Autonomic Cardiovascular Responses to Heme Oxygenase Inhibition in Conscious Rats

Haruhisa Hirakawa, Yoshiaki Hayashida

Abstract—Carbon monoxide (CO) is produced in the course of heme degradation from biliverdin by heme oxygenase (HO) in various tissues, including the central nervous system. Recent studies suggest the inhibition of HO activity increases arterial pressure mediated by the autonomic nervous system. The present study was designed to investigate the autonomic regulation of cardiovascular responses to inhibition of endogenous CO production by the HO inhibitor Zinc deuteroporphyrin 2, 4-bis glycol (ZnDPBG) by using direct sympathetic nerve recordings in conscious, chronically instrumented rats. ZnDPBG induced increases in mean arterial pressure (MAP) (P<0.05) and renal sympathetic nerve activity (RSNA) (P<0.05) but no significant change in heart rate (P>0.05) in intact rats. In atropine-treated rats, ZnDPBG also induced increases in MAP (P<0.05) and RSNA (P<0.05) but no change in heart rate (P>0.05). In sinoaortic denervated rats, ZnDPBG induced increases in MAP (P<0.05), heart rate (P<0.05), and RSNA (P<0.05). ZnDPBG shifted the baroreflex curve for RSNA upward and to the right, which was characterized by increases in the maximum and minimum response and midpoint pressure without altering the maximum gain. These results indicate that inhibition of HO activity within the central nervous system causes sympathoexcitation, resulting in an increase in arterial pressure. We conclude that the CO/HO system plays an important role in cardiovascular regulation by modulating sympathetic tone. (Hypertension. 2006;48:1124-1129.)

Key Words: carbon monoxide ■ heme oxygenase ■ autonomic nervous system ■ baroreflex ■ arterial pressure ■ heart rate ■ renal sympathetic nerve activity

Carbon monoxide (CO) is a gaseous molecule generated as a byproduct of enzymatic degradation of heme by heme oxygenase (HO). HO is the rate-limiting enzyme in the catabolism of heme-generating biliverdin, CO, and iron.1,2 Three forms of HO have been characterized.2–4 HO-1 is induced by numerous stressors and expressed at high concentration in the spleen and liver. HO-2 is not inducible and is widely distributed throughout the body with a high concentration in the brain. HO-2 is normally expressed in the endothelial and the smooth muscle of the blood vessels and in the carotid body chemoreceptor. The recently discovered HO-3 has a 90% homology with HO-2 in amino acid structure and is a poor heme catalyst.3 Accumulating evidence suggests that CO exerts a physiological role in various tissues. For example, systemic administration of HO substrates, such as heme-L-arginate and heme-L-lysinate, acutely decrease arterial pressure (AP) in conscious spontaneously hypertensive rats.5 Intracerebroventricular administration of HO inhibitor attenuates endotoxin-induced fever in conscious rats.6 Systemic administration of HO inhibitors increases renal vascular resistance and lowers renal blood flow in anesthetized rats.7 Zinc deuteroporphyrin 2, 4-bis glycol (ZnDPBG) is an inhibitor of HO activity. It has been reported that systemic administration of ZnDPBG induces a sustained increase in AP,5,8 which is abolished by autonomic ganglionic blockade or α1-adrenoreceptor blockade.9 Accordingly, it has been suggested that the pressor response elicited by the inhibition of HO activity is mediated by the autonomic nervous system. However, no direct evidence of HO inhibition on sympathetic nerve activity has been reported.

In the central nervous system (CNS), it has been suggested the CO/HO system plays an important role in the regulation of cardiovascular function. Johnson et al8 reported that microinjection of ZnDPBG into the nucleus tractus solitarii (NTS) caused an increase in AP, and this effect was reversed by an injection of CO into the NTS in conscious rats. They also showed that systemic administration of ZnDPBG attenuated baroreflex control of heart rate (HR). Lo et al10 reported that injection of ZnDPBG into the NTS attenuated reflex bradycardia in response to an increase in AP by phenylephrine (PE). However, whether inhibition of HO activity modulates baroreflex control of sympathetic nerve activity has not been elucidated.
Accordingly, the present study was designed to investigate the role of the CO/HO system in the regulation of the autonomic nervous system and its contribution to the cardiovascular system in conscious rats. To achieve this objective, cardiovascular and renal sympathetic nerve activity (RSNA) responses were measured continuously before and after intraperitoneal injection of ZnDPBG in conscious intact and sinoaortic denervated (SAD) rats. The contribution of parasympathetic nervous system to the HR response induced by ZnDPBG was also examined in rats treated with atropine. Furthermore, the effect of ZnDPBG on the baroreflex control of HR or RSNA was investigated.

Methods

Surgical Procedures
Male Wistar rats (weighing between 300 and 350 g) were divided into 2 groups: 16 intact and 7 SAD rats. Each animal was anesthetized with sodium pentobarbital (50 mg/kg IP), and heparin-filled polyethylene catheters were inserted into the abdominal aorta from the right femoral artery to measure AP and into the inferior vena cava from the right femoral vein to deliver drugs. A silicone catheter was placed within the intraperitoneal space through a midline abdominal incision to administer ZnDPBG. The electrode for the renal sympathetic nerve recording was implanted as described previously. In brief, through a left flank incision, a branch of the left renal nerve running on or beside the renal artery was exposed and dissected free from surrounding tissue. Stainless-steel bipolar electrodes were placed around the dissected renal nerve. The nerve and electrodes were stabilized with 2-component silicone rubber gel (Semicosil 932, Wacker Silicones Corp). After the gel hardened, the incision was closed. All of the catheters and electrodes were routed subcutaneously to exit at the nape.

Sinoaortic Denervation
Sinoaortic denervation was performed using the method of Krieger as modified by Schreihofer and Sved. The details are as described previously.

To prevent infection, all of the rats were treated with an intraperitoneal injection of ampicillin (30 mg/kg) after surgery. All of the other drugs were dissolved into normal saline on the day of the experiment. All of the rats were allowed 30 to 60 minutes to adjust to their environment, and control measurements commenced when stable MAP, HR, and RSNA were observed.

Protocol 1: MAP, HR, and RSNA Responses to ZnDPBG in Intact Rats (n=8)
We examined the effect of systemic administration of ZnDPBG on MAP, HR, and RSNA in intact rats. After 30 minutes of control data collection, rats were given an intraperitoneal injection of ZnDPBG (45 μmol/kg) or drug vehicle only (50 mmol/L Na₂CO₃). MAP, HR, and RSNA were measured continuously for the control period and 2 hour after administration of ZnDPBG.

Protocol 2: MAP, HR, and RSNA Responses to ZnDPBG in Atropine-Treated Rats (n=8)
To access the influence of parasympathetic nerve on HR response to ZnDPBG, 8 rats were pretreated by atropine methyl nitrate (4 mg/kg IV). All of the other experimental procedures were the same as performed in protocol 1.

Protocol 3: MAP, HR, and RSNA Responses to ZnDPBG in SAD Rats (n=7)
In this protocol, we examined the cardiovascular and sympathetic nerve responses after the denervation of the sinus and aortic nerves, which are the afferent pathways from the peripheral chemoreceptors and baroreceptors. Experiments were conducted in a same manner as protocol 1 in SAD rats.

Protocol 4: Relationship Between MAP and HR or RSNA
To examine the relationship between MAP and HR or RSNA, ramped increases and decreases in MAP were performed before and ≥30 minutes after the administration of ZnDPBG. The ramp increase in MAP (0.32 to 0.80 mm Hg/s) was produced by infusion of PE (0 to 1.0 mL/min of 20 μg/mL), and the ramp decrease in MAP (0.47 to 0.96 mm Hg/s) was produced by infusion of nitroprusside (0.1 mg/mL/min of 100 μg/mL) with an infusion pump (CMA/100, CMA/Microdialysis). PE or nitroprusside was administered in random order with an interadministration interval of ≥10 minutes, during which MAP, HR, and RSNA returned to their preadministration levels. The analog signals of MAP and HR or MAP and RSNA were digitized every 1.4 ms and averaged every second. The data relating HR or RSNA to the increase or decrease in MAP were subjected to a logistic function using an equation based on the mathematical model described by Kent et al., where P1 is the range of HR or RSNA (maximum value−minimum value), P2 is the slope coefficient, P3 is MAP at the midrange of the curve, and P4 is the minimum HR or RSNA. Baroreflex sensitivity was defined as the maximum gain of the curve, which was calculated as \(-P1\times P2\times 0.25\).

Data Analysis
Data are presented as the mean±SEM. Statistical analysis was performed by first determining overall significance with ANOVA. Significance within the group was determined post hoc with Fisher’s least-significant difference test. Comparisons between the 2 groups were analyzed by paired or unpaired t test. P<0.05 was considered statistically significant.

Results

Protocol 1: Effects of ZnDPBG on MAP, HR, and RSNA in Intact Rats
Original recordings of AP, HR, and RSNA responses to ZnDPBG in an intact rat are shown in Figure 1. ZnDPBG caused increases in systolic and diastolic AP and RSNA but not in HR. The averaged values of these parameters before and after injection of ZnDPBG from this protocol are shown in Figure 2A. The mean values for 30 minutes
of the control period and last 30 minutes of the experimental period are also shown in Table 1. When ZnDPBG was injected intraperitoneally, MAP, HR, and RSNA increased transiently. After the transient increases, MAP and RSNA increased gradually and reached a steady state within 60 minutes after the injection of ZnDPBG, but HR did not change significantly. Vehicle treatment also induced transient increases in MAP, HR, and RSNA but no further effect on these parameters.

Protocol 2: Effects of ZnDPBG on MAP, HR, and RSNA in Atropine-Treated Rats

The control values of MAP in intact and atropine-treated rats were not significantly different. There were also no significant differences in MAP and RSNA responses to ZnDPBG between intact and atropine-treated rats. HR during the control period in atropine-treated rats was significantly higher than that in intact rats. ZnDPBG caused no significant effect on HR in atropine-treated rats (Figure 2B).

Protocol 3: Effects of ZnDPBG on MAP, HR, and RSNA in SAD Rats

Figure 2C shows the averaged values of MAP, HR, and RSNA before and after the injection of ZnDPBG in SAD rats. When ZnDPBG was injected, transient increases followed by gradual increases in MAP, HR, and RSNA were observed in SAD rats.

Protocol 4: Effects of ZnDPBG on the Relationship Between MAP and HR or RSNA

The baroreflex curve for HR tended to be shifted to the right by ZnDPBG (Figure 3 and Table 2). The midpoint of the reflex curve (P3) tended to increase, but the response range (P1), slope of the curve (P2), minimum response (P4), or maximum gain did not change significantly after ZnDPBG administration. On the other hand, ZnDBPG shifted the baroreflex curve for RSNA toward the right and upward (Figure 3 and Table 2), which was characterized by significant increases in the midpoint of the curve (P3) from 92.0±2.5 to 103.9±3.1 mm Hg, maximum response (P1+P4) from 231.6±11.0% to 377.6±47.0%, and a minimum response (P4) from 17.0±7.35 to 99.8±23.4%. ZnDPBG induced no significant changes in either the response range (P1) or maximum gain.

Discussion

The major findings in the present study are: (1) inhibition of HO activity with ZnDPBG induces sustained increases in MAP and RSNA without significant HR change in conscious intact and atropine-treated rats; (2) ZnDPBG caused significant increases in MAP, HR, and RSNA in SAD rats; and (3) ZnDPBG shifted the baroreflex curve for RSNA upward and to the right with significant increases in the maximum response, midpoint of the reflex, and minimum response but induced no significant changes in the slope of the curve or maximum gain.
In intact and SAD rats, the MAP or HR responses to ZnDPBG administration are in accord with previous reports that an HO inhibitor induced a sustained increase in MAP without bradycardia.9,10 Because this pressor response was blocked by pretreatment of α1-adrenoceptor blockade with prazosin or ganglionic blockade with chlorisondamine, it has been suggested that the increase in MAP after ZnDPBG treatment was attributable to an activation of the autonomic nervous system.9 However, a ZnDPBG-induced RSNA increase had not been described previously. The present study provides direct evidence, for the first time, of RSNA increase in response to ZnDPBG treatment in conscious rats and also demonstrates that the time course of the MAP increase is in parallel to the RNSA increase. A previous study reported that HO-2 was expressed in carotid bodies and that inhibition of HO activity augmented the sensory discharge.15 It is not likely that the increase in RNSA was mediated via its action on the carotid bodies, because the ZnDPBG increased RNSA in SAD rats, where the afferent input from peripheral chemoreceptors is abolished. These findings suggest that ZnDPBG crosses the blood–brain barrier and directly stimulates the sympathetic nervous pathway.

### TABLE 2. Effect of ZnDPBG on Arterial Baroreflex Control of HR or RSNA in Intact Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>ZnDPBG</th>
</tr>
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<tbody>
<tr>
<td><strong>MAP-HR reflex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1, bpm</td>
<td>244.9±20.4</td>
<td>256.3±29.6</td>
</tr>
<tr>
<td>P2</td>
<td>0.079±0.010</td>
<td>0.090±0.011</td>
</tr>
<tr>
<td>P3, mm Hg</td>
<td>104.5±4.2</td>
<td>115.4±3.1</td>
</tr>
<tr>
<td>P4, bpm</td>
<td>222.3±7.3</td>
<td>227.6±13.7</td>
</tr>
<tr>
<td>Maximum gain, bpm/mm Hg</td>
<td>−4.8±0.6</td>
<td>−5.5±0.4</td>
</tr>
<tr>
<td><strong>MAP-RSNA reflex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1, %</td>
<td>214.6±16.0</td>
<td>277.8±24.1*</td>
</tr>
<tr>
<td>P2</td>
<td>0.122±0.008</td>
<td>0.132±0.025</td>
</tr>
<tr>
<td>P3, mm Hg</td>
<td>92.0±2.5</td>
<td>103.9±3.1*</td>
</tr>
<tr>
<td>P4, %</td>
<td>17.0±7.3</td>
<td>99.8±23.4*</td>
</tr>
<tr>
<td>Maximum gain, %/mm Hg</td>
<td>−6.6±0.6</td>
<td>−9.1±1.9</td>
</tr>
</tbody>
</table>

Values are mean±SEM. P1 indicates range of HR or RSNA; P2, slope of reflex response; P3, MAP at midrange of the curve; P4, minimum RSNA.

*Significantly different (P<0.05) from control.

To our knowledge, the effect of ZnDPBG on baroreflex control of sympathetic nerve activity has not been studied before. In the present study, the most marked effect of ZnDPBG on the baroreflex control of RSNA was an increase in maximum response. This indicates that ZnDPBG caused an increase in the amount of sympathetic output to the peripheral organs. This result supports the previous notion that ZnDPBG inhibits brain HO activity when administered intraperitoneally.16 and the ZnDPBG induced pressor response may emanate from a primary effect of the inhibition of CO production in the CNS.9 However, the present study does not provide direct evidence for the sites of the action of the HO inhibition. Further experiments are needed to delineate the complex interaction between HO and the sympathetic nervous systems.

ZnDPBG induced marked increases in the maximum and minimum responses of RSNA in the baroreflex curve but did not significantly change those of HR. There may be a number of possible explanations for the difference between the baroreflex control of HR and RSNA. First, it has been reported that there is a dissociation of baroreflex control of HR and RSNA during hypertension. For example, Barrett et al.17 showed that angiotensin II–induced hypertension resulted in a reduction in the maximum response in RSNA but did not make a significant change in the maximum response in HR. Vitela et al.18 reported that the sensitivity of baroreflex control of RSNA was unchanged but that of HR was attenuated in hypertensive rats. They suggested that this dissociation might be the result of alterations within the central pathways regulating RSNA and HR or the result of changes in the descending modulation of the medullary neurons in the baroreflex circuit. Second, ZnDPBG might regulate HR by modulating β-adrenoceptor activity in the heart. Under control conditions, the expression of HO-2 in rat atria has been demonstrated.19 In addition, it has been reported that inhibition of HO activity reduced dilator responses of cerebral arteries to a β-adrenoceptor agonist, isoproterenol,20 suggesting that ZnDPBG inhibits β-adrenoceptor function. It is also known that β-adrenoceptor blockade has relatively little effect on the normal heart of an individual at rest but a profound effect when the sympathetic nervous system is activated.21 Furthermore, sympahtoexcitation exerts a positive chronotropic effect mainly by activating β-adrenoceptors.
in the sino-atrial (SA) node. Thus, it is reasonable to speculate the positive chronotropic effect because of sympathetic excitation might be abolished by direct inhibition of β-adrenoceptor activity by ZnDPBG in the SA node. Third, it is generally considered that the SA node is densely innervated by postganglionic parasympathetic neurons, and parasympathetic tone predominates over sympathetic tone in HR regulation. Therefore, it seems possible that the positive chronotropic effect of sympathetic excitation does not directly reflect HR responses during the post-ZnDPBG period, when there is no difference in parasympathetic tone between the control and post-ZnDPBG period.

In intact rats, ZnDPBG increased RSNA but did not induce a significant change in HR. ZnDPBG may simultaneously activate the sympathetic and parasympathetic nervous system. If so, parasympathetic blockade might have induced an HR increase in response to ZnDPBG administration in atropine-treated rats, but ZnDPBD caused no such change in these animals. This result indicates that the HR response was independent of both sympathetic and parasympathetic nervous activities. On the other hand, ZnDPBG produced an increase in HR in rats with baroreceptor denervation (SAD), suggesting that the mechanism of HR increase involves arterial baroreflex control of HR. Acute resetting of the arterial baroreceptor occurs within seconds to minutes of a change in AP and stabilizes within 5 to 15 minutes after the pressure change. 

In this study, the baroreflex curve for HR tended to shift to the higher MAP level. This shift might result in an attenuation of the change in the resting HR during a significant increase in the resting MAP after ZnDPBG administration. It is also possible that ZnDPBG inhibited β-adrenergic function, as stated above. Taken together, the attenuation of HR change by baroreflex resetting in response to AP change and the negative chronotropic effect of β-adrenoceptor blockade might result in the absence of change in HR when ZnDPBG induced an increase in RSNA in intact rats. Further study is necessary to elucidate the underlying mechanisms of ZnDPBG in relationship to HR.

Perspectives

There are numerous studies that other neurotransmitters, such as NO or angiotensin II, are involved in in the central regulation of sympathetic outflow. However, the effects of NOS inhibition or angiotensin II receptor blockade in these studies are conflicting. The important factors that affect these results are acute surgical stress and the use of anesthetics. As far as inhibition of HO activity is concerned, it has been suggested that postsurgical stress may influence the pressor mechanism involved in the regulation of AP. A major strength of the present experiment lay in maintaining the high quality of the nerve recordings for ≥4 days after the surgery so that it was possible to demonstrate sympathoexcitation by the systemic administration of an HO activity inhibitor.

In summary, the present study demonstrates that ZnDPBG, an inhibitor of HO activity, increased in MAP and RSNA but did not affect HR in intact and atropine-treated rats. On the other hand, ZnDPBG caused increases in MAP, HR, and RSNA in SAD rats. ZnDPBG caused a marked increase in the maximum and minimum responses of the MAP–RSNA curve. Accordingly, we conclude that inhibition of CO production within the CNS caused an increase in AP because of sympathoexcitation in conscious rats. This would suggest that the central HO/CO system plays an important role in the central regulation of AP by modulating sympathetic nerve activity.

Disclosures

None.

References


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