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Carbon Monoxide as a Novel Central Pyrogenic Mediator

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Carbon monoxide (CO) are produced by heme oxygenase (HO), and HO was detected in hypothalamus. However, the roles of CO produced in hypothalamus was not fully elucidated. So, we tested the effects of CO on body temperature because preoptic-anterior hypothalamus was known as the presumptive primary fever-producing site. CO-saturated aCSF (4 μl, i.c.v.) and hemin (10 μg, i.c.v.) elicited marked febrile response. Pretreatment with indomethacin completely inhibited CO- and hemin-induced fever. Zinc protoporphyrin-IX (10 μg, i.c.v.) or ODQ (50 μg, i.c.v.) partially reduced hemin-induced febrile response. Dibutyryl-cGMP (100 μg, i.c.v.) produced profound febrile response and this febrile response was attenuated by indomethacin. These results indicate that endogenous CO may have a role as a pyrogenic mediator in CNS and CO-mediated pyresis is dependent on prostaglandin production and partially on activation of soluble guanylate cyclase.

Key words: Carbon monoxide, Pyresis, Heme oxygenase, Pyrogen

INTRODUCTION

Inflammation or administration of exogenous pyrogens (e.g., bacterial endotoxin) leads to production of endogenous pyrogenic cytokines (LeMay *et al.*, 1990; Kluger, 1991; Van Zee *et al.*, 1991; Roth *et al.*, 1993; Jansky *et al.*, 1995) and subsequent stimulation of PGs production in the central nervous system (CNS) (Cao *et al.*, 1995; 1996; Quan *et al.*, 1998; Yermakova & O'Banion, 2000). The ending step in febrile response is the action of PGE_2 in the preoptic area of the anterior hypothalamus, which is considered to be the thermoregulatory site of CNS (Coceani, 1990; Dinarello, 1990; Matsuda *et al.*, 1992). Various substances (interleukin (IL)-1β, IL-6, interferons, and tumor necrosis factor) have been suggested as being involved in the thermoregulatory pathways within the brain (Klunger, 1991 Klit *et al.*, 1994; Dinarello, 1999) but the various steps are still unknown. Of all the cytokines released within the central nervous system, interleukin-6 seems to be a critical mediator of fever generation (Chai *et al.*, 1999).

In addition to this common mechanism underlies fever, the nitric oxide (NO) (Redford *et al.*, 1995; Lin & Lin, 1996; Scammell *et al.*, 1996a; Roth *et al.*, 1998; Jung *et al.*, 2001) and carbon monoxide (CO) (Jung & Lee, 1999; Steiner *et al.*, 1999; Steiner & Branco, 2000; 2001) were proposed as another mediator in febrile responses. However, the described effects of NO on thermore-gulation and fever were not consistent in all investigated species of experimental animals (Gerstberger, 1999) and the effects of CO were not sufficiently investigated.

Endogenous CO shares with NO a role as a putative neural messenger in the brain (Verma *et al.*, 1993). Like NO, CO is believed to modulate CNS function mainly via an increase in cytoplasmic cyclic GMP (cGMP) concentrations secondary to the activation of soluble guanylate cyclase (Kharitonov *et al.*, 1995). CO is produced by heme oxygenase (HO) which catalyzes the metabolism of heme to biliverdin and iron. Three distinct isoforms of HO were have been identified : HO-1, HO-2, and HO-3. Although all isoforms were shown to be present in several areas of the rat CNS (Ewing & Maines., 1992), most of the heme catabolism in the brain is accounted for by HO-2 (Sun *et al.*, 1990). HO-2 was detected as a significant levels in hypothalamus, and although HO-1 activity in the brain is usually low, it can be induced by endotoxin (Rizzardini *et al.*, 1994; Yet *et al.*, 1997) and cytokines (Rizzardini et al., 1993; Terry *et al.*, 1998) which were known as a pyrogen. However, the roles of HO and its

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product CO were not fully understood yet. In this study, we studied the effects of exogenous and endogenous CO on body temperature to elucidate the roles of HO/CO system in thermoregualtion and got the results which differ from those of other investigators (Steiner & Branco, 2000; 2001)

MATERIALS AND METHOD

Chemicals

Hemin, zinc protoporphrine IX (ZnPP IX), dibutyryl-cGMP, 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ), and indomethacin were purchased from Sigma (U.S.A.). Hemin, and ZnPP IX were dissolved in 0.1 N NaOH and diluted in artificial CSF solution (aCSF) containing of (mM) NaCl 138, KCl 50, $NaHCO_3$ 11, KH_2PO_4 1, $CaCl_2$ 1.1, $MgCl_2$ 1, pH 7.4. ODQ was dissolved in dimethyl sulfoxide (DMSO) solution and diluted in aCSF. Indomethacin was dissolved with 4% sodium bicarbonate solution.

Surgery

Adult male Sprague-Dawley rats, weighing 250-300 g, were used and were housed individually at an ambient temperature of 22 ± 1°C with a 12 h light-dark cycle. Animal water and food *ad libitum* were allowed. After anesthetia with secobarbital sodium (30 mg/kg, i.p), a cannula (0.8 mm o.d.) was stereotaxically implanted in the lateral ventricle (P: 0.9 mm, L: 1.5 mm, V: 3.5 mm) for intracerebroventricular (i.c.v.) injection of drugs, according to Paxinos and Watson (1997). The cannula was anchored with dental cement to the calvarium surface. The reflected muscles and skin were replaced around the mound containing the cannula and were sutured. These animals were used for experiment after the recovery period for 5 days.

Determination of effects on body temperature

Experiments were conducted between 10:00 a.m. and 7:00 p.m.. The rectal temperature of each rats was measured at every 30 min in conscious state. Only animals whose body temperature was stable and in the range of 37.0-37.5°C were used to determine the effect of drug applications.

Thermal indexes were calculated as areas under the curves (°C/h) after the treatments for a total period of 6 hrs.

Statistics

Temperature responses were assessed as changes from pre-injection values (△°C). Results were expressed as the means ± S.E.M. for experiments. The significance of the difference between groups was determined by Student's *t*-test.

RESULTS

Effects of exogenous CO

I.c.v. administration of CO-saturated aCSF (5 μl) elicited the profound increase in body temperature with a maximal response 3 hrs after administration, whereas vehicle didn't cause significant change in body temperature. The thermal index of exogenous CO-treated rats (TI=1.76 ± 0.10°C/hr) was significantly higher than of group injected with vehicle (TI=0.25 ± 0.02°C/hr). Intraperitoneal pretreatment of indomethacin significantly abolished the increase in body temperature elicited by the exogenous CO with 0.26 ± 0.02°C/hr of thermal index which was not significantly different from of control group (Fig. 1).

Effects of hemin

I.c.v. injection of hemin (10 μg, n=4), a substrate of HO and potent inducer of HO-1, produced febrile response that started to increase 1 hr after injection and peaked 3 hr after injection (Fig. 2). Thermal index was significantly increased by hemin (TI=1.56 ± 0.03°C/hr). Indomethacin

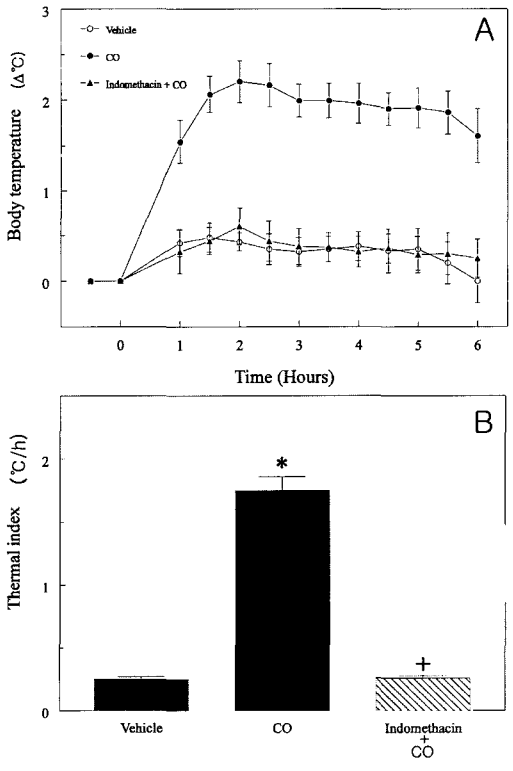

Fig. 1. Changes of body temperature (A) and thermal indexes (B) for 6 hrs after i.c.v. injection of CO-saturated artificial cerebrospinal fluid (4 μl). Indomethacin (10 mg/kg, i.p.) was administrated 30 min before CO injection. Each value represents mean ± S.E.M. of 4-6 experiments. Vehicle: 4 μl of artificial cerebrospinal fluid, * P<0.01 compared with vehicle, + P<0.01 compared with CO alone group.

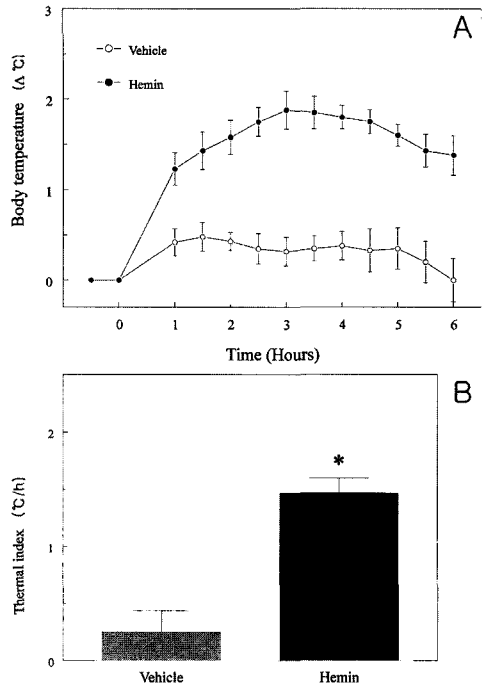

Fig. 2 Body temperature (A) and thermal indexes (B) for 6 hrs after i.c.v. injection of hemin 10 μg. Each value represents mean ± S.E.M. of 4-5 experiments. Vehicle: 4 μl of artificial cerebrospinal fluid, * P<0.01 compared with vehicle.

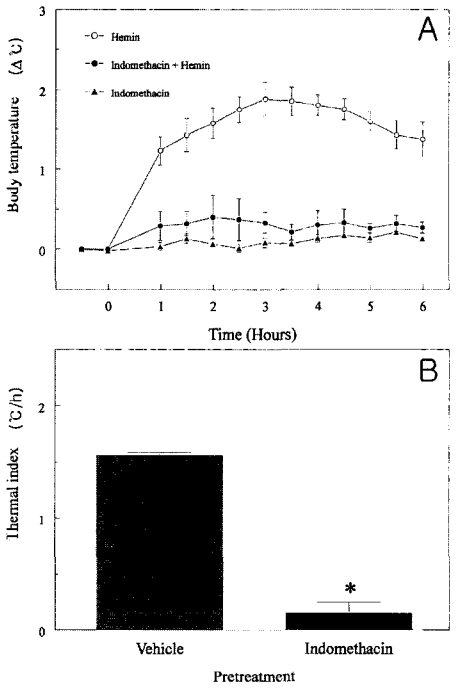

Fig. 3. Effects of pretreatment with indomethacin on hemin-induced febrile response. Changes of body temperature (A) and thermal indexes (B) for 6 hrs after i.c.v. injection of hemin 10 μg. Indomethacin (10 mg/kg, i.p.) was administrated 30 min before hemin injection. Each value represents mean ± S.E.M. of 4 experiments. Vehicle: 0.2 ml of saline (i.p.), * P<0.01 compared with vehicle.

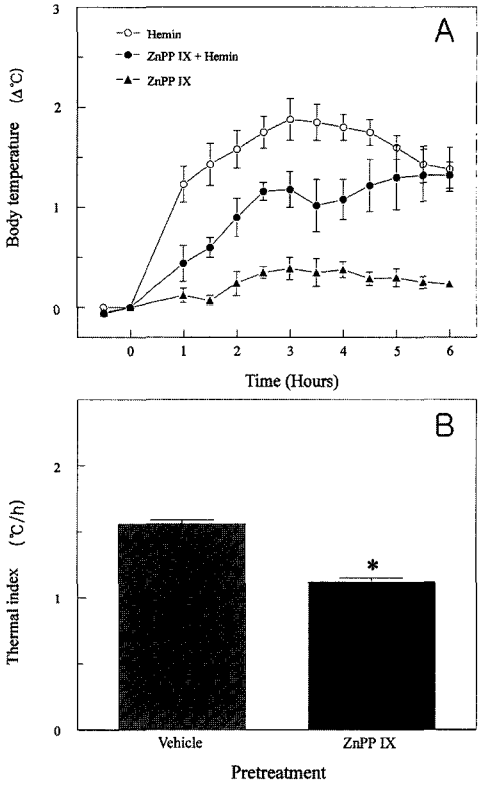

Fig. 4. Effects of pretreatment with zinc protoporphrine IX (ZnPP IX) on hemin-induced febrile response. Changes of body temperature (A) and thermal indexes (B) for 6 hrs after i.c.v. injection of hemin 10 μg. ZnPP IX (10 μg, i.c.v.) was administrated 30 min before hemin injection. Each value represents mean ± S.E.M. of 5-6 experiments. Vehicle: 4 μl of artificial cerebrospinal fluid (i.c.v.), * P<0.01 compared with vehicle.

(10 mg/kg, i.p.) had no significant effect in the body temperature of rats. But, hemin-induced febrile response was blocked by indomethacin. Thermal index indicated hemin-induced febrile response (TI=1.56 ± 0.03) was blocked by 90% (TI=0.15 ± 0.10) (Fig. 3).

ZnPP IX (10 μg, i.c.v.) alone did not elicite significant changes of body temperature. However, pretreatment of ZnPP IX 30 min before significantly attenuated the hemin-induced fever. Thermal index indicated hemin-induced febrile response (TI=1.56 ± 0.03) was blocked by 28% (TI=1.12 ± 0.03) (Fig. 4).

To examine the relationship between guanylate cyclase and febrile response by hemin, rats were injected ODQ (an inhibitor of guanylate cyclase, 50 μg, i.c.v.) intracerebroventricularly 30 min before hemin (10 μg, i.c.v.) administration. Hemin-induced febrile response was partially blocked by ODQ (Fig. 5). Thermal index indicated hemin-induced febrile response (TI=1.56 ± 0.03) was blocked by 42% (TI=0.91 ± 0.07) (Fig. 5).

Also the membrane permeable analogue of cyclic GMP, dibutyryl cGMP (100 g, n=5) produced marked febrile response in rats. The fever induced by i.c.v injection of

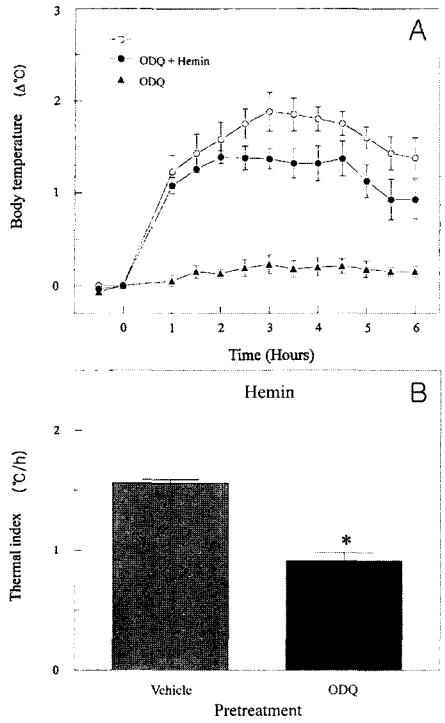

Fig. 5. Effects of pretreatment with ODQ on hemin-induced febrile response. Changes of body temperature (A) and thermal indexes (B) for 6 hrs after i.c.v. injection of hemin 10 μg. ODQ (50 μg, i.c.v.) was administrated 30 min before hemin injection. Each value represents mean ± S.E.M. of 4 experiments. Vehicle: 4 μl of artificial cerebrospinal fluid (i.c.v.), * P<0.01 compared with vehicle.

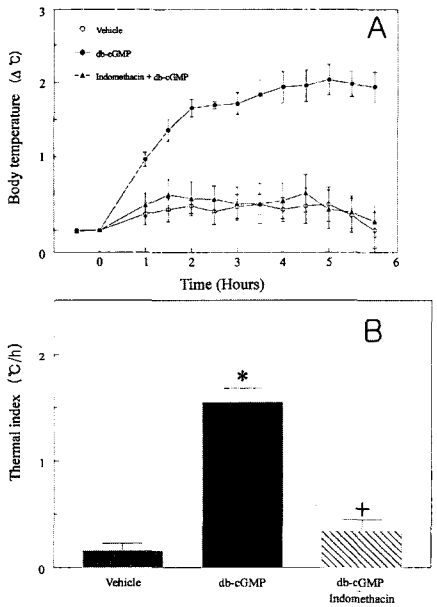

Fig. 6. Changes of body temperature (A) and thermal indexes (B) for 6 hrs after i.c.v. injection of dibutyryl cyclic GMP (db-cGMP) 100 μg. Indomethacin (10 mg/kg, i.p.) was administrated 30 min before db-cGMP injection. Each value represents mean ± S.E.M. of 4-6 experiments. Vehicle: 4 μl of artificial cerebrospinal fluid, * P<0.01 compared with vehicle, + P<0.01 compared with db-cGMP alone group.

dibutyryl cGMP was also attenuated by pretreatment with indomethacin (10 mg/kg, i.p., n=5) in rats (Fig. 6).

DISCUSSION

The present results show that both CO-saturated aCSF and hemin induced fever when they were administered into i.c.v.. Although hemin can be oxidatively cleaved by the HO system to CO (Maines *et al.*, 1997), in the present study, pretreatment with ZnPP IX, an inhibitor of heme oxygenase (Grundemar *et al.*, 1997), attenuated the fever induced by i.c.v injection of hemin. This suggests that CO produced by heme oxygenase in the hypothalamus of the rat brain can be involved in the development of the fever. However, CO produced by HO-2 which constitutively expressed in the brain seems to be unimportant in thermoregulation mechanism because there was no significant changes in the basal body temperature by i.c.v. administration of ZnPP IX. Except the more profound pyretic effect of exogenous CO in our experiment, these results is consistent with of other group (Steiner & Branco, 2000, 2001).

The present results show that pretreatment with ODQ, an inhibitor of guanylyl cyalase (Schramnel *et al.*, 1996b), did partially attenuate the fever induced by i.c.v. injection of CO (data not shown) or hemin. Soluble guanylyl cyclase is virtually absent from the rat hypothalamus (Burgunder *et al.*, 1994; Matsuoka, 1992). As far as the lack of CO effect on cyclic GMP production is concerned, a fundamental factor may be that the gas is only a weak activator of soluble guanylyl cyclase, producing only 1- or 2-fold increases in enzymatic activity (Kharitonov *et al.*, 1995; Burstyn *et al.*, 1995). Therefore, effects of CO mediated by its activation of soluble guanylyl cyclase are likely to be detected only in those brain areas, such as the hippocampus, endowed with high levels of soluble guanylyl cyclase (Zhuo *et al.*, 1993). On the contrary, two major studies have shown very low soluble guanylyl cyclase levels in the rat hypothalamus (Burgunder *et al.*, 1994; Matsuoka, 1992), which is not consistent with our result. It remains difficult to explain the partial inhibition by ODQ of the febrile response elicited by hemin. But, i.c.v injection of dibutyryl cyclic GMP, a membrane permeable analogue of cyclic GMP, causes a febrile response. The increase in cyclic GMP can activate phospholipase A_2 to provide arachidonic acid, the substrate for conversion by the activated cyclooxygenase to prostaglandin E_2 (Canteros *et al.*, 1995). These results raise the prospect that cyclic GMP formed within the tissue of the brain has a supplementary role of a fever.

Arachidonate metabolites, mostly prostaglandins, are thought to be involved in the central mechanism of the development of a fever (Milton & Wendlandt, 1970; Veale

& Cooper, 1974). It was also shown that the febrile response of rats to prostaglandin E_2 was potent when i.c.v injected (Minano *et al.*, 1997). In the present study, the fever induced by i.c.v injection of hemin, CO-saturated aCSF or dibutyryl cyclic GMP was completely attenuated by pretreatment with indomethacin, an inhibitor of cyclooxygenase. These results are different from those of other investigators that CO-induced pyresis is not dependent on prostaglandin and cyclooxygenase. (Steiner & Branco, 2000, 2001). The precursor of CO through the heme oxygenase pathway, hemin, induced concentration-dependent increases in prostaglandin E_2 from the rat hypothalamic explants (Mancuso *et al.*, 1997). Moreover CO-saturated solutions increased prostaglandin E_2 release from the rat hypothalamic explants. CO signals in the rat hypothalamus via the activation of cyclooxygenase (Mancuso *et al.*, 1998). Our results were supported by the reports.

These results propose that endogenous CO may have a role as a pyrogenic mediator in CNS and CO-mediated pyresis is dependent on prostaglandin production and partially on activation of soluble guanylate cyclase.

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