Development of hyperthermia following intracerebroventricular administration of endotoxin in the rat: effect of kinin B₁ and B₂ receptor antagonists

1Katharine Walker, Andy Dray & Martin Perkins

Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN

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) B₂ 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 B₂ receptor antagonists, [des-Arg⁹, Leu⁸]BK (0.1 – 1 nmol, i.c.v.) or [des-Arg⁹]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 B₂ 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-Arg⁹]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 B₂ or B₁ receptors, respectively. The action of bradykinin has been extensively studied in models of peripheral inflammation. Stimulation of B₂ 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, B₂ 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; Geczy et al., 1989; Rang et al., 1991). Unlike the B₂ receptor, which is constitutively expressed in many tissues, the B₁ 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 B₁ 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 (Fujiiwara 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; Maier-Hauff 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 determine 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 B₁ and B₂ 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 anaesthetised with enflurane throughout the microinjection procedure (duration: 5–10 min). Solutions were

1 Author for correspondence.
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 anesthetized, 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-[Hyp3, Thr5, D-Tic7, Oic8]-BK; synthesized at the Sandoz Institute for Medical Research), [des-Arg4]Hoe 140 (Peninsula Labs, Europe) and [des-Arg4, Leu5]BK (Bachem A.G.) were administered in saline (as above). Indomethacin (Sigma) was dissolved in 2% Na2CO3 (buffered to pH 7 with NaH2PO4). 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 pretreatment 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; F1,34 = 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; 0.1 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.

Effect of bradykinin B2 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 B₂ 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: $0.08 \pm 0.1\textdegree C$; Hoe 140 30 pmol: $0.2 \pm 0.3\textdegree C$) 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).

**Effect of i.c.v. co-administration of bradykinin B₂ 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 B₂ receptor antagonists, [des-Arg⁹, Leu⁸]BK or [des-Arg⁹⁰]Hoe 140. Neither of the B₂ antagonists produced a significant reversal of LPS-induced hyperthermia at 2–6 h following administration. Administration of either [des-Arg⁹, Leu⁸]BK (1 nmol, i.c.v.) or [des-Arg⁹⁰]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-
peripheral B1 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 (Anderson et al., 1993).

The present study provides direct evidence for the participation of kinins in LPS-induced thermogenesis. The role of central B2 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 B2 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 B2 receptor-mediated effects if administered locally to an inflamed tissue. In peripheral models of inflammation, B2 receptor-mediated effects are observed 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 B2 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 μg kg⁻¹ did not affect LPS-receptor-mediated responses. 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 B2 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 Dascome, 1985).

In contrast to the effect of the B2 receptor antagonist, central administration of two selective B1 receptor antagonists, [des-Arg9-Leu²]BKB (Barabe et al., 1980; Marceau & Regoli, 1991) or the highly potent [des-Arg⁹]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 B1 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-Arg⁹]Hoe 140 inhibited peripheral B1 receptor-mediated vasodepression at systemic doses lower than those injected i.c.v. in the present study (Wirth et al., 1992).

Although B2 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 B2 receptor-mediated hyperthermia following systemically administered LPS. Peripheral studies on B2 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 B2 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 B1 receptor-mediated responses are not detected following the acute co-administration of B2 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 B2 receptor agonist [des-Arg⁹]BK, but not BK itself, induced an increase in rat core temperature. Further experiments will determine respective roles of kinin B1 and B2 receptors in the induction and maintenance of endotoxin-induced hyperthermia.

All components of the kallikrein-kinin system, B2 receptors and bradykinin-immunoreactivity are 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, 1992; Pela et al., 1995) and this hyperthermia appeared to be mediated by the formation of prostanooids and 5-HT, as the inhibition of 5-HT or prostanooid 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; Geese 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 B2 receptor antagonist in the present study supports this hypothesis.

References


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