Interaction of Carbon Monoxide and Adenosine in the Nucleus Tractus Solitarii of Rats

Chia-Hui Lin, Wan-Chen Lo, Michael Hsiao, Ching-Jiunn Tseng

Abstract—Carbon monoxide has been identified as an endogenous biological messenger in the brain. Heme oxygenase catalyzes the metabolism of heme to carbon monoxide and biliverdin. Previously, we have shown the involvement of carbon monoxide in central cardiovascular regulation, baroreflex modulation, and glutamatergic neurotransmission in the nucleus tractus solitarii of rats. We also showed that adenosine increased the release of glutamate in the nucleus tractus solitarii. In this study, we investigated the possible interactions of carbon monoxide and adenosine in the nucleus tractus solitarii. Male Sprague-Dawley rats were anesthetized with urethane, and blood pressure were monitored intra-arterially. Unilateral microinjection of increasing doses of hemin (0.01 to 3.3 nmol), a heme molecule cleaved by heme oxygenase to yield carbon monoxide, produced a significant decrease in blood pressure and heart rate in a dose-dependent manner. In addition, similar cardiovascular effects were observed after injection of adenosine (2.3 nmol). These cardiovascular effects of hemin were attenuated by prior administration of the adenosine receptor antagonist 1,3-dipropyl-8-sulfophenylxanthine. Similarly, pretreatment of the heme oxygenase inhibitor zinc protoporphyrin IX or zinc deuteroporphyrin 2,4-bis glycol also attenuated the depressor and bradycardic effects of adenosine. These results indicate that the interaction between carbon monoxide and adenosine may contribute to the activation of heme oxygenase in central cardiovascular regulation. (Hypertension. 2003;42:380-385.)

Key Words: central nervous system ■ blood pressure ■ adenosine ■ heart rate ■ neuroregulators

The gaseous compound carbon monoxide (CO), a new neuromodulator agent, has been shown to play a role as a neurotransmitter. In animals, the predominant source of autonomic control of the cardiovascular system. It has been studied for its potential role as a neuromodulator in a number of autonomic functions. Recent evidence has indicated that adenosine can also affect cardiovascular function. Adenosine has been known to be a potent vasodilator acting through purinergic receptors both on 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. It has recently been pointed out that CO formed within the NTS subserves a vasodepressor mechanism that is tonically active in awake rats. We have previously reported that unilateral microinjection of hematin, a heme molecule cleaved by HO to yield CO, into the NTS of anesthetized rat produce dose-related depressor and bradycardic effects. On the other hand, systemic administration or direct microinjection into the NTS of HO inhibitor zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) attenuates the baroreceptor reflex. Taken together, these findings suggest that CO within the NTS may play an important role in the regulation of cardiovascular function. Adenosine has been known to be a potent vasodilator acting through purinergic receptors both on 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. Recent 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), heart rate (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 electrical stimulation\textsuperscript{16} or microinjection of excitatory substances\textsuperscript{16–18} results in decreased sympathetic tone, hypotension, and bradycardia.

Previously we have reported that the gaseous neurotransmitter nitric oxide (NO) is involved in central cardiovascular regulation and interacts with glutamate and adenosine in the NTS of the rat.\textsuperscript{17,19,20} CO is a second simple diatomic gas molecule that shares some physicochemical properties of NO. These studies suggest that CO may be a neurotransmitter in the nervous system. We and others have shown that the cardiovascular effects of CO in the NTS are strikingly similar to adenosine. In addition, both adenosine and CO are involved in baroreflex regulation.\textsuperscript{9,14} Adenosine acts by facilitating glutamate release from baroreceptor afferents,\textsuperscript{14} and CO may affect NTS glutamatergic neurotransmission to participate in cardiovascular control.\textsuperscript{16,17} Furthermore, CO also induces relaxations of vascular tissues\textsuperscript{21} and cultured vascular smooth muscle cells.\textsuperscript{22} Taken together, it appears that CO and adenosine might have some degree of interaction in the CNS.

The purpose of the present study was to provide pharmacological evidence as to whether CO and adenosine are reciprocally released in the NTS. Our results indicate an interaction between CO and adenosine in central cardiovascular regulation.

**Methods**

**Materials**

Experimental drugs such as urethane, L-glutamate, and adenosine were from Sigma Chemical Co. Zinc protoporphyrin IX (ZnPPIX) and (R,S)-3,5-dihydroxyphenylglycine (DHPG) were obtained from Tocris Cookson Ltd. ZnDPBG was obtained from Prophyrin Products. ZnPPIX and ZnDPBG were dissolved in 50 mmol/L Na\textsubscript{2}CO\textsubscript{3} (pH 8.8 to 9.4) immediately before use. Hemin (ICN Biochemicals Inc) was dissolved in 30% 0.1N NaOH (pH 8.6 to 9). 1,3-Dipropyl-8-sulfophenylxanthine (DSPX) was purchased from Research Biochemicals. All other drugs were dissolved in normal saline on the day of the experiment.

**Animals**

Male Sprague-Dawley rats (250 to 350 g) were obtained from National Science Council Animal Facility and housed in the animal room of Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan, ROC). The rats were kept in individual cages in a room in which lighting was controlled (12 hours on/12 hours off), and temperature maintenance was maintained at 23\degree C to 24\degree C. The rats were given Purina Laboratory Chow and tap water ad libitum.

**Experimental Procedures**

All animal protocols were approved by the Research Animal Facility Committee at Kaohsiung Veterans General Hospital. Humane treatment was administered at all times. 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 the NTS have been described previously.\textsuperscript{19} In this study, each injection volume was restricted to 60 nl.

In the first group of animals, BP and HR changes were monitored after intra-NTS microinjection of hemin (0.01, 0.1, 0.33, 1, 3.3 nmol). The changes of BP and HR were measured through unilateral microinjection of hemin (1 nmol) before and 10 minutes after intra-NTS administration with HO inhibitor (ZnPPIX or ZnDPBG; 0.1, 0.33, 1 nmol) or vehicle alone (50 mmol/L Na\textsubscript{2}CO\textsubscript{3}). To investigate the effect of preadministration of the HO inhibitor ZnPPIX and ZnDPBG on cardiovascular responses to adenosine in the NTS, animals were first injected with adenosine (2.3 nmol) into the unilateral NTS. The rats were then allowed to rest for at least 30 minutes until the BP and HR had returned to basal levels. The changes in BP and HR were then monitored by microinjection of the same doses of adenosine 10 minutes after intra-NTS administration with different doses (0.1, 0.33, and 1 nmol) of ZnPPIX, ZnDPBG, or vehicle. Similar experimental procedures were used to study the effects of pretreatment with the adenosine receptor antagonist DSPX (0.92 nmol) on hemin (1 nmol) or vehicle (30% 0.1N NaOH in saline) into the NTS in different groups of animals. During the study, a negative control experiment was performed. The effects of prior administration with ZnPPIX (1 nmol) on DHPG (0.03 nmol) were studied by using the same procedures as described above.

**Statistics**

A paired \textit{t} test (before and after pretreatments), unpaired \textit{t} test (for control and study group comparisons), or repeated-measures ANOVA followed by the Dunnett test for significant differences was applied to compare group differences. Differences with a probability value of <0.05 were considered significant. All data are expressed as mean±SEM.

**Results**

In Sprague-Dawley rats, the basal mean blood pressure (MBP) and HR were 92±8 mm Hg and 303±17 bpm, respectively. Unilateral intra-NTS microinjection of increasing doses of hemin (0.01, 0.1, 0.33, 1, and 3.3 nmol) into the NTS produced dose-dependent depressor and bradycardic effects (data not shown). Maximal effect was observed at 1 nmol (−34±2 mm Hg and −68±5 bpm, respectively). Pretreatment with different doses of ZnPPIX and ZnDPBG attenuated the depressor and bradycardic responses to hemin in a dose-dependent manner (Figures 1A and 1B) (\(P<0.05\) compared with control value). However, prior administration of vehicle did not modify the cardiovascular effects of hemin (Figures 1A and 1B). For negative control, prior administration of ZnPPIX (1 nmol) did not modify the depressor and bradycardic effects of the group 1 metabotropic glutamate receptor agonist DHPG (Figure 1C).

Previously we have shown that intra-NTS microinjection of adenosine (2.3 nmol) caused hypotension and bradycardia.\textsuperscript{12,20} However, the cardiovascular effects of adenosine were significantly attenuated by pretreatment with adenosine receptor antagonist DSPX (0.92 nmol). To test whether the CO system was involved in the cardiovascular effects of adenosine, we used the HO inhibitor ZnPPIX and ZnDPBG. Figure 2A shows significant attenuation of the adenosine-induced cardiovascular effects by previous intra-NTS administration of ZnPPIX (1 nmol). Pretreatment with different doses of ZnPPIX (0.1, 0.33, and 1 nmol) attenuated the depressor and bradycardic responses to adenosine dose-dependently, as shown in Figure 2B (\(P<0.05\) compared with control value). Similarly, prior treatment with the different type of HO inhibitor, ZnDPBG, also significantly attenuated the cardiovascular effects of adenosine. Pretreatment with different doses of ZnDPBG (0.1, 0.33, and 1 nmol) also attenuated the depressor and bradycardic responses to adenosine dose-dependently (\(P<0.05\) compared with control value, Figure 2C). Therefore, our studies demonstrated that both ZnPPIX and ZnDPBG significantly attenuated the cardiovascular effects of adenosine. The attenuated cardiovascular-
cular effects of ZnPPIX or ZnDPBG on adenosine reached a maximum effect in 10 minutes and lasted for at least 60 minutes (data not shown). However, prior administration of vehicle did not modify the cardiovascular effects of adenosine (Figures 2B and 2C).

To investigate reciprocal interactions between purinergic mechanisms and CO in the NTS, we tested the cardiovascular effects of hemin on prior administration of the adenosine receptor antagonist DPSPX in the NTS. Unilateral microinjection of hemin (1 nmol) into the NTS resulted in hypotension and bradycardia (Figure 3A, $-37\pm4$ mm Hg and $-73\pm8$ bpm, respectively). After pretreatment with DPSPX (0.92 nmol), the depressor and bradycardic responses to hemin were attenuated significantly (Figure 3B, $-11\pm2$ mm Hg and $-18\pm4$ bpm, respectively, $P<0.05$ compared with vehicle group).

**Discussion**

In the present study, we showed that intra-NTS microinjection of hemin, a substrate for CO production, dose-dependently induced depressor and bradycardic effects similar to that induced by NO system. Prior microinjection of the HO inhibitor ZnPPIX or ZnDPBG significantly suppressed the cardiovascular effects of hemin (Figures 1A and 1B). The results suggest that hemin was transformed into the CO by the presence of HO in NTS. These results were in agreement with our previous findings. One report has shown that administration of an HO inducer such as heme-arginate or heme-lysinate causes a marked decrease in BP in spontaneously hypertensive rats (SHR). Furthermore, there is evidence that HO inhibitors increase BP and peripheral resistance, suggesting that endogenous CO subserves a tonic vasodepressor function. In addition, CO may influence some forms of synaptic plasticity, because hippocampal long-term potentiation is inhibited by HO inhibitor and enhanced by exogenous administration of CO. Such observations and other evidence indicate that the HO/CO system may be involved in the regulation of various neural and cardiovascular functions.

Endogenous CO shares a similar role with NO as a putative neural messenger in the brain. Both gases are believed to modulate CNS function through an increase in cytoplasmic cGMP concentrations secondary to the activation of sGC. HO can act as a source of CO in neurons. In mammalian tissues, endogenous CO mainly derives from the degradation of heme by heme oxygenase (HO-2), and this accounts for almost 95% of the total CO produced in normal condition. The amount of CO can be increased enormously when inducible HO-1 is upregulated in response to a variety of stressful agents that challenge a particular organ or tissue. Both forms of the HO enzymes are found abundantly in the brain stem. Although hemin can induce HO-1, both ZnPPIX and ZnDPBG can inhibit the functions of HO-1 and HO-2. In our study, endogenous production of CO was promoted experimentally by treating with hemin. During the experiment, we noticed that microinjection of ZnPPIX or ZnDPBG into the NTS produced a decrease in arterial pressure and heart rate, which was opposite to the findings in conscious rats as described previously. Such observations also were found in our previous reports. The decreasing responses are nonspecific effects, as apparently is also observed with the solvent. The phenomenon is not known and is not necessarily inhibition of a putative tonic effect of CO.

Iron, biliverdin, and CO are the products of HO-mediated metabolism of heme. It has been suggested that free iron can stimulate the generation of free radicals and promote lipid peroxidation. It has been suggested that some free radicals may promote vasodilation through direct activation of guanylyl cyclase. However, pretreatment with deferoxamine to

**Figure 1.** Inhibitory effect of HO inhibitors on hemin and DHPG in NTS. A, Hemin (1 nmol) was injected in the absence (open bars), presence of vehicle, or ZnPPIX. B, Hemin (1 nmol) was injected in the absence (open bars), presence of vehicle, or ZnDPBG. C, DHPG (0.03 nmol) was injected in the absence (open bars), presence of ZnPPIX (1 nmol). Vertical bars represent SEM change from baseline values, which were 94±10 mm Hg for MBP and 327±16 bpm for HR. Each point represents average of 8 rats. *Significantly different from corresponding control (*$P<0.05$).
Chelate free iron did not prevent HO substrate–induced decreases in BP in rats. Bilirubin, an antioxidant, is converted from biliverdin after heme degradation by HO. Therefore, the BP-lowering responses to HO substrates may be linked to their antioxidative actions. However, a recent study by Johnson et al demonstrated that treatment with biliverdine did not induce BP decrease in SHR. Their results suggested that the vasodepressor effect of heme substrates is not directly related to enhanced formation of biliverdine of free iron.

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. Autoradiographic studies have demonstrated that the highest density of central adenosine uptake sites is in NTS. In this study, we showed that adenosine exerted excitatory cardiovascular effects in the NTS of anesthetized animals. Our findings were in agreement with previous reports.

In addition, we showed that 2 selective HO inhibitors, ZnPPIX and ZnDPBG, significantly attenuate the depressor and bradycardic responses induced by adenosine dose-dependently (Figures 2B and 2C). This provides sufficient evidence that adenosine in some way works through the HO/CO pathway.

On the contrary, prior administration of the adenosine receptor antagonist DPSPX significantly attenuated the depressor and bradycardic responses of hemin in the NTS (Figure 3). These observations might suggest that the cardiovascular effect of hemin is mediated through adenosine in the NTS. A recent study has demonstrated that CO dilates...
cerebral arterioles by priming Ca\(^{2+}\)-activated K\(^+\) channels for activation by Ca\(^{2+}\) sparks.\(^{34}\) CO-induced dilations are inhibited by blockers of the large-conductance Ca\(^{2+}\)-activated K\(^+\) (K\(\text{Ca}\)) channel in tail, gracilis muscle, renal, and cerebral arteries.\(^{3,35}–37\) These findings support an important role for K\(\text{Ca}\) channels in CO dilations. It has been shown that adenosine stimulated the increase of intracellular Ca\(^{2+}\) concentration and mobilized cytosolic Ca\(^{2+}\) of neurons.\(^{38,39}\) Therefore, our finding that the adenosine receptor antagonist attenuated the cardiovascular effects of CO in the NTS suggests that the activation of adenosine receptors by adenosine may modulate the cardiovascular effects of CO in the NTS. This indicates that subtle interactions of CO and adenosine might occur in the central cardiovascular regulation of rats.

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**References**


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