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Bradykinin B_1 and B_2 receptors, tumour necrosis factor α and inflammatory hyperalgesia

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1 The effects of BK agonists and antagonists, and other hyperalgesic/antihyperalgesic drugs were measured (3 h after injection of hyperalgesic drugs) in a model of mechanical hyperalgesia (the end-point of which was indicated by a brief apnoea, the retraction of the head and forepaws, and muscular tremor).

2 DALBK inhibited responses to carrageenin, bradykinin, DABK, and kallidin.

3 Responses to kallidin and DABK were inhibited by indomethacin or atenolol and abolished by the combination of indomethacin + atenolol.

4 DALBK or HOE 140, given 30 min before, but not 2 h after, carrageenin, BK, DABK and kallidin reduced hyperalgesic responses to these agents.

5 A small dose of DABK + a small dose of BK evoked a response similar to the response to a much larger dose of DABK or BK, given alone.

6 Responses to BK were antagonized by HOE 140 whereas DALBK antagonized only responses to larger doses of BK. The combination of a small dose of DALBK with a small dose of HOE 140 abolished the response to BK.

7 The hyperalgesic response to LPS (1 μg) was inhibited by DALBK or HOE 140 and abolished by DALBK + HOE 140. The hyperalgesic response to LPS (5 μg) was not antagonized by DALBK + HOE 140.

8 These data suggest: (a) a predominant role for B_2 receptors in mediating hyperalgesic responses to BK and to drugs that stimulate BK release, and (b) activation of the hyperalgesic cytokine cascade independently of both B_1 and B_2 receptors if the hyperalgesic stimulus is of sufficient magnitude.

Keywords: Inflammatory hyperalgesia; bradykinin; [des-Arg^9]BK; [des-Arg^9, Leu^8]BK; tumour necrosis factor alpha; interleukin-8; prostaglandin E_2

Abbreviations: ATEN, Atenolol; BK, bradykinin; DABK, [des-Arg^9]BK; DALBK, [des-Arg^9, Leu^8]BK; Cg, carrageenin; D-Arg^0-Hyp^3-Thi^5-Dtic^7-Oic^8-BK, HOE 140; INDO, indomethacin; IL, interleukin; IU, international unit; i.pl., intraplantar; LPS, lipopolysaccharide; PGE_2, prostaglandin E_2; TNFα, tumour necrosis factor α

Introduction

Bradykinin (BK) is an important inflammatory mediator involved in oedema formation and serves as a trigger for inflammatory pain (Steranka *et al*., 1988; Dray & Perkins, 1993; Hall, 1997). BK activates and sensitizes pain receptors (nociceptors). Activation of nociceptors causes immediate overt pain (Armstrong *et al*., 1957; Sicuteri *et al*., 1965; Ferreira, 1972; Whalley *et al*., 1987), whereas sensitization of nociceptors is responsible for the development of inflammatory hyperalgesia (Ferreira, 1972; Ferreira *et al*., 1978a). BK exerts its biological activities by stimulating two receptor subtypes, B_1 and B_2 (see Hall, 1997, for a review). B_2 receptors have been localized to sensory neurones (Steranka *et al*., 1988; Nagy *et al*., 1993). B_1 receptors have been reported to be present on sensory neurones or ganglia, especially after induction, in some models (Segond-von Banchet *et al*., 1996; Seabrook *et al*., 1997) but not in a model of persistent inflammatory hyperalgesia (Davis *et al*., 1996).

BK has been shown to be hyperalgesic in models of inflammation, in both behavioural and electrophysiological studies (Dray *et al*., 1988; Lang *et al*., 1990; Handwerker & Reeh, 1991). Until a few years ago it was believed that the acute inflammatory events, such as oedema and inflammatory pain, were mediated solely by B_2 receptors, since HOE 140, a B_2 receptor antagonist, inhibited the hyperalgesic effect of BK and was anti-hyperalgesic in experimental models of inflammatory pain, including carrageenin-evoked hyperalgesia (Beresford & Birch, 1992; Heapy *et al*., 1991; Ferreira *et al*., 1993). More recently, it was shown that both B_1 and B_2 receptors play an important role in mechanical and thermal hyperalgesia, notably when the hyperalgesia is persistent and subsequent to an earlier inflammatory insult, suggesting the induction of B_1 receptors at the site of injury by inflammatory mediators, such as cytokines (Perkins *et al*., 1993; Perkins & Kelly, 1993). Consistent with this notion, in models of mechanical and thermal hyperalgesia, hyperalgesic responses to IL-1β were inhibited by the BK B_1 receptor antagonist DALBK (Davis & Perkins, 1994; Perkins & Kelly, 1994; Perkins *et al*., 1995)

In a model of inflammatory (mechanical) hyperalgesia, responses to carrageenin and bacterial endotoxin (lipopolysaccharide, LPS) were initiated by BK, acting on BK_2

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receptors, which stimulated the release of tumour necrosis factor α (TNFα). The TNFα induced the production of IL-1β and IL-6, which stimulated the production of cyclo-oxygenase products, and IL-8, which stimulated production of sympathomimetic amines (Cunha *et al*., 1992; Ferreira *et al*., 1993). These data are consistent with data obtained in another model of inflammatory hyperalgesia: inflammatory hyperalgesia caused by Freund's complete adjuvant, to which TNFα and IL-1β contributed (Garabedian *et al*., 1995; Woolf *et al*., 1996, 1997; Banner *et al*., 1998) and with a report that BK evoked the release of TNFα from macrophage monolayers (Tiffany & Burch, 1989).

The aim of the present study was to investigate further, in a model of inflammatory (mechanical) hyperalgesia, the BK-initiated, TNFα-driven, cytokine cascade and to investigate the relative contributions of the two BK receptor subtypes, B_1 and B_2 to this stimulation.

Methods

Animals

Male Wistar rats, weighing 130–180 g, were housed in temperature controlled rooms (22–25°C) with water and food *ad libitum* until use.

Nociceptive test

A constant pressure of 20 mmHg (measured using a sphygmomanometer), was applied (*via* a syringe piston moved by compressed air) to an area of 15 mm^2 of the dorsal surface of the hind paws of rats, and discontinued when they presented a typical 'freezing reaction'. The freezing reaction was signalled by a brief apnoea, concomitant with the retraction of the head and forepaws and a reduction in the escape movements which animals frequently made to escape from the position imposed by the experimental situation. Usually, the apnoea was associated with successive waves of muscular tremor. For each animal, the latency to the onset of the freezing reaction (from the time of first application of the pressure) was measured before administration (zero time) and again, 3 h after administration of a hyperalgesic agent. The intensity of hyperalgesia was quantified as the reduction in reaction time, calculated by subtracting the value of the second measurement from that of the first (Ferreira *et al*., 1978b, 1988, Cunha *et al*., 1998). Reaction times were typically 32–34 s (with s.e.means of 0.5–1.0 s) before injection and 2–4 s after stimulation with hyperalgesic agents. Multiple paw treatments did not alter basal reaction times. Different individuals prepared the solutions to be injected, made the injections, and measured the reaction times.

Experimental protocol

Hyperalgesia was measured 3 h after injection of hyperalgesic agents (agonists), each injected in 100 μl, into the hind paws (intraplantar, i.p.l.) of rats. Anti-hyperalgesic agents (antagonists and antibodies) were injected i.pl. (50 or 100 μl, into the paw to be injected with a hyperalgesic agent) except for HOE

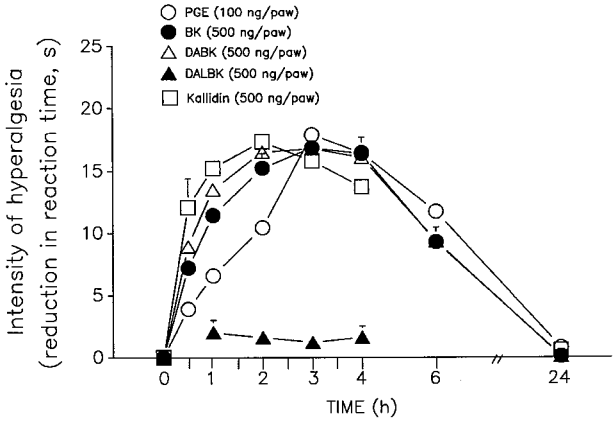

Figure 2 Time course of the development of responses to hyperalgesic agents. The intensity of hyperalgesia was measured in injected paws 0.5–24 h after injection of PGE_2 (PGE, 100 ng paw^{-1}, open circles), BK (500 ng paw^{-1}, filled circles), DABK (500 ng paw^{-1}, open triangles), DALBK (500 ng paw^{-1}, filled triangles), kallidin (500 ng paw^{-1}, open squares). All substances were injected i.pl. in a volume of 100 μl. Vertical bars are s.e.means in groups of five animals.

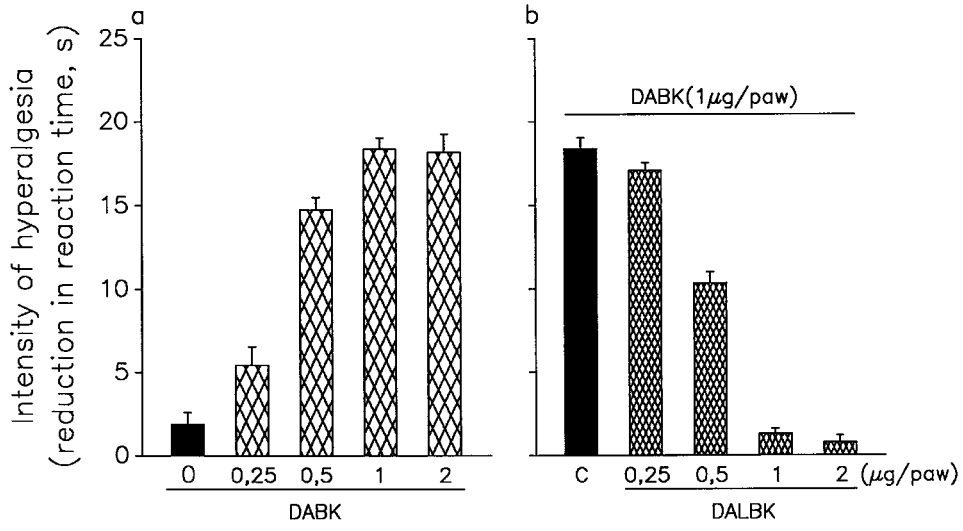

Figure 1 Dose-dependence of hyperalgesia evoked by DABK and its inhibition by DALBK. (a) The intensity of hyperalgesia in injected paws after injection of DABK (0.25–2.0 μg paw^{-1}). (b) The inhibitory effect of DALBK (0.25–2.0 μg paw^{-1}) on the hyperalgesic response to DABK (1 μg,), injected into the same paws, 30 min later. Hyperalgesia was measured 3 h after injection of DABK. Vertical bars are s.e.means in groups of five animals.

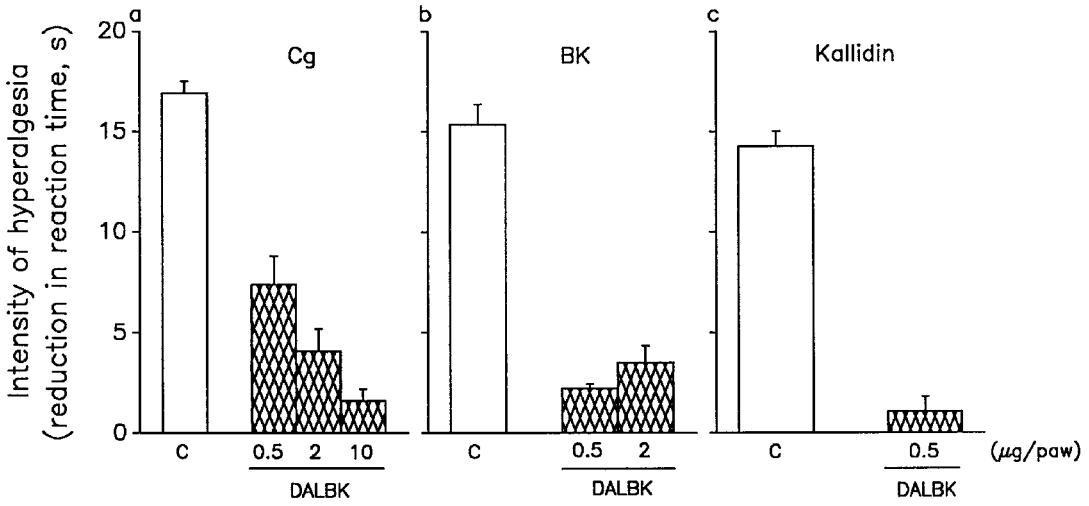

Figure 3 The inhibitory effect of DALBK (0.5–10.0 μg paw^{-1}) on the hyperalgesic responses to (a) carrageenin (100 μg), (b) BK (500 ng) and (c) kallidin (500 ng). DALBK was injected into paws to be injected with one of the three hyperalgesic agents, 30 min before the hyperalgesic agent. Hyperalgesia was measured 3 h after injection of hyperalgesic agents. Vertical bars are s.e.means in groups of five animals.

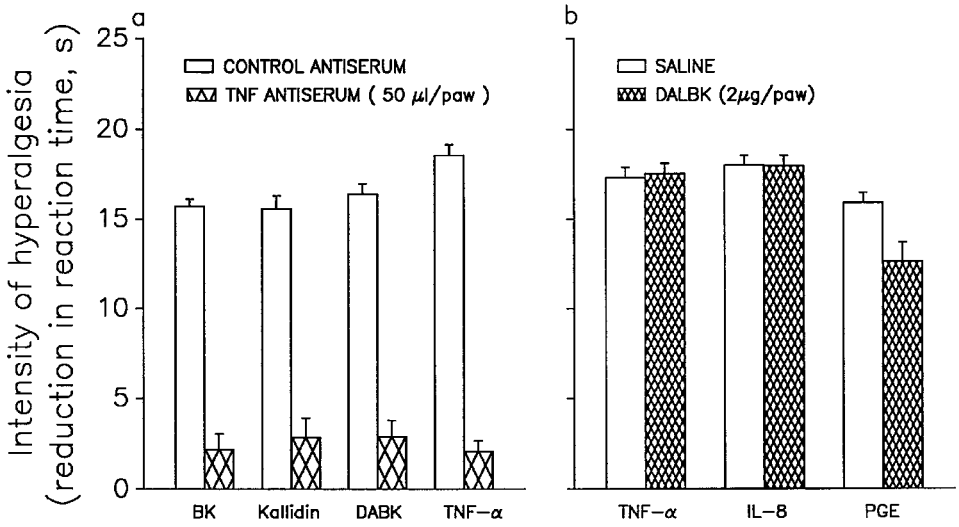

Figure 4 (a) Inhibition by a sheep anti-murine TNFα serum (50 μl paw^{-1}) of the hyperalgesic effects of BK (500 ng), kallidin (500 ng), DABK (500 ng) and murine TNFα (2.5 pg). Anti-murine TNFα serum (hatched columns) or pre-immune control serum (open columns) was injected into paws to be injected with one of the four hyperalgesic agents, 30 min before the hyperalgesic agent. (b) Effect of DALBK (2.0 μg paw^{-1}) on the hyperalgesic responses to TNFα (2.5 pg), IL-8 (0.1 ng) and PGE_2 (100 ng). DALBK (2 μg paw^{-1}) was injected into paws to be injected with one of the three hyperalgesic agents, 30 min before the hyperalgesic agent. Hyperalgesia was measured 3 h after injection of hyperalgesic agents. Vertical bars are s.e.means in groups of five animals.

140, which was injected subcutaneously (s.c., 200 μl), 30 min before or 2 h after hyperalgesic agents. Results are presented as means $\pm$ s.e.means of groups of five animals.

Drugs

The following drugs were obtained from the sources indicated. Recombinant murine TNFα (NIBSC preparation coded 88/532, 200,000 Units 1 μg ampoule^{-1}), human recombinant interleukin-8 (72 amino acids, NIBSC preparation coded 89/520, 1000 International Units 1 μg ampoule^{-1}), atenolol, BK, [des-Arg^9]BK (DABK), [des-Arg^9, Leu^8]BK (DALBK), kallidin, prostaglandin E_2, PGE_2, (Sigma Chemical Co., St. Louis, MO, U.S.A.), HOE 140 (D-Arg^0-Hyp^3-Thi^5-DTic^7-Oic^8-BK, Hoechst AG, Frankfurt, Germany), sheep anti-murine recombinant TNFα antiserum and pre-immune serum (Dr T. Meager, Division of Immunobiology, NIBSC, Mahadevan *et al.*, 1990), indomethacin (Merck, Sharpe & Dohme Ltd, Hoddesdon, Herts, U.K.), carrageenin (FMC Corporation, Philadelphia, U.S.A.).

Results

Inhibition by DALBK of hyperalgesia evoked by DABK

DABK (0.25–2.0 μg) injected into one hind paw of rats evoked dose-dependent hyperalgesia in injected paws with a dose of DABK (1 μg) evoking the maximum hyperalgesic response to this drug (Figure 1). DALBK (0.25–2.0 μg)

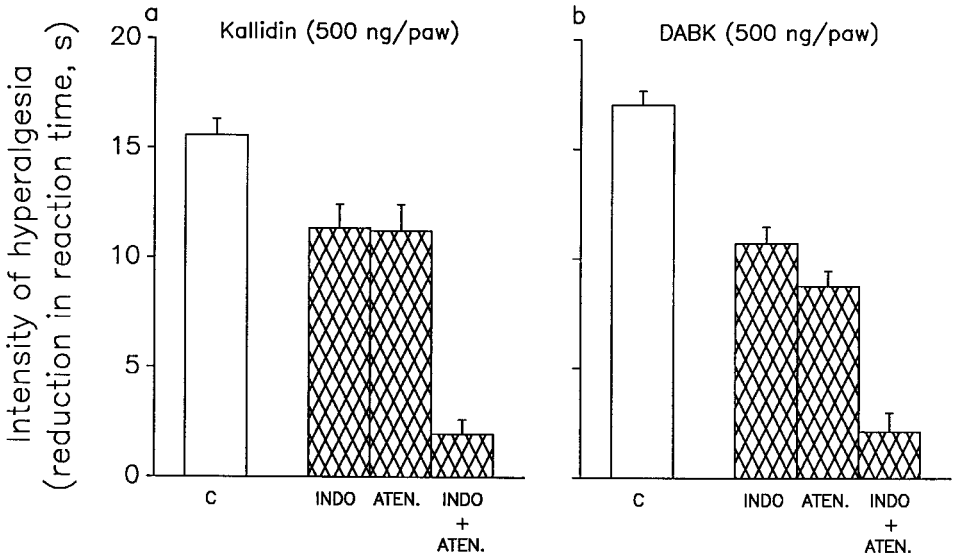

Figure 5 Inhibition by indomethacin (INDO, 100 μg), atenolol (25 μg) and indomethacin + atenolol (INDO, 100 μg + ATEN, 25 μg) of hyperalgesic responses to (a) Kallidin (500 ng) and (b) DABK (500 ng). Indomethacin or atenolol or both were injected into paws to be injected with Kallidin or DABK, 30 min before the hyperalgesic agents. Hyperalgesia was measured 3 h after injection of hyperalgesic agents. Vertical bars are s.e.means in groups of five animals.

injected into hind paws to be injected, 30 min later, with DABK inhibited, in a dose-dependent manner, responses to DABK (1.0 μg) with a dose of DALBK (1.0 μg) abolishing the hyperalgesic response to DABK (1 μg, Figure 1).

Time course of hyperalgesic responses

Injection of the hyperalgesic agents PGE_2 (100 ng), BK (500 ng), DABK (500 ng), and kallidin (500 ng), into one hind paw of rats evoked time-dependent hyperalgesia in injected paws, which began within 30 min of injection, reached a plateau within 2 h (3 h for PGE_2) and was maximum at 3 h after injection (Figure 2). Responses had begun to decline at 6 h (3 h for kallidin) and had returned to pre-injection values within 24 h. The B_1 receptor antagonist DALBK (500 ng, i.pl.) was without effect.

Inhibition by DALBK of responses to carrageenin, BK and kallidin

Carrageenin (100 μg), BK (500 ng) and kallidin (500 ng) evoked hyperalgesic responses, measured 3 h after their injection (i.pl., Figure 3). DALBK, injected into paws to be injected 30 min later with one of the hyperalgesic agents, antagonized the hyperalgesic responses: DALBK (0.5 μg) abolished responses to BK and kallidin whereas DALBK (10.0 μg) was required to abolish the response to carrageenin (Figure 3).

Inhibition by sheep anti-murine TNFα of responses to BK, kallidin, DABK and murine TNFα

BK (500 ng), kallidin (500 ng), DABK (500 ng) and murine TNFα (2.5 pg) evoked hyperalgesic responses, measured 3 h after their injection (i.pl., Figure 4a). The responses were abolished by injection of a sheep anti-murine TNFα serum (50 μl), injected into paws to be injected with one of the four hyperalgesic agents, 30 min before the hyperalgesic agent.

Effect of DALBK on responses to murine TNFα, IL-8 and PGE_2

TNFα (2.5 pg), IL-8 (0.1 ng) and PGE_2 (100 ng) evoked hyperalgesic responses, measured 3 h after their injection (Figure 4b). The responses to TNFα, IL-8 and PGE_2 were little affected by DALBK (2.0 μg, injected into paws to be injected with one of the hyperalgesic agents, 30 min before the hyperalgesic agent).

Inhibition by indomethacin and atenolol of responses to kallidin and DABK

Hyperalgesic responses to kallidin (500 ng, Figure 5a) and DABK (500 ng, Figure 5b) were inhibited by 27 and 37%, respectively by indomethacin (100 μg) and by 27 and 48%, respectively by atenolol (25 μg, each injected into paws to be injected with either kallidin or DABK, 30 min before the hyperalgesic agent). The responses to kallidin and DABK were abolished (-88%) by the combination of indomethacin + atenolol (100 + 25 μg, respectively, Figure 5).

Effects of B_1 and B_2 receptor antagonists on hyperalgesic responses to B_1 and B_2 receptor agonists

DALBK (2 μg, i.pl.), given 30 min before, but not 2 h after, injections (i.pl.) of carrageenin (100 μg), BK (500 ng), DABK (500 ng) and kallidin (500 ng) reduced (by -66 to -92%) the hyperalgesic responses to these agents (Figure 6). Similarly, HOE 140 (1 mg kg^{-1}, s.c.) given 30 min before, but not 2 h after, injections (i.pl.) of carrageenin (100 μg), BK (500 ng), and kallidin (500 ng) reduced (by -72 to -88%) the hyperalgesic responses to carrageenin, BK and kallidin; responses to DABK (500 ng) were not antagonized by HOE 140 (1 mg kg^{-1}, s.c.), given either 30 min before or 2 h after the DABK (Figure 6).

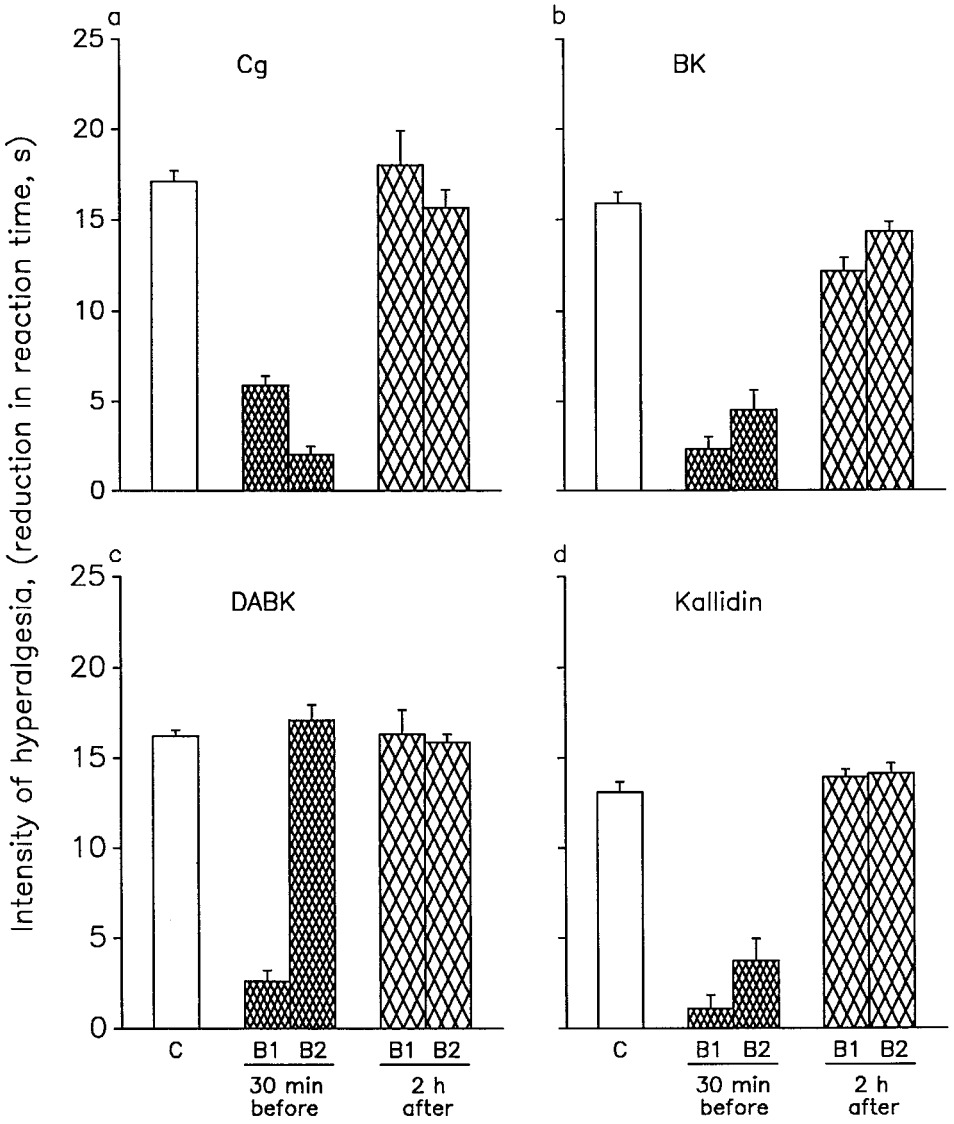

Figure 6 Effects of B_1 and B_2 receptor antagonists on hyperalgesic responses to carrageenin and B_1 and B_2 receptor agonists. DALBK (B_1 antagonist, 2 μg, i.pl.) or HOE 140 (B_2 antagonist, 1 mg kg^{-1}, s.c.) was given either 30 min before or 2 h after injections (i.pl.) of (a) carrageenin (Cg, 100 μg), (b) BK (500 ng), (c) (500 ng) and (d) kallidin (500 ng). Hyperalgesia was measured 3 h after injection of hyperalgesic agents, vertical bars are s.e.means in groups of five animals.

Synergy between the B_1 agonist DABK and BK

The combination of a small dose of the B_1 agonist DABK (100 ng, i.pl.) and BK (25 ng, i.pl.) evoked a hyperalgesic response of 17.9$\pm$0.6 s, compared with responses to DABK (100 ng, i.pl.) and BK (25 ng, i.pl.), each given alone, of 4.1$\pm$0.6 and 5.0$\pm$0.3 s, respectively (Figure 7a). The response to this combination of the two drugs (17.9$\pm$0.6 s) exceeded by 97% the addition of the two individual responses (4.1+5.0=9.1 s) and was similar to the responses to a much larger dose (500 ng) of either DABK or BK, given alone: 16.1$\pm$0.7 and 18.0$\pm$0.6 s, respectively (Figure 7a).

Inhibition of responses to BK by HOE 140 and DALBK

Hyperalgesic responses to BK (25–500 ng, i.pl.) were antagonized by HOE 140 (1 mg kg^{-1}, s.c.), with responses to the smaller doses of BK (25 and 50 ng, i.pl.) abolished and responses to larger doses of BK (100 and 500 ng, i.pl.) reduced by 88 and 67%, respectively (Figure 7b). In contrast, hyperalgesic responses to small doses of BK (25 and 50 ng, i.pl.) were not antagonized by DALBK (500 ng, injected into paws to be injected with BK, 30 min before the BK) whereas hyperalgesic responses to larger doses of BK (100 and 500 ng, i.pl.) were reduced by 27 and 53%, respectively following DALBK (500 ng, Figure 7b). DALBK, even at a small dose (200 ng, i.pl.), was a much more effective antagonist of BK (500 ng) when combined with a small dose of HOE 140 (0.1 mg kg^{-1}, s.c., Figure 7c). When given alone, each antagonist had a marginal effect (−15 and −17%, respectively) upon the response to BK whereas the combination of the two antagonists abolished the response to BK (Figure 7c).

Effects of B_1 and B_2 receptor antagonists on hyperalgesic responses to LPS

Hyperalgesic responses to LPS (1 μg) were inhibited by DALBK (500 ng, i.pl.) and HOE 140 (0.1 mg kg^{-1}, s.c.) by −34 and −76%, respectively. When these doses of the two antagonists were combined, the response to BK was reduced by 88%. In contrast, hyperalgesic responses to LPS (5 μg) were little affected by DALBK (500 ng, i.pl., +13%), HOE 140

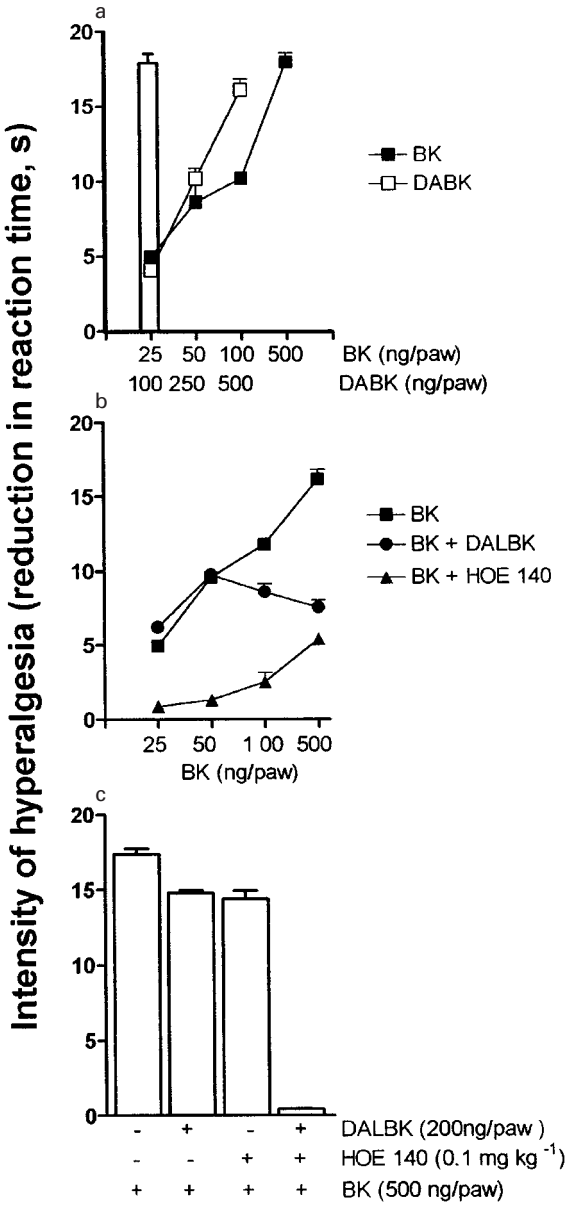

Figure 7 (a) Hyperalgesic responses to injections of BK (25–500 ng, i.pl., filled squares) and DABK (100–500 ng, open squares), each given alone or together (BK, 25 ng + DABK, 100 ng, open column). (b) Inhibition of hyperalgesic responses to BK (25–500 ng, i.pl., filled squares) by DALBK (500 ng, injected into the paws to be injected with BK, filled circles) or HOE 140 (1 mg kg^{-1}, s.c., filled triangles), each given 30 min before the BK. (c) Inhibition of the hyperalgesic response to BK (500 ng, i.pl.) by DALBK (200 ng, injected into the paws to be injected with BK) and HOE 140 (0.1 mg kg^{-1}, s.c.), each given alone (+ −, − +) or together (+ +). Hyperalgesia was measured 3 h after injection of hyperalgesic agents. Vertical bars are s.e.means in groups of five animals.

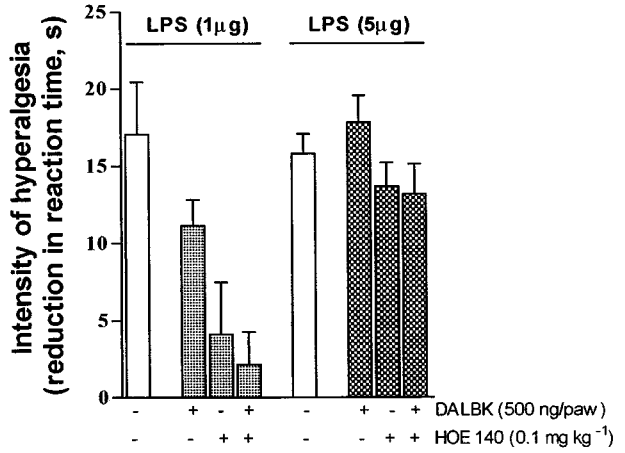

Figure 8 Effects of B_1 and B_2 receptor antagonists on hyperalgesic responses to LPS. DALBK (500 ng, i.pl.) or HOE 140 (0.1 mg kg^{-1}, s.c.), or both were given 30 min before injection (i.pl.) of LPS (1 μg or 5 μg). Hyperalgesia was measured 3 h after injection of LPS. Vertical bars are s.e.means in groups of five animals.

(0.1 mg kg^{-1}, s.c., −13%) or by the combination of these doses of the two antagonists (−17%).

Discussion

In the model of mechanical hyperalgesia utilized in the present study, the inflammatory agent carrageenin induced production of TNFα, which initiated a cascade of cytokine release (Cunha *et al*., 1992). IL-1β and IL-6, induced by TNFα, stimulated the release of hyperalgesic cyclo-oxygenase products (inflammatory hyperalgesia), whereas IL-8, induced by TNFα, stimulated the release of hyperalgesic sympathomimetics (sympathetic hyperalgesia). An early and crucial role for TNFα was proposed because a single injection of this cytokine mimicked the response to carrageenin by inducing production of IL-1β, IL-6 and IL-8, and a single injection of antiserum neutralizing endogenous TNFα abolished the response to carrageenin (Cunha *et al*., 1992). Subsequently, BK, acting upon BK_2 receptors, was identified as a trigger for the induction of TNFα (Ferreira *et al*., 1993): the BK B_2 receptor antagonist HOE 140 (1 mg kg^{-1}) inhibited hyperalgesic responses to BK, carrageenin and smaller doses of LPS (0.5–2 μg). However, the failure of even a large dose of HOE 140 (10 mg kg^{-1}) to inhibit hyperalgesic responses to a larger dose of LPS (5 μg) revealed that the (hyperalgesic) cytokine cascade could be activated independently of BK_2 receptors if the hyperalgesic stimulus were of sufficient magnitude (Ferreira *et al*., 1993). The possible involvement of BK B_1 receptors in the induction of the cytokine cascade was not addressed in the study.

The results of a series of experiments, utilizing a variety of models of hyperalgesia, including mechanical hyperalgesia, indicate a role for BK B_1 receptors in mediating hyperalgesic responses, notably persistent responses to inflammatory stimuli (Perkins & Kelly, 1993, 1994; Davis & Perkins, 1994; Perkins *et al*., 1993, 1995). The present study was undertaken to gain more information about the characteristics of the BK-initiated, TNFα-driven cytokine cascade delineated previously (Cunha *et al*., 1992; Ferreira *et al*., 1993) and to investigate the relative contributions of the two BK receptor subtypes, B_1 and B_2, in this model of mechanical hyperalgesia. The B_1 agonist DABK is here shown to evoke hyperalgesic responses of similar magnitude and duration to responses to BK and kallidin. Also, the B_1 antagonist DALBK antagonized not only hyperalgesic responses to the B_1 agonist DABK, but responses to BK, kallidin and carrageenin. The effect of BK on B_1 receptors is likely to have been due to endogenous conversion of BK to DABK. Consistent with previous results (Dray & Perkins, 1993), these data suggest a role for both B_1 and B_2 receptors in mediating responses to BK, kallidin and carrageenin.

Evidence that the B_1-mediated hyperalgesic effects of DABK, BK, kallidin and carrageenin resulted from the triggering of the same TNFα-driven cascade of cytokines and other mediators shown previously to be induced subsequent to activation of B_2 receptors (Ferreira *et al*., 1993) came from three different experiments. Firstly, an antiserum neutralizing rat TNFα abolished hyperalgesic responses to BK, kallidin and DABK, and the B_1 antagonist DALBK failed to inhibit responses to TNFα, IL-8 and PGE_2. Secondly, indomethacin and atenolol inhibited hyperalgesic responses to kallidin and DABK, and indomethacin + atenolol (given together) abolished responses to kallidin and DABK. Thirdly, the B_1 antagonist DALBK, in common with the B_2 antagonist HOE 140, was effective against BK, kallidin, and carrageenin only when given (30 min) before but not (2 h) after these hyperalgesic agents. Thus, B_1 receptors can have a role both in initiating the (TNFα-driven) cascade of hyperalgesic cytokines (and other mediators) and in mediating responses to one of those cytokines, namely IL-1β (Davis & Perkins, 1994, Perkins & Kelly, 1994; Perkins *et al*., 1995).

The capacity of both the B_1 agonist DABK and BK to evoke maximum hyperalgesic responses suggests a role for both B_1 and B_2 receptors in initiating BK-dependent hyperalgesic responses. This notion is supported by the synergy between the B_1 agonist DABK and BK, with a small dose of each combining to give a response equivalent to that of a $\geqslant 5$ fold larger dose of either drug given alone. The property of the B_2 antagonist HOE 140 to antagonize responses to both small and large doses of BK, in contrast to the B_1 antagonist DALBK, which antagonized only responses to larger doses of BK, suggests that, in the above model, responses to small doses of BK were mediated predominantly *via* B_2 receptors, whereas responses to larger doses of BK activated B_1 receptors in addition to B_2 receptors. Further evidence for a role for B_1 receptors in mediating hyperalgesic responses to larger doses of BK came from the finding that the combination of ineffectual doses (when given alone) of the B_1 antagonist DALBK and the B_2 antagonist HOE 140 abolished the response to a large dose of BK. However, the predominant role of the B_2 receptor in mediating hyperalgesic responses to larger doses of BK (Ferreira *et al*., 1993) was confirmed in that a large dose of the B_2 antagonist HOE 140 was itself sufficient to abolish the hyperalgesic response to a large dose of BK.

The earlier finding (Ferreira *et al*., 1993) that the B_2 antagonist HOE 140 inhibited the hyperalgesic response to a small dose of LPS (1 μg) but not that to a larger dose (5 μg) was confirmed in the present study. Similarly, the B_1 antagonist DALBK has now been shown to also inhibit the hyperalgesic response to a small dose of LPS (1 μg) but not that to a larger dose (5 μg). Indeed, the hyperalgesic response to the larger dose of LPS (5 μg) was not inhibited even by the combination of the B_1 and B_2 antagonists. These data reveal that the (hyperalgesic) cytokine cascade can be activated independently of both B_1 and B_2 receptors if the hyperalgesic stimulus is of sufficient magnitude. Nevertheless, the demonstration of a powerful synergy between (small doses of) B_1 and B_2 receptor antagonists in antagonizing hyperalgesic responses to BK suggests that the combination of B_1 and B_2 receptor antagonists merits further evaluation. This is particularly so in circumstances in which a BK agonist is believed to play a role but in which a B_1 or a B_2 antagonist, administered alone, fails to inhibit the response.

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