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Nitric oxide synthase-cyclo-oxygenase pathways in organum vasculosum laminae terminalis: possible role in pyrogenic fever in rabbits

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1 Fever was induced in rabbits by administration of *Escherichia coli* endotoxin (lipopolysaccharide; LPS; 0.001–10 μg) into the organum vasculosum laminae terminalis (OVLT). Deep body temperature was evaluated over a period of 7 h.

2 The LPS-induced febrile response was mimicked by intra-OVLT injection of the nitric oxide (NO) donors, S-nitroso-acetylpenicillamine (SNAP, 1–10 μg), sodium nitroprusside (SNP, 50 μg), or hydroxylamine (10 μg), the cyclic GMP analogue 8-bromo-cyclic GMP (8-Br-cyclic GMP, 10–100 μg), or prostaglandin E_2 (PGE_2, 0.2 μg).

3 Dexamethasone (Dex, a potent inhibitor of the transcription of inducible NO synthase, iNOS, 10 μg), anisomycin (a protein synthesis inhibitor, 100 μg), L-N^5-(1-iminoethyl)ornithine (L-NIO; an irreversible NOS inhibitor, 10–200 μg), aminoguanidine (a specific iNOS inhibitor, 1000 μg), or N^G-methyl-L-arginine acetate (L-NMMA, a NOS inhibitor, 100 μg) inhibited fever induced by LPS when injected into the OVLT 1 h before LPS injection. An intra-OVLT dose of 1000 μg of N^G-nitro-L-arginine methyl ester (L-NAME, a potent inhibitor of constitutive NOS) did not exhibit antipyretic effects.

4 Methylene blue (an inhibitor of NOS and soluble guanylate cyclase, 1–10 μg), 6-(phenylamino)-5,8-quinolinedione (LY-83583; an inhibitor of soluble guanylate cyclase and NO release, 20 μg), or indomethacin (an inhibitor of cyclo-oxygenase, COX, 400 μg) inhibited fever induced by LPS when injected into the OVLT 1 h before LPS injection. Pretreatment with methylene blue or haemoglobin (a NO scavenger, 100 μg) attenuated the fever induced by intra-OVLT injection of SNAP.

5 The PGE_2-induced fever was potentiated, rather then attenuated, by pretreatment with an intra-OVLT dose of animoguanidine (1000 μg), L-NMMA (100 μg), or L-NIO (200 μg).

6 These results suggest that iNOS-COX pathways in the OVLT represent an important mechanism for modulation of pyrogenic fever in rabbits.

Keywords: Nitric oxide synthase; cyclo-oxygenase; nitric oxide; cyclic GMP; prostaglandin; lipopolysaccharide; organum vasculosum laminae terminalis (OVLT); fever

Introduction

The organum vasculosum laminae terminalis (OVLT) is a site through which signals which increase body temperature are transferred from the blood to the brain in animals (Blatteis *et al.* 1983; Hashimoto *et al.*, 1994; Stitt, 1985). Circulating pyrogens including bacterial endotoxin (or lipopolysaccharide, LPS) and pyrogenic cytokines cause the formation of prostaglandins (PG) within the OVLT region, which in turn activate adjacent neurones. The resulting neuronal signal is conducted to the thermoregulatory centre in the preoptic area of the anterior hypothalamus, which is in close proximity to the OVLT region (Hashimoto *et al.*, 1994). The production of pyrogenic cytokines such as interleukin-1 (IL-1) occurs in the rabbit OVLT during fever induced by endotoxin (Nakamori *et al.*, 1994).

Nitric oxide (NO) is generated from the terminal guanidine nitrogen atom(s) of the amino acid L-arginine by the enzyme NO synthase (NOS; for review see Moncada *et al.*, 1991). Three different isoforms of NOS have been described. Two constitutive forms producing small amounts of NO have been found in endothelial (eNOS) and neuronal (nNOS) cells (Moncada *et al.*, 1991). A cytokine inducible form of NOS (iNOS) which produces large quantities of NO has been described in activated macrophages (Lowenstein *et al.*, 1992; Lyons *et al.*, 1992). In the CNS, NO has been implicated as both a mediator of neurotoxicity and a neuromodulator (Bredt & Snyder, 1992). Exposure of cells in culture to LPS causes the release of inflammatory cytokines such as IL-1, IL-6, and tumour necrosis factor (TNF). In addition, LPS or cytokines cause the synthesis of NOS and cyclo-oxygenase (COX) *in vitro* and *in vivo* (Simmons & Murphy, 1993; Akarasereenont *et al.*, 1994; Minc-Golomb *et al.*,1994; Salvemini *et al.*, 1993; 1995; Sautebin *et al.*, 1995; Swierkosz *et al.*, 1995). COX exists in at least 2 isoforms (Xie *et al.*, 1992). COX-1 is present constitutively in various types of cells including endothelial cells (Mitchell *et al.*, 1993), whereas the expression of COX-2 is induced by inflammatory stimuli including cytokines (Maier *et al.*, 1990) and LPS (Lee *et al.*, 1992). NO-dependent cyclic GMP production in glial cells can also be induced and regulated by LPS or pyrogenic cytokines (Simmons & Murphy, 1993). Recently, preliminary results show that the IL-1 β-induced fever was affected by treatment with NOS inhibitors in rats (Reimers *et al.*, 1994), but not affected in rabbits (Kapás *et al.*, 1994).

In the light of the above findings, we have investigated whether the NOS and COX pathways in the OVLT region are involved in the development of pyrogenic fever in rabbits. The purpose of this study was to examine the effects of LPS, PGE_2, NO donors or a cyclic GMP analogue on body temperature in rabbits after micro-injection into the OVLT. In addition, experiments were carried out to investigate the effects of intra-OVLT injection of the inhibitors of NOS, COX, or guanylate cyclase on the fever induced by intra-OVLT administration of LPS.

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Methods

Experimental animals

Adult male New-Zealand White rabbits, weighing between 2.5 and 3.0 kg at the start of the study, were used. The animals had been previously implanted with cannula guide tubes under general anaesthesia, but the thermal experiments were carried out in unanaesthetized animals restrained in rabbit stocks. Between experiments the animals were housed individually at an ambient temperature of 22±1°C with a 12 h light-dark cycle and the lights being switched on at 06 h 00 min. Animal chow and water *ad libitum* were allowed.

Surgical techniques

An intracerebral cannula (0.81 mm o.d.) was implanted into each animal under general anaesthesia (pentobarbitone sodium, 30 mg kg^{-1}, i.v.). Standard aseptic techniques were employed. The stereotaxic atlas and coordinates of Sawyer *et al.* (1954) were used. The cannula was located in the OVLT (A:4.5 mm, L:0.0 mm, and V:14 mm). The animal was placed in the stereotaxic apparatus, and the frontal and parietal bones were exposed by a midline incision on the scalp. After the appropriately located craniotomy had been trephined, two self-tapping screws were inserted into the parietal or frontal bones and the cannula was inserted to the depth through the craniotomy hole. The cannula was anchored with dental acrylic cement to the calvarium surface, which had been scraped clean of periosteum. The reflected muscles and skin were replaced around the acrylic mound containing the cannula and screws and were sutured with chromic gut (000). A period of 1 week was allowed for the animals to recover before they were used.

Experimental procedures

Experiments were conducted between 09 h 00 min and 17 h 00 min, each animal being used at intervals of not less than 5 days. Throughout the experiment, colonic temperature (T_{co}) was measured every minute with a copper-constantan thermocouple connected to a thermometer (YOKOGAWA, HR1300). The colonic temperature of each animal was allowed to stabilize for at least 90 min before any injections. Only animals whose body temperature was stable and in the range of 38.8–39.8°C were used to determine the effect of drug applications. Microinjections were made into the OVLT through a 26 gauge cannula passed through the implanted guide tube to a site 0.5 mm beyond the tip of the outer tube. The volume of fluid injected over about 5 s was 1 μl unless otherwise stated. Procedures minimizing contamination by extraneous pyrogens were used as described previously for the preparation and injection of drug solutions (Won & Lin, 1991), with the exception that solutions were not passed through a 0.22 mm filter.

The effects of intra-OVLT injection of LPS, PGE_2, NO donors, or 8-Br-cyclic GMP on thermoregulatory responses were assessed in an animal partitional Calorimeter as previously described (Won & Lin, 1988). Briefly, metabolic rate was calculated from the oxygen consumption of the animals. Respiratory evaporative heat loss was calculated by measuring the increase in water vapour content in the expired air over that of the inspired air. Estimated ear skin blood flow was used as a sensitive index of ear vasomotor activity in terms of ml min^{-1}.

Drugs

The following drugs were used: *Escherichia coli* lipopolysaccharide (LPS, 026:B6, 0.001–10 μg), dexamethasone (Dex, 1–10 μg), anisomycin (10–100 μg), hydroxylamine (1–10 μg), sodium nitroprusside (SNP, 1–50 μg), N^G-nitro-L-arginine methyl ester (L-NAME 100–1000 μg), methylene blue (1–10 μg), 8-bromo-cyclic GMP (8-Br-cyclic GMP, 1–100 μg), prostaglandin E_2 (PGE_2, 0.2 μg), indomethacin (50–400 μg), and haemoglobin (100 μg) were purchased from Sigma Chemical Company. S-nitroso-acetylpenicillamine (SNAP, 0.1–10 μg), aminoguanidine (100–1000 μg), L-N^5-(1-iminoethyl)ornithine (L-NIO, 1–200 μg), N^G-methyl-L-arginine acetate (L-NMMA, 100 μg), and 6-(phenylamino)-5,8-quinolinedione (LY-83583, 1–20 μg) were purchased from the company of Research Biochemicals International. SNAP and LY-83583 were dissolved in dimethyl sulphoxide (DMSO) solution and diluted to 5% with saline for use. Indomethacin and dexamethasone were dissolved in phosphate buffered saline. All other compounds were dissolved in saline. According to the data available, these NOS inhibitors required a pre-incubation time of about 1 h to exert their actions. Therefore, in the present study, all drugs were administered 1 h before intra-OVLT injection of LPS, SNAP, PGE_2, or 8-Br-cyclic GMP.

Histological verification

After the completion of the experiments, an aliquot of methylene blue was injected down the cannula to measure the spread of the injected solution. The head of each animal was perfused with PBS solution, followed by 4% paraformaldehyde (PFA) fixative solution. After perfusion, the brain was removed and placed in a well-labelled glass vial filled with 4% PFA fixative, and fixed at 4°C for 0.5–1 h after which the solution was changed to 15% sucrose in PBS until the brain sank in the vial. Later, the fixed brains were cut in 50 mm sections so that stereotaxic coordinates of injection site in each rabbit were verified.

Statistical analysis

Temperature responses were assessed as changes from pre-injection values (Δ°C). Results are expressed as the mean± s.e.mean for n experiments. The probability (P) of the significance of the difference between different groups was determined by Student's 2-tailed t test (Armitage, 1971).

Results

Studies with protein synthesis inhibitors

LPS administration intra-OVLT (0.001–10 μg) produced a dose-dependent rise in deep body temperature of rabbits. A typical pattern of fever induced by an intra-OVLT dose (0.2 μg, $n=12$) of LPS is depicted in Figure 1. Fever was maximal about 3 h after injection and was associated with heat conservation by ear skin vasoconstriction and increased heat production. Body temperature returned to pre-injection values about 16 h after intra-OVLT injection of LPS. Intra-OVLT injection of doses of 0.01–10 μg of LPS resulted in a significant increase in body temperature which lasted for up to 6 h (end of the observation period).

The fever induced by intra-OVLT injection of LPS was significantly attenuated by pretreatment with intra-OVLT injection of Dex (a potent inhibitor of the transcription of inducible NO synthase, 10 μg, $n=8$) or anisomycin (a protein synthesis inhibitor, 100 μg, $n=8$) when given 1 h before LPS administration ($P<0.05$; Figure 1). Control injection of Dex ($n=6$), anisomycin ($n=6$), or saline ($n=12$) into the OVLT of rabbits produced no significant change in body temperature (data not shown).

Studies with NO synthase inhibitors

In addition, the fever induced by intra-OVLT administration of LPS was significantly attenuated by pretreatment 1 h before LPS with intra-OVLT injection of L-NIO (10–200 μg, $n=6$ for each; Figure 2a), aminoguanidine (1000 μg, $n=6$; Figure 2b), or L-NMMA (100 μg, $n=6$; Figure 2b) in rabbits

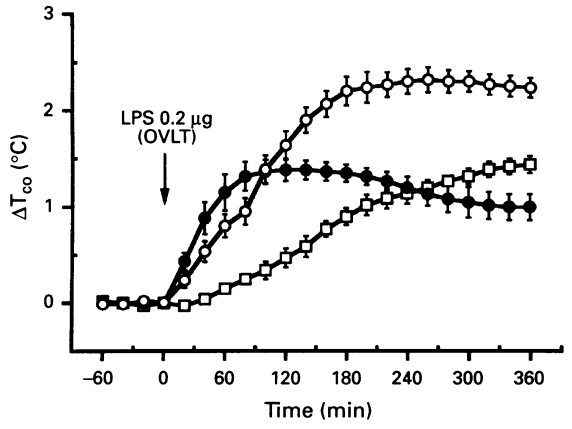

Figure 1 Effects of dexamethasone or anisomycin on the fever induced by LPS in rabbits at an ambient temperature of 22±1°C. At the time indicated by the arrow (↓), LPS solution was injected into the OVLT. An equivalent volume of saline (0.9%, ○, $n=12$), dexamethasone (10 μg, ●, $n=8$), or anisomycin (10 μg, □, $n=8$) was given 1 h before the LPS injection. Points represent the mean (with s.e.mean) change in temperature (Δ°C) for n experiments. Dexamethasone and anisomycin caused a significant inhibition of the increase in body temperature caused by LPS from 120 to 360 min and from 20 to 360 min, respectively.

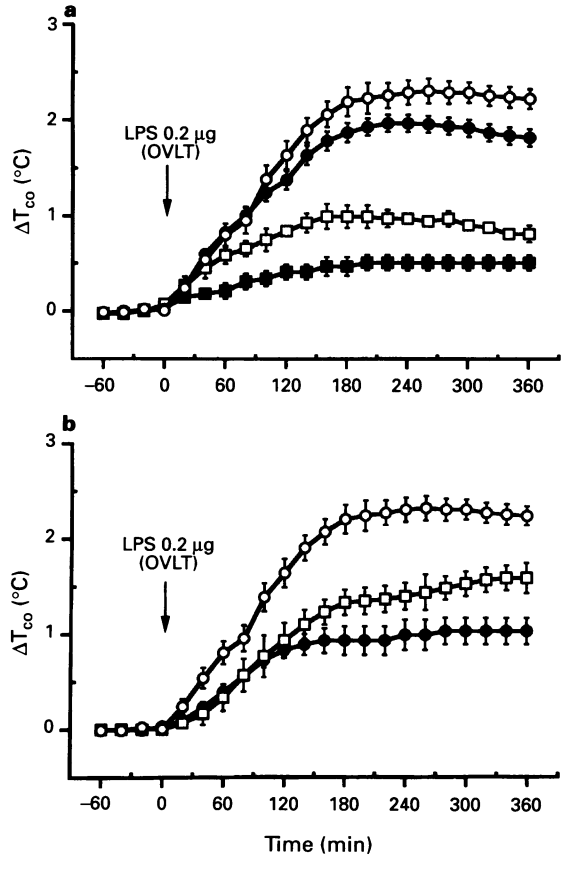

Figure 2 Effects of NOS inhibitors on the fever induced by LPS in rabbits at an ambient temperature of 22±1°C. At the time indicated by the arrow (↓), LPS solution was injected into the OVLT. (a) An equivalent volume of saline (0.9% ○, $n=12$), three different doses of L-NIO (10 μg, ●, $n=6$; 100 μg, □, $n=6$; 200 μg, ■, $n=6$), or (b) saline (0.9%, ○, $n=12$), aminoguanidine (10 μg, ●, $n=6$), or L-NMMA (10 μg, □, $n=6$) was given 1 h before the LPS injection. Points represent the mean (with s.e.mean) change in temperature (Δ°C) for n experiments. L-NIO, aminoguanidine or L-NMMA caused a significant inhibition of the increase in body temperature caused by LPS from 40 to 360 min.

($P<0.05$). However, the LPS-induced fever was not significantly reduced by pretreatment with L-NAME (1000 μg, $n=6$; data not shown). Again, control injection of either one of these NO synthase inhibitors had no effect on body temperature of rabbits.

Studies with NO donors and a cyclic GMP analogue

Intra-OVLT administration of the NO donors SNAP (1–10 μg, $n=6$ for each; Figure 3a), SNP (10–50 μg, $n=5$ for each; data not shown), or hydroxylamine (1–10 μg, $n=5$; data not shown), or the membrane permeable analogue of cyclic GMP, 8-Br-cyclic GMP (10–100 μg, $n=6$ for each; Figure 3b) produced febrile responses in rabbits. The fever was associated with ear skin vasoconstriction and increased heat production (data not shown).

Studies with cyclic GMP inhibitors

Pretreatment of rabbits with intra-OVLT injection of methylene blue (an inhibitor of soluble guanylate cyclase, 10 μg, $n=6$) or LY-83583 (an inhibitor of soluble guanylate cyclase and NO release, 20 μg, $n=6$), although showing no effect on the basal levels of body temperature, significantly attenuated the fever induced by intra-OVLT injection of LPS ($P<0.05$; Figure 4a). Methylene blue (10 μg, $n=6$, Figure 4b), or haemoglobin (a NO scavenger; 100 μg, $n=4$; Figure 4b) pretreatment also significantly attenuated the fever induced by intra-OVLT injection of SNAP ($P<0.05$).

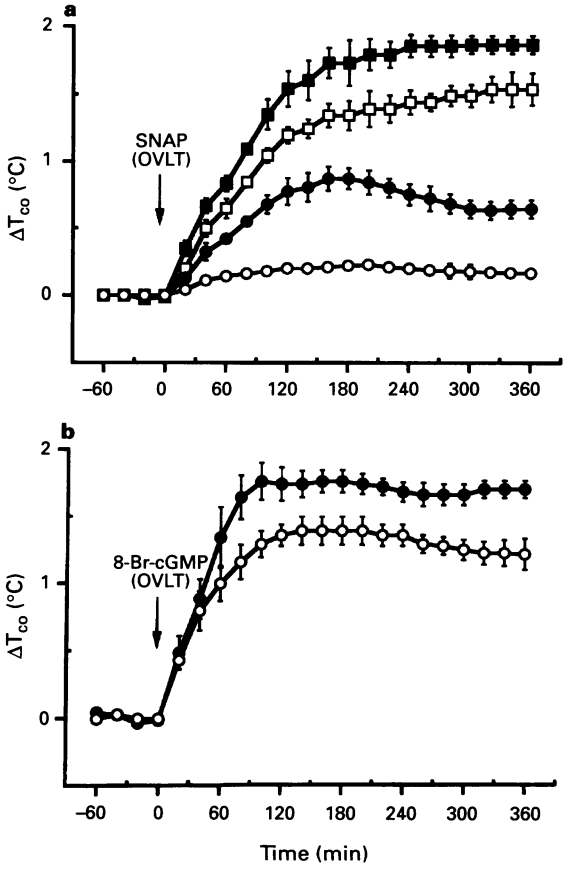

Figure 3 Febrile responses produced by SNAP or 8-Br-cyclic GMP in rabbits at an ambient temperature of 22±1°C. At the time indicated by the arrow (↓), (a) an equivalent volume of vehicle (○, $n=6$), or three different doses of SNAP (1 μg, ●, $n=6$; 5 μg, □, $n=6$; 10 μg, ■, $n=6$) were given into the OVLT, or (b) two different doses of 8-Br-cyclic GMP (10 μg, ○, $n=6$; 100 μg, ●, $n=6$) were given into the OVLT. Points represent the mean (±s.e.mean) change in temperature (Δ°C) for n experiments. SNAP or 8-Br-cyclic GMP caused a significant increase in body temperature from 30 to 360 min.

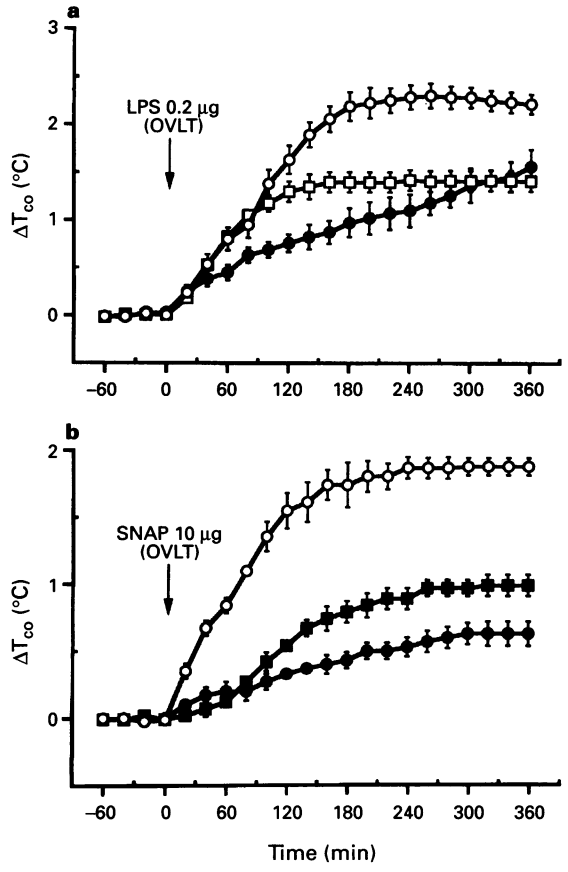

Figure 4 Effects of methylene blue, LY-83583, or haemoglobin on the febrile responses induced by LPS or SNAP in rabbits at an ambient temperature of 22±1°C. At the time indicated by the arrow (↓), (a) LPS, or (b) SNAP solution was injected into the OVLT. An equivalent volume of vehicle (○, $n=9$), methylene blue (10μg, ●, $n=6$), LY-83583 (20μg, □, $n=6$), or haemoglobin (100μg, ■, $n=4$) was given 1 h before the LPS and SNAP injection. Points represent the mean (±s.e.mean) change in temperature (Δ°C) for n experiments. Methylene blue caused a significant inhibition of the increase in body temperature caused by LPS or SNAP from 30–60 to 360 min. LY-83583 caused a significant inhibition of the increase in body temperature caused by LPS from 120 to 360 min, while haemoglobin caused a significant inhibition of the increase in body temperature caused by SNAP from 15 to 360 min.

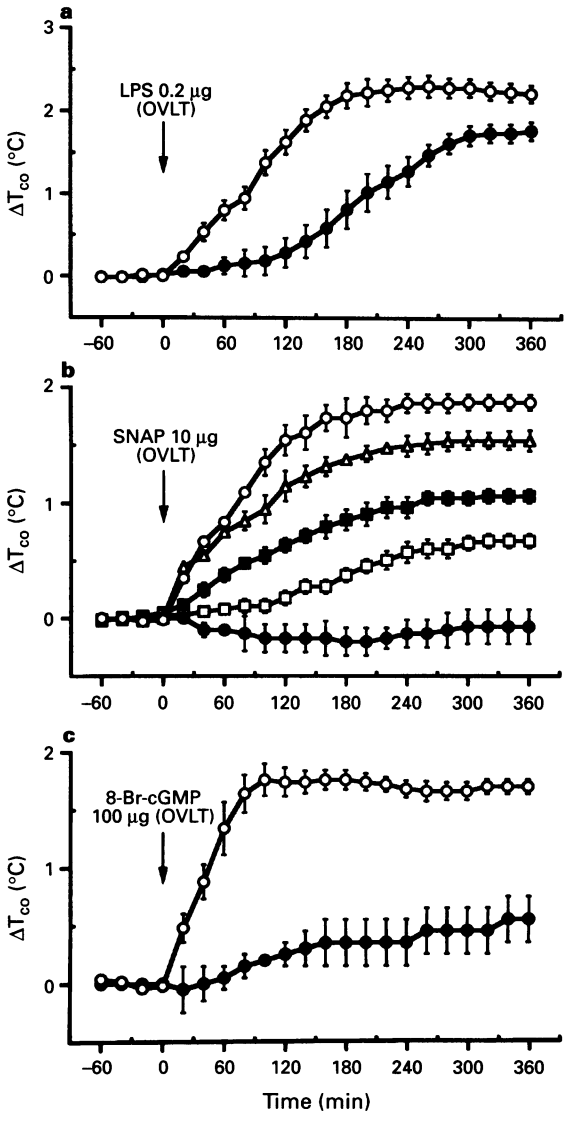

Figure 5 Effects of indomethacin on the fever induced by LPS, SNAP, or 8-Br-cyclic GMP in rabbits at an ambient temperature of 22±1°C. At the time indicated by the arrow (↓), (a) LPS, (b) SNAP, or (c) 8-Br-cyclic GMP solution was injected into the OVLT. An equivalent volume of vehicle (○, $n=9$), or four different doses of indomethacin (400μg, ●, $n=9$; 200μg, □, $n=4$; 100μg, ■, $n=4$; 50μg, △, $n=4$) were given 1 h before the LPS, SNAP, or 8-Br-cyclic GMP injection. Points represent the mean (±s.e.mean) change in temperature (Δ°C) for n experiments. Indomethacin caused a significant inhibition of the increase in body temperature caused by LPS, SNAP or 8-Br-cyclic GMP from 15–75 to 360 min

Studies with a COX inhibitor

Figure 5a shows that pretreatment of rabbits with indomethacin (400 μg, $n=9$) significantly attenuated the fever induced by intra-OVLT injection of LPS ($P<0.05$). Indomethacin appears to lose its protective effect from the 2nd hour onward. Even when indomethacin was added back at 2 h, a full inhibition was not observed. In addition, the fever induced by intra-OVLT injection of SNAP was attenuated by pretreatment with indomethacin (50–400 μg, $P<0.05$; Figure 5b) in a dose-dependent manner. The fever induced by intra-OVLT injection of 8-Br-cyclic GMP was also attenuated by pretreatment with indomethacin (400 μg, $P<0.05$; Figure 5c). Control injection of vehicle into the OVLT of rabbits produced no significant change in body temperature ($n=6$; data not shown). However, micro-injection of indomethacin (400 μg, $n=6$) into the OVLT produced a slight decrease in body temperature (0.5°C, data not shown).

Studies with PGE_2

Like LPS, NO donors, or cyclic GMP analogue, intra-OVLT administration of PGE_2 (0.2 μg) produced fever in rabbits (Figure 6). The fever induced by PGE_2 was significantly enhanced, rather than attenuated, by pretreatment of rabbits with intra-OVLT injection of NOS inhibitors including aminoguanidine (1000 μg, $n=6$), L-NMMA (100 μg, $n=6$), and L-NIO (200 μg, $n=6$) ($P<0.05$; Figure 6).

Discussion

In conscious rabbits, injection into the OVLT of (i) LPS, (ii) several chemically different NO donors, (iii) the cyclic GMP analogue 8-Br-cyclic GMP or (iv) PGE_2 caused a dose-related increase in body temperature (fever), which appeared to be secondary to a decrease in heat loss due to peripheral vasoconstriction and/or increased heat production (thermogenesis) secondary to shivering. The LPS-induced fever was attenuated by Dex, a potent inhibitor of the transcription of iNOS (Rees *et al.*, 1990; Szabó *et al.*, 1994; Wu *et al.*, 1995), as well as the

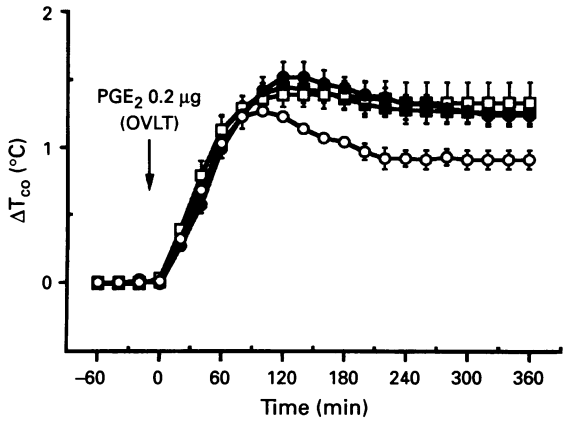

Figure 6 Effects of NO synthase inhibitors on the febrile responses produced by PGE_2 in rabbits at an ambient temperature of 22±1°C. At the time indicated by the arrow (↓), PGE_2 solutions were injected into the OVLT. An equivalent volume of saline (0.9%, ○, $n=12$), aminoguanidine (1000 μg, ●, $n=6$), L-NMMA (100 μg, □, $n=6$), or L-NIO (200 μg, ■, $n=6$) was given 1 h before the PGE_2 injection. Points represent the mean (±s.e.mean) change in temperature (Δ°C) for n experiments. The increase in body temperature caused by PGE_2 was significantly increased by aminoguanidine, L-NMMA or L-NIO from 120 to 360 min

protein synthesis inhibitor, anisomycin. This suggests that transcription of the iNOS gene and protein synthesis in the OVLT of the rabbit brain are involved in the development of fever induced by intra-OVLT administration of LPS. In addition, the fever induced by intra-OVLT injection of LPS was attenuated by several NOS inhibitors, including aminoguanidine, L-NMMA, and L-NIO. In contrast, pretreatment with intra-OVLT injection of L-NAME (1000 μg) did not attenuate the LPS-induced fever. Similarly, the IL-1-induced fever was not affected by intracerebroventricular administration of a high dose (5000 μg) of L-NAME in rabbits (Kapás *et al.*, 1994). L-NAME and L-NMMA are equally potent inhibitors of eNOS from porcine endothelial cells (Rees *et al.*, 1990). Aminoguanidine is more than 20 fold less potent than L-NMMA on the activity of nNOS purified from rat cerebellum (Misko *et al.*, 1993) and 40 fold less potent than L-NMMA in blocking eNOS mediated vasodilatation in rats (Corbett *et al.*, 1992). In contrast, aminoguanidine is a 2 fold more potent inhibitor of iNOS from LPS-treated RAW 264.7 macrophages than L-NMMA (Misko *et al.*, 1993), and L-NAME is a 3 to 5 fold less potent inhibitor of iNOS in the murine macrophage cell-line J774 compared to L-NMMA (McCall *et al.*, 1991). Hence, aminoguanidine preferentially inhibits iNOS while L-NAME is the most powerful inhibitor of eNOS. These observations suggest that NO produced by the iNOS in the OVLT mediates the LPS-induced fever in rabbits.

In addition, the present results show that pretreatment with methylene blue, an inhibitor of NOS and soluble guanylate cyclase (Ignarro 1984; Mayer *et al.*, 1993) or LY-83583, an inhibitor of soluble guanylate cyclase and NO release (Mülsch *et al.*, 1988; Schmidt *et al.*, 1985) attenuate the fever induced by intra-OVLT administration of LPS or SNAP. This suggests that the NOS-dependent production of cyclic GMP in the OVLT is involved in pyrogenic responses. The contention is supported by the findings reported by many investigators. For example, several kinds of reticuloendothelial cells are found in the OVLT regions. The intravenous injection of LPS caused the synthesis of IL-1 in the OVLT during fever in rabbits (Nakamori *et al.*, 1994). The present results also show that intra-OVLT injection of 8-Br-Cyclic GMP, a membrane permeable analogue of cyclic GMP, also causes a febrile response. The effects of NO on PG production have to date been described as cyclic GMP-independent (Salvemini *et al.*, 1993), yet the pyrogenic effect of SNAP obtained in the present results appears to be cyclic GMP-dependent. In fact, NO binds to the haeme-Fe^{2+} prosthetic group of the soluble guanylate cyclase and results in increase in the levels of cyclic GMP (Mellion *et al.*, 1981; Ignarro, 1991). This increase in cyclic GMP activates phospholipase A_2 to provide arachidonic acid, the substrate for conversion by the activated COX to PGE_2 (Canteros *et al.*, 1995). Thus, the synergistic production of prostaglandins by NO and cyclic GMP may be involved in the pyrogenic effect of SNAP.

Arachidonate metabolites, mostly prostaglandins, are thought to be involved in the central mechanism of the development of fever (Milton & Wendlandt, 1970; Veale & Cooper, 1974). It was also shown that the febrile responses of rats to prostaglandins were more potent when injected into the OVLT region than into the anterior hypothalamic area (Stitt, 1991). Furthermore, a significantly higher incidence of inhibitory responses to prostaglandins was found among the temperature-sensitive neurones in the OVLT than in the hypothalamic region (Matsumura *et al.*, 1990; Matsuda *et al.*, 1992). From these lines of evidence it may be inferred that prostaglandin production is induced by circulating pyrogens somewhere in the OVLT region. This hypothesis is strengthened by our results. Indeed, in the present study, the fever induced by intra-OVLT injection of either LPS, NO donors, or 8-Br-cyclic GMP was attenuated by pretreatment with intra-OVLT injection of indomethacin (an inhibitor of COX). In contrast, intra-OVLT administration of NOS inhibitors failed to attenuate the fever induced by intra-OVLT injection of PGE_2 in rabbit. Inhibition of guanylate cyclase with methylene blue or interaction of NO with haemoglobin (Kanner *et al.*, 1992) also attenuated the fever induced by a NO donor. NO is derived from L-arginine by the enzyme NOS whereas COX converts arachidonic acid to prostaglandins. The inducible isoforms (iNOS and COX-2) are not normally expressed but are induced following stimulation with LPS (Fu *et al.*, 1990; Rees *et al.*, 1990; Moncada *et al.*, 1991; Masferrer *et al.*, 1992; Szabó *et al.*, 1994; Wu *et al.*, 1995). Many effectors of NO production lead to the simultaneous release of mediators (such as PGE_2) from the COX pathway. This is true for the agents such as LPS or IL-1β (Salvemini *et al.*, 1990; Stabler *et al.*, 1991). NO or PGE_2 increases the levels of cyclic GMP or cyclic AMP in effector cells. This synergistic effect may be one mechanism(s) through which the NOS and COX systems operate to induce a pyrogenic response. Another possible interaction is at the level of the enzyme. In this respect, the COX enzymes are potential targets for NO because they contain an iron-haeme centre at their active site (Hemler & Lands, 1976; Kalyanaraman *et al.*, 1982). We therefore propose that the LPS-induce fever or prostaglandin production is due to the induction of COX-2 (which would also be prevented by dexamethasone) and/or secondary to an activation of either COX-1 or COX-2 by NO (produced by iNOS). Indomethacin is a more potent inhibitor of COX-1 than COX-2 (Mitchell *et al.*, 1993). Thus it is conceivable that the early inhibition by indomethacin (within the first 2 h) of the febrile response elicited by LPS is due to inhibition of COX-1 activity, while the lack of effect of indomethacin on the late febrile response is due to the inability of indomethacin to inhibit COX-2 activity.

The fever induced by intracerebroventricular injection of PGE_2 in rabbits is blocked by an EP_1 receptor antagonist (Cranston *et al.*, 1976). In a human erythroleukaemia cell line, EP_1 receptor subtypes activate G proteins (Schwaner *et al.*, 1995). In addition, NO exerts its neuronal effects through modification of G proteins (Hess *et al.*, 1994). Thus, it appears that NOS inhibition acts through modification of the G proteins-mediated EP_1 receptor sensitivity to potentiate the pyrogenic effect of PGE_2 in rabbits.

In the present results, the fever induced by intra-OVLT injection of LPS was mimicked by intra-OVLT injection of NO donors, a cyclic GMP analogue, or PGE_2. The LPS-induced fever was attenuated by intra-OVLT injection of either a transcription inhibitor, a protein synthesis inhibitor, NOS inhibitors, a guanylate cyclase inhibitor, a cyclic GMP inhibitor,

or a COX inhibitor. Aminoguanidine (a specific iNOS inhibitor) inhibits the LPS-induced fever, while L-NAME (a specific eNOS inhibitor) does not affect the LPS-induced fever. Furthermore, the fever induced by NO donors was attenuated by intra-OVLT injection of methylene blue, haemoglobin, or indomethacin. The 8-Br-cyclic GMP-induced fever was also attenuated by indomethacin. It is concluded that LPS may act through the NOS-COX pathways in the OVLT region of rabbit brain to induce febrile responses.

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