British Journal of Pharmacology (1996) 117, 684-688

Development of hyperthermia following intracerebroventricular administration of endotoxin in the rat: effect of kinin B_1 and B_2 receptor antagonists

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1 *E. coli* lipopolysaccharide (LPS) produced a dose-dependent (dose range: 0.02-150 μg) increase in rat core temperature that was maximal 6 h after intracerebroventricular (i.c.v.) administration. LPS (200 ng) increased core temperature by 1.0±0.2°C, 6 h following administration, as compared to vehicle-treated controls (−0.2±0.2°C).

2 LPS-induced (200 ng) hyperthermia was prevented by co-administration of the bradykinin (BK) B_2 receptor antagonist, Hoe 140 (10 and 30 pmol, i.c.v.) or by indomethacin (10 nmol, i.c.v.).

3 Systemic administration of Hoe 140 at doses up to 1 μmol kg^{-1}, s.c., did not attenuate LPS-induced (200 ng, i.c.v.) hyperthermia. However, LPS hyperthermia was significantly reduced by systemic administration of indomethacin (1 μmol kg^{-1}, i.v.).

4 Co-administration of the selective B_1 receptor antagonists, [des-Arg^9, Leu^8]BK (0.1-1 nmol, i.c.v.) or [des-Arg^{10}] Hoe 140 (0.1-1 nmol, i.c.v.), did not prevent LPS-induced hyperthermia.

5 It is concluded that the development of hyperthermia following central administration of endotoxin requires activation of central, but not peripheral bradykinin B_2 receptors. The formation of kinins within the CNS may be an important initial component of CNS inflammation following infection.

Keywords: Kinin; bradykinin receptors; hyperthermia; lipopolysaccharide; cerebral inflammation

Introduction

Bradykinin (BK) and its active metabolite, [des-Arg^9]BK, are peptides that are produced at the site of tissue injury or infection (Bhoola *et al.*, 1992) where they induce a variety of pro-inflammatory effects through the activation of specific B_2 or B_1 receptors, respectively. The action of bradykinin has been extensively studied in models of peripheral inflammation. Stimulation of B_2 receptors in peripheral tissues has been shown to mimic many aspects of the acute inflammatory response including vasodilatation, oedema formation and pain (see Hall, 1992; Bhoola *et al.*, 1992; Dray & Perkins, 1993). Furthermore, B_2 receptor stimulation causes the release of a number of other pro-inflammatory mediators, such as arachidonic acid metabolites (Nielsen *et al.*, 1988; Burch & Tiffany, 1989; Gecse *et al.*, 1989; Rang *et al.*, 1991). Unlike the B_2 receptor, which is constitutively expressed in many tissues, the B_1 receptor is normally expressed to a very limited extent. However, many studies have now demonstrated that during chronic inflammation the expression and activation of the B_1 receptor is increased (Regoli *et al.*, 1986; Hall, 1992; Perkins *et al.*, 1993; Perkins and Kelly, 1994; Davis & Perkins, 1994).

The brain and spinal cord contain all of the components necessary for kinin formation, including kinin precursors, and the necessary activation and degradation enzymes (see Walker *et al.*, 1995b). In addition, autoradiographic studies have identified specific bradykinin binding sites in the brain and spinal cord (Fujiwara *et al.*, 1989; Privitera *et al.*, 1992). Kinin formation and activity has been demonstrated during different forms of CNS trauma, including brain lesion injury and cerebral ischaemia (Maier-Hauff *et al.*, 1984; Unterberg *et al.*, 1986; Ellis *et al.*, 1987, 1988; Kamiya *et al.*, 1990, 1993; Wahl *et al.*, 1993).

Bradykinin is also a pyrogenic agent (Rao & Bhattacharya, 1988; Pela *et al.*, 1995). Intracerebroventricular administration of bradykinin has been shown to produce a dose-related increase in the core body temperature of rats that can be reduced by inhibiting brain 5-hydroxytryptamine (5-HT) or prostaglandin activity (Rao & Bhattacharya, 1988). However, while a large number of peptides have been shown to induce hyperthermia when injected directly into the brains of animals, few of them have subsequently been shown to have a physiological role in thermogenesis (see Rothwell, 1994). In order to dermine whether kinins are important in thermogenesis it is first necessary to demonstrate kinin receptor activation in a model of endotoxin-induced hyperthermia (see Rao & Bhattacharya, 1988; Pela *et al.*, 1995). In this study we address this issue directly by examining the effects of kinin B_1 and B_2 receptor blockade in endotoxin-induced hyperthermia. Furthermore, since kinin receptors are present both in the CNS and widespread throughout the periphery, it would be valuable to determine whether centrally and/or peripherally located kinin receptors contribute to the development of fever in this model. These findings have been published in abstract form (Walker *et al.*, 1995a).

Methods

Animals and experimental procedures

Male Sprague-Dawley rats (160-200 g) from Charles River Ltd. (Kent, UK) were housed in groups of 6 in a temperature-controlled room (24°C), that was kept on a 12/12 h light/dark cycle. Experiments were performed during the light phase of the cycle. Animals were provided with food and water *ad libitum*.

Standard stereotaxic surgical techniques were used to administer intracerebroventricular (i.c.v.) injections of lipopolysaccharide (LPS), test compounds or vehicle solution to rats. Rats were anaesthetized with enflurane throughout the microinjection procedure (duration: 5-10 min). Solutions were

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injected via a sterile stainless steel 30 guage needle attached to a Hamilton syringe with a Kopf (Tujunga, CA, U.S.A.), model 5000, Micro Injector Unit that was fixed to a Kopf stereotaxic frame. The injection needle tip was placed at coordinates: 0.8 mm posterior to bregma, 1.4 mm lateral to the sagittal suture and 3.7 mm below the surface of the skull in the right lateral ventricle. Following the removal of the injection needle the hole in the skull was sealed with sterile bone wax and the scalp was sutured. Immediately following the i.c.v. injection, while still anaesthetized, rats received an i.v. drug or vehicle injection via the tail vein.

A Physitemp (Clifton, NJ, U.S.A.) RET-2 rectal probe for rats connected to a Physitemp BAT-12 microprobe thermometer was used to measure rat core body temperature before and at 2, 4, 6, 8 or 24 h following i.c.v. injections of LPS.

Drugs

E. coli lipopolysaccharide (Sigma, serotype 0111:B4) was administered in saline (sterile, pyrogen free 0.9% saline, Sigma) and dilutions were made up in silicon coated vials. Hoe 140 (D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK; synthesized at the Sandoz Institute for Medical Research), [des-Arg^{10}] Hoe 140 (Peninsula Labs, Europe) and [des-Arg^9, Leu^8]BK (Bachem A.G.) were administered in saline (as above). Indomethacin (Sigma) was dissolved in 2% Na_2CO_3 (buffered to pH 7 with NaH_2PO_4). Compounds were administered either i.v. (injection volume = 1 ml kg^{-1}) or co-administered i.c.v. with LPS (injection volume = 10 μl).

Statistical analyses

Results were confirmed by parametric overall analyses of variance (ANOVA) followed by individual group comparisons (Tukey, Dunnetts, Students t test; $P<0.05$) according to the methods described by Kirk (1968). The difference between the pre- and post-treatment scores were calculated for each rat and the data are presented as the mean ± s.e.mean change from pre-treatment baseline.

Results

Development of hyperthermia following i.c.v. LPS administration

Figure 1a illustrates the time course of LPS-induced hyperthermia. A significant increase ($P<0.001$) in rat core temperature was produced by LPS following i.c.v. administration of doses from 2 ng (4 h following administration) to 150 μg (2–24 h following administration) with the highest dose producing the greatest overall increase in core temperature. The temperatures of all LPS-treated groups had returned to baseline 24 h following administration, except for the highest dose (150 μg) which had returned to baseline by 48 h after administration (Figure 1a). Comparisons of individual groups ($P<0.05$) indicated that the maximal increase in body temperature was produced 4–6 h after administration. A linear regression ANOVA indicated a significant linear effect of LPS dose (correlation coefficient: $r=0.78$; $F_{1,34}=55.71$, $P<0.001$) on hyperthermia 4 h following LPS administration (Figure 1b).

In order to compare the effects of central vs. systemic routes of LPS administration on the development of hyperthermia, subcutaneous administration of LPS was tested in two additional groups of rats, as shown in Figure 2. When administered s.c. LPS doses of 0.15 or 150 μg both produced a significantly smaller increase ($P<0.001$) in core temperature 4 (0.2 μg, i.c.v.: 1.0 ± 0.2°C; 0.2 μg, s.c.: −0.08 ± 0.1°C; 150 μg, i.c.v.: 1.6 ± 0.2°C; 150 μg, s.c.: 0.8 ± 0.1°C) or 6 h (Figure 2) following administration, as compared to the effects of the same doses following i.c.v. administration.

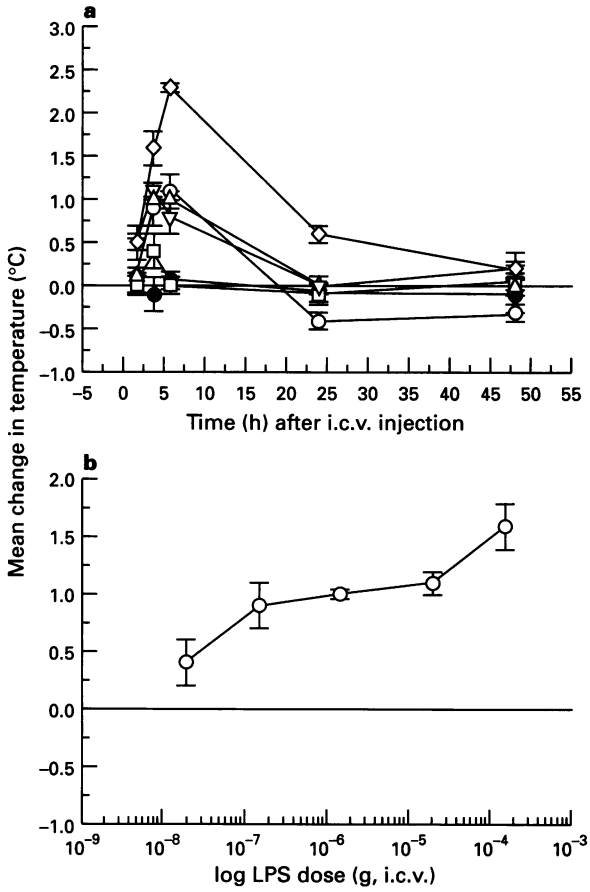

Figure 1 Mean (±s.e.mean) change in rat core body temperature at 2, 4, 6, 24 and 48 h following (a) or 4 h following (b) i.c.v. administration of various doses of LPS or vehicle. (a) Depicts the time course of core temperature changes in groups treated with different doses of LPS: 0.02 μg (□), 0.15 μg (○), 1.5 μg (▽), 15 μg (△), and 150 μg (◇) or vehicle (●); (b) depicts the effect of LPS dose on changes in core temperature in these same dose groups 4 h following LPS administration. *$P<0.05$ compared to the vehicle-treated control ($n=6$/group).

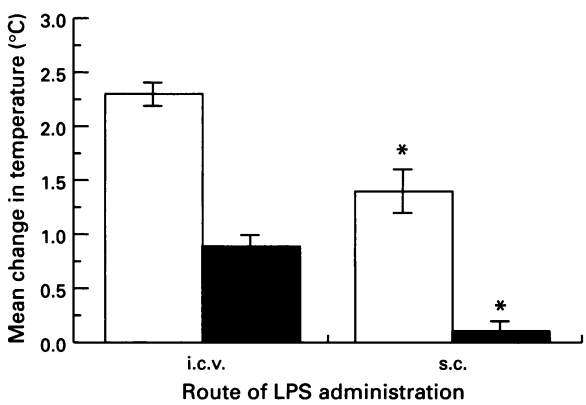

Figure 2 Mean (±s.e.mean) change in rat core body temperature 6 h following either i.c.v. or s.c. administration of 150 μM (open columns) or 0.15 μM (solid columns) LPS. *$P<0.05$ compared to i.c.v. treated group. ($n=6$/group).

Effect of bradykinin B_2 receptor antagonist treatment on LPS-induced hyperthermia

LPS- (0.2 μg, i.c.v.) induced an increase in rat core temperature 4 (0.9 ± 0.2°C) and 6 h (Figure 3) after i.c.v. administra-

tion. Co-administration of LPS with the selective bradykinin B_2 receptor antagonist, Hoe 140 (10 or 30 pmol, i.c.v.) significantly prevented LPS-induced hyperthermia up to 6 h after administration. ANOVA revealed a significant effect of Hoe 140 treatment ($P<0.001$) and comparisons of individual groups vs. the LPS-treated group indicated a significant ($P<0.05$) inhibition of LPS-induced hyperthermia by 10 and 30 pmol Hoe 140 4 h (Hoe 140, 10 pmol: 0.08 ± 0.1°C; Hoe 140 30 pmol: 0.2 ± 0.3°C) 6 h (Figure 3) after administration. Co-administration of LPS and indomethacin (10 nmol, i.c.v.) also prevented the rise in core temperature 6 h following administration (Figure 3). Administration of Hoe 140 (30 pmol, i.c.v.) or indomethacin (10 nmol, i.c.v.) alone did not produce a significant change from baseline core temperature ($n=6$/group; data not shown).

Table 1 shows the effects of systemically administered Hoe 140 or indomethacin on the development of hyperthermia in rats 6 h following i.c.v. administration of LPS (0.2 μg, i.c.v.). All LPS-treated groups displayed significant increases in core temperature and there was no significant effect of systemic Hoe 140 treatment up to 6 h following administration. In contrast to the effects of systemically administered Hoe 140, i.v. administered indomethacin (1 μmol kg^{-1}) significantly reversed LPS-induced hyperthermia ($P<0.001$) at 4 and 6 h following administration. Administration of indomethacin or Hoe 140 alone (1 μmol kg^{-1}, i.v.) had no effect on baseline core temperature (Table 1).

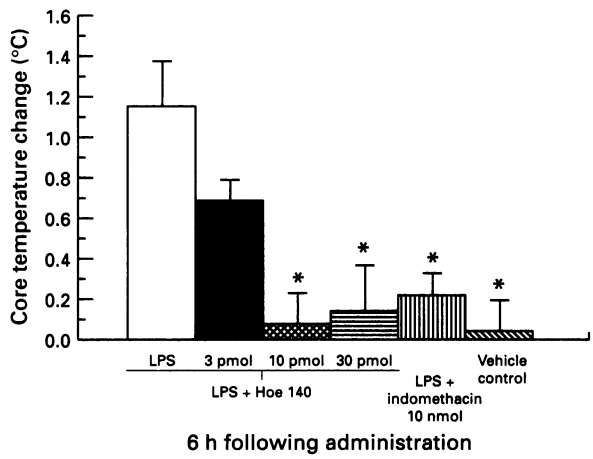

Figure 3 Mean (±s.e.mean) change in rat core body temperature 6 h following i.c.v. administration of 200 ng LPS, vehicle or i.c.v. co-administration of 200 ng LPS and various doses of Hoe 140 (3, 10 and 30 pmol) or indomethacin (10 nmol). *$P<0.05$ compared to the group treated with LPS alone ($n=6$/group).

Table 1 Mean (±s.e.mean) change in rat core temperature (°C) following central administration of LPS and systemic administration of Hoe 140 or indomethacin

Treatment group	*6 h*
LPS (0.2 μg, i.c.v.)	0.9 (±0.2)
Vehicle control	0 (±0.1)*
LPS + Hoe 140 (0.01 μmol kg^{-1}, i.v.)	0.7 (±0.1)
LPS + Hoe 140 (0.1 μmol kg^{-1}, i.v.)	0.7 (±0.1)
LPS + Hoe 140 (1 μmol kg^{-1}, i.v.)	0.9 (±0.2)
Saline (i.c.v.) + Hoe 140 (1 μmol kg^{-1}, i.v.)	−0.2 (±0.1)*
LPS + indomethacin (0.01 μmol kg^{-1}, i.v.)	1.3 (±0.2)
LPS + indomethacin (0.1 μmol kg^{-1}, i.v.)	0.7 (±0.3)
LPS + indomethacin (1 μmol kg^{-1}, i.v.)	−0.2 (±0.2)*
Saline (i.c.v.) + indomethacin (1 μmol kg^{-1}, i.v.)	−0.2 (±0.2)*

*$P<0.05$ compared to the LPS-treated group. ($n=6$/group)

Effect of i.c.v. co-administration of bradykinin B_1 receptor antagonist on LPS-induced hyperthermia

Figure 4 illustrates the development of hyperthermia following i.c.v. administration of LPS alone, or 6 h after the co-administration of LPS with one of two selective B_1 receptor antagonists, [des-Arg^9, Leu^8]BK or [des-Arg^{10}] Hoe 140. Neither of the B_1 antagonists produced a significant reversal of LPS-induced hyperthermia at 2–6 h following administration. Administration of either [des-Arg^9, Leu^8]BK (1 nmol, i.c.v.) or [des-Arg^{10}] Hoe 140 (1 nmol, i.c.v.) alone had no effect on baseline core temperature ($n=12$/group; data not shown).

Discussion

Bacterial endotoxin, when administered i.c.v., produces a rapid increase in body temperature that reaches a maximum between 4–8 h after administration. The time course, magnitude and dose-related effect of LPS presented in this study are consistent with previous reports of LPS-induced hyperthermia in rats (see Dascombe, 1985). Luheshi *et al.* (1993) reported that an i.c.v. injection of 1 μg LPS induced an increase in rat core temperature of approximately 1°C, that was maintained for up to 6–8 h following administration.

It has been previously established that lower doses of LPS are required to produce hyperthermia when administered centrally than when administered peripherally. Direct evidence indicates that the preoptic/anterior hypothalamic nuclei are the central locus for the pyrogenic response in various mammalian species (see Dascombe, 1985 for review). Accordingly, in this study LPS evoked a substantial and reliable increase in body temperature when administered i.c.v.; however, peripheral administration of the same doses produced a significantly smaller increase. The lower dose of LPS, 0.15 μg, produced a significant increase in body temperature only when administered i.c.v. The higher dose of LPS (150 μg) produced a significant increase in core temperature when administered either s.c. or i.c.v., although the increase was greater 6 h following i.c.v. administration. These results indicate that whereas hy-

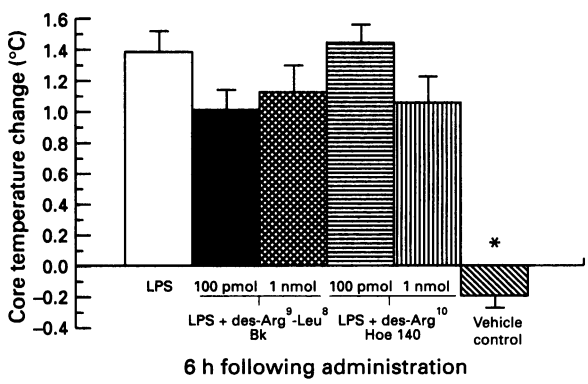

Figure 4 Mean (±s.e.mean) change in rat core body temperature 6 h following i.c.v. administration of 200 ng LPS, vehicle or i.c.v. co-administration of 200 ng LPS and either [des-Arg^9, Leu^8]BK (100 pmol and 1 nmol) or [des-Arg^{10}] Hoe 140 (100 pmol and 1 nmol). *$P<0.05$ compared to the group treated with LPS alone ($n=12$/group).

perthermia might result from leakage of 150 μg LPS into the systemic circulation, the hyperthermia induced by 0.15 μg LPS can only be explained by the induction of thermogenic mechanisms within the CNS. For this reason, we used only a lower dose of LPS to examine the effects of kinin receptor antagonists on LPS-induced hyperthermia. It is important to note that this lower dose (0.2 μg) of LPS has been shown to induce a pronounced acute inflammatory response characterized by the infiltration of neutrophils and macrophages within the ventricles, choroid plexus and subarachnoid space, but not the CNS parenchyma, following intracerebral or i.c.v. administration (Andersson *et al.*, 1992).

The present study provides direct evidence for the participation of kinins in LPS-induced thermogenesis. The role of central B_2 receptors in hyperthermia is also supported by the recent work of Pela *et al.* (1995) demonstrating that i.c.v. administration of 2–8 nmol of the highly potent and selective B_2 receptor antagonist, Hoe 140 (see Knolle *et al.*, 1992) also prevents the hyperthermia induced by systemically administered LPS. In this study, central co-administration of 10–30 pmol of Hoe 140 completely inhibited the development of hyperthermia following i.c.v. administration of LPS. Furthermore, the prevention of LPS-induced hyperthermia was produced by doses of Hoe 140 in the range that would be expected to reverse B_2 receptor-mediated effects if administered locally to an inflamed tissue. In peripheral models of inflammation, B_2 receptor-mediated effects were reversed by the local administration of Hoe 140 within the dose range of 1–10 pmol (Knolle *et al.*, 1992; Davis & Perkins, 1994; Davis *et al.*, 1994). Peripheral B_2 receptor-mediated effects could also be inhibited by intravenous administration of Hoe 140 in the dose range of 10–100 nmol kg^{-1} (Davis & Perkins, 1994). However, in the present study intravenous administration of doses of Hoe 140 up to 1 μmol kg^{-1} did not affect LPS-induced hyperthermia. Hoe 140, a peptide antagonist, was likely to be poorly bioavailable in the CNS following systemic administration. Accordingly, the differences in the effectiveness of Hoe 140 following central vs. systemic administration can be explained by its action at central, but not peripheral B_2 receptors. This is further illustrated by the observation that indomethacin (which crosses the blood-brain barrier) reversed hyperthermia when administered systemically. However, lower doses of indomethacin were more effective in reversing hyperthermia when administered i.c.v. Indomethacin and other non-steroidal anti-inflammatory drugs are well known for their anti-pyrogenic effects which are more effective following central administration (see Dascombe, 1985).

In contrast to the effect of the B_2 receptor antagonist, central administration of two selective B_1 receptor antagonists, [des-Arg^9, Leu^8]BK (Barabe *et al.*, 1980; Marceau & Regoli, 1991) or the highly potent [des-Arg^{10}] Hoe 140 (Wirth *et al.*, 1992), did not affect the hyperthermia induced by i.c.v. administered LPS. Co-administration of doses that have been shown to reverse peripheral B_1 receptor-mediated effects (Barabe *et al.*, 1980; Marceau & Regoli, 1991; Wirth *et al.*, 1992) did not reverse LPS-induced hyperthermia. Indeed, the potent and long lasting [des-Arg^{10}] Hoe 140 inhibited peripheral B_1 receptor-mediated vasodepression at systemic doses lower than those injected i.c.v. in the present study (Wirth *et al.*, 1992).

Although B_2 receptor antagonists are clearly effective in reversing the development of hyperthermia when administered in the initial or acute phase of LPS-induced cerebral inflammation, recent evidence from Pela *et al.* (1995) suggests a later induction of B_1 receptor-mediated hyperthermia following systemically administered LPS. Peripheral studies on B_1 receptor-mediated activity have demonstrated that while it is expressed to a limited extent in normal tissues, during a chronic or 'persistent' inflammation the expression of the B_1 receptor-mediated activity is increased (Regoli *et al.*, 1986; Hall, 1992; Perkins *et al.*, 1993; Davis & Perkins, 1994). Consistent with these peripheral studies it appears that although B_1 receptor-mediated responses are not detected following the acute co-administration of B_1 antagonists, they are detected at later stages of the CNS inflammatory response. Pela *et al.* (1995) demonstrated that 24 h following systemic administration of LPS i.c.v. administration of the B_1 receptor agonist [des-Arg^9]BK, but not BK itself, induced an increase in rat core temperature. Further experiments will determine respective roles of kinin B_1 and B_2 receptors in the induction and maintenance of endotoxin-induced hyperthermia.

All components of the kallikrein-kinin system, B_2 receptors and bradykinin-immunoreactivity are widely distributed throughout the rodent CNS (see Walker *et al.*, 1995b). Indeed, high concentrations of bradykinin- and kininogen-immunoreactivity have been demonstrated in the hypothalamus and pituitary (Kariya *et al.*, 1985; Richoux *et al.*, 1991) which coincides with the locus for thermoregulation. In addition, bradykinin increased core temperature in rats following i.c.v. administration (Rao & Bhattacharya, 1988; Pela *et al.*, 1995) and this hyperthermia appeared to be mediated by the formation of prostanoids and 5-HT, as the inhibition of 5-HT or prostanoid release prevented BK-induced hyperthermia (Rao & Bhattacharya, 1988). Kinins are potent stimulators of arachidonic acid metabolism in CNS microglia (Nielsen *et al.*, 1988; Burch & Tiffany, 1989; Gecse *et al.*, 1989) and intracerebral injections of bradykinin have been shown to increase brain prostaglandin synthesis (Bhattacharya *et al.*, 1986). Finally, the activation of the CNS immune system, and the production and release of pyrogenic cytokines, such as interleukin-1 (IL-1) IL-6 and tumour necrosis factor (TNFα) (Tiffany & Burch, 1989; Banati *et al.*, 1993), is known to be an important early component of thermogenesis (Hopkins & Rothwell, 1995; Rothwell & Hopkins, 1995; Rothwell, 1994). Kinin formation is also likely to be one of the early events, possibly preceding even the release of pyrogenic cytokines, following infection or injury within the CNS. The dramatic reversal of hyperthermia following the co-administration of endotoxin and a B_2 receptor antagonist in the present study supports this hypothesis.

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(Received May 31, 1995
Revised October 6, 1995
Accepted October 17, 1995)