitroxidergic mechanism. Taken together, our results indicate that NO mechanisms operating in the NTS differentially contribute to the pattern

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Table 6. Comparison of first responses to microinjections of CGS-21680 (20 pmol/50 nl) following pretreatment with ACF (100 nl, volume control) vs. L-NAME (20 nmol/100 nl): parallel design

<table>
<thead>
<tr>
<th></th>
<th>Maximal Responses</th>
<th>Integral Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP, Δ%</td>
<td>HR, Δbeats/min</td>
</tr>
<tr>
<td>ACF + CGS</td>
<td>17 -18.42±2.8 -53.4±6.1 -47.4±5.5 50.8±8.0</td>
<td>-98.7±25.6 -310.7±40.3 281.6±47.2 318.5±54.5</td>
</tr>
<tr>
<td>L-NAME + CGS</td>
<td>7 13.45±3.3* -22.65±7.4* 29.8±6.5* 52.5±4.6</td>
<td>55.7±54.4* -72.85±61.7* 118.3±93.3* 302.4±47.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. *P < 0.05 vs. ACF + CGS, ACF + CGS (n = 17) combined first, control responses in L-NAME, TRIM, and time control groups.
of regional sympathetic responses evoked by stimulation of NTS A₂a receptors.

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