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Central involvement of kinin B_1 and B_2 receptors in the febrile response induced by endotoxin in rats

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1 The effect of central injection of selective kinin B_1 and B_2 receptor antagonists on the febrile response induced by endotoxin (*E. coli* lipopolysaccharide, LPS) in rats was investigated.

2 Intracerebroventricular (i.c.v.) injection of a selective B_2 receptor antagonist (Hoe-140, 8 nmol) reduced the early (0–2 h), but increased the late phase (4–6 h) of the febrile response induced by intravenous (i.v.) injection of LPS (0.5 μg kg^{-1}).

3 Co-administration of Hoe-140 (8 nmol, i.c.v.) with LPS (0.5 μg kg^{-1}, i.v.), followed 2.5 h later by the i.c.v. injection of a selective B_1 receptor antagonist [des-Arg^9-Leu^8]-bradykinin (BK, 8 nmol), significantly reduced the febrile response induced by LPS throughout the whole experimental period.

4 Intravenous injection of Hoe-140 (1 mg kg^{-1}) significantly reduced the febrile response induced by LPS (0.5 μg kg^{-1}, i.p.).

5 Pretreatment (24 h) with LPS (0.5 μg kg^{-1}, i.v.) reduced the febrile response induced by BK or [Tyr^8]-BK (both, 5 nmol, i.c.v.), but markedly increased the febrile response induced by [des-Arg^9]-BK (5 nmol, i.c.v.). The response induced by [des-Arg^9]-BK in LPS-pretreated rats was significantly inhibited by co-injection of [des-Arg^9-Leu^8]-BK (15 nmol, i.c.v.).

6 The results suggest that kinins are involved in the induction of LPS-induced fever and that central B_2 and B_1 receptors are activated during the initial and late phase of this response, respectively. The results also suggest that downregulation and/or desensitization of B_2 receptors and induction and/or upregulation of B_1 receptors in LPS-pretreated animals may have a significant pathophysiologcal role in the induction and maintenance of fever. These observations may be specifically important in the case of chronic inflammatory conditions, because the BK metabolite [des-Arg^9]-BK, so far considered an inactive metabolite, acquires an active and relevant role with the progressive expression of B_1 receptors that occurs in such states.

Keywords: Kinins; fever; lipopolysaccharide; B_1 and B_2 receptors; B_1 and B_2 agonists and antagonists

Introduction

Bradykinin (BK), its precursor and the enzymes involved in its synthesis and inactivation have been characterized in the central nervous system (CNS) (for a review see Bhoola *et al*., 1992). The suggestion that BK acts as a modulator and neurotransmitter in the CNS (Graeff, 1971; Kariya *et al*., 1985; Miller, 1987) has been further strengthened by the demonstration of the existence of high density BK-immunoreactive neurones in the hypothalamus, mainly the paraventricular and dorsomedial nuclei, and in several other brain structures (Corrêa *et al*., 1979; Perry & Snyder, 1984; Kariya *et al*., 1985).

In addition to the B_1 and B_2 receptors described by Regoli and Barabé (1980), recent studies have suggested the existence of B_{2A} and B_{2B} receptor subtypes (Regoli *et al*., 1993). The complementary DNA encoding the B_2 receptor has been cloned in tissues of several animal species, including mouse (McIntyre *et al*., 1993; Hess *et al*., 1994), rat (McEachern *et al*., 1991) and man (Hess *et al*., 1992; Eggerickx *et al*., 1993; Powell *et al*., 1993). B_2 receptors are widely distributed in the periphery and CNS and seem to mediate most of the kinin actions under normal conditions. They exhibit high affinity for BK, Lys-BK and [Tyr^8]-BK (Bhoola *et al*., 1992; Farmer & Burch, 1992; Hall, 1992; Regoli *et al*., 1993) and can be selectively and competitively antagonized by several, potent B_2 receptor antagonists, including Hoe-140 (Lembeck *et al*., 1991). Although a human B_1 receptor has been recently cloned from lung fibroblasts (Menke *et al*., 1994), this receptor is normally restricted to some tissues and expressed during pathological states, after tissue injury, or during *in vitro* incubation. B_1 receptors exhibit greater affinity for the kinin metabolites [des-Arg^9]-BK and [des-Arg^{10}]-kallidin than BK and can be selectively and competitively antagonized by the B_1 receptor antagonists [des-Arg^9-Leu^8]-BK or [des-Arg^9-Leu^8]-kallidin.

Central injection of BK has been shown to induce many responses, including behavioural excitability followed by sedation (Graeff *et al*., 1969), noradrenaline depletion (Graeff *et al*., 1969), antidiuretic effects (Rocha e Silva & Malnic, 1964; Hoffman & Schimid, 1978), catatonia (Da Silva & Rocha e Silva, 1971), hypertension (Corrêa & Graeff, 1974; Lindsey *et al*., 1989; Fior *et al*., 1993), antinociception (Ribeiro *et al*., 1971; Ribeiro & Rocha e Silva, 1973; Laneuville & Couture, 1987; Laneuville *et al*., 1989; Pelá *et al*., 1996) and antiaversive effect (Burdin *et al*., 1992).

Indirect evidence showing the involvement of BK in the regulation of body temperature and induction of fever has also been obtained. Pelá *et al*. (1975) demonstrated that the kinin-like activity, mainly in the hypothalamus, decreased during lipopolysaccharide (LPS)-induced fever in rabbits, suggesting the involvement of the brain kininogen-kinin system in this response. In addition, an increase in body temperature induced by intracerebroventricular (i.c.v.) injection of BK has also been demonstrated in rabbits (Almeida e Silva & Pelá, 1978) and in rats (Mohan Rao & Bhattacharya, 1988). Recently, Walker *et al*. (1996) have addressed the involvement of kinin receptors in fever and demonstrated the involvement of kinin B_2 receptors in the response induced by endotoxin. However, these authors

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have used the i.c.v. route for the injection of endotoxin but, according to Stitt & Bernheim (1985), the febrile response obtained by this route may not be compared to the response induced by the i.v. route, because there are several distinct differences in the characteristics of the two fevers.

The present study aimed to investigate the effect of selective kinin B_1 and B_2 receptor antagonists on the febrile response induced by i.v. LPS in rats, in order to give further insights into the role of kinins in this response and the receptor subtypes involved.

Methods

Animals

Male Wistar rats with body weights ranging from 180–200 g were used. The animals were housed at 24±1°C and kept in a 12 h light : 12 h dark cycle (lights on at 06 h 00 min). The animals had access to water and standard laboratory rat diet throughout *ad libitum*.

Surgery

Under pentobarbitone anaesthesia (40 mg kg^{-1}, i.p.), a stainless steel guide cannula (0.8 mm outer diameter) directed to the right lateral ventricle was implanted stereotaxically according to the coordinates AP 0.8 mm, L 1.5 mm, and V 4 mm related to the bregma (Paxinos & Watson, 1986), one week before the experiments. Animals that showed weight loss, signs of infection or a misplaced cannula were excluded from the study. Drugs were injected i.c.v. in a volume of 2 μl over a period of 20 s.

Body temperature measurements

Colonic temperature was measured in unrestrained rats with a plastic coated thermistor probe inserted 5 cm beyond the anal sphincter and connected to a telethermometer (Yellow Spring Instruments, U.S.A.). Temperatures were measured for two hours before and up to five or six hours after treatment, at 30 or 60 min intervals. One day before the experiment, the rats were handled and colonic temperature measured to minimize stress-induced changes of body temperature. All experiments were conducted at the thermoneutral zone for rats (28±1°C) (Gordon, 1990) between 08 h 00 min and 17 h 00 min. Only animals with basal temperatures between 36.8 and 37.5°C were used. On the day of the experiment, the basal temperature of each animal was determined four times, at 30 min intervals, before each injection.

Drugs

E. coli lipopolysaccharide 0111 : B4 (LPS), [des-Arg^9-Leu^8]-BK, [des-Arg^9]-BK, [Tyr^8]-BK (Sigma); bradykinin (BK; Fundap, Escola Paulista de Medicina-EPM, Brazil or Sigma)

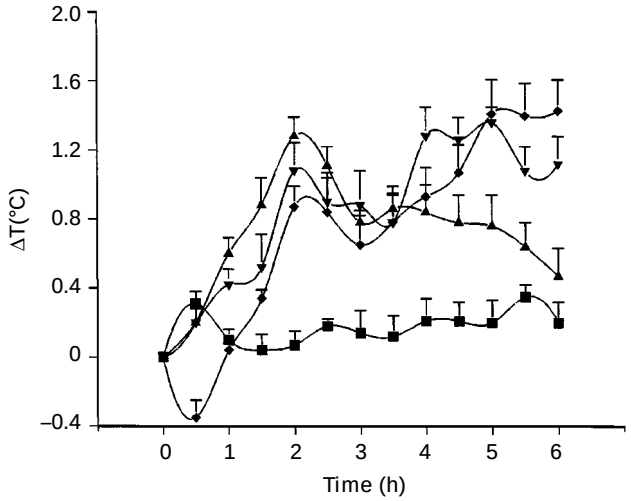

Figure 1 Effect of co-administration of Hoe-140 (4 or 8 nmol, i.c.v.) on the febrile response induced by LPS (0.5 μg kg^{-1}, i.v.) in rats. The figure indicates the time course of the response and the values represent change from baseline temperature; vertical lines show s.e.mean. (■) CSF 2 μl, i.c.v./saline 1 ml kg^{-1}, i.v. ($n=7$); (▲) CSF 2 μl, i.c.v./LPS ($n=7$); (▼) Hoe-140 4 nmol/LPS ($n=5$); (◆) Hoe-140 8 nmol/LPS ($n=7$). Basal temperatures of the groups were: 37.15±0.11, 36.98±0.11, 36.92±0.03, 37.04±0.05°C, respectively.

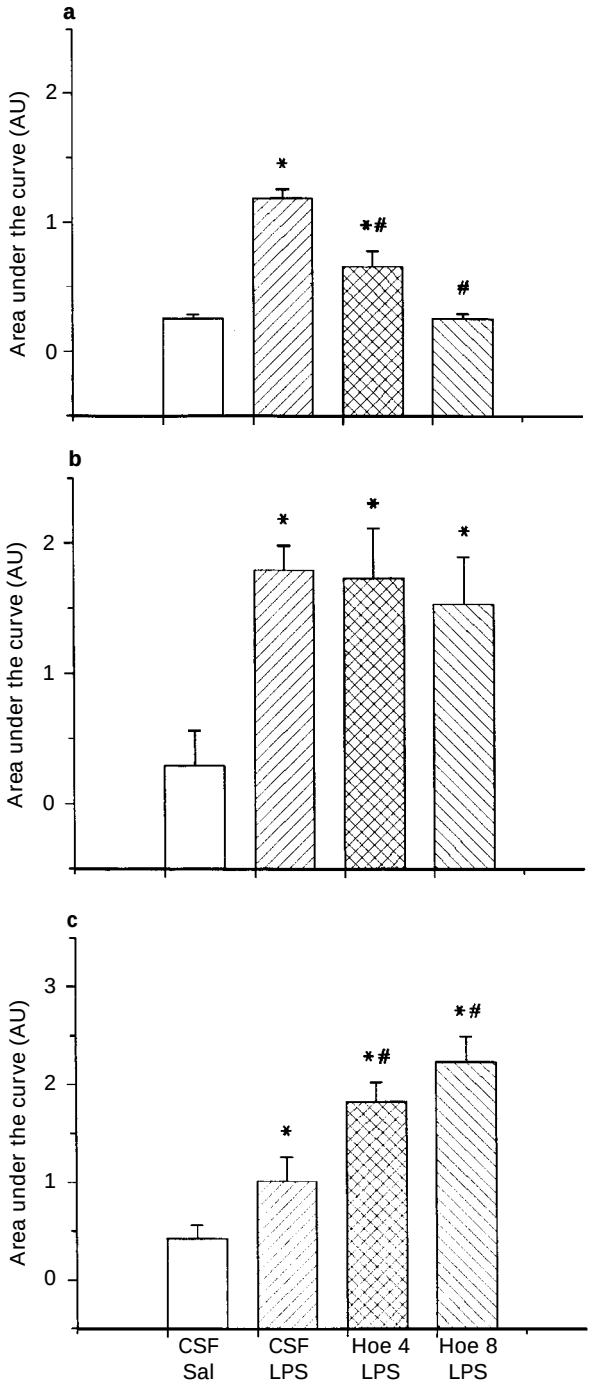

Figure 2 Effect of co-administration of Hoe-140 (4 or 8 nmol, i.c.v.) on the febrile response induced by LPS (0.5 μg kg^{-1}, i.v.) in rats. The panels represent the area under the curve at the specified times. The data were obtained from the results indicated in Figure 1. (a), (b) and (c) Represent the area under the curves of the groups shown in Figure 1 at 0–2 h, 2–4 h and 4–6 h, respectively. *Significantly different from Sal/Sal ($P<0.05$). # Significantly different from Sal/LPS ($P<0.05$).

and Hoe-140 (DArg{Hyp^3-Thi^5-DTic^7-Oic^8}BK) (kindly donated by Dr K.J. Wirsth - Hoechst, Germany) were used. Vehicle used was sterile saline from commercial sources and artificial cerebrospinal fluid (CSF).

Statistical analysis

Results are presented as means ± s.e.mean of temperature changes or area under the curves (expressed in computer arbitrary units - AU). Degree of significance was tested by one-way analysis of variance followed by Scheffe's test by use of SPSS statistical software. $P<0.05$ was considered significant for all comparisons.

Results

LPS (0.5 μg kg^{-1}, i.v.) induced a prolonged fever in rats, with a rapid onset and a peak after 2 h. Body temperature remained stable throughout the experiment in animals treated with vehicle (Figure 1). Co-administration of Hoe-140 (4 and 8 nmol, i.c.v.) significantly reduced the initial phase (0–2 h) (Figure 2a), did not change the intermediate phase (2–4 h) (Figure 2b) and significantly increased the late phase (4–6 h) (Figure 2c) of LPS-induced fever.

Co-administration of Hoe-140 (1 mg kg^{-1}, i.v.) significantly reduced the febrile response induced by intraperitoneal (i.p.) injection of LPS (0.5 μg kg^{-1}) (Figure 3).

Next, the effect of treatment with both B_1 and B_2 receptor antagonists on the febrile response induced by LPS (0.5 μg kg^{-1}, i.v.) was investigated. [des-Arg^9-Leu^8]-BK was injected 2.5 h after Hoe-140 for two main reasons: first, because Hoe-140 inhibited only the initial phase (0–2 h) of the response induced by endotoxin and, second, because it has been demonstrated that B_1 expression occurs later after the injection of an inflammatory stimulus (Campos *et al*., 1996). Figure 4 shows that the co-administration of Hoe-140 (8 nmol, i.c.v.), followed 2.5 h later by [des-Arg^9-Leu^8]-BK (8 nmol, i.c.v.), significantly reduced LPS-induced fever throughout the whole experimental period.

In order to investigate the effect of desensitization or induction of BK receptors following LPS treatment on the febrile response induced by BK and the B_1 agonist [des-Arg^9]-BK, the next experiments were carried out. The febrile

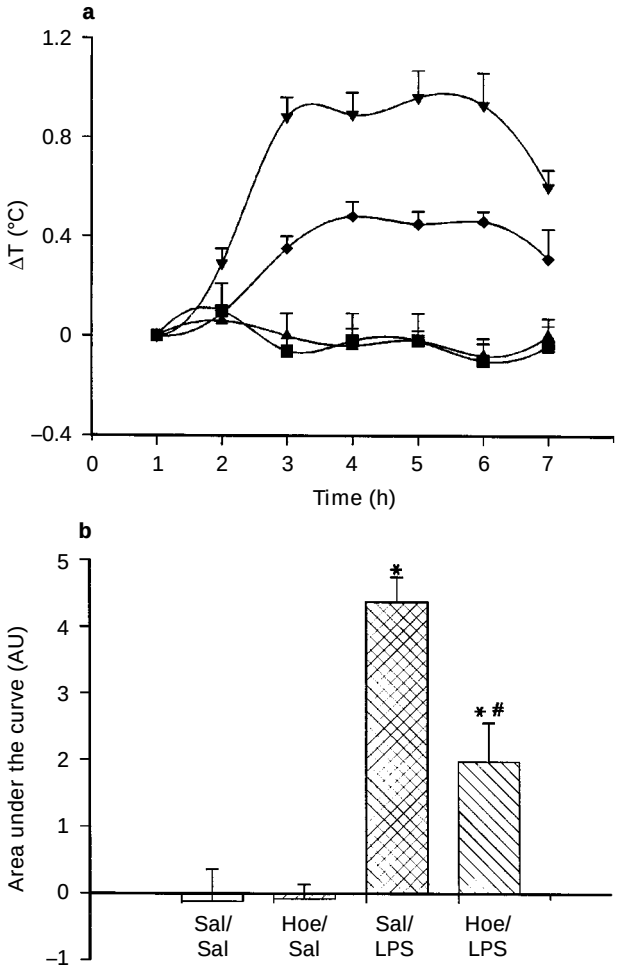

Figure 3 Effect of co-administration of Hoe-140 (1 mg kg^{-1}, i.v.) on the febrile response induced by LPS (0.5 μg kg^{-1}, i.p.). (a) Indicates the time course of the response and the values represent change from baseline temperature; vertical lines show s.e.mean. (b) Represents the area under the curve of the groups shown in (a). In (a): (■) saline 1 ml kg^{-1}, i.v./saline 1 ml kg^{-1}, i.p. ($n=5$); (▲) Hoe-140/saline 1 ml kg^{-1}, i.p. ($n=5$); (▼) saline 1 ml kg^{-1}, i.v./LPS ($n=8$); (◆) Hoe/LPS ($n=8$). Basal temperatures of the groups were: 37.32±0.04, 37.34±0.03, 37.34±0.02, 37.46±0.01 °C, respectively. *Significantly different from Sal/Sal ($P<0.05$). # Significantly different from Sal/LPS ($P<0.05$).

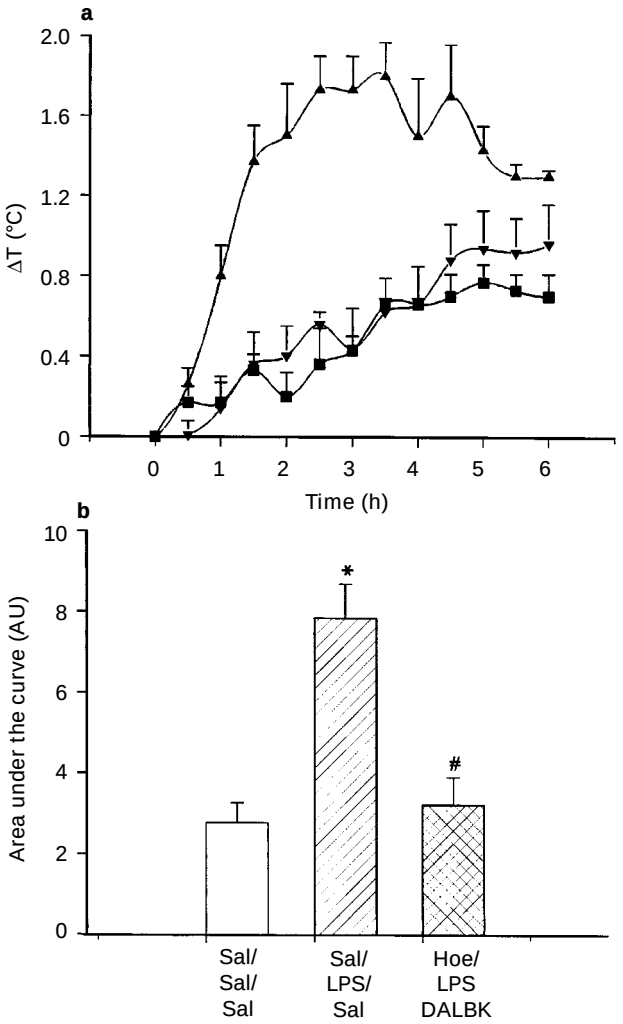

Figure 4 Effect of co-administration of Hoe-140 (8 nmol, i.c.v.), followed 2.5 h later by i.c.v. administration of [des-Arg^9-Leu^8]-BK (DALBK, 8 nmol), on the febrile response induced by LPS (0.5 μg kg^{-1}, i.v.). (a) Indicates the time course of the response and the values represent change from baseline temperature; vertical lines show s.e.mean. (b) Represents the area under the curve of the groups shown in (a). In (a) (■) saline 2 μl, i.c.v./saline 1 ml kg^{-1}, i.v. ($n=4$); (▲) saline 2 μl, i.c.v./LPS ($n=5$); ▼ Hoe-140/LPS/DALBK ($n=5$). Basal temperatures of the groups were: 37.16±0.08, 37.00±0.08, 37.14±0.01 °C, respectively. *Significantly different from Sal/Sal/Sal ($P<0.05$). # Significantly different from Sal/LPS/Sal ($P<0.05$).

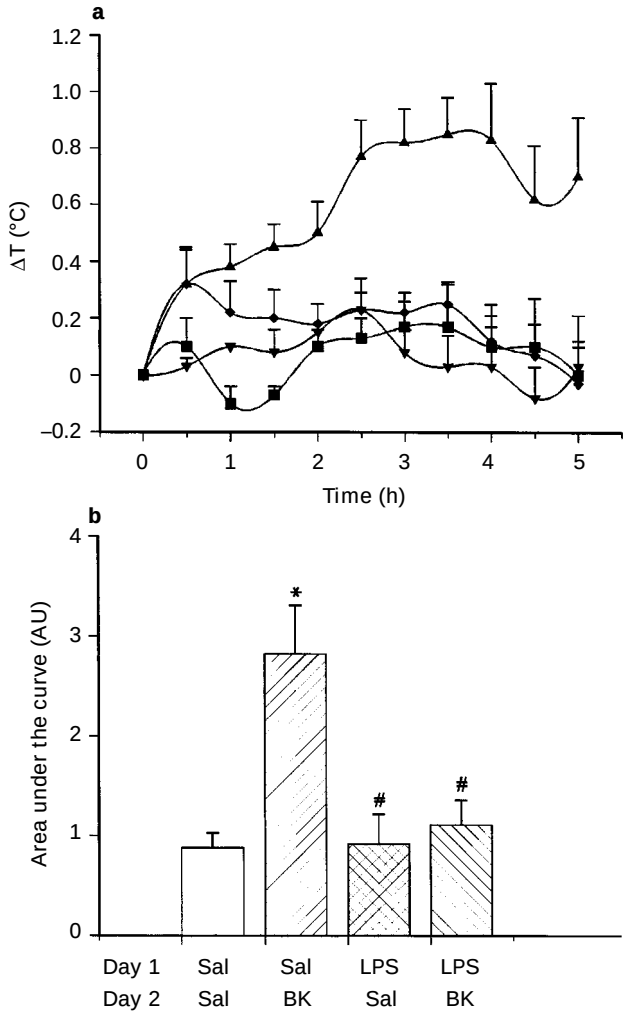

Figure 5 Effect of prior (24 h) treatment with LPS (0.5 μg kg^{-1}, i.p.) on the body temperature changes induced by BK (5 nmol, i.c.v.)., (a) Indicates the time course of the response and the values represent change from baseline temperature; vertical lines show s.e.mean. (b) Represents the area under the curve of the groups shown in (a). In (a) (■) saline 1 ml kg^{-1}, i.p./saline 2 μl, i.c.v. ($n=3$); (▲) saline 1 ml kg^{-1}, i.p./BK ($n=6$); (▼) LPS/saline 2 μl, i.c.v. ($n=4$); (◆) LPS/BK ($n=6$). Basal temperatures of the groups were: 37.27±0.13, 37.04±0.08, 37.18±0.08, 37.13±0.11 °C, respectively. *Significantly different from Sal/Sal ($P<0.05$). # Significantly different from Sal/BK ($P<0.05$).

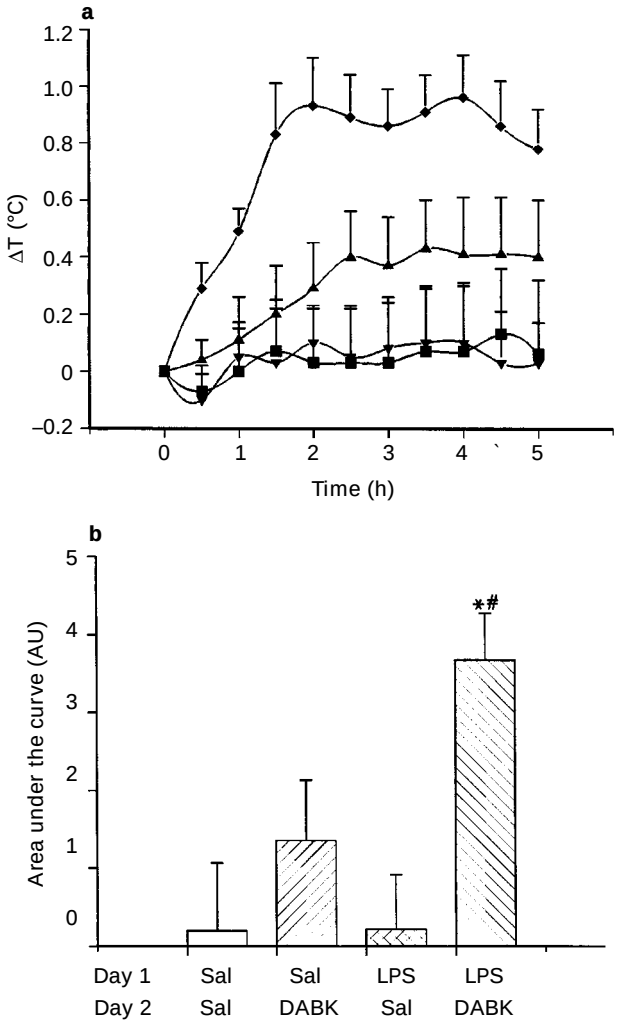

Figure 6 Effect of prior (24 h) treatment with LPS (0.5 μg kg^{-1}, i.p.) on the body temperature changes induced by [des-Arg^9]-BK (DABK, 5 nmol, i.c.v.). (a) Indicates the time course of the response and the values represent change from baseline temperature; vertical lines show s.e.mean. (b) Represents the area under the curve of the groups shown in (a). In (a) (■) saline 1 ml kg^{-1}, i.p./saline 2 μl, i.c.v. ($n=3$); (▲) saline 1 ml kg^{-1}, i.p./DABK ($n=7$); (▼) LPS/saline 2 μl, i.c.v. ($n=4$); (◆) LPS/DABK ($n=8$). Basal temperatures of the groups were: 37.06±0.02, 37.20±0.03, 37.25±0.03, 37.13±0.03 °C, respectively. *Significantly different from Sal/Sal ($P<0.05$). # Significantly different from Sal/DABK ($P<0.05$).

response induced by BK (5 nmol, i.c.v.) was significantly reduced in animals treated with LPS (0.5 μg kg^{-1}, i.v.) 24 h earlier (Figure 5). A similar reduction of the magnitude of fever by prior treatment with LPS was also observed when [Tyr^8]-BK, another selective B_2 agonist, was used (data not shown). However, the fever induced by [des-Arg^9]-BK (5 nmol, i.c.v.), a response of reduced magnitude in animals previously treated with saline, was markedly enhanced by prior (24 h) treatment with LPS (0.5 μg kg^{-1}, i.v.) (Figure 6). Co-administration of [des-Arg^9-Leu^8]-BK (15 nmol, i.c.v.) significantly reduced the febrile response induced by [des-Arg^9]-BK (5 nmol, i.c.v.) in animals pretreated with LPS (0.5 μg kg^{-1}, i.p.) (Figure 7).

Discussion

The present results show that activation of kinin B_2 and B_1 receptors in the CNS is relevant for the induction and maintenance of LPS-induced fever in rats.

Central injection of the selective B_2 receptor antagonist Hoe-140 resulted in an attenuation of the early phase and an increase of the late phase of LPS-induced fever in rats. However, i.c.v. co-administration of Hoe-140, followed 2.5 h later by i.c.v. injection of the B_1 receptor antagonist [des-Arg^9-Leu^8]-BK, significantly inhibited the febrile response induced by i.v. injection of LPS, throughout the whole experimental period analysed. These results suggest that a different temporal activation of central kinin receptors occurs during the febrile response induced by LPS, with the initial and late phases of this response involving activation of B_2 and B_1 receptors, respectively. As BK has been shown to interact mainly with B_2 receptors (Regoli *et al*., 1993), it seems likely that during the late phase of LPS-induced fever, B_1 receptors may be activated by one of the BK metabolites formed by the action of carboxypeptidases found in rat brain wall microvessels (Bausback & Ward, 1988). The interpretation of the late increase of LPS-induced fever in animals treated with Hoe-140 is complex based solely on the present results. However, it may be speculated that the oc-

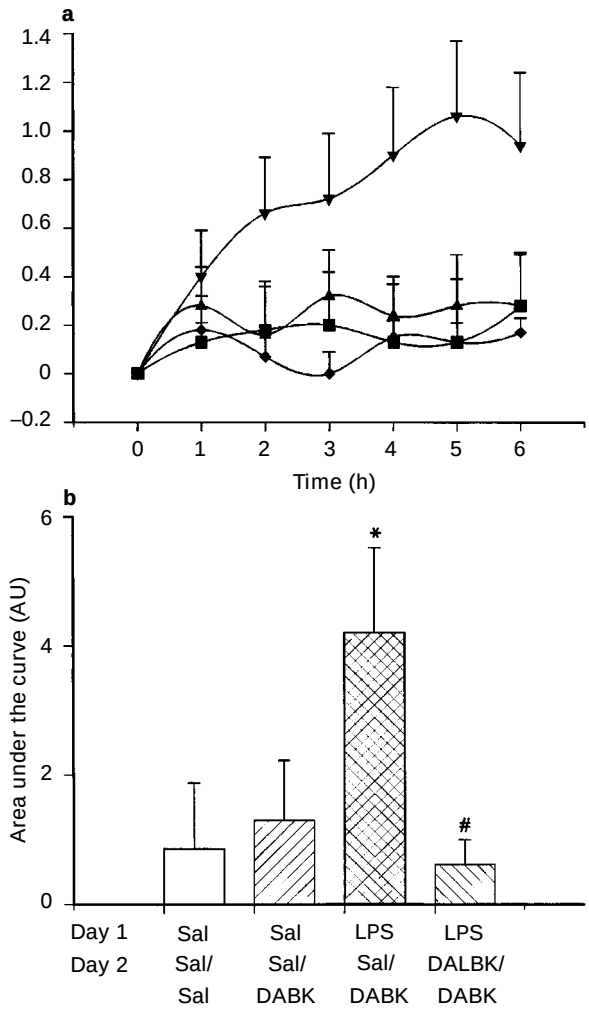

Figure 7 Effect of co-administration of [des-Arg^9-Leu^8]-BK (DALBK, 15 nmol, i.c.v.) on the body temperature changes induced by [des-Arg^9]-BK (DABK, 5 nmol, i.c.v.) in animals that were previously (24 h) treated with saline (1 ml kg^{-1}, i.p.) or endotoxin (0.5 μg kg^{-1}, i.p.). (a) Indicates the time course of the response and the values represent change from baseline temperature; vertical lines show s.e.mean. (b) Represents the area under the curve of the groups shown in (a). In (a) (■) Day 1: saline - Day 2: saline 2 μl, i.c.v./saline 2 μl, i.c.v.; (▲) Day 1: saline - Day 2: saline 2 μl, i.c.v./DABK ($n=8$); (▼) Day 1: LPS - Day 2: saline 2 μl, i.c.v./ DABK ($n=8$); (◆) Day 1: LPS - Day 2: DALBK 15 nmol, i.c.v./DABK ($n=8$). Basal temperatures of the groups were 37.00 ± 0.03, 37.20 ± 0.02, 37.13 ± 0.04, 37.15 ± 0.02°C, respectively. *Significantly different from Sal/Sal/Sal ($P=<0.05$). #Significantly different from LPS/Sal/DABK ($P<0.05$).

cupation of the receptors by Hoe-140 may favour the metabolism of kinins, hence, causing the accumulation of [des-Arg^9]-BK in the CNS, and consequently, resulting in the late increase of LPS-induced fever. Although our results suggest the involvement of both B_2 and B_1 kinin receptors in LPS-induced fever, it seems likely that the interaction of BK with B_2 receptors is essential for the induction, while B_1 receptors activated by BK metabolites are relevant for the maintenance of this response.

In support of the present results, Walker *et al*. (1996) have recently demonstrated the involvement of central B_2 receptor activation in the febrile response induced by central injection of LPS in rats. Nevertheless, they did not observe inhibition of LPS-induced fever in animals treated with a B_1 receptor antagonist. This difference may be related to the time of administration of the B_1 receptor antagonist, concomitant with LPS and consequently very early in the development of fever, the reduced doses (0.1 – 1 nmol) of the antagonist and the route of administration of LPS.

The present study also showed that the febrile response induced by BK was greatly reduced when the animals were treated with LPS 24 h earlier. This effect may be related to CNS B_2 receptor desensitization and/or downregulation, similar to data found in LPS-induced rat paw oedema (Campos *et al*., 1996). It has also been demonstrated that seven daily intraplantar injections of BK or [Tyr^8]-BK resulted in progressive desensitization of paw oedema in rats (Campos & Calixto, 1995; Campos *et al*., 1995). This desensitization may be correlated with a downregulation and/or internalization of B_2 receptors, possibly associated with a decreased coupling of activated receptors to G-proteins (Roscher *et al*., 1984; 1990; Muñoz & Leeb-Lundberg, 1992; Wolsing & Rosenbaum, 1993). However, the occurrence of such mechanisms in the CNS has not been demonstrated and their role in the reduced febrile response to BK following pretreatment with LPS is still a matter of speculation.

Central injection of a selective B_1 receptor agonist, [des-Arg^9]-BK, was not as effective as BK in inducing an increase of body temperature. A similar result has also been observed in relation to the central antinociceptive effect of this peptide (Pelá *et al*., 1996). These observations are in agreement with the results of many studies showing that [des-Arg^9]-BK does not induce marked effects (Regoli & Barabé, 1980; Marceau *et al*., 1983; Steranka & Burch, 1991). However, the response induced by [des-Arg^9]-BK was greatly enhanced when the animals were treated with LPS 24 h earlier. The febrile response induced by [des-Arg^9]-BK in LPS-treated animals was inhibited by central co-administration of a selective B_1 receptor antagonist, [des-Arg^9-Leu^8]-BK, suggesting that activation of central B_1 receptors are important for the induction of this response. It is well known that, under physiological circumstances, B_1 agonists, including [des-Arg^9]-BK, induce minor effects, as the tissues normally express B_2 receptors (Regoli & Barabé, 1980; Marceau *et al*., 1983; Steranka & Burch, 1991). Nevertheless, expression of B_1 receptors can be induced by a variety of conditions and may exert an important role in certain pathological states, including oedema and hyperalgesia (Marceau *et al*., 1983; Farmer *et al*., 1991; Dray & Perkins, 1993; Perkins & Kelly, 1993; Corrêa & Calixto, 1993; Campos *et al*., 1995; 1996; Campos & Calixto, 1995).

The present results suggest that pretreatment of animals with LPS may be associated with B_2 receptor desensitization and/or downregulation and B_1 receptor upregulation in the CNS. Such suggestions are based on the observations that following LPS-treatment, the response to BK and [Tyr^8]-BK, which acts mainly by activation of B_2 receptors, was reduced and the response to [des-Arg^9]-BK, a B_1 agonist, was enhanced.

The inhibition of LPS-induced fever by i.v. injection of Hoe-140 suggests that peripherally produced BK, acting by stimulation of B_2 receptors, may also play an important role in the induction of this response. However, i.v. injection of Hoe-140, did not increase the late phase of LPS-induced fever. These results suggest that kinin receptors may have different roles in the febrile response induced by i.v. and i.c.v. injection of LPS. It has been shown that BK stimulates the production of some cytokines, such as interleukin-1 and tumour necrosis factor-α (Tiffany & Burch, 1989; Ferreira *et al*., 1993). These cytokines have an important role in the induction of LPS-induced fever (Kluger, 1991) and also stimulate the expression of B_1 receptors (Deblois *et al*., 1991). Furthermore, it has been demonstrated that BK stimulates the production of prostaglandin E_2 (PGE_2, Bathon *et al*., 1992) and synergistically interacts with this eicosanoid (Campos & Calixto, 1995). Prostaglandins are important mediators of the febrile response induced by different pyrogens and their production is also stimulated by cytokines (Kluger, 1991). In addition, PGE_2 significantly potentiates [des-Arg^9]-BK induced paw oedema in animals that had been previously treated with LPS (Campos *et al*., 1996).

In conclusion, the present results, with a different pharmacological approach, give further support to our previous re-

sults (Pelá *et al*., 1975; Almeida e Silva & Pelá, 1978) showing the involvement of kinins in the induction of LPS-induced fever. The present results indicate that activation of central B_2 and B_1 kinin receptors may be involved in the induction and maintenance of LPS-induced fever, respectively. Finally, desensitization and/or downregulation of B_2 receptors and upregulation and/or induction of B_1 receptors in the CNS after treatment with LPS, may also play a relevant pathophysiological role in the induction and maintenance of fever. These observations may be specifically important in the case of chronic inflammatory conditions, because the BK metabolite [des-Arg^9]-BK, so far considered an inactive metabolite, acquires an active and relevant role with the progressive expression of B_1 receptors that occurs in such states.

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