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Carbon monoxide and metabotropic glutamate receptors in rat nucleus tractus solitarii: participation in cardiovascular effect

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Abstract

Carbon monoxide (CO) has been identified as an endogenous biological messenger in the brain. Heme oxygenase catalyzes the metabolism of heme to biliverdin and CO. Recent studies have demonstrated that CO is involved in central cardiovascular regulation and modulates the baroreflex in the nucleus tractus solitarii of rats. The purpose of the present study was to investigate the possible interaction of CO and excitatory amino acids in the nucleus tractus solitarii. In anesthetized male Sprague–Dawley rats, unilateral intranucleus tractus solitarii microinjection of hematin, a heme molecule cleaved by heme oxygenase to yield CO, or excitatory amino acids L-glutamate produced depressor and bradycardiac effects. Similar cardiovascular effects were observed with several agonists for ionotropic glutamate receptors such as *N*-methyl-D-aspartate (NMDA), (±)-α-amino-3-hydroxyl-5-methylisoxazole-4-propanoic acid (AMPA), kainic acid and for metabotropic glutamate (mGlu) receptors, *trans*-(±)-1-amino-(1*S*,3*R*)-cyclopentanedicarboxylic acid (ACPD). Among these agonists, prior administration of the heme oxygenase inhibitor, zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) (1 nmol), significantly attenuated the cardiovascular effects of hematin, L-glutamate and ACPD. Furthermore, the cardiovascular effects of ACPD were prevented by the selective mGlu receptors antagonist L-2-amino-3-phosphonoprionate (L-AP3). However, pretreatment with ZnDPBG failed to prevent the cardiovascular responses to microinjection of NMDA, AMPA and kainic acid. On the other hand, prior administration of the NMDA receptor antagonist, diazocilpine (MK-801), or (±)-2-amino-5-phosphonopentanoic acid (APV) attenuated the depressor and bradycardiac effect of hematin. These results demonstrated that mGlu receptors may couple to the activation of heme oxygenase via the liberation of CO to participate in central cardiovascular regulation. They also suggested that CO and excitatory amino acids may interact in the nucleus tractus solitarii of rats.

Keywords: Carbon monoxide; Heme oxygenase; Glutamate receptor, Metabotropic; Nucleus tractus solitarii

1. Introduction

A new neuromodulatory agent, the gaseous compound carbon monoxide (CO), has been shown to play a role as neurotransmitter or neuromodulator (Dawson and Snyder, 1994). In animals, the predominant source of CO generation is from heme degradation. Heme oxygenase is the rate-limiting enzyme responsible for the catabolism of heme and subsequent production of CO and bilirubin. Two forms of heme oxygenase have been identified. Heme oxygenase-1, induced by heme and numerous oxidative stressors, is enriched in spleen and liver. In contrast, heme oxygenase-2 is present abundantly in the brain and testis as a constitutive enzyme (Maines, 1988). Studies have suggested that CO arising from heme via metabolism by heme oxygenase stimulates soluble guanylate cyclase activity and promotes an increase in cGMP in neural and cardiovascular tissues (Maines, 1993; Morita et al., 1995). Zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) appeared to be a very potent inhibitor of both forms of heme oxygenase, and was shown to inhibit almost completely human liver heme oxygenase at concentrations as lower as 0.5 μm (Chernick et al., 1989). It has also been shown to cause a lowering of blood pressure in hypertensive rats, via the heme oxygenase-mediated formation of CO (Johnson et al., 1996). These results implicate the heme oxygenase–CO system as a

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potential regulator of various neural (Maines, 1993; Verma et al., 1993) and cardiovascular functions (Maines, 1993; Morita et al., 1995; Ewing et al., 1994).

Heme oxygenase is widely expressed in the brain and is responsible for the impressive CO-generating ability of the brain, including brainstem (Maines, 1993; Ewing and Maines, 1992). In the central nervous system, the nucleus of the solitary tract is the site where afferent fibers arising from arterial baroreceptor, chemoreceptors, cardiopulmonary receptors and other visceral receptors make the first central synapses (Spyer, 1981), and thus play an important role in the integration of autonomic control of the cardiovascular system (Reis, 1984). It has recently been pointed out that CO formed within the nucleus tractus solitarii subserves a vasodepressor mechanism that is tonically active in awake rats (Johnson et al., 1997). We have reported that unilateral microinjection of hematin, a heme molecule cleaved by heme oxygenase to yield CO, into the nucleus tractus solitarii produce dose-related depressor and bradycardiac effects (Lo et al., 2000). On the other hand, systemic administration (Johnson et al., 1997) or direct microinjection into the nucleus tractus solitarii (Lo et al., 2000) of heme oxygenase inhibitor, zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG), attenuates the baroreceptor reflex. Taken together, these findings suggest that CO within the nucleus tractus solitarii may play an important role in the regulation of cardiovascular function.

The excitatory amino acid, glutamate, is the principal transmitter of vagal projections to the nucleus tractus solitarii that mediates the afferent arm of the baroreceptor reflex (Leone and Gordon, 1989; Meeley et al., 1989). Glutamate acts on two main classes of receptor, i.e., (1) ionotropic glutamate receptors, which can be distinguished pharmacologically by their selective activation by *N*-methyl-D-aspartate (NMDA), (±)-α-amino-3-hydroxyl-5-methylisoxazole-4-propanoic acid (AMPA) and kainic acid, and (2) metabotropic glutamate (mGlu) receptors, which can be selectively activated by *trans*-(±)-1-amino-(1*S*,3*R*)-cyclopentanedicarboxylic acid (ACPD) (Sugiyama et al., 1989; Tanabe et al., 1992). The mGlu receptors are widely distributed in the central nervous system. A number of signal transduction pathways linked to mGlu receptor activation have been identified (Cartmell et al., 1992; Charpak et al., 1990; Harvey and Collingridge, 1993). Nevertheless, the mechanisms underlying mGlu receptors effects remain to be elucidated. A recent study has provided evidence that a heme oxygenase product, such as CO, may affect nucleus tractus solitarii glutamatergic neurotransmission and thus to participate in cardiovascular control in conscious animals (Silva et al., 1999). However, it remains unclear as to which glutamate receptor subtypes(s) are involved. In addition, several lines of evidence reported that blocking of CO production with zinc protoporphyrin IX, a heme oxygenase inhibitor, prevents the induction of long-term potentiation in hippocampal slices (Stevens and Wang, 1993; Zhuo et al., 1993). Moreover, CO plays a pivotal role in the glutamate release in synapse, and may be a retrograde messenger in long-term potentiation (Shinomura et al., 1994). Based on these various findings, it appears that CO and excitatory amino acids might interact in the nucleus tractus solitarii.

The aim of the present study was to provide pharmacological evidence as to whether CO and excitatory amino acids interact with each other in the nucleus tractus solitarii. In addition, the role of mGlu receptors in central cardiovascular regulation by CO was examined.

2. Materials and methods

2.1. Experiment procedures

Male Sprague–Dawley rats (250–350 g) were housed in the animal room of Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan, ROC). The rats were anesthetized with urethane (1.0 g/kg i.p. and 300 mg/kg i.v. if necessary). A polyethylene cannula was inserted into the femoral vein for administration of drugs, and blood pressure was measured directly via a cannula placed into the femoral artery and connected to a pressure transducer (P23 ID; Gould) and a polygraph (AT5000; Gould). Heart rate was monitored continuously with a tachograph preamplifier (13-4615-65; Gould). Tracheostomy was performed to preserve airway patency during the experiment.

The animals were then placed in a stereotaxic instrument (Kopf) with the head downward at a 45° angle. The dorsal surface of the medulla was exposed via limited craniotomy, and the animals were allowed to rest for at least 1 h before experiments. For microinjections into the nucleus tractus solitarii, single-barrel glass pipettes with external tip diameters of 40 μm were prepared (0.031-in. OD, 0.006-in. ID; Richland Glass, New Jersey). The pipette was fixed on the stereotaxic holder and connected to a Hamilton microsyringe through polyvinyl tubing.

For functional identification of the nucleus tractus solitarii, pipettes were filled with the lower dose of L-glutamate (0.154 nmol/60 nl) for injection into the nucleus tractus solitarii with anteroposterior coordinates 0.0 mm, mediolateral, 0.5 mm, and vertical, 0.4 mm with the obex used as reference (Tseng et al., 1996; Lo et al., 1997). Injections were given over 10 s by air pressure generated by a hand-held syringe while the pipette tip was positioned in the nucleus tractus solitarii. A specific decrease in blood pressure and heart rate ($\geqq -30$ mm Hg and -50 bpm) was demonstrated after microinjection of L-glutamate in the nucleus tractus solitarii. The response was restricted to the intermediate third of the nucleus tractus solitarii, and administration of the same dose of L-glutamate in areas adjacent to the nucleus tractus solitarii failed to elicit the response. In this study, each injection volume in the nucleus tractus solitarii was restricted to 60 nl and microinjections were limited to six to eight times in a rat. To replace drugs in microinjection pipettes, the single-barrel pipette was lifted and then washed with distilled water three times. It was then

filled with the next drug and reinserted into an identical position in the nucleus tractus solitarii according to the coordinates formerly determined with L-glutamate.

To investigate the effect of pre-administration of the heme oxygenase inhibitor, ZnDPBG, on cardiovascular responses to L-glutamate and hematin in the nucleus tractus solitarii, different groups of animals were first injected with L-glutamate (2.3 nmol) or hematin (1 nmol) into the unilateral nucleus tractus solitarii. The rats were then allowed to rest for at least 30 min until the mean blood pressure and heart rate had returned to basal levels. After this, the changes in mean blood pressure and heart rate were observed on microinjection of the same doses of L-glutamate or hematin 10 min after intranucleus tractus solitarii administration with ZnDPBG (1 nmol) or vehicle.

In additional experiments, to evaluate which class of glutamate receptors link to heme oxygenase, we tested the cardiovascular effects of several agonists for ionotropic glutamate receptors and mGlu receptors such as NMDA (0.01 nmol), AMPA (1 pmol), kainic acid (0.1 pmol) and ACPD (0.01 nmol) in different groups of animals. The mean blood pressure and heart rate were monitored before and after unilateral microinjections of ZnDPBG (1 nmol) or vehicle in the nucleus tractus solitarii. The cardiovascular action of these agonists was observed 10–90 min after microinjection of ZnDPBG. We also examined the influence of the mGlu receptors antagonist, L-2-amino-3-phosphonoprionate (L-AP3) (1 nmol), on the cardiovascular effects of ACPD. Furthermore, experiments were designed to investigate whether cardiovascular effects of hematin in the nucleus tractus solitarii were affected by pretreatment with a NMDA receptor antagonist. A similar experimental procedure was used to study the effects of pretreatment with

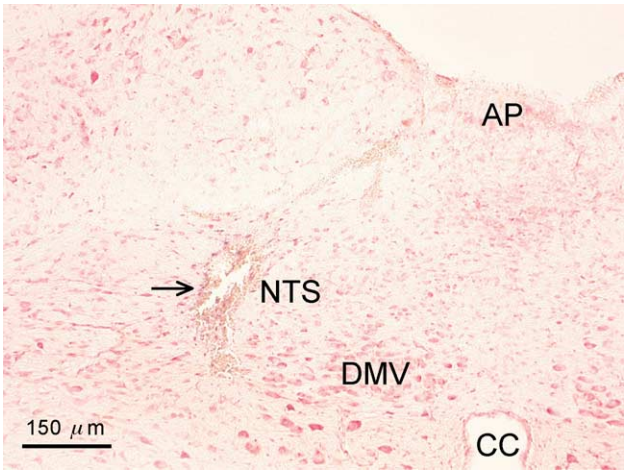

Fig. 1. Histological section showing the injection site in the brainstem nucleus of rats in coronal section. Shown are coronal sections 14.08 mm caudal to the bregma. Arrowheads indicate the site of injection in the nucleus tractus solitarii (NTS). Maps and coordinates (from bregma) are taken from the atlas of Paxinos and Watson, 1986. Scale bar: 150 μm. AP indicates area postrema; DMV, dorsal motor nucleus of vagus; CC, central canal.

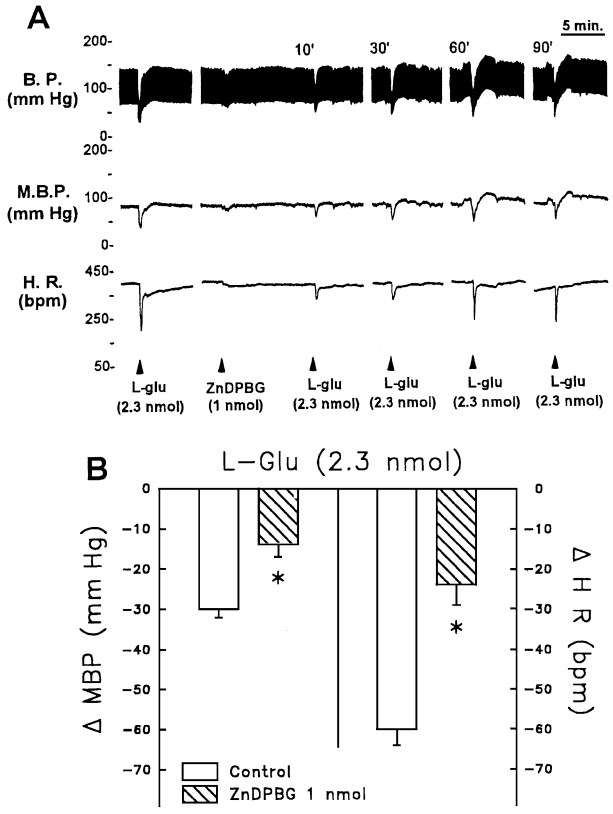

Fig. 2. (A) Cardiovascular effects of unilateral injection of L-glutamate (L-glu, 2.3 nmol) into the nucleus tractus solitarii before and after ZnDPBG (1 nmol) in anesthetized rats. L-Glutamate and ZnDPBG were injected at the indicated time points. Blood pressure (BP), mean blood pressure (MBP) and heart rate (HR) recordings were made at a paper speed of 3 mm/min. Horizontal bar represents recording during 5-min intervals. (B) Comparative MBP and HR effects of L-glutamate (2.3 nmol) by ZnDPBG (1 nmol) on unilateral intranucleus tractus solitarii administration of the substances. L-Glutamate was injected after the vehicle (control) or after ZnDPBG (1 nmol). Vertical bars represent S.E.M. change from baseline values, which were 93 ± 4 mm Hg for MBP and 392 ± 5 bpm for HR. Each bar represents the average data from 12 rats. *$P < 0.05$, compared with control value.

diazocilpine (MK-801) (0.1–1 nmol) or (±)-2-amino-5-phosphonopentanoic acid (APV) (1 nmol) on hematin (1 nmol) in the nucleus tractus solitarii of rat.

After completion of experiments, 60 nl of sky blue was injected through the cannula and the rats were perfused intracardially with saline followed sequentially by a solution of 4% formaldehyde and 30% sucrose solution. Sections of 40 μm of the brainstem were stained with cresyl violet, and proper placement of the pipette tip in the nucleus tractus solitarii was verified by histological sections under the microscope. The injection sites in the nucleus tractus solitarii are presented in Fig. 1.

2.2. Materials

Experimental drugs such as urethane, L-glutamate, hematin, NMDA, ACPD, L-AP3 and MK-801 were purchased from Sigma; AMPA, kainic acid and APV were purchased

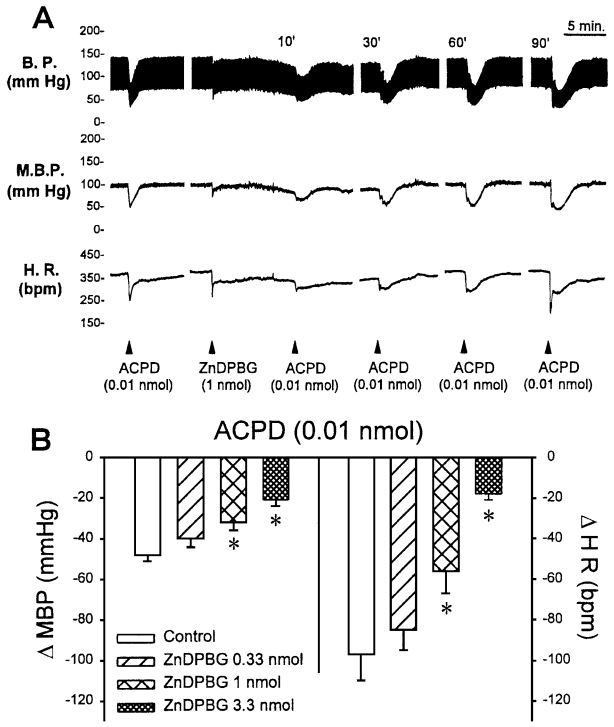

Fig. 3. (A) Cardiovascular effects of unilateral injection of ACPD (0.01 nmol) into the nucleus tractus solitarii before and after ZnDPBG (1 nmol) in anesthetized rats. ACPD and ZnDPBG were injected at the indicated time points. Blood pressure (BP), mean blood pressure (MBP) and heart rate (HR) recordings were made at a paper speed of 3 mm/min. Horizontal bar represents recording during 5-min intervals. (B) Comparative MBP and HR effects of ACPD (0.01 nmol) by different doses of ZnDPBG (0.33–3.3 nmol) on unilateral intranucleus tractus solitarii administration of the substances. ACPD was injected after the vehicle (control) or after ZnDPBG. Vertical bars represent S.E.M. change from baseline values, which were 105 ± 4 mm Hg for MBP and 367 ± 5 bpm for HR. Each bar represents the average data from 10 rats. *$P<0.05$, compared with control value.

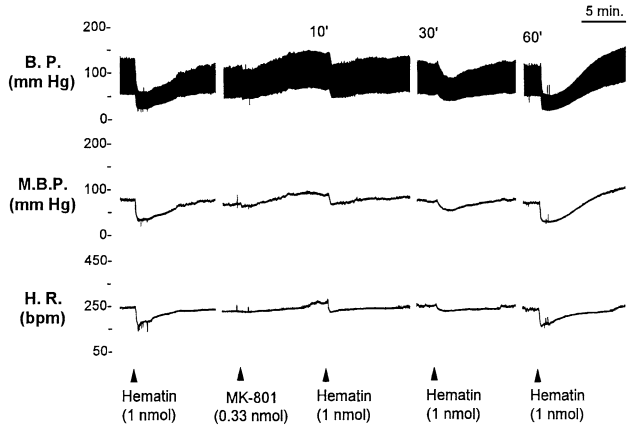

Fig. 4. Cardiovascular effects of unilateral injection of hematin (1 nmol) into the nucleus tractus solitarii before and after MK-801 (0.33 nmol) in anesthetized rats. Hematin and MK-801 were injected at the indicated time points. Blood pressure (BP), mean blood pressure (MBP) and heart rate (HR) recordings were made at a paper speed of 3 mm/min. Horizontal bar represents recording during 5-min intervals.

from RBI. ZnDPBG was obtained from Prophyrin Products. ZnDPBG was dissolved in 50 mmol/l Na_2CO_3 (pH 8.8–9.4) immediately before use. Hematin was dissolved in 30% 0.1 N NaOH (pH 8.6– 9). All other drugs were dissolved in normal saline on the day of the experiment.

2.3. Statistical analysis

A paired *t*-test (before and after intranucleus tractus solitarii microinjection) and unpaired *t*-test (for control and study group comparisons) or repeated-measures analysis of variance (ANOVA) followed by Dunnett's test for significant differences were used. Differences with $P<0.05$ were considered significant. All data are presented as means ± S.E.M.

3. Results

In agreement with our previous findings, intranucleus tractus solitarii microinjection of hematin (1 nmol) resulted in hypotension and bradycardia (Lo et al., 2000). The administration of vehicle (30% 0.1 N NaOH) itself elicit only slight cardiovascular effects, and the pattern of response was different from that to hematin. After pretreatment with a heme oxygenase inhibitor, ZnDPBG (1 nmol), for 10 min, the depressor and bradycardiac responses to hematin were attenuated significantly (from − 50 ± 4 mm Hg and − 96 ± 11 bpm to − 21 ± 3 mm Hg and − 30 ± 7 bpm, respectively; $P<0.05$, paired *t*-test). Pretreatment with the vehicle of ZnDPBG did not affect the cardiovascular responses to hematin. (from − 48 ± 4 mm Hg and − 92 ± 12 bpm to − 40 ± 5 mm Hg and − 81 ± 9 bpm, respectively; $P>0.05$, paired *t*-test). Unilateral microinjection of L-glutamate (2.3 nmol) into the nucleus tractus solitarii produced remarkable depressor and bradycardiac effects (Fig. 2A). Pretreatment

Table 1
Cardiovascular effects of NMDA (0.01 nmol), AMPA (1 pmol), kainic acid (0.1 pmol) and ACPD (0.1 nmol) on prior intranucleus tractus solitarii administration of ZnDPBG (1 and 3.3 nmol)

	Control		ZnDPBG (1 nmol)		Control		ZnDPBG (3.3 nmol)	
	ΔMBP (mm Hg)	ΔHR (bpm)	ΔMBP (mm Hg)	ΔHR (bpm)	ΔMBP (mm Hg)	ΔHR (bpm)	ΔMBP (mm Hg)	ΔHR (bpm)
NMDA	− 43 ± 3	−108± 7	− 43 ± 6	−117± 10	− 48 ± 2	− 98 ± 11	− 51 ± 3	− 85 ± 10
AMPA	− 51 ± 3	− 95 ± 9	− 50 ± 3	− 90 ± 11	− 49 ± 2	− 98 ± 12	− 46 ± 5	− 87 ± 13
Kainic acid	− 35 ± 3	− 61 ± 7	− 36 ± 4	− 60 ± 5	− 37 ± 2	− 59 ± 6	− 37 ± 4	− 48 ± 5
ACPD	− 48 ± 2	− 97 ± 13	− 32 ± 4[a]	− 56 ± 11[a]	− 47 ± 3	− 97 ± 12	− 21 ± 3[a]	− 18 ± 3[a]

[a] Significantly different from corresponding control response ($n=10$), $P<0.05$.

with ZnDPBG (1 nmol) for 10 min attenuated significantly the cardiovascular responses to L-glutamate (from -30 ± 2 mm Hg and -60 ± 4 bpm to -13 ± 3 mm Hg and -24 ± 5 bpm, respectively; $P<0.05$, paired t-test) (Fig. 2B). However, prior administration of the vehicle of ZnDPBG into the nucleus tractus solitarii did not modify the cardiovascular effects of L-glutamate (from -29 ± 3 mm Hg and -65 ± 5 bpm to -26 ± 3 mm Hg and -60 ± 4 bpm, respectively; $P>0.05$, paired t-test).

In addition, to evaluate which class of glutamate receptors link to heme oxygenase, we tested the cardiovascular effects of several agonists for ionotropic glutamate receptors and mGlu receptors such as NMDA (0.01 nmol), AMPA (1 pmol), kainic acid (0.1 pmol) and ACPD (0.01 nmol) in different groups of rats. Microinjection of these agonists into the nucleus tractus solitarii all produced prominent depressor and bradycardiac effects. Pretreatment with increasing doses of ZnDPBG (0.33–3.3 nmol), dose-dependently attenuated the cardiovascular effects produced by microinjection of ACPD into the nucleus tractus solitarii (Fig. 3A,B). In this experiment, the ACPD-mediated depressor and bradycardiac effect was prevented by the relatively selective antagonist of the mGlu receptors, L-AP3 (1 nmol) (from -37 ± 3 mm Hg and -70 ± 7 bpm to -22 ± 2 mm Hg and -33 ± 4 bpm, respectively; $P<0.05$, paired t-test). However, prior administration of ZnDPBG (1–3.3 nmol) did not affect the cardiovascular responses to NMDA, AMPA and kainic acid (Table 1).

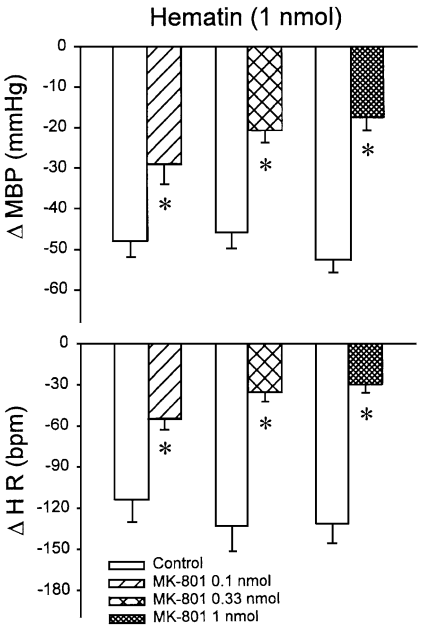

Fig. 5. Comparative mean blood pressure (MBP) and heart rate (HR) effects of hematin (1 nmol) by MK-801 on unilateral intranucleus tractus solitarii administration of the substances. Hematin was injected after the vehicle (control) or after MK-801. Vertical bars represent S.E.M. change from baseline values, which were 97 ± 5, 92 ± 4 and 102 ± 5 mm Hg for MBP, and 275 ± 6, 253 ± 5 and 289 ± 5 bpm for HR. Each bar represents the average data from 12 rats. *$P<0.05$, compared with control value.

To investigate whether the interaction between NMDA receptors and CO exists in the nucleus tractus solitarii, we examined the effects of NMDA receptor antagonists, MK-801 and APV, on hematin. After pretreatment with different doses of MK-801 (0.1–1 nmol) for 10 min, the depressor and bradycardiac responses to hematin were attenuated significantly and dose-dependent attenuation effects were observed (Figs. 4 and 5). Moreover, prior administration of APV (1 nmol) also attenuated the cardiovascular effects of hematin (from -49 ± 3 mm Hg and -105 ± 8 bpm to -34 ± 3 mm Hg and -47 ± 6 bpm, respectively; $P<0.05$, paired t-test).

The attenuated cardiovascular effects of heme oxygenase inhibitor and NMDA receptor antagonists on L-glutamate, ACPD and hematin had recovered 60–90 min after injection of the antagonists (Figs. 2–4).

4. Discussion

The CO system and excitatory amino acids seem to have interrelated effects in the regulation of cardiovascular responses. In this study, we demonstrated that microinjection of hematin, a substrate for CO production, into the nucleus tractus solitarii induced depressor and bradycardiac effects. These effects of hematin require heme oxygenase because prior administration of the heme oxygenase inhibitor, ZnDPBG, significantly suppressed the cardiovascular effects of hematin. Microinjection of L-glutamate into the nucleus tractus solitarii also produced depressor and bradycardiac effects. These results were similar to those of previous studies which suggested that the excitatory amino acid, L-glutamate, and CO may be potential neurotransmitters of baroreceptor information in the rat nucleus tractus solitarii (Lo et al., 2000; Talman et al., 1980). In addition, prior administration of ZnDPBG significantly attenuated the cardiovascular effects of intranucleus tractus solitarii microinjection of L-glutamate. Such findings are consistent with the possibility that the function of heme oxygenase may be linked with L-glutamate neurotransmission and its effects on blood pressure (Silva et al., 1999). Thus, these observations might suggest that activation of one of the multiple glutamate receptors by L-glutamate is mediated through heme oxygenase activation which produces CO in the nucleus tractus solitarii of rats. During the experiment, we noticed that microinjection of ZnDPBG into the NTS produced a decrease in arterial pressure and heart rate, which was opposite to the findings in conscious rats (Johnson et al., 1997; Silva et al., 1999). We assumed that the anesthetics we used during the study might have produced this difference.

Results of various studies have suggested that CO stimulates soluble guanylate cyclase activity and promotes elevation of cGMP in neural and cardiovascular tissues (Maines, 1993; Verma et al., 1993; Ewing et al., 1994; Ewing and Maines, 1992). These results have implicated the

heme oxygenase–CO system as a potential regulator of various neural (Maines, 1993; Verma et al., 1993) and cardiovascular functions (Morita et al., 1995; Ewing et al., 1994). Previous observations showed that CO formed by brain heme oxygenase plays a significant role in central cardiovascular regulation (Johnson et al., 1997; Glaum and Miller, 1993), and we also reported that inhibition of heme oxygenase attenuated baroreflex activation (Lo et al., 2000). Furthermore, it has been suggested that heme oxygenase may be involved with the cardiovascular effects of L-glutamate in the nucleus tractus solitarii (Silva et al., 1999). However, which glutamate receptor subtype(s) is/are involved in the stimulation of CO synthesis in the nucleus tractus solitarii by glutamate or other neurotransmitter receptor agonists remains to be demonstrated.

The excitatory amino acid, glutamate, is the main transmitter of vagal projections to the nucleus tractus solitarii, mediating the afferent arm of the baroreceptor reflex (Leone and Gordon, 1989; Meeley et al., 1989). Recently, glutamate receptors have been classified into ionotropic glutamate receptors, such as NMDA, AMPA and kainic acid subtypes, and mGlu receptors which are coupled with GTP-binding proteins such as G_i and G_q and can be selectively activated by (1*S*,3*R*)-ACPD (Hollmann and Heinemann, 1994). Heme oxygenase inhibitor has been reported to block the effects of mGlu receptor activation, and CO is thought to be primarily responsible for mediating glutamate action at metabotropic receptors (Glaum and Miller, 1993). In the present study, we investigated the possibility that the diffusible second messenger, CO, which might be coupled to mGlu receptors activation to modulate the central cardiovascular effects. Consistent with this idea, microinjection of the mGlu receptor agonist, ACPD, into the nucleus tractus solitarii produced depressor and bradycardiac effects. These cardiovascular effects of ACPD were strongly blocked by ZnDPBG in a dose-dependent manner. Similarly, pretreatment with the relatively selective antagonist of mGlu receptors, L-AP3, reduced the cardiovascular responses to intranucleus tractus solitarii administration of ACPD. Moreover, microinjection of the other excitatory amino acids NMDA, AMPA and kainic acid, into the nucleus tractus solitarii, also produced depressor and bradycardiac effects. Nevertheless, prior administration of the same or greater doses of ZnDPBG did not affect these cardiovascular effects of NMDA, AMPA and kainic acid. In the present study, ZnDPBG attenuated the cardiovascular effects of L-glutamate, and ACPD are relatively specific and provides evidence that heme oxygenase might be activated by stimulation of mGlu receptors to produce CO to participate in central cardiovascular regulation.

CO could well function as both an intracellular and an intercellular messenger, because CO is a readily diffusible gas and it is produced by the heme oxygenase system in every cell. Zhuo et al. (1993) examined a possible role of CO in long-term potentiation. Zinc protoporphyrin IX (ZnPP), which inhibits heme oxygenase, blocked the induction of long-term potentiation in a dose-dependent manner. Long-term potentiation is a sustained increase in synaptic efficacy that is thought to contribute to certain forms of learning in mammals (Hawkins et al., 1993). In the CA1 region of the hippocampus, the induction of long-term potentiation generally requires Ca^{2+} influx through postsynaptic NMDA glutamate receptor channels, maintenance of long-term potentiation apparently involves in part a presynaptic increase in transmitter release, implying that the postsynaptic cell must send one or more retrograde messages to the presynaptic terminals (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991). There is evidence to indicate that several molecules may act as such retrograde messengers during long-term potentiation in the hippocampus, including the soluble gases nitric oxide (Zhuo et al., 1993; O'Dell et al., 1991) and CO (Stevens and Wang, 1993; Zhuo et al., 1993). Shinomura et al. (1994) reported that the glutamate release mechanism in synaptic transmission is regulated by CO production and CO may be a retrograde messenger in long-term potentiation. It is interesting to hypothesize that a similar mechanism of CO as a retrograde messenger might be involved in the cardiovascular responses to glutamate in the nucleus tractus solitarii.

In the present study, unilateral microinjection of hematin into the nucleus tractus solitarii produced depressor and bradycardiac effects. Pretreatment with MK-801, the most potent NMDA receptor antagonist, dose-dependently attenuated the cardiovascular effects of hematin. Furthermore, the inhibitory effects also were observed with another NMDA receptor antagonist, APV. These observations might suggest that cardiovascular responses to hematin were mediated through NMDA receptors in the nucleus tractus solitarii. The mechanism is still unclear. However, it has been reported that the glutamate release mechanism in synaptic transmission is regulated by CO production (Shinomura et al., 1994). As nitric oxide is presumed to be a retrograde messenger (Bliss and Collingridge, 1993), postsynaptic CO could also be a candidate as retrograde messenger. It may regulate glutamate release from presynaptic terminals, although the location of CO synthesis in the synapse requires further investigation. Furthermore, it has been reported that CO and nitric oxide potentiate long-term potentiation, which is blocked by heme oxygenase inhibitor ZnDPBG or hemoglobin, a scavenger of nitric oxide (Zhuo et al., 1993). Long-term potentiation in the hippocampus is thought to be initiated by activation of the postsynaptic NMDA receptor and the entry of calcium, whereas the maintenance of long-term potentiation is mediated by a sustained increase in glutamate release (Bliss et al., 1986). Therefore, our finding that NMDA receptor antagonists attenuated the cardiovascular effects of CO in the nucleus tractus solitarii of rats may be explained by a retrograde effect of CO on glutamate release to modulate the central cardiovascular effects.

In conclusion, mGlu receptors may couple to the activation of heme oxygenase via the liberation of CO to participate in central cardiovascular regulation and suggested that

CO and excitatory amino acids are likely to have subtle interactions in the nucleus tractus solitarii.

Acknowledgements

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