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Bradykinin initiates cytokine-mediated inflammatory hyperalgesia

[1]S.H. Ferreira, B.B. Lorenzetti & *S. Poole

Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil and *Division of Endocrinology National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG

1 The hyperalgesic activities in rats of bradykinin, carrageenin and lipopolysaccharide (LPS) were investigated in a model of mechanical hyperalgesia.

2 Bradykinin and carrageenin evoked dose-dependent hyperalgesia with maximum responses of similar magnitude to responses to LPS (1 and 5 μg).

3 Hoe 140, an antagonist of BK_2 receptors, inhibited in a dose-dependent manner hyperalgesic responses to bradykinin, carrageenin and LPS (1 μg) but not responses to LPS (5 μg), prostaglandin E_2, dopamine, tumour necrosis factor α (TNFα), IL-1, IL-6 and IL-8.

4 Responses to bradykinin and LPS (1 and 5 μg) were inhibited by the cyclo-oxygenase inhibitor, indomethacin and by the β-adrenoceptor antagonist, atenolol. The effects of indomethacin and atenolol were additive: their combination abolished responses to bradykinin and LPS (1 μg) and markedly attenuated the response to LPS (5 μg).

5 Antiserum neutralizing endogenous TNFα abolished the response to bradykinin whereas antisera neutralizing endogenous IL-1β, IL-6 and IL-8 each partially inhibited the response. The combination of antisera neutralizing endogenous IL-1β + IL-8 or IL-6 + IL-8 abolished the response to bradykinin.

6 Antisera neutralizing endogenous TNFα, IL-1β, IL-6 and IL-8 each partially inhibited responses to LPS (1 and 5 μg). Increasing the dose of antiserum to TNFα or giving a combination of antisera to IL-1β + IL-8 or IL-6 + IL-8 further inhibited responses to LPS (1 and 5 μg).

7 These data show that bradykinin can initiate the cascade of cytokine release that mediates hyperalgesic responses to carrageenin and endotoxin (1 μg). The lack of effect of Hoe 140 on hyperalgesic responses to LPS (5 μg) suggests that the release of hyperalgesic cytokines can be initiated independently of bradykinin BK_2 receptors.

Keywords: Bradykinin; inflammatory hyperalgesia; tumour necrosis factor; interleukin-1; interleukin-6; interleukin-8

Introduction

Bradykinin has two roles in the development of inflammatory pain: activation and sensitization of pain receptors (nociceptors). Activation of nociceptors causes immediate, overt pain whereas sensitization of nociceptors is responsible for the development of inflammatory hyperalgesia. Bradykinin was established as a pain mediator because it produced immediate, overt pain in man (Armstrong *et al.*, 1957; Sicuteri *et al.*, 1965; Ferreira, 1972; Whalley *et al.*, 1987). This immediate effect of bradykinin has been the subject of numerous behavioural and electro-physiological studies (Dray *et al.*, 1988; Lang *et al.*, 1990; Handwerker & Reeh, 1991) and appears to result from the activation of the high threshold nociceptors associated with C fibres. In contrast, little attention has been given to the delayed and long-lasting hyperalgesic effect of bradykinin. This effect was inhibited in rat paws by a bradykinin receptor antagonist and by the cyclo-oxygenase inhibitor, indomethacin (Steranka *et al.*, 1987; 1988; Taiwo & Levine, 1988).

Bradykinin is believed to participate in various models of inflammatory hyperalgesia (Steranka *et al.*, 1988; Costello & Hargreaves, 1989; Fujiyoshi *et al.*, 1989; Chau *et al.*, 1991) and recently a specific BK_2 receptor antagonist, Hoe 140, was shown to inhibit the delayed hyperalgesic effect of bradykinin. Hoe 140 produced analgesia in experimental models of inflammatory pain, particularly in carrageenin induced hyperalgesia (Beresford & Birch, 1992).

Previously we showed that in a rat paw pressure test, carrageenin-evoked hyperalgesia resulted from the combined effect of the release of cyclo-oxyenase products and sympathomimetic amines (Nakamura & Ferriera, 1987). More recently we showed that in this model, a cascade of cytokine release preceded the release of the cyclo-oxygenase products and sympathomimetics (Ferreira *et al.*, 1988; Cunha *et al.*, 1991; 1992). The proposed sequence of events was that carrageenin stimulated the release of TNFα, which: (i) induced IL-1β and IL-6, which stimulated the production of cyclo-oxygenase products and (ii) induced IL-8 which stimulated production of sympathomimetics (Cunha *et al.*, 1992).

Given the capacity of the bradykinin antagonist Hoe 140 to inhibit carrageenin-evoked inflammatory hyperalgesia and a recent report that bradykinin evoked the release of TNFα and IL-1 from macrophage monolayers (Tiffany & Burch, 1989) we investigated the possibility that bradykinin was involved in the cytokine-mediated hyperalgesic pathways activated by carrageenin and lipopolysaccharide (LPS).

(A preliminary account of this work was given at the 14th European Workshop on Inflammation/British Inflammation Research Association joint meeting, London, July 1992).

Methods

Nociceptive test

A constant pressure of 20 mmHg was applied to the hind paws of rats and discontinued when they presented a typical freezing reaction (reaction time). This reaction was characterized by a reduction of escape movements: animals usually made several attempts to escape from the position imposed by the experimental situation. These were followed by alterations in respiratory frequency with the onset of a typical

[1] Author for correspondence.

shivering reaction. The intensity of hyperalgesia was quantified as the variation in reaction time (Δ reaction time) obtained by subtracting values measured 3 h after administration of hyperalgesic substances from (control) reaction times (measured before injection at zero time, Ferreira *et al.*, 1978).

Experimental protocol

Hyperalgesia was induced by bradykinin (0.01–1 μg), carrageenin (30–300 μg), LPS (0.5–5 μg), PGE_2 (100 ng), dopamine (10 μg), TNFα (2.5 pg), IL-1β (0.1 iu), IL-6 (1.0 ng), and IL-8 (0.1 ng), injected in 100 μl into the hind paws of rats (intraplantar, i.pl.). Hoe 140 (0.1–10 mg kg^{-1}) was given s.c. and indomethacin (100 μg) and atenolol (25 μg) were injected in 100 μl into the (same) hind paws, 30 min before the hyperalgesic substances. Antisera neutralising rat TNFα, IL-1β, IL-6 and IL-8 and mixtures of antisera were injected in 50–150 μl into the (same) hind paws, 30 min before the hyperalgesic agents. Results are presented as means with s.e.mean of groups of 5 animals. The statistical tests used are indicated in the text. Formal statistical tests are not reported for differences where means differed by three times the larger s.e.mean.

Materials

Drugs IL-1β, IL-6, IL-8 (72 amino acids) and TNFα were NIBSC preparations coded 86/680, 88/514, 89/520 and 87/650, respectively. Indomethacin was a gift from Merck, Sharpe & Dohme Ltd (Hoddesdon, Herts). Carrageenin was a gift from the FMC Corporation (Philadelphia, U.S.A.). Atenolol, bradykinin, lipopolysaccharide from *E. coli* 0111: B4 (LPS) and dopamine were purchased from Sigma (St. Louis, U.S.A.). PGE_2 was a gift from the Upjohn Co (U.S.A.) and Hoe 140 ([D-Arg^1, Thi^6, (1,2,3,4-tetrahydroisoquinolin-2-yl-carbonyl)^8, ((3as,7as)-octahydroindol-2-ylcarbonyl)^9]bradykinin) was a gift from Hoechst AG (Frankfurt, Germany).

Antisera Sheep anti-rat IL-1β; rabbit anti-rat IL-6, a generous gift from Professor J. Gauldie (McMaster University, Hamilton, Ontario, Canada); sheep anti-human IL-8 serum and sheep anti-murine TNFα serum were kindly provided by Dr R. Thorpe and Dr T. Meager, Division of Immunobiology, NIBSC.

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

Results

Time course of hyperalgesic responses to bradykinin

Injection of bradykinin into one hind paw of rats (0.01–1.0 μg/paw, i.pl.) evoked dose-dependent hyperalgesia in injected paws, which began within 30 min of injection, reached a plateau within 2 h and was maximum at 3 h after injection (Figure 1a). Responses had begun to decline at 4 h and had returned to pre-injection values within 24 h.

Inhibition by Hoe 140 of the hyperalgesic responses to bradykinin

Hoe 140 (0.1–10 mg kg^{-1}) given s.c., 30 min before bradykinin (500 ng/paw), dose-dependently inhibited bradykinin evoked hyperalgesia (Figure 1b). Maximum inhibition was achieved with 1 and 10 mg kg^{-1} Hoe 140, there being no significant difference (ANOVA, $P > 0.05$) in responses to bradykinin in the presence of these two doses of antagonist. In contrast to its capacity to abolish the effect of bradykinin,

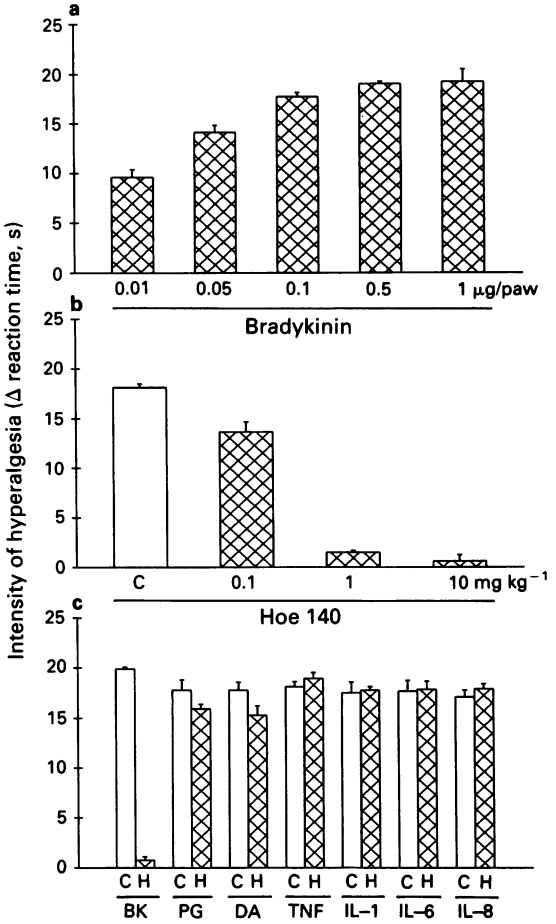

Figure 1 Antagonism by Hoe 140 of the hyperalgesic effects of bradykinin. Responses were measured 3 h after injection (in 100 μl, i.pl.) of bradykinin and other hyperalgesic substances. Hoe 140 was given s.c. 30 min before hyperalgesic substances. Panel (a) shows the intensity of hyperalgesia in injected paws in response to bradykinin (0.01–1.0 μg). Panel (b) shows the effect of saline (C, open column) and Hoe 140 (0.1–10 mg kg^{-1}, cross-hatched columns) on responses to bradykinin (500 ng). Panel (c) shows the effect of saline (C, open columns) and Hoe 140 (H, 1 mg kg^{-1}, cross-hatched columns) on hyperalgesic responses to bradykinin (BK, 500 ng), and prostaglandin E_2 (PG, 100 ng), dopamine (DA, 10 μg), TNFα (TNF, 2.5 μg), IL-1β (IL-1, 0.1 i.u.), IL-6 (1.0 ng) and IL-8 (0.1 ng). Mean ± s.e.mean in groups of 5 rats are shown.

Hoe 140 (1 mg kg^{-1}, s.c.) did not inhibit hyperalgesic responses to prostaglandin E_2 (100 ng), dopamine (10 μg), TNFα (2.5 μg), IL-1β (0.1 iu), IL-6 (1.0 ng) and IL-8 (0.1 ng, Figure 1c). The doses of these agents were the smallest that evoked maximum responses.

Inhibition by Hoe 140 of the hyperalgesic responses to carrageenin and LPS

Hoe 140 (0.1–10 mg kg^{-1}) injected s.c., 30 min before carrageenin (100 μg/paw) and LPS (1.0 μg/paw), dose-dependently inhibited hyperalgesic responses to these substances (Figure 2a) but not responses to a larger dose of LPS (5 μg/paw, Figure 2a and 2c). There was no significant difference between the antinociceptive effects of 1 and 10 mg kg^{-1} Hoe 140 (ANOVA, $P > 0.05$). Hoe 140 at 1 mg kg^{-1} (s.c.) also inhibited hyperalgesic responses to carrageenin (30 and 300 μg/paw, Figure 2b) and LPS (0.5 and 2 μg/paw, Figure 2c). (The volumes of paws injected with carrageenin alone (100 μg) were 700 ± 57 μl, compared with 430 ± 72 μl after Hoe 140 at 0.1 mg kg^{-1}, 316 ± 49 μl after Hoe 140 at 1.0 mg kg^{-1} and 330 ± 56 μl after Hoe 140 at 10 mg kg^{-1}. Bradykinin and LPS did not cause oedema at the doses tested).

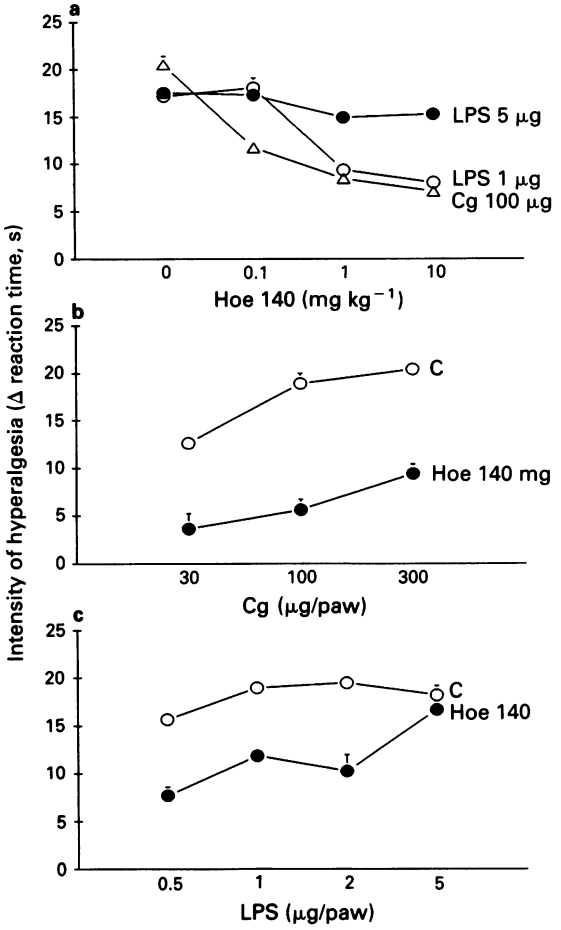

Figure 2 The effect of Hoe 140 on hyperalgesic responses to carrageenin and lipopolysaccharide (LPS). Responses were measured 3 h after injection (in 100 μl, i.pl.) of carrageenin and LPS. Hoe 140 was given s.c. 30 min before carrageenin and LPS. Panel (a) shows the effect of Hoe 140 (0.1–10 mg kg^{-1}) on responses to carrageenin (100 μg, Δ), LPS (1 μg, O) and LPS (5 μg, ●). Panels (b) and (c) show the effects of saline (C, O) and Hoe 140 (Hoe 1 mg kg^{-1}, s.c., ●) on hyperalgesic responses to carrageenin (30–300 μg) and LPS (0.5–5 μg), respectively. Mean ± s.e.mean (frequently within the dimensions of the symbols) in groups of 5 rats are shown.

Inhibition by atenolol and indomethacin of the hyperalgesic responses to bradykinin and LPS

Local pretreatment with indomethacin (100 μg/paw) or atenolol (25 μg/paw), 30 min before the injection of bradykinin (500 ng/paw) or LPS (1 and 5 μg/paw) into the same paw, inhibited the hyperalgesic responses to these substances (Figure 3). The effects of indomethacin and atenolol were additive: their combination markedly attenuated responses to bradykinin and LPS (1 and 5 μg).

Inhibition by anti-cytokine sera of the hyperalgesic responses to bradykinin

Hyperalgesia evoked by bradykinin (500 ng/paw) was inhibited by local pretreatment with anti-cytokine sera, injected into the same paw, 30 min before the bradykinin (Figure 4a). Responses to bradykinin were effectively abolished (i.e., Δ reaction times were reduced by ⩾ 85%) by pretreatment with antiserum neutralising TNFα (50 μl) and inhibited by antisera neutralising IL-1β (50 μl), IL-6 (50 μl) and IL-8 (50 μl). The effects of anti-IL-1β + anti-IL-6 sera (50 μl of each) were not additive whereas the effects of anti-IL-1β + anti-IL-8 sera

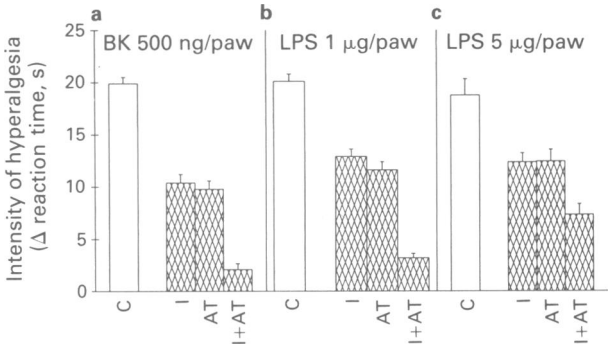

Figure 3 Inhibition by atenolol and indomethacin of the hyperalgesic responses to bradykinin and lipopolysaccharide (LPS). Responses were measured 3 h after injection (in 100 μl, i.pl.) of (a) bradykinin (BK, 500 ng), (b) LPS (1 μg), and (c) LPS (5 μg). Pretreatments were given (in 100 μl, i.pl.) 30 min before bradykinin or LPS. C = saline; I = indomethacin, 100 μg; AT = atenolol, 25 μg. Mean ± s.e.mean in groups of 5 rats are shown.

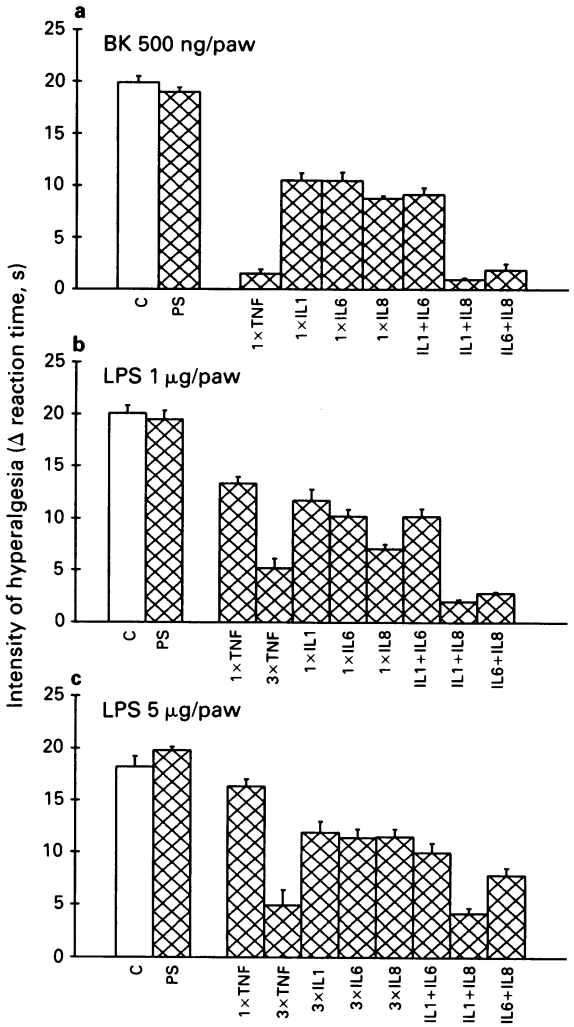

Figure 4 Inhibition of the hyperalgesic responses to bradykinin and lipopolysaccharide (LPS) by anti-cytokine sera. Responses were measured 3 h after injection (in 100 μl, i.pl.) of (a) bradykinin (BK, 500 ng), (b) LPS (1 μg), and (c) LPS (5 μg). Antisera were injected (in a total volume of 150 μl) 30 min before bradykinin or LPS. C = saline; PS = preimmune serum; 1 × TNF = 50 μl anti-TNFα serum; 3 × TNF = 150 μl anti-TNFα serum; 1 × IL1 = 50 μl anti-IL-1β serum; 3 × IL1 = 150 μl anti-IL-1β serum; 1 × IL6 = 50 μl anti-IL-6 serum; 3 × IL6 = 150 μl anti-IL-6 serum; 1 × IL8 = 50 μl anti-IL-8 serum; 3 × IL8 = 150 μl anti-IL-8 serum; 1 + 6 = 50 μl anti-IL-1β + 50 μl anti-IL-6 sera; 1 + 8 = 50 μl anti-IL-1β + 50 μl anti-IL-8 sera; 6 + 8 = 50 μl anti-IL-6 + 50 μl anti-IL-8 sera. Mean ± s.e.mean in groups of 5 rats are shown.

(50 μl of each) or anti-IL-6 + anti-IL-8 sera (50 μl of each) were additive: these combinations effectively abolished the response to bradykinin.

Inhibition by anti-cytokine sera of the hyperalgesic responses to LPS

The pattern of inhibition by the anti-cytokine sera of responses to LPS (1 μg) was similar to that of responses to bradykinin except that a larger dose of anti-TNFα serum (150 μl) was required to attenuate markedly responses to LPS (1 μg, Figure 4b). Hyperalgesic responses to LPS (5 μg) were also markedly attenuated by the larger dose (150 μl) but not by the smaller dose (50 μl) of anti-TNFα serum (Figure 4c). Responses to LPS (5 μg) were inhibited by antisera neutralising IL-1β (150 μl), IL-6 (150 μl) and IL-8 (150 μl). The effects of anti-IL-1β + anti-IL-6 sera (50 μl of each) were not additive whereas the effects of anti-IL-1β + anti-IL-8 sera (50 μl of each) or anti-IL-6 + anti-IL-8 sera (50 μl of each) were additive, especially the former (Figure 4c).

Discussion

Bradykinin has been shown to cause a dose-dependent hyperalgesia that is inhibited in a dose-dependent manner by the BK_2 receptor antagonist Hoe 140 (Heapy *et al.*, 1991). The lack of effect of Hoe 140 on responses to PGE_2, dopamine, TNFα IL-1, IL-6 and IL-8 confirmed the specificity of Hoe 140 reported in other systems (Heapy *et al.*, 1991; Beresford & Birch, 1992) and provided evidence that BK_2 receptors are not involved in the mediation of responses to these other hyperalgesic stimuli.

Previously it was shown that the inflammatory agent, carrageenin, induced production of TNFα, which activated a cascade of cytokine release (Cunha *et al.*, 1992). IL-8, induced by TNFα, stimulated the release of hyperalgesic sympathomimetics (sympathetic hyperalgesia), whereas IL-1β and IL-6, induced by TNFα, stimulated the release of hyperalgesic cyclo-oxygenase products (inflammatory 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).

In the present study, bradykinin-evoked hyperalgesia was abolished by pretreatment with antiserum neutralizing endogenous TNFα whereas TNFα-evoked hyperalgesia was not inhibited by Hoe 140, suggesting that bradykinin induced TNFα. Consistent with this hypothesis was the finding that drugs and antisera shown previously to inhibit responses to TNFα (Cunha *et al.*, 1992) also inhibited responses to bradykinin with a pattern of inhibition similar to that shown previously for TNFα. Also consistent with the hypothesis is a report that bradykinin induced production of TNF and IL-1 in cultures of murine macrophage cell lines (Tiffany & Burch, 1989).

Recent studies showing that IL-1β but not TNFα could induce BK_1 receptor-mediated hyperalgesia in rats in thermal (Perkins & Kelly, 1993) and mechanical (Davies & Perkins, 1993) models more severe than that used in the present study suggest roles for both types of bradykinin receptor in a complex network of hyperalgesic mediators in which bradykinin can induce cytokines and *vice versa*. It has been suggested that BK_2 receptors may play a more significant part in the earlier stages of inflammatory pain, with BK_1 receptors maintaining the hyperalgesic state during inflammation and injury (Dray & Perkins, 1993). Knowledge of the precise location of the two receptor types (e.g., on nociceptive neurones, macrophages, vascular smooth muscle and synovial cells) and of the conditions required for their expression should improve our understanding of the kinin/cytokine pathways.

The pattern of inhibition of hyperalgesic responses to LPS (1 and 5 μg) by indomethacin, atenolol, and the anti-cytokine sera was similar to that shown previously for carrageenin (Cunha *et al.*, 1991; 1992), suggesting that LPS, like carrageenin, evoked hyperalgesia by activating the cytokine cascade. However, the finding that Hoe 140 inhibited hyperalgesic responses to the smaller dose of LPS (1 μg) but not responses to the larger dose (5 μg) shows that the cytokine cascade can be activated independently of BK_2 receptors if the hyperalgesic stimulus is of sufficient magnitude. Subsequent studies should investigate a possible role for BK_1 receptors in the development of hyperalgesia in response to stimuli such as large doses of LPS.

There is a substantial literature showing that carrageenin and LPS activate the plasma kinin system (Rothschild & Gascon, 1966; Damas & Remacle-Volon, 1992) and that LPS is a potent, 'direct' activator of cytokine production, especially from cells of the monocyte/macrophage lineage. Therefore, the contribution of bradykinin to the development of inflammatory hyperalgesia in certain pathological processes may well be overshadowed by either 'direct' stimulation of cytokine production by stimuli such as LPS or by other intermediates in hyperalgesic pathways. Consequently, the usefulness of bradykinin antagonists as analgesics will depend upon the relative contribution of bradykinin to the release of cytokines and other hyperalgesic mediators. Bradykinin antagonists are most likely to be useful analgesics in conditions such as mumps, pancreatitis, intense burns and gram-negative infections in which kinin systems are activated.

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