Modulatory Effects of Carbon Monoxide on Baroreflex Activation in Nucleus Tractus Solitarii of Rats

Wan-Chen Lo, Chung-Ren Jan, Hung-Ting Chiang, Ching-Jiunn Tseng

Abstract—Recent studies suggest that carbon monoxide (CO), which is produced in significant quantities in many brain regions, may function as a neurotransmitter. Heme oxygenase catalyzes the metabolism of heme to CO and biliverdin; however, the physiological role of CO in central cardiovascular regulation was not well understood. In the present study, we evaluated the baroreflex response of CO in the nucleus tractus solitarii (NTS) of rats. Male Sprague-Dawley rats were anesthetized with urethane, and blood pressure and heart rate were monitored intra-arterially. Unilateral microinjection (60 nL) of hematin, a heme molecule cleaved by heme oxygenase to yield CO, into the NTS produced prominent dose-related depressor and bradycardic effects. Baroreflex responses were elicited by increasing doses of phenylephrine (10 to 30 μg/kg IV) before and after intra-NTS administration of zinc deuteroporphyrin 2,4-bis-glycol (ZnDPBG) (1 nmol), an inhibitor of heme oxygenase activity, or vehicle alone. The reflex bradycardia elicited by phenylephrine was significantly inhibited by pretreatment with ZnDPBG. Furthermore, the inhibitory effect of ZnDPBG on baroreflex activation was dose dependent. These results suggest CO formed by brain heme oxygenase plays a significant role in central cardiovascular regulation and that inhibition of heme oxygenase attenuated baroreflex activation. (Hypertension. 2000;35:1253-1257.)

Key Words: bradycardia ■ brain ■ baroreflex

Carbon monoxide (CO), previously thought to be a toxic gas and biological waste product, is now being considered a likely candidate in the new class of gaseous neural messengers.1 In animals, the predominant source of CO generation is heme degradation. Heme oxygenase (HO) is the rate-limiting enzyme responsible for the catabolism of heme and subsequent production of CO and bilirubin. Two forms of HO have been identified. HO-1 is enriched in spleen and liver and is induced by heme and numerous oxidative stressors. In contrast, HO-2 is a constitutive enzyme and is present abundantly in the brain and testis.2 Recent studies have suggested that CO stimulates soluble guanylate cyclase activity and promotes elevation of cGMP in neural and cardiovascular tissues.3–8 These results have implicated the HO-CO system as a potential regulator of various neural5,6 and cardiovascular functions.4,7,8 HO is widely expressed in the brain and is responsible for the impressive CO-generating ability of the brain, including brain stem.3,6 Zinc deuteroporphyrin 2,4-bis-glycol (ZnDPBG) is an inhibitor of HO activity.5,9,10 ZnDPBG has been shown to inhibit endogenous CO production in rats11 and thus has been applied in in vivo studies of the physiological actions of HO.

In the central nervous system, the nucleus of the solitary tract (NTS) is the site where afferent fibers arising from arterial baroreceptor, chemoreceptors, cardiopulmonary receptors, and other visceral receptors make the first central synapses12 and thus play an important role in the integration of autonomic control of the cardiovascular system.13 A recent study has provided evidence that CO formed within the NTS subserves a vasodepressor mechanism that is tonically active in awake rats.14 It has been indicated that a subset of glutamate receptors that are involved in the function of the afferent arm of the baroreceptor reflex may be coupled with HO-mediated production of CO.15 We have reported in a previous study that microinjection of hematin, a heme molecule cleaved by HO to yield CO, into the NTS produced prominent dose-related depressor and bradycardic effects, and prior administration of ZnDPBG abolished the cardiovascular effects of hematin in the NTS.16 These results suggest that CO formed by brain HO plays a role in the central cardiovascular regulation. However, the role of CO in the baroreflex regulation in the brain stem nuclei remains unclear.

In the present study, we have evaluated further the baroreflex response of CO in the NTS of rats. Our results indicate that endogenous CO might be involved in baroreflex regulation.

Methods

Materials
ZnDPBG was obtained from Porphyrin Products. All other drugs were from Sigma Chemical Co. ZnDPBG was dissolved in

Received September 7, 1999; first decision October 21, 1999; revision accepted January 21, 2000.
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50 mmol/L Na₂CO₃ immediately before use. Hematin was dissolved in 30% 0.1N NaOH (pH 8.6 to 9). All the other drugs were dissolved in normal saline to the final concentrations in a volume not exceeding 60 nL. For each drug, only 60 nL was pressure-microinjected into the NTS.

Animals
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, ROC). 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° to 24°C. The rats were given Purina Laboratory Chow and tap water ad libitum.

Experimental Procedure
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 have been described previously.⁷-¹⁸

The changes of arterial pressure and heart rate were measured intra-arterially in anesthetized rats through unilateral microinjection of hematin (0.33 nmol/60 nL) before and 10 minutes after intra-NTS administration with HO inhibitor (ZnDPBG; 1 nmol/60 nL) or vehicle alone (50 mmol/L Na₂CO₃, 60 nL). Baroreflex responses were elicited by increasing doses of phenylephrine (10 to 30 µg/kg IV) before and after intra-NTS administration of different doses of ZnDPBG (0.1 to 3.3 nmol) or vehicle to test the induced baroreflex responses of phenylephrine (10 to 30 µg/kg IV). To study the reversible effects of hematin, 10 minutes after intra-NTS administration of ZnDPBG, hematin (1 nmol) was applied intra-NTS in the same animal.

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

The peak reflex bradycardia elicited by phenylephrine was expressed as pulse period (ms) and plotted against the respective peak increase in systolic blood pressure (BP) for the 3 different doses of phenylephrine in each animal. The slope of the curve from the linear regression obtained between the increases in pulse period and BP was used to express baroreflex sensitivity. The differences in slope values between control and the experimental groups were analyzed by a 2-way ANOVA followed by Duncan’s multiple range test or by the Student’s unpaired or paired t test when appropriate. Significance was accepted at a level of P < 0.05.

Results
Consistent with our previous findings, unilateral microinjection of ascending doses of hematin (0.1 to 1 nmol) into the NTS produced dose-dependent depressor and bradycardic effects (Figure 1A). After pretreatment with an HO inhibitor

Figure 1. A, Line graph showing cardiovascular effects of increasing hematin doses microinjected into NTS. Vertical bars represent SEM change from vehicle pretreatment values, which were 105±5 mm Hg for mean blood pressure (MBP) and 338±8 bpm for heart rate (HR). ○, Changes of MBP; □, changes of HR. Each point represents average of 8 rats. *P<0.05 compared with control. B, Bar graphs showing inhibition of cardiovascular effects of hematin by ZnDPBG in NTS. Hematin (0.33 nmol) was injected in absence (open columns) or presence (hatched columns) of ZnDPBG (1 nmol). Vertical bars represent SEM (n=8). *Significant difference from corresponding control hematin response. *P<0.05.

Figure 2. Tracings showing attenuation of baroreflex responses of intravenous injection of phenylephrine (Phe: 10, 20, 30 µg/kg) before and after intra-NTS microinjection of ZnDPBG (1 nmol) in anesthetized rats. Phenylephrine and ZnDPBG were injected as indicated. Blood pressure (BP), mean blood pressure (MBP), and heart rate (HR) recordings were made at paper speed of 3 mm/min. Horizontal bar represents time period of 5 minutes.
ZnDPBG (1 nmol) for 10 minutes, the depressor and bradycardic responses to hematin (0.33 nmol) were attenuated significantly (from 243±6 mm Hg and 272±8 bpm to 213±6 mm Hg and 223±6 bpm, respectively) (Figure 1B).

Prior administration of vehicle did not modify the cardiovascular effects of hematin.

Intravenous injection of phenylephrine increased BP and pulse period in anesthetized animals in a dose-dependent manner (Figure 2). Administration of vehicle (60 nL) into the NTS did not modify the reflex bradycardia elicited by phenylephrine or the slope of the baroreflex curve. In contrast, a similar increase in BP elicited less bradycardia after microinjection of ZnDPBG (1 nmol), with a significant lowering in the baroreflex slope (from 5.4±0.7 to 1.6±0.6 ms/mm Hg, n=8, Figure 3, A and B). Prior administration of vehicle did not modify the cardiovascular effects of hematin.

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ZnDPBG (1 nmol) for 10 minutes, the depressor and bradycardic responses to hematin (0.33 nmol) were attenuated significantly (from −43±4 mm Hg and −72±8 bpm to −13±3 mm Hg and −23±6 bpm, respectively) (Figure 1B). Prior administration of vehicle did not modify the cardiovascular effects of hematin.

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The time course of intra-NTS administration of ZnDPBG on the baroreflex response was investigated. As shown in Figure 5, intra-NTS injection of 1 nmol of ZnDPBG considerably attenuated the baroreflex slope, reaching a maximum effect in 10 minutes. This inhibition of baroreflex effect persisted for >90 minutes. However, intra-NTS injection of the vehicle did not significantly alter the baroreflex sensitivity.

In an ancillary study, we investigated the effects of the CO precursor hematin on the baroreflex response after intra-NTS injection of ZnDPBG. The attenuated baroreflex sensitivity was not significantly reversed by intra-NTS injection of hematin (0.33 nmol) (data not shown).

**Discussion**

In the present study, we have provided evidence that micro-injection of hematin, a substrate for CO production, into the NTS dose-dependently induced depressor and bradycardic effects. These effects of hematin required HO because prior administration of a HO inhibitor (ZnDPBG) significantly suppressed the cardiovascular effects of intra-NTS microinjection of hematin. (0.33 nmol) (data not shown).

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jection with hematin. These results were similar to our previous findings.\textsuperscript{16} Thus, our data suggested that the activation of HO by hematin might be mediated by CO in the NTS of rats.

It has been shown that CO shares some of the chemical and biological properties of nitric oxide.\textsuperscript{1} Endogenous CO production could lead to cGMP synthesis through activation of guanylyl cyclase.\textsuperscript{1,3} The enzyme HO could act as a source of CO in neurons. Two forms of this enzyme are found in the brain. HO-1 normally shows only a limited distribution, but its synthesis can be selectively increased in certain neurons and glia through activation of heat shock elements by diverse stimuli.\textsuperscript{2,3} Conversely, HO-2 is widely distributed in the brain under all conditions.\textsuperscript{2,3} Notably, both forms of the enzyme are found abundantly in the brain stem.\textsuperscript{3}

There are several reports on CO inhibition of the contractility of vascular smooth muscle and relaxation of coronary vascular smooth muscle.\textsuperscript{19–21} Moreover, administration of inducers of HO such as heme arginate or heme lysinate cause a marked decrease in BP in spontaneously hypertensive rats and an increase of coronary blood flow in isolated perfused hearts.\textsuperscript{22,23} Furthermore, there is evidence that HO inhibitors increase BP and peripheral resistance, suggesting that endogenous CO subserves a tonic vasodepressor function.\textsuperscript{24} 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.\textsuperscript{25,26} Such observations and other evidence now indicate that this heme–HO-CO system may be involved in regulation of various neural and cardiovascular functions.

The NTS is the site where afferent fibers arising from the arterial and cardiopulmonary baroreceptors make the first central synapses.\textsuperscript{12,13} Experimental lesions of the NTS lead to loss of baroreflex control of BP, sympathetic activation, and severe hypertension in animals. Johnson et al\textsuperscript{14} have reported that microinjections of ZnDPBG directly into the NTS of conscious rats also increase arterial pressure. Because this pressor response is reversed by ipsilateral administration of CO-saturated saline, it appears most likely that ZnDPBG-induced pressor effects result from impaired formation of the HO product CO within the NTS. We have shown previously that unilateral microinjection of hematin into the NTS and area postrema of anesthetized rats produced prominent dose-related depressor and bradycardic effects.\textsuperscript{16} Conversely, microinjection of hematin into the rostral ventrolateral medulla produces pressor and tachycardic effects. In the present study, we found that ZnDPBG also attenuated the cardiovascular effects of hematin in the NTS. These data suggest that CO production by HO activation may participate in central cardiovascular regulation.

In the modulation of baroreceptor and chemoreceptor functions, L-glutamate is an important neurotransmitter.\textsuperscript{27,28} Microinjection of L-glutamate into the NTS can mimic baroreflex responses (hypotension and bradycardia) in anesthetized rats\textsuperscript{29}; however, these cardiovascular effects are similar to hematin. Thus, it is of interest to know whether CO is involved in central baroreflex regulation. Systemic administration of ZnDPBG has been shown to interfere with the baroreceptor reflex.\textsuperscript{14} In addition, we further examined whether microinjection of HO inhibitor into the NTS exerted its effects by inhibiting central transmission of the baroreflex. We found that reflex bradycardia elicited by phenylephrine was markedly inhibited by pretreatment with ZnDPBG. Furthermore, the inhibitory effects of ZnDPBG on baroreflex activation were dose dependent. The present study demonstrates that HO inhibition decreased the baroreflex slope, implicating an attenuation of baroreflex sensitivity. These results suggest that endogenous CO may play a facilitatory role in central baroreflex regulation.

The mechanism by which an HO inhibitor attenuates baroreflex sensitivity is unclear. However, a recent study has provided evidence that a subset of glutamate receptors, which are involved in the function of the afferent arm of the baroreceptor reflex, may associate with HO-mediated CO production.\textsuperscript{15} In other experiments, it has been shown that ZnDPBG pretreatment attenuated the depressor and bradycardic response to L-glutamate, implying that CO may affect the effect of glutamatergic neurotransmission in cardiovascular control.\textsuperscript{30} Thus, this interaction may be physiologically significant in the control of the baroreflex.

In conclusion, our results indicate microinjection of an HO inhibitor into the NTS decreases baroreflex sensitivity in rats. Our findings suggested that central endogenous CO might be involved in medullary regulation of BP and that inhibition of HO could attenuate baroreflex activation.

Acknowledgments

This work was supported by grants from the Kaohsiung Veterans General Hospital research program (VGHKS-88-30, 89-101) and grant NSC-89-2320-B-075B-005 to Dr Ching-Jiunn Tseng.

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Hypertension. 2000;35:1253-1257
doi: 10.1161/01.HYP.35.6.1253

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