Abstract—It has been shown that nitric oxide (NO) is synthesized in the central nervous system as well as in vascular endothelial cells. We recently reported that NO was involved in central cardiovascular regulation, modulated the baroreflex, and was involved in a reciprocal release with excitatory amino acids in the nucleus tractus solitarii (NTS) of rats. We also reported previously that adenosine increased the release of glutamate in the NTS. The purpose of the present study was to investigate the possible interaction of NO and adenosine in the NTS. Male Sprague-Dawley rats were anesthetized with urethane, and blood pressure was monitored intra-arterially. Unilateral microinjection of L-arginine (3.3 nmol/60 nL) into the NTS produced decreases in blood pressure and heart rate. Microinjection of adenosine (2.3 nmol/60 nL) also produced depressive and bradycardic effects. These cardiovascular effects were attenuated by prior administration of the specific adenosine receptor antagonist DPSPX (0.92 nmol). Similarly, prior administration of NO synthase inhibitor L-nitro-L-arginine methyl ester significantly attenuated the depressive and bradycardic effects of adenosine. These results demonstrate a reciprocal attenuation of adenosine receptor antagonist and NO synthase inhibitor on L-arginine and adenosine responses, respectively, in the NTS and implicate an interaction between NO and adenosine in central cardiovascular regulation. (Hypertension. 1998;32:1034-1038.)

Key Words: nitric oxide ▪ adenosine ▪ nucleus tractus solitarii

Previous studies have shown that nitric oxide (NO), which largely accounts for the biological effects of endothelium-derived relaxing factors, is synthesized from L-arginine in the central nervous system (CNS) as well as in other tissues, including vascular endothelial cells. NO may play a physiological role in local transcellular communication by facilitating cGMP formation in adjacent cells through the activation of soluble guanylate cyclase.

The nucleus tractus solitarii (NTS) processes information from a visceral afferent receptor, including the baroreceptor afferent nerves, and plays an important role in the integration of autonomic control of the cardiovascular system. NO synthase exists in intrinsic neurons within the NTS and in central and primary afferent terminals within this nucleus. Tagawa et al. demonstrated that NO increases the neuronal activity of adjacent neurons in the NTS through an increase in cGMP. We recently reported that microinjection of L-arginine unilaterally into the NTS of anesthetized rats produced a pronounced concentration-dependent decrease in mean blood pressure (MBP), heart rate (HR), and renal sympathetic nerve activity. We also found that the microinjection of NO synthase inhibitors attenuated the hemodynamic effects produced by activation of the baroreceptor reflex. Furthermore, NO and excitatory amino acids reciprocally release each other in the NTS. These findings suggest that NO is involved in neural transmission and central cardiovascular regulation.

Adenosine has been known to be a potent vasodilator acting through purinergic receptors on both vascular smooth muscle and endothelial cells. This endogenous nucleoside has been studied for its potential role as a neuromodulator in a number of autonomic functions. Much evidence has indicated that adenosine can also affect cardiovascular function within the CNS. The highest density of adenosine uptake sites in the CNS has been observed in the NTS. In this nucleus, adenosine decreases blood pressure (BP), HR, and renal sympathetic nerve activity and modulates baroreflex responses. Furthermore, it has been reported that perfusion of adenosine through a microdialysis probe can increase the release of glutamate in the NTS. These effects are compatible with activation (rather than inhibition) of neuronal cells in the NTS, in which either electric stimulation or microinjection of excitatory substances results in decreased sympathetic tone, hypotension, and bradycardia.

The cardiovascular effects of adenosine in the NTS are strikingly similar to those of NO. In addition, both adenosine
Numerous studies have reported that adenosine stimulates the release of NO from endothelial cells through adenosine receptor activation and that the peripheral vasodilator response to adenosine is mediated by NO. In the CNS, NO participates in the hypotensive effect by adenosine A1 receptor stimulation. Nevertheless, there is evidence indicating that endogenous NO and NO donors enhance adenosine release from ventral striatum and hippocampal slices. Taken together, it appears that NO and adenosine have some degree of interaction in the CNS.

The purpose of the present study was to provide pharmacological evidence of whether NO and adenosine are reciprocally released in the NTS. Our results implicate an interaction between NO and adenosine in central cardiovascular regulation.

**Methods**

Male Sprague-Dawley rats (weight, 250 to 350 g; Charles River) were obtained and housed in the animal room of Veterans General Hospital–Kaohsiung (Kaohsiung, Taiwan, Republic of China). The rats were kept in individual cages in a room in which lighting was controlled (12 hours on/12 hours off), and temperature was maintained at 23°C to 24°C. The rats were given Purina Laboratory Chow and tap water ad libitum.

Rats were anesthetized with urethane (1.0 g/kg IP and 300 mg/kg IV if necessary). The preparation of animals for intra-NTS microinjection and the methods used in the localization of NTS were described previously.

In the first group of animals, the effects of intra-NTS administration of adenosine (2.3 nmol/60 nL) on BP and HR were observed 10 minutes after intra-NTS microinjection of 60 nL saline. Thirty minutes after recovery, the animals were pretreated with different doses (1, 33, and 100 nmol) of NO synthase inhibitors (N\textsuperscript{\textguillemotleft}G\textsuperscript{-}monomethyl-L-arginine [L-NMMA], N\textsuperscript{\textguillemotleft}G\textsuperscript{-}nitro-L-arginine methyl ester [L-NAME], and N\textsuperscript{\textguillemotleft}G\textsuperscript{-}nitro-arginine methyl ester [D-NAME]) and the adenosine receptor antagonist DPSPX (0.92 nmol), respectively. The cardiovascular action of the same dose of adenosine was observed after 10 to 90 minutes. In different groups of animals, a similar experimental procedure was used to study the effects of pretreatment with the adenosine receptor antagonist DPSPX (0.92 nmol) on L-arginine (3.3 nmol) in the NTS.

After completion of the experiment, 120 nL ink was injected through the cannula, and the rats were perfused with saline followed by a solutions of 4% formaldehyde and 30% sucrose solution sequentially. Sections (40 μm) of the brain stem were stained with cresyl violet, and proper placement of the pipette tip in the NTS was verified with histological sections under the microscope.

For NTS microinjection, the drugs were dissolved in sterile saline to the final concentrations in a volume not exceeding 60 nL. For each drug, only 60 nL was pressure-microinjected into the NTS. The following drugs were used: urethane, L-glutamic acid, L-arginine, L-NMMA, L-NAME, D-NAME (Sigma Chemical Co), and DPSPX (Research Biochemicals).

For statistical analysis, paired t test (before and after intra-NTS microinjection) and unpaired t test (for control and study group comparisons) or repeated-measures ANOVA followed by Dunnett’s test for significant differences were used. Differences with P<0.05 were considered significant. All data were presented as mean±SEM.

**Results**

In agreement with our previous findings, intra-NTS microinjection of adenosine (2.3 nmol) resulted in hypotension and bradycardia. However, the cardiovascular effects of adenosine were significantly attenuated by previous intra-NTS administration of 0.92 nmol of DPSPX (from −26±2 to −5±1 mm Hg for MBP and from −30±5 to −10±2 bpm for HR, respectively). Prior administration of saline into the NTS did not modify the effects of adenosine on MBP and HR. To test whether the NO system was involved in the cardiovascular effects of adenosine, we used the NO synthase inhibitors L-NMMA and L-NAME. Pretreatment with different doses of L-NMMA or L-NAME (10, 33, and 100 nmol) for 10 minutes significantly attenuated the depressor and bradycardic responses to adenosine (Figures 1 through 3). In addition, we examined the attenuation efficiency of different doses of L-NMMA and L-NAME (10, 33, and 100 nmol) on adenosine. L-NMMA and L-NAME attenuated the cardiovascular effects of adenosine dose dependently (Figures 2 and 3). In contrast, the attenuation effects of L-NAME were more potent than those of L-NMMA on an equimolar basis (Figures 2 and 3). However, pretreatment with D-NAME (33 nmol/60 nL) did not modify the cardiovascular effects of adenosine in any way.

To investigate whether reciprocal interactions between a purinergic mechanism and NO are present in the NTS, we tested the cardiovascular effects of L-arginine on prior administration of adenosine receptor antagonist DPSPX in the NTS. Unilateral microinjection of L-arginine (3.3 nmol) into the NTS resulted in hypotension and bradycardia.
These effects were similar to our previous findings. After pretreatment with DPSPX (0.92 nmol) for 10 minutes, the depressor and bradycardic responses to L-arginine were attenuated significantly (−21 ± 1 mm Hg and −19 ± 3 bpm, respectively) (Figure 4). These effects were similar to our previous findings. After pretreatment with DPSPX (0.92 nmol) for 10 minutes, the depressor and bradycardic responses to L-arginine were attenuated significantly (−21 ± 1 mm Hg and −19 ± 3 bpm, respectively) (Figure 4).

The attenuated cardiovascular effects of NO synthase inhibitors and adenosine receptor antagonist on adenosine and L-arginine had recovered 60 to 90 minutes after injection of the antagonists (Figures 1 and 4).

**Discussion**

The NO system and adenosine seem to have interrelated effects in the regulation of cardiovascular response. In the present study we demonstrated that microinjection of adenosine into the NTS induced depressor and bradycardic effects. These results were similar to our previous findings. Prior administration of adenosine receptor antagonist DPSPX and NO synthase inhibitors L-NMMA or L-NAME significantly attenuated the cardiovascular effects of intra-NTS microinjection with adenosine. Thus, the results of the present study suggest that the activation of adenosine receptors by adenosine might be mediated through NO in the NTS of rats.

Adenosine is a potent vasodilator of vascular smooth muscle. Endothelial cells modulate vascular tone through the release of NO, which also elicits vasodilation. Several reports have demonstrated that inhibition of NO synthesis significantly decreased coronary vasodilatory response attributed to adenosine, suggesting that adenosine-induced vasodilation is accomplished by action on vascular smooth muscle and stimulation of NO release by endothelial cells. In the CNS, adenosine exerts neuroprotective and neuromodulatory effects on presynaptic, postsynaptic, and possible extrasynaptic A₁ and A₂ receptors. Furthermore, adenosine depresses the release of both excitatory and inhibitory neurotransmitters and has a marked depressant action on the firing of neurons. Autoradiographic studies have also demonstrated that the highest density of central adenosine uptake sites is in the NTS. Mosqueda-Garcia et al demonstrated that activation of adenosine receptors produced the subsequent release of glutamate. Microinjection of adenosine into the NTS produced a dose-dependent decrease in systolic and diastolic BP, HR, and sympathetic nerve activity. In addition, adenosine antagonists such as DPSPX and caffeine can inhibit baroreflex activation.

We have reported recently that NO was involved in central cardiovascular regulation, and the depressor effect of NO in the NTS and rostral ventrolateral medulla might be through inhibition of renal sympathetic nerve activity. Furthermore, the NO synthase inhibitor also attenuated baroreflex activation in the NTS. These cardiovascular effects of NO are similar to the effects of adenosine in the NTS. Stella et al demonstrated that NO partially mediated the hypotensive
NO-mediated cardiovascular effects. Because the brain constitutive NO synthase is Ca\(^{2+}\)-calmodulin dependent, adenosine receptor–stimulated rise in intracellular Ca\(^{2+}\) concentration of neurons may involve adenosine-mediated NO production.\(^{32}\)

NO may serve as a messenger in the CNS and peripheral nervous system, much like a neuromodulator and transmitter with a widespread signaling mechanism and function.\(^{33}\) The production of NO may be linked to the activation of the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptor.\(^{34}\) Activation of postsynaptic NMDA receptors leads to intracellular synthesis of NO and a rise in the cGMP level,\(^{35}\) whereas NO has also been suggested to participate in retrograde synaptic signaling.\(^{36}\) We also recently reported that in the NTS, NO may have a retrograde effect on NMDA and non-NMDA receptors to modulate excitatory amino acid release.\(^{37}\) It has been shown that NO modulates neurotransmitter release from several brain regions\(^{21,22,24–26}\) and both NO and adenosine A\(_{2A}\) receptor agonist are capable of acting presynaptically within the rat NTS to modulate the release of glutamate.\(^{37,38}\) However, it is not clear whether the purinergic mechanism is involved in the central cardiovascular regulation of NO.

In the present study, prior administration of the adenosine receptor antagonist DPSPX significantly attenuated the depressor and bradycardic responses of L-arginine in the NTS. These observations might suggest that the cardiovascular effects of L-arginine were mediated through adenosine in the NTS. Fisher et al\(^{23}\) demonstrated that superfusion with a NO donor increases the extracellular concentration of adenosine, most likely by enhancing its release instead of inhibiting its metabolism in the ventral striatum. In the hippocampus, NO evokes a concentration-dependent release of adenosine from both stimulated and unstimulated nerves.\(^{39}\) It has been demonstrated that NO may increase transmitter release by stimulating guanylate cyclase and the production of cGMP.\(^{21}\) Therefore, in our studies the fact that adenosine antagonist attenuated the cardiovascular effects of NO in the NTS of the rat suggests that NO may modulate adenosine release in central cardiovascular function, and the data presented herein may represent evidence for a physiologically relevant interaction between adenosine and NO within the NTS.

In conclusion, the present data demonstrated that NO and adenosine reciprocally release each other in the NTS and are likely to have subtle interactions in the central cardiovascular regulation of rats.

**Acknowledgments**

This work was supported by National Science Council 86-2314-B-075B-013 and Veterans General Hospital-Kaohsiung 86-68 to Dr Ching-Jiunn Tseng.

**References**


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**Figure 4.** A, Cardiovascular effects of unilateral injection of L-arginine (L-Arg) (3.3 nmol) into the NTS before and after DPSPX (0.92 nmol) in anesthetized rats. L-Arginine and DPSPX were injected at the indicated time points. BP, MBP, and HR recordings were made at a paper speed of 3 mm/min. Horizontal bar represents recording during 5-minute intervals. B, Comparative MBP and HR effects of L-arginine (3.3 nmol) by the adenosine receptor antagonist DPSPX (0.92 nmol) on unilateral intra-NTS administration of the substances. L-Arginine was injected in the absence (control) or presence of DPSPX (0.92 nmol). Vertical bars represent SEM change from baseline values, which were 104±5 and 97±4 mm Hg for MBP and 312±4 and 315±5 bpm for HR. Each bar represents the average data from 8 rats. *Significantly different from corresponding control L-arginine response.
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Wan-Chen Lo, Chung-Ren Jan, Sheng-Nan Wu and Ching-Jiunn Tseng

Hypertension. 1998;32:1034-1038
doi: 10.1161/01.HYP.32.6.1034

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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