Mechanisms mediating NTS P$_{2x}$ receptor-evoked hypotension: cardiac output vs. total peripheral resistance

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The nucleus tractus solitarius (NTS) is a major integrative center in the brain stem involved in reflex control of the cardiovascular system and autonomic function (9). All of the major organs have afferent inputs that synapse in the NTS (14). In addition, both carotid and aortic baroreceptors in the rat terminate and converge in the subpostremal region of the NTS. The primary neurotransmitter utilized by baroreflex afferents in the NTS is glutamate (9, 13, 25). Activation of arterial baroreceptor afferents or glutamatergic receptors in the subpostremal NTS produces stimulus/dose-dependent decreases in mean arterial pressure (MAP) and heart rate (HR) (25). Because MAP is the product of cardiac output (CO) and total peripheral resistance (TPR), changes in one or both of these variables are responsible for the decreases in MAP that occur after baroreflex activation. Olivier and Stephen-son (16) documented that the relative contributions of CO and TPR to these baroreflex-evoked changes in MAP are dependent on the strength of the afferent stimulus.

In addition to basic glutamatergic transmission in baroreflex activation at the level of the NTS, recent studies (8, 10, 13, 17, 19–24) from our laboratory and others strongly suggest that extracellular ATP plays an important role as an independent neurotransmitter or as a cotransmitter with glutamate in these mechanisms. For example, stimulation of P$_{2x}$ purinoceptors located in the subpostremal NTS, via the selective agonist α,β-methylene ATP, produced dose-dependent reductions in MAP, HR, and efferent sympathetic nerve activity (5, 10, 19, 22, 23). The responses consist of a fast- and short-lasting “neuromediator-like” component followed by a less-pronounced and longer-lasting “neuromodulator-like” component (22, 23). The time course of the fast response to stimulation of NTS P$_{2x}$ purinoceptors closely resembles the response to stimulation of glutamatergic receptors in the same site of the NTS (22). Blockade of ionotropic glutamatergic receptors, involved in baroreflex transmission at the level of the NTS, markedly attenuated the fast “neurotransmitter-like” component of the response to stimulation of NTS P$_{2x}$ purinoceptors, indicating that these two mechanisms are linked together (23). In addition, blockade of the NTS P$_{2}$ purinoceptor with suramin, a P$_{2}$ purinoceptor antagonist, virtually abolished the baroreflex control of HR (21). Finally, St. Lambert et al. (24) recently demonstrated that ATP within the NTS participates in mediating the cardiovascular responses to stimulation of the hypothalamic defense area.

Although the studies described above strongly support the importance of P$_{2x}$ purinoceptors in mediating/modulating baroreflex responses at the level of the NTS, the mechanisms mediating the depressor response to stimulation of NTS P$_{2x}$ purinoceptors remain unknown. Because stimulation of NTS P$_{2x}$ purinoceptors decreases both HR and efferent sympathetic nerve...
activity (5, 19, 22), it is likely that decreases in both CO and TPR contribute to the depressor response. However, a decrease in HR does not necessarily lead to a decrease in CO, because, with greater filling time, stroke volume (SV) may increase such that CO may be unchanged. Therefore, the purpose of the present study was to determine the relative contributions of CO and TPR to the hypotension evoked by NTS P2x purinoceptor activation. We hypothesized that this depressor response would be primarily due to reductions in TPR.

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 relative contributions of CO and TPR in mediating the hypertensive response to activation of subpostremal NTS P2x purinoceptors were studied using 13 male Sprague-Dawley rats (325–375 g). P2x purinoceptors located in the NTS were activated via microinjection of α,β-methylene ATP, a selective P2x purinoceptor agonist, at the approximate minimal hypertensive dose (25 pmol/50 nl, n = 8) and the maximal hypertensive dose (100 pmol/50 nl, n = 7) (5, 10). Two animals received microinjections on both the right and the left side of the NTS.

Instrumentation and measurements. Implantation of a Doppler ultrasonic flow probe to measure CO (minus coronary blood flow) was performed using aseptic procedures. The rats were anesthetized with pentobarbital sodium (50 mg/kg), and supplemental doses were administered as needed. A right thoracotomy was performed through the third intercostal space, the ascending aorta was dissected free of surrounding tissue, with care taken to avoid damage to nerves, and an appropriately sized Doppler ultrasonic flow probe was placed around the vessel. The flow probe wires were tunneled subcutaneously and exteriorized at the back of the neck. The incision was closed in layers, and the animals were allowed at least 3 days to recover. During the recovery period, animals were monitored for any signs of infection, weight loss, and irregularities in breathing. In preliminary studies, we found that the success rate was markedly improved when the thoracotomy was performed several days before the experiment.

On the day of the experiment, the rats were anesthetized with a combination of α-chloralose (80 mg/kg) and urethane (500 mg/kg) administered intraperitoneally, intubated endotracheally, and allowed to respire spontaneously. Rectal temperature was maintained between 37 and 38°C by a water heating pad (model TP-500, Gaymer industries). A catheter (polyethylene-50) was placed in the right femoral artery and connected to a TXX-R Viggo-spectramped pressure transducer to monitor arterial pressure and HR. A catheter was also placed in the right femoral vein to continuously infuse anesthetics [α-chloralose (8–16 mg·kg⁻¹·h⁻¹) and urethane (50–100 mg·kg⁻¹·h⁻¹, ~0.5–1 ml/h)]. The pressure transducer was connected to a Beckman Dynograph (R711), and the flow probe was connected to a pulsed Doppler flowmeter (Baylor Electronics). These signals were transmitted to an analog-to-digital converter (Modular Instruments) interfaced to a laboratory computer. MAP, HR, and CO were recorded continuously using Biowindows software (Modular Instruments).

The entire procedure for discrete microinjections into the subpostremal NTS has been described previously (3–7, 10, 19, 22). Briefly, the animals were mounted in a cranial stereotaxic apparatus. The dorsal medulla was exposed at the level of the obex after dissection of the neck muscles and the atlantocippal membrane. Animals were allowed to stabilize for at least 30 min before microinjection of α,β-methylene ATP. After the stabilization period, unilateral microinjections were performed using multibarrel glass micropipettes (15- to 20-μm tip diameter for each barrel) into the middle to caudal one-third of the subpostremal NTS via a pneumatic picopump (model PV820, WPI). A total volume of 50 nl was injected over 5–10 s. In several studies, we (5, 19, 22) demonstrated that this volume of vehicle does not affect MAP, HR, peripheral blood flow, or efferent sympathetic nerve activity. With the pipette tip at an angle of 22° from the vertical plane and the rat skull tilted 45°, the surface coordinates for insertion of the micropipette relative to the caudal tip of the area postrema were as follows: anterior-posterior, -0.1 mm; mediolateral, 0.3 mm; and dorso-ventral, 0.35 mm, from the dorsal surface of the brain stem.

The carbocyanine dye 1,1'-dioctadecyl-3,3,3',3'-tetramethylindo-carbocyanine perchlorate (DiI; 0.1% solution in DMSO, Molecular Probes) was delivered from a separate barrel of the micropipette to mark the injection site for histological analysis. At the completion of the experiments, the animals were perfused transcardially with 10% buffered formalin, and the brains were subsequently processed histologically in 64-μm coronal sections. The unstained tissue sections were examined via fluorescence microscopy to determine the site of injection marked by the DiI lipophilic dye. The injection sites were plotted on schematic representations of coronal sections of the rat subpostremal NTS according to the atlas of Barraco et. al (2). The injection sites are shown in Fig. 1.

Fig. 1. Microinjection sites in the subpostremal nucleus tractus solitarius (NTS) for all experiments. A schematic diagram of the transverse section of the medulla oblongata from a rat brain is shown. The NTS is shown at the level of the caudal tip of the area postrema (AP), C, central canal; 10, dorsal motor nucleus of the vagus nerve; 12, nucleus of the hypoglossal nerve; Gr, gracile nucleus; Cu, cuneate nucleus. The numbers on the left side of the schematic diagram denote the rostrocaudal position (in mm) of the section relative to the obex according to the atlas of the rat subpostremal NTS (2).
Data analysis. TPR was calculated as CO/MAP, and SV was calculated as CO/HR. Responses for MAP, HR, CO, TPR, and SV were quantified in two ways: 1) maximal change compared with the 60-s basal control period immediately before microinjection, and 2) integration of the response over the time until 80% recovery of MAP (19, 22). A t-test was used to determine statistical significance between the two doses.

To estimate the relative roles of CO and TPR in mediating the depressor response evoked by NTS P2x purinoceptor stimulation, calculations were made using the following equations

\[
\text{MAP}_{\text{TPR}} = \frac{\text{CO}_{\text{Control}} \times \text{TPR}_{\text{Observed}}}{(1)}
\]

\[
\text{MAP}_{\text{CO}} = \frac{\text{CO}_{\text{Observed}} \times \text{TPR}_{\text{Control}}}{(2)}
\]

where MAP_{TPR} reflects the predicted level of MAP if only changes in TPR occurred, and MAP_{CO} reflects the predicted level of MAP if only changes in CO occurred. These calculations are similar to those we have used previously (1). A two-way ANOVA was performed to determine significance between the observed trace of MAP and the two calculated traces of MAP_{CO} and MAP_{TPR} vs. time. Further comparisons were made with the individual points using a modified Bonferroni test (Fig. 4).

RESULTS

There were no significant differences between the control values for the two doses; therefore, basal values measured before microinjections of the low- and high-doses of α,β-methylene ATP were averaged and are shown in Table 1. Figure 2 shows tracings of MAP, CO, and TPR from individual experiments before and after microinjection of α,β-methylene ATP at both the low (25 pmol/50 nl) and high dose (100 pmol/50 nl). Although MAP, CO, and TPR decreased in both experiments, note that the maximal decreases in TPR were similar between the doses, whereas the maximal decrease in CO was over fourfold greater at the high dose versus the low dose.

Figure 3 shows the averaged maximal (top) and integral responses (bottom) for MAP, CO, HR, SV, and TPR after activation of NTS P2x purinoceptors. Stimulation of P2x purinoceptors in the subpostremal NTS produced dose-dependent decreases in MAP, CO, and HR. The changes in MAP and HR are consistent with those reported in previous studies. All of the responses except the integrals of SV were significantly different from no change. The SV responses tended to be biphasic, decreasing initially and then increasing beyond the control level as CO recovered more rapidly than HR. In addition, the responses at the high dose were significantly larger than those at the low dose with the exception of the maximal changes in TPR and SV. There was a tendency for the decrease in SV to be greater at the high dose, although the differences did not reach statistical significance (\( P = 0.055 \)). The greatest differences between low- and high-dose re-

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<th>MAP, mmHg</th>
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<td>90.9 ± 2.5</td>
<td>364 ± 11</td>
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Data are presented as means ± SE; n = no. of rats. MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; TPR, total peripheral resistance; SV, stroke volume.

Table 1. Control levels of hemodynamic variables
sponses were observed for CO. The TPR response to the high dose of agonist lasted markedly longer than that evoked by the small dose. Therefore, the integral of TPR for the high dose was markedly and significantly greater than that for the low dose. The high dose of α,β-methylene ATP also had a much longer-lasting effect than the low dose for MAP, CO, and HR.

The relative roles of CO and TPR in producing the hypotension evoked by P2x purinoceptor stimulation are presented in Fig. 4. In Fig. 4, the observed MAP response is plotted versus time along with the predicted level of MAP calculated if either CO or TPR remained constant. At the low dose (Fig. 4A), MAP_{TPR} followed the observed MAP very closely, and at no point were these curves significantly different. In contrast, MAP_{CO} showed only a small decrease with activation of NTS P2x purinoceptors. This is consistent with a minimal contribution of CO to the decrease in MAP at the low dose. At the high dose (Fig. 4B), both CO and TPR contributed similarly to the decrease in MAP. MAP_{CO} and MAP_{TPR} were not significantly different at any time point after microinjection of the high dose of α,β-methylene ATP, indicating that the relative contributions of changes in CO and TPR in mediating this depressor response were similar.

**DISCUSSION**

This is the first study to examine the relative roles of CO and TPR in mediating the hypotension evoked by stimulation of P2x purinoceptors in the subpostremal NTS. The major finding was that the mechanisms mediating the P2x purinoceptor-induced hypotension are dependent on the level of NTS P2x purinoceptor activation. At the low dose of the P2x purinoceptor agonist, changes in CO contribute very little to the depressor response; this decrease in MAP is mainly
due to the large reduction in TPR. In contrast, both CO and TPR mediate the P2x purinoceptor-induced hypotension at the high dose. These data suggest that there is a much higher threshold to elicit large changes in CO versus that for TPR.

At the high dose, the maximal reduction in TPR was not statistically greater than at the low dose. However, in this setting, a much larger decrease in CO occurred, and, therefore, the larger reduction in MAP observed at the high dose stemmed from a much greater CO response. Thus the genesis of the MAP dose-response relationship appears to be mainly due to a markedly greater dose-response relationship for CO than for TPR.

The relative roles of HR and SV in mediating the changes in CO also appear to be related to the level of NTS P2x purinoceptor activation. Both doses of P2x purinoceptor agonist caused significant reductions in both HR and SV. However, at the low dose, the changes in HR were two times those observed for SV on a percent basis (8.5 ± 1.2 vs. 4.2 ± 1.5%). In contrast, at the high dose, the changes in HR and SV were more comparable (23.8 ± 2.2 vs. 17.2 ± 8.0% for HR and SV, respectively). Thus, at the low dose of P2x purinoceptor agonist, the reduction in CO stemmed more from the decrease in HR, whereas at the higher dose, decreases in both HR and SV contributed similarly to the fall in CO.

Role of NTS P2x purinoceptors in cardiovascular control. Recent studies (8, 10, 12, 13, 17, 19–24) support the concept that ATP acts as a fast neurotransmitter or cotransmitter within the central nervous system and is involved in the regulation of autonomic activity by the NTS. St. Lambert et al. (24) showed that ATP release within the NTS mediates, in part, the cardiovascular responses to stimulation of the hypothalamic defense area. A recent study (23) from our laboratory has shown that the rapid sympathoinhibitory responses to P2x purinoceptor stimulation are markedly attenuated by previous blockade of inotropic glutamatergic receptors, indicating that ATP may act via increasing the release of glutamate (23). Inasmuch as ATP may be a cotransmitter with glutamate in the NTS and that glutamate is the primary neurotransmitter utilized by baroreceptor afferents (9, 13, 25), it is possible that ATP may also be involved in the processing of baroreceptor information within the NTS. In support of this concept, activation of NTS P2x purinoceptor yields regional sympathoinhibitory responses qualitatively similar to those observed with stimulation of NTS inotropic glutamatergic receptors as well as activation of arterial baroreceptors (20, 23). In addition, arterial baroreflex control of HR is markedly impaired after blockade of NTS P2 purinoceptors (21). The results from the present study also show that stimulation of NTS P2x purinoceptors yields changes in CO and TPR similar to those observed by the arterial baroreflex inasmuch as the baroreflex regulation of arterial pressure stems more via regulation of TPR than CO (11, 15, 16, 18). However, the exact extent to which ATP acts in the NTS in the integration of afferent as well as descending information and the subsequent control of autonomic output is not well understood.

Limitations. This study was performed using an anesthetized animal preparation and thus is limited by the potential complications inherent in such models. Anesthesia may modulate the baseline levels of efferent autonomic tone and thereby the ability to elicit changes in CO and TPR. Thus the relative roles of CO and TPR in mediating the responses to NTS P2x purinoceptor stimulation may be different in the conscious animal. We performed the thoracotomy for measurement of CO as a recovery procedure. In preliminary experiments, the success rate and the stability of the preparation were low when all of the surgery was attempted in one acute experiment. However, studying the chronically prepared animal markedly improved the stability of the preparation and significantly shortened the duration of the subsequent acute experiment. This approach is an important consideration because we did not measure blood gases in this study, and marked alterations in normal blood gases could affect autonomic responses. We do not feel that this is a major concern, however, because the baseline levels of all hemodynamic variables were quite stable, within normal levels, and the experiments were completed generally in <2 h. Thus within the confines of these potential limitations, we are confident in the relative roles of CO and TPR in mediating the hypotensive response evoked by P2x purinoceptor stimulation.

In conclusion, the hypotension evoked by P2x purinoceptor stimulation in the subpostmedial NTS is mediated by decreases in both CO and TPR. The relative roles of CO and TPR are dependent on the extent of receptor activation. At the high dose of P2x purinoceptor agonist, both CO and TPR contribute similarly to the response, but at the low dose, there is almost no contribution from CO. The dose-response relationship between the level of NTS P2x purinoceptor stimulation and the depressor response resides mainly in the modulation of the CO component of the response.

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REFERENCES


