Purinergic Receptors in the Brainstem Mediate Hypotension and Bradycardia

CHING-JIUNN TSENG, ITALO BIAGGIONI, MARTIN APPALSAMY, AND DAVID ROBERTSON

SUMMARY Adenosine acts at many sites to modulate neuronal activity. The purpose of this study was to investigate a possible role for adenosine as a neuromodulator of brainstem cardiovascular control. Microinjections of adenosine (0-2.3 nmol) were made stereotaxically into various brainstem sites. Injection of adenosine into the nucleus tractus solitarii (NTS) produced dose-related decreases in heart rate and systolic and diastolic blood pressures. Maximal changes occurred 90 seconds after injection. Injection into the area postrema also produced decreased heart rate and systolic and diastolic blood pressures. No significant effect occurred following injection into the C1 area. Adenosine 5'-triphosphate and its analogue, βγ-methylene adenosine 5'-triphosphate also produced dose-related and potent vasodepressor and bradycardia effects in the NTS. Injection of 1,3-dipropyl-8-p-sulfophenylxanthine (0.92 nmol), a potent adenosine receptor antagonist, produced no effect itself, but abolished for 45 minutes the actions of further injections of adenosine and adenosine 5'-triphosphate (but not L-glutamate) in both the NTS and area postrema. Thus, NTS and area postrema injections of adenosine decrease blood pressure and heart rate in anesthetized normotensive rats through adenosine receptors located in these areas. These findings support a role for endogenous adenosine as a central modulator in cardiovascular control. (Hypertension 11: 191-197, 1988)

KEY WORDS • adenosine • adenosine 5'-triphosphate • central nervous system • nucleus tractus solitarii • area postrema • rats • nucleosides • purines • purinergic neurotransmission

AUTONOMIC cardiovascular control is achieved in the medulla oblongata through integration of input from higher centers with afferent input from receptors in chest and neck structures. This afferent input travels in the vagus and glossopharyngeal nerves and terminates principally in the nucleus tractus solitarii (NTS). NTS neurons have projections to many rostral and ventral sites. The C1 area in the ventrolateral medulla, which contributes to the tonic control over sympathetic outflow, receives some of these NTS projections. Stimulation of the NTS produces inhibition of sympathetic tone, while stimulation of the C1 area leads to an increase in sympathetic activation.

Because of the importance of central mechanisms in the autonomic control of the heart and vasculature, the identification of the relevant neurotransmitters and neuromodulators has been a priority of investigators. Data have been developed to support a role for L-glutamate as a neurotransmitter in the nucleus of the solitary tract. Likewise, γ-aminobutyric acid (GABA) appears to be an important inhibitory neurotransmitter in the C1 area. Both phenylethanolamine-N-methyltransferase and neuropeptide Y have been found in C1 area neurons that presumably innervate the intermediolateral column of the spinal cord.

In spite of the progress that has been made in the identification of these neurotransmitters and neuromodulators, additional ones probably also contribute to medullary cardiovascular control. Adenosine is a ubiquitous product in the metabolism of adenosine 5'-triphosphate (ATP). Specific receptors for adenosine have been found in the central nervous system and appear to modulate intracellular levels of cyclic adenosine 3',5'-monophosphate. Tissue levels of adenosine in the central nervous system have been shown to increase during hypoxia and seizures.

Adenosine inhibits the release of many neurotransmitters, including acetylcholine, norepineph-
rine,15,16 dopamine,17 GABA,18 and L-glutamate,19 both in the central nervous system and in peripheral tissues. Adenosine also has a marked depressive action on the firing of neurons in virtually all regions of the central nervous system tested.20 It has also been proposed that adenosine, or ATP, may act as a neurotransmitter or cotransmitter in some nerves.21 ATP is co-stored with norepinephrine in sympathetic postganglionic neurons.22 ATP has been proposed to function as a cotransmitter in some of these neurons23 through actions on P1 and P2 purinergic receptors.24 Because of the ubiquity of adenosine and ATP and their potential physiological importance, we investigated their possible role as neuromodulators of brainstem cardiovascular control.

Materials and Methods
Male Sprague-Dawley rats (Sasco Sprague-Dawley, Omaha, St. Louis, MO, USA) weighing 300 ± 50 g were used in this study. The rats were kept in individual cages in a room where lighting was controlled (12 hours on, 12 hours off) and room temperature was kept between 23 and 24°C. The animals were given Purina Laboratory Chow (St. Louis, MO, USA) and tap water ad libitum.

Rats were anesthetized with urethane (1.5 g/kg i.p.). Femoral artery and vein catheterizations were then performed for physiological recording and drug administration. The arterial catheter was connected to a Hewlett-Packard strain-gauge transducer (Model 1280, Waltham, MA, USA) to measure blood pressure and heart rate and a Hewlett-Packard physiological recorder (Model 7754B). Rats were then placed prone in the Kopf Instruments stereotaxic instrument (Tujunga, CA, USA) with head flexion at 15 degrees. Occipital craniotomy was performed to expose the dorsal medulla. Rats were stabilized for more than 1 hour after completion of the operation. Agents were then microinjected into brainstem nuclei through micropipettes fabricated from glass capillary tubing (outside diameter, 0.006 in.; inside diameter, 0.0006 in.; Richmond, NJ, USA) with tips (50 μm) shaped by a standard laboratory micropipette puller. Pipettes were carried in a stereotaxic micromanipulator and connected by polyvinyl tubing to a Hamilton microsyringe (Reno, NV, USA). The volume of the injectate was 60 nl. Different concentrations of agents were then injected intramedullarily into the NTS, the area postrema, or the C1 area of the ventrolateral medulla. The following coordinates were used for the NTS: mediolateral (ML) 0.8, anteroposterior (AP) 0.4, dorsoventral (DV) 0.8 mm; for the area postrema: ML 0.0, AP 0.33, DV 0.15 mm; for the C1 area: ML 2.0, AP 2.5, DV 2.6 mm with the fourth ventricle as zero. Only one drug was tested in each rat. The injection sites were confirmed by responsiveness to L-glutamate administration and by histological confirmation. All injections were unilateral.

Drugs
The following drugs were used: adenosine (Sigma Chemical, St. Louis, MO, USA), ATP (Sigma), β-y-methylene ATP (Volga, 1,3-dipropyl-8-p-sulfophenylxanthine (Research Biochemicals, Wayland, MA, USA), L-glutamic acid (NBC, Cleveland, OH, USA), adenosine deaminase from calf intestine (Boehringer Mannheim Biochemicals, Indianapolis, IN, USA), 5′-N-ethylcarboxamidoadenosine (NECA; Research Biochemicals), (−)-N6-(R-phenyl-isopropyl)adenosine (PIA; Boehringer Mannheim Biochemicals, Mannheim, West Germany), and urethane (Aldrich, Milwaukee, WI, USA). All drugs injected into the medulla were dissolved in artificial cerebrospinal fluid (NaCl, 123 mM; CaCl2, 0.86 mM; KCl, 3.0 mM; MgCl2, 0.89 mM; NaHCO3, 25 mM; NaH2PO4, 0.50 mM; Na2HPO4, 0.25 mM; aerated with 95% O2/5% CO2, pH 7.4). NECA and PIA were dissolved in artificial cerebrospinal fluid with 10% dimethyl sulfoxide.

Statistical Analysis
Results were compared by analysis of variance (Clinfo System, Clinical Research Center). When the analysis of variance revealed a difference, the residual mean square was applied in Dunnett’s test to characterize which values were different from the control. Group differences were analyzed by unpaired t test. All null hypotheses were two-tailed, and a p value less than 0.05 was considered significant. All data are presented as means ± SEM.

Results
Microinjection (60 nl) of adenosine (0, 0.23, 1.12, 2.3 nmol in artificial cerebral spinal fluid) in the NTS produced a dose-related decrease in heart rate and in systolic and diastolic blood pressures (Figure 1). Maximal changes occurred 90 seconds after injection. The injection of 2.3 nmol adenosine reduced heart rate by 10 ± 2 beats/min and reduced blood pressure by 24/14 ± 2/2 mm Hg (n = 7, p < 0.001). A somewhat greater effect was observed with injection into the area postrema. Heart rate fell 18 ± 1 beats/min, while blood pressure fell 29/25 ± 4/3 mm Hg (n = 6, p < 0.001).

![Figure 1](https://hyper.ahajournals.org/)

**Figure 1.** Effects of increasing doses of adenosine injected into the NTS on systolic (SBP) and diastolic blood pressure (DBP) and heart rate (HR). Asterisk denotes a significant change compared with baseline value.
Similarly, microinjection of ATP (0.01–1.1 nmol) and its analogue, β,γ-methylene ATP (0.01–1.1 nmol), were made into the NTS area. The injection of 1.1 nmol ATP reduced heart rate by 55 ± 2 beats/min and reduced blood pressure by 34 ± 1 mm Hg (n = 6, p < 0.001; Figure 2). Injection of 1.1 nmol β,γ-methylene ATP decreased heart rate by 73 ± 2 beats/min and reduced blood pressure by 40/38 ± 2/1 mm Hg (n = 6, p < 0.001; Figure 3).

To determine the specificity of the effects of adenosine and ATP, we used a potent and specific adenosine receptor antagonist, 1,3-dipropyl-8-p-sulfophenylxan-
thine (0.92 nmol). The injection of the antagonist itself into the NTS had no effect on heart rate or blood pressure. However, the antagonist abolished the effect of further injections of adenosine or ATP in the NTS (Figure 4). During the time when the adenosine antagonist was preventing any response to injected adenosine or ATP, the NTS responsiveness to l-glutamate was unimpaired. The actions of adenosine or ATP following the administration of antagonist were restored in approximately 45 minutes (Figures 5 and 6). Parallel studies showed similar results in the area postrema. We also found that adenosine deaminase (6 ng) locally injected into the NTS and area postrema completely blocked the effect of adenosine, ATP, and β,γ-methylene ATP, but not those of L-glutamic acid. Injection of adenosine deaminase alone produced a slight and transient increase in blood pressure (Figure 7). On the contrary, adenosine deaminase previously denatured by boiling did not alter the effects of β,γ-methylene ATP (Table 1, Figure 7). This finding further supports our view that ATP and β,γ-methylene ATP were converted to adenosine and act through P1 purinergic receptors.

In contrast to the significant bradycardic and vasodepressor effects of adenosine and ATP in the NTS and area postrema, the injection of similar amounts of adenosine and ATP into the C1 area of the ventrolateral medulla was devoid of pharmacological effect on blood pressure or heart rate.

We also studied the effects of two adenosine analogues (PIA and NECA) with relative specificity to A1 and A2 adenosine receptors in an attempt to determine the receptor type present in these medullary centers. We previously determined that the solution necessary to dissolve these compounds (10% dimethyl sulfoxide) had no effect when injected into the NTS or area postrema. Furthermore, 10% dimethylsulfoxide did not affect the hypotension produced by L-glutamic acid or adenosine, implying that the function of these receptors was not affected. Injection of 0.23 nmol NECA reduced heart rate by 22 ± 2 beats/min and reduced blood pressure by 30/20 ± 2/2 mm Hg (n = 8). Equimolar injections of PIA were significantly less effective than NECA (p < 0.001, by unpaired t test) in reducing heart rate (8 ± 1 beats/min) and blood pressure (11/7 ± 2/1 mm Hg; n = 6). As expected, both NECA and PIA were more potent than adenosine on an equimolar basis, since these are stable analogues of adenosine. The greater potency of NECA seems to imply the presence of an A2 (R1) adenosine receptor in the medullary centers. However, differences in liposolubility or in other pharmacodynamic characteristics between these agents could partially contribute to the difference in potency observed.

**Discussion**

Since the identification of the NTS as an important nucleus in cardiovascular control, a variety of putative neurotransmitters and drugs have been tested for their potency in altering blood pressure and heart rate in this site. Norepinephrine, acetylcholine, epinephrine, L-glutamate, dopamine, and other agents are known to reduce blood pressure and heart rate when injected into the NTS. In contrast, certain agents, such as angiotensin II, vasopressin, and yohimbine, have been shown to raise blood pressure and heart rate when injected into this site.

The presence of adenosine receptors in many sites in the central nervous system encouraged us to consider the possibility that this agent might also exert important effects on cardiovascular control. We and others have recently shown that adenosine increases heart rate and systolic blood pressure and stimulates respiration in humans. These actions are probably due to adenosine-induced carotid body chemoreceptor activation. In contrast to these peripheral actions of adenosine, intracerebroventricular injection of adenosine has a depressor effect on respiratory control. Furthermore, the injection of the adenosine receptor agonists NECA and PIA has recently been shown to exert substantial depressor effects in some medullary sites, including the NTS and the area postrema. The fact that adenosine has such potent hypotensive actions in the area postrema is especially interesting, since this area is essential in the development of hypertension in two-kidney, one clip rats and caffeine, an adenosine receptor antagonist, has been shown to enhance the pressor effect of angiotensin II in this model of hypertension.
Our studies demonstrate that adenosine and ATP both reduce blood pressure and heart rate when injected into the NTS and the area postrema. The finding that an adenosine receptor antagonist blocked the depressor effect of adenosine but not that of L-glutamate is supportive evidence for the presence of specific adenosine receptors capable of modulating blood pressure in these medullary centers. Moreover, ATP was considerably more potent on a molar basis in eliciting these responses than was adenosine itself. Burnstock and his collaborators have provided evidence that ATP may function as a neurotransmitter or cotransmitter in various neuron terminals, including especially neuron terminals that release catecholamines. The finding of relatively greater depressor potency for ATP than for adenosine might suggest that an ATP receptor (a P2 purinergic receptor) mediates these effects. However, since in our studies the effects of ATP were effectively blocked both by adenosine receptor antagonism with 1,3-dipropyl-8-p-sulfophenylxanthine and by enzymatic degradation of adenosine with adenosine deaminase.

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
<th>After</th>
</tr>
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<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>-33±4</td>
<td>+3±1*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>-20±3</td>
<td>+3±1*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>-34±5</td>
<td>+4±2*</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM (n = 7).

SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate.

*p<0.001, compared with preinjection values (by paired t test).
deaminase, adenosine receptors may mediate the responses observed. Adenosine is known to be very rapidly taken up into cells. When it is thus removed from the membrane receptor sites at which many of its effects are mediated, it cannot, of course, continue to exert its pharmacological effect. Therefore the injection of adenosine into the NTS may be followed by a relatively rapid transit of adenosine to intracellular sites. ATP, which may not be so readily transported into the cells, may thus penetrate more effectively to sites distant from the injection needle and release adenosine locally onto membrane receptors in those sites. This latter mechanism could account for the greater potency of ATP compared with adenosine, simply on the basis of its improved delivery of adenosine to its relevant receptor sites.

The administration of an adenosine antagonist into the NTS or AP had little effect on blood pressure in our model, which could imply that adenosine receptors do not play a major role in cardiovascular control in the basal state. However, since anesthesia greatly alters prevailing autonomic tone, different results might be obtained in the awake state. In any case, it seems premature to rule out a role for adenosine receptors in the basal state on the basis of data from anesthetized animals.

In conclusion, we have shown that adenosine and ATP decrease blood pressure and heart rate when injected into the NTS and the area postrema. These effects appear to be mediated through adenosine receptors located in these areas, since they are abolished by a specific adenosine receptor antagonist. The effects of adenosine are specific for these areas since microinjections of adenosine in other regions of the medulla, including the C1 area, had no effect. These findings are consistent with a role for endogenous adenosine in the central regulation of autonomic outflow to the cardiovascular system.

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