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Tyrosine Kinase is Involved in Hemin-Induced Pyresis

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To investigate the mechanisms involved in hemin-induced febrile response, the rectal temperature of rats were measured after intracerebroventricular (i.c.v.) injections of hemin, with or without antagonists. Hemin (10 μg) elicited a significant febrile response, which lasted from 30 min, to more than 6 h, after its administration, but this was not the case with biliverdin (i.c.v.) and bilirubin (i.c.v.). The hemin-induced febrile response was significantly inhibited by pretreatment with an inhibitor of tyrosine kinase (genistein), but not by pretreatment with an inhibitor of protein kinase C (chelerythrine) and a scavenger of iron (deferoxamine). These results suggest that tyrosine kinase is involved in the hemin-induced febrile response.

Key words: Hemin, Carbon monoxide, Tyrosine kinase

INTRODUCTION

Fever is a phenomenon characterized by a raised thermoregulatory set point, which leads to an elevation in body temperature (Kluger, 1991). Fever is a common manifestation of injury, tumor, infection and inflammation (Atkins & Bodel, 1979). Considerable efforts have been made to identify the mechanisms of fever, and evidence suggests that a common pathway underlies fevers during a variety of disorders. This common mechanism is believed to involve the induction of pyrogenic cytokines, such as interleukin IL-1 and IL-6 (Kluger *et al.*, 1995), by an exogenous pyrogen (ex., bacterial endotoxin), with the subsequent stimulation of prostaglandins generation, in particular, prostaglandin E_2 (PGE_2) (Milton, 1989; Blatteis & Sehic, 1997; Charles, 1999). PGE_2 is thought to act as the final mediator of fever. Although it seems definite that central PGE_2 is an essential mediator of fever, the mechanisms by which peripheral pyrogens signal the brain to raise body temperature is still unclear.

Carbon monoxide (CO) has recently been recognized to act as a signaling molecule, as it is a vasoactive substance (Johnson *et al.*, 1999), and a neurotransmitter or neuromodulator (Dawson & Snyder, 1994; Snyder *et al.*, 1998, 2001), and has been proposed as a possible mediator of the febrile response in the hypothalamus (Jung & Lee, 1999; Steiner *et al.*, 1999; Steiner & Branco, 2000, 2001). CO is produced by heme oxygenase (HO), which catalyzes the metabolism of heme to biliverdin and iron (Abraham *et al.*, 1988; Maines, 1993, 1997). Our previous report showed that hemin, a substrate and potent inducer of HO, produced profound pyresis and HO, with prostaglandin and cGMP also involved in this effect (Jang *et al.*, 2002). However, the precise mechanisms mediating the hemin-induced pyresis remain to be elucidated.

Cytokines are known to be important mediators in several types of pyresis, and many cytokines receptors have an intracellular tyrosine kinase domain (Ullrich & Schlessinger, 1990), the activation of which results in the phosphorylation of proteins following ligand binding. It was recently reported that the activation of tyrosine kinase, in the central nervous system, by lipopolysaccharide (LPS) leads to the generation of fever (Hiromi & Mayumi, 2000; Lin & Lin, 2000). Protein kinase C (PKC), which plays a pivotal role in the signaling and regulation of multiple cellular processes, was also reported to be involved in the regulation of LPS fever (Kozak *et al.*, 1997).

In this study, in order to elucidate the mechanism of hemin-induced pyresis, the involvement of the products of HO, tyrosine kinase and PKC were examined.

MATERIALS AND METHODS

Chemicals

The hemin chloride, bilirubin, biliverdin, deferoxamine,

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chelerythrine and genistein were purchased from Sigma (U.S.A.). The hemin was dissolved in 0.1 M NaOH and diluted in artificial cerebrospinal fluid (aCSF), containing in (mM) NaCl 138, KCl 50, $NaHCO_3$ 11, KH_2PO_4 1, $CaCl_2$ 1.1 and $MgCl_2$ 1 at pH 7.4. The bilirubin and biliverdin were dissolved in 4 mM NaOH, and the deferoxamine in pyrogen-free sterile saline.

Surgery

Adult male Sprague-Dawley rats, weighing 250-300 g, were used for experiments, and were housed individually at an ambient temperature of 22±1°C, with a 12 h light-dark cycle. The Animals were allowed free access to food and water. After anesthesia, with pentobarbital (40 mg/kg, i.p), a cannula (0.8 nm o.d., 0.4 nm i.d.) was stereotaxically implanted in the lateral ventricle (P: 0.9 mm, L: 1.5 mm, V: 3.5 mm) for the intracerebroventricular (i.c.v.) injection of the drugs, according to the procedure of Paxinos and Watson (Paxinos & 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 sutured. After surgery, the animals were treated intramuscularly with Unasyn (80 mg/kg), twice a day, for 2 days. These animals were used for the experiment, following a 5 day recovery period.

Measurement of body temperature

The experiments were conducted between 10:00 a.m. and 7:00 p.m. The rectal temperature of each rat was measured every 30 min, in a conscious state. Only rats with stable body temperatures, in the range 37.0-37.5°C, were used to determine the effects of the drug applications. Room temperature was 22±1°C.

The thermal indexes were calculated as the areas under the curves (Δ/h) after total treatment periods of 6 h.

Statistics

The temperature responses were assessed as the changes in the pre-injection values (Δ), and expressed as the mean±S.E.M. of the experiments. Any significant difference between the groups was determined by the Student's *t*-test.

RESULTS

Effect of hemin

Hemin is a physiological substrate and the inducer of HO. Hemin, or aCSF, was injected intracerebroventricularly, and the hemin evoked a fever, which started to increase 1 h after injection, peaked 2 h later and was maintained for a further 3 h. The thermal index indicated that the febrile response induced by hemin (TI = 1.346±0.100) was ten-times higher than that by aCSF (TI = 0.130±0.176) (Fig. 1).

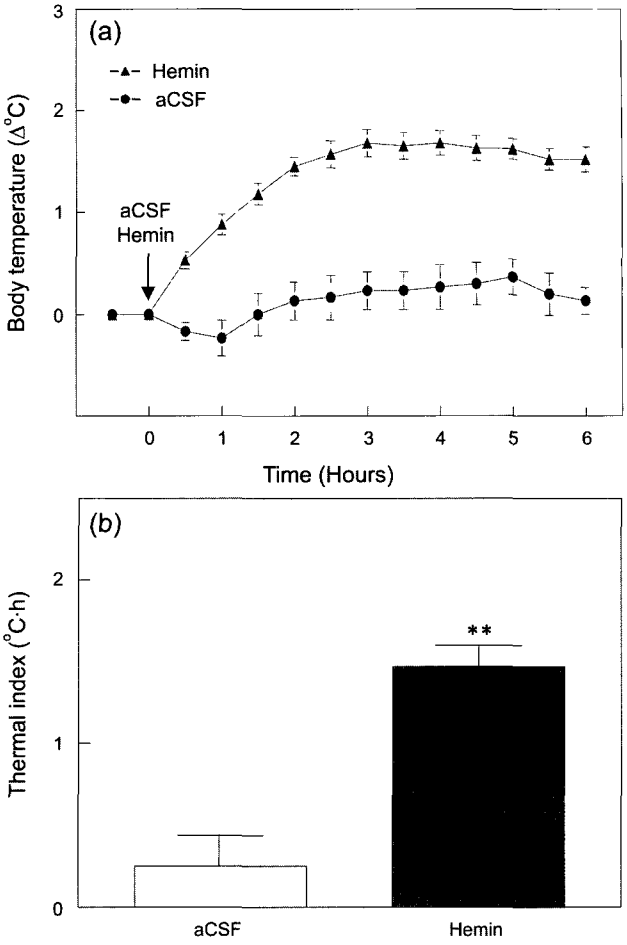

Fig. 1. The effect of hemin on the body temperature. (A) Changes in the body temperature of rats injected with hemin (10 μg, i.c.v., n=6) or a vehicle (aCSF, i.c.v., n=3). Arrows indicate the time of injection. (B) Thermal index (TI) between 0 and 6 h after the hemin or vehicle injection in rats. Each value represents the mean±S.E.M. **P<0.01.

Effects of products of HO

To examine whether other heme products (biliverdin, bilirubin and iron) are related to the febrile response caused by hemin, rats were intracerebroventricularly injected with biliverdin, bilirubin and an iron chelator. The biliverdin and bilirubin evoked no febrile response. Deferoxamine, the iron chelator used, did not block the hemin-induced pyresis (Fig. 2, 3 and 4).

Effects of tyrosine kinase inhibitor and protein kinase C inhibitor in hemin-induced pyresis

Genistein, an inhibitor of tyrosine kinase, was also intracerebroventricularly injected 30 min before the administration of the hemin. Fig. 5 shows the hemin-induced febrile response was partially blocked by the genistein. The thermal index indicated the hemin-induced febrile response (TI = 1.346±0.1) was significantly blocked by the genistein (TI = 0.579±0.138).

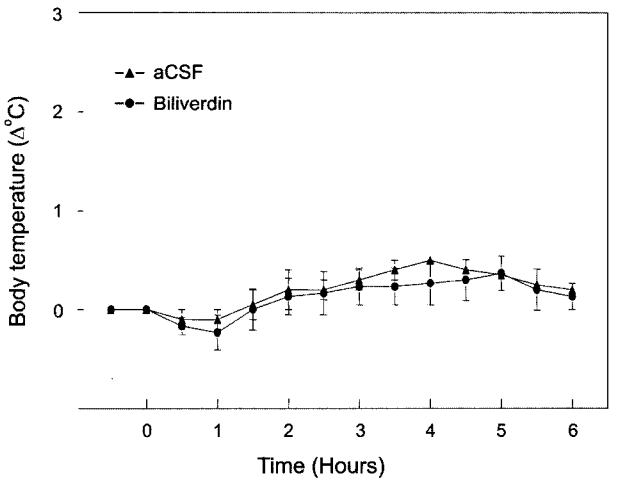

Fig. 2. Changes in the body temperature of rats injected with biliverdin (152 nmol, i.c.v., n=4) or a vehicle (aCSF, i.c.v., n=4). Each value represents the mean±S.E.M.

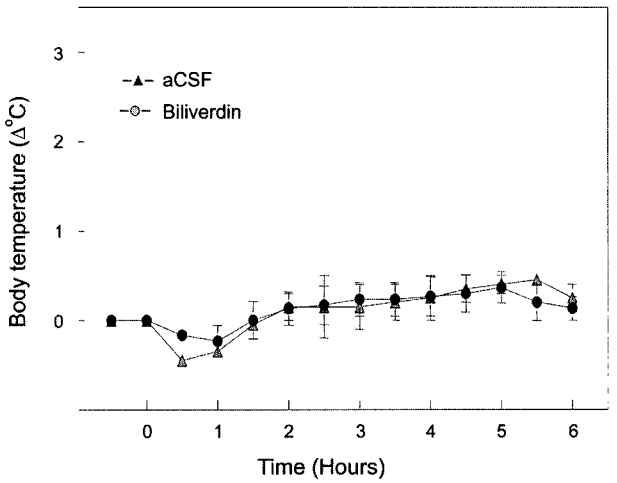

Fig. 3 Changes in the body temperature of rats injected with bilirubin (152 nmol, i.c.v., n=4) or a vehicle (aCSF, i.c.v., n=4). Each value represents the mean±S.E.M.

Chelerythrine, an inhibitor of protein kinase C, was intracerebroventricularly injected 30 min before the administration of the hemin. It also did not block the hemin-induced febrile responses (Fig. 6).

DISCUSSION

HO is the enzyme responsible for the catalytic oxidation of heme to iron, biliverdin and CO, *in vivo,* and seems to be extensively distributed throughout the body, including the central nervous system (Maines, 1988; Marks *et al.*, 1991). Biliverdin is subsequently converted to bilirubin, by biliverdin reductase (Maines, 1988). All the products of the heme catabolism by HO are physiologically active. Iron can catalyze the formation of reactive oxygen metabolites,

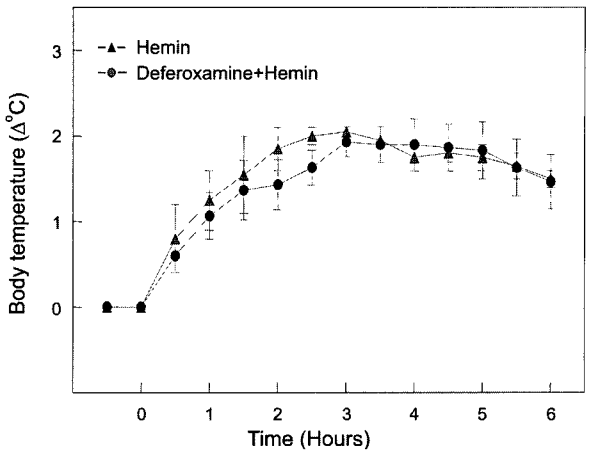

Fig. 4. Effect of the iron chelator, deferoxamine, on the hemin-induced pyresis. Changes in the body temperature of rats injected with hemin (10 μg,, i.c.v. n=4) are represented. Deferoxamine (150 ng, i.c.v.) was administered 30 min before the administration of the hemin. Each value represents the mean±S.E.M.

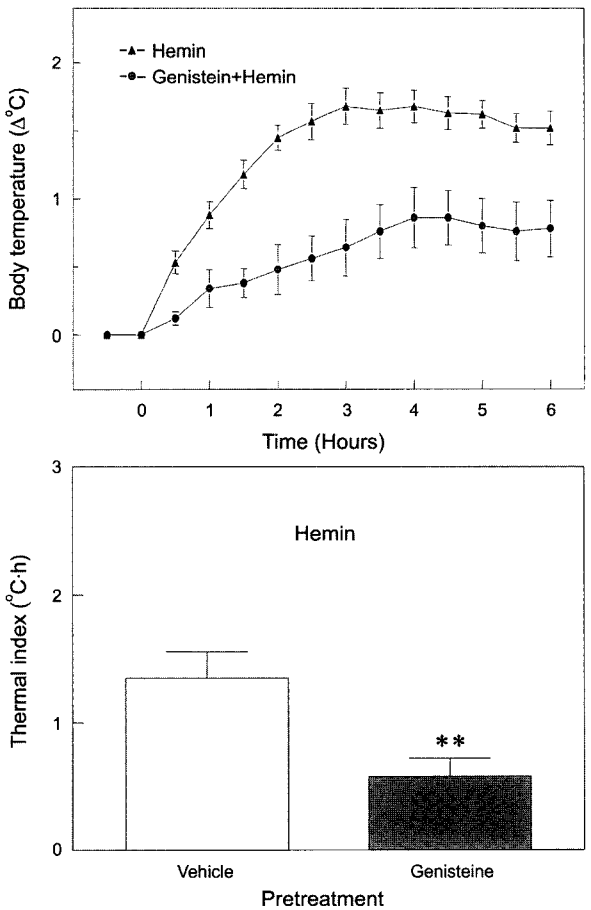

Fig. 5. Effect of the tyrosine kinase inhibitor, genistein, on the hemin-induced pyresis. Genistein (10 μg, i.c.v.) was administered 30 min before the administration of the hemin. (A) Changes in the body temperature of rats injected with hemin (10 μg, i.c.v., n=6) are represented. (B) Thermal index (TI) for 6 h after the hemin injection in rats. Each value represents the mean± S.E.M. ** $P<0.01$.

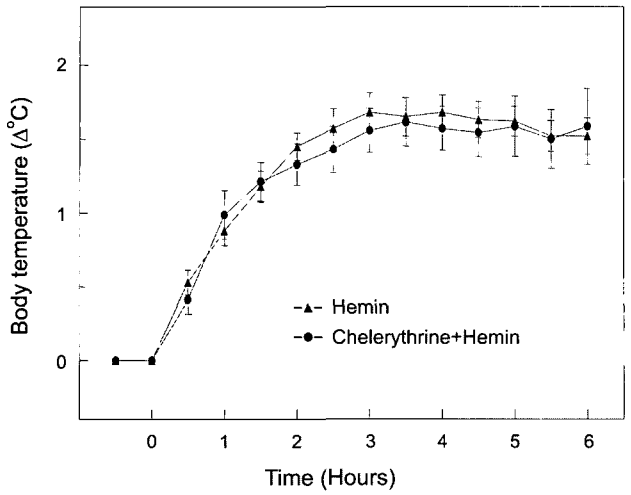

Fig. 6. Effect of the protein kinase C inhibitor, chelerythrine, on the hemin-induced pyresis. Changes in the body temperature of rats injected with hemin (10 μg, i.c.v. n=7) are represented. Chelerythrine (3 μg, i.c.v.) was administered 30 min before the administration of the hemin.

and regulate the genes, and biliverdin and its derivative, bilirubin, have long been known as antioxidants (Stocker *et al.*, 1987a, b; Belanger *et al.*, 1997). CO is recognized to act as a signaling molecule, being a vasoactive substance (Johnson *et al.*, 1999) and a neurotransmitter or neuromodulator (Dawson & Snyder, 1994; Snyder *et al.*, 1998, 2001).

In this study, whether the products of the catabolism of hemin are involved in the hemin-induced pyresis was also studied. Similarly to the effect of hemin, the i.c.v. administration of either biliverdin or bilirubin induced no significant changes in body temperature, and the pretreatment with deferoxamine, as an iron chelator, did not change the pyreactive activity of hemin. In our previous report (Jang *et al.*, 2001), hemin-induced pyresis was reduced by the pretreatment with an HO inhibitor, and CO elicited a similar pyretic effect to that of hemin. These results indicate that the hemin-induced pyresis may be mediated by CO, and not by biliverdin, bilirubin or iron.

Several kinds of cytokine, interleukin IL-1α, IL-1β, IL-6, IL-8, tumor necrosis factor-α and interferon-γ, act as endogenous pyrogens, which activate the membrane receptors that signal through the tyrosine kinases of the Janus kinase (JAK) family (Kluger, 1991; Rothwell, 1994; Ihle, 1995). There are a few reports that have studied the involvement of tyrosine kinase in the generation of the febrile response (Lin & Lin, 2000; Pela *et al.*, 2000; Tsushima & Mori, 2000, 2001). Tyrosine kinase was proposed to be involved in the febrile response elicited by LPS (Lin & Lin, 2000; Tsushima & Mori, 2000). In this study, genestein, an inhibitor of tyrosine kinase, significantly blocked the hemin-induced pyretic effect. In another of our recent studies, genestein also showed a similar effect on the CO-induced febrile response (Data were not shown). These results suggest that the hemin-induced febrile response may involve the production of CO and the activation of tyrosine kinase.

LPS is well known as an exogenous pyrogen. Kozak *et al.* (1997) reported that PKC mediated the LPS-induced febrile response. However, in the report of Tsushima & Mori (2000), the pretreatment with a PKC inhibitor did not affect the LPS-induced febrile response. The blocking action of a PKC inhibitor may depend on the administration site. The subcutaneous administration of a PKC inhibitor attenuated the LPS-induced fever (Kozak *et al.*, 1997), but the i.c.v. administration did not (Tsushima & Mori, 2000). To study whether the hemin-induced febrile response involves PKC, a PKC inhibitor, chelerythrine, was intracerebroventricularly administered 30 min before the administration of the hemin. However, the chelerythrine did not change the hemin-induced febrile response. These results indicate that PKC is not involved in the hemin-induced pyresis.

In conclusion, the hemin-induced febrile response involves the activation of tyrosine kinase, but not the production of biliverdin, bilirubin and iron, or the activation of PKC.

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