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Differential sensitivity of antinociceptive assays to the bradykinin antagonist Hoe 140

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1 The antinociceptive activity of the bradykinin (BK) BK_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]BK (Hoe 140) was determined in a range of mouse abdominal constriction assays.

2 Hoe 140 potently inhibited the response induced by i.p. injection of 10 μg BK/mouse, and 1 μg BK/mouse in mice pre-sensitized by i.p injection of prostaglandin E_2 (PGE_2). The ED_{50} values in these assays were 1.9 and 3.7 μg kg^{-1} respectively. This confirms that Hoe 140 is a potent antagonist of BK *in vivo*.

3 Hoe 140 produced potent, but incomplete inhibition of the responses evoked by i.p injection of kaolin or 0.25% acetic acid. ED_{25} values in these assays were 2.7 and 16.1 μg kg^{-1}, and the maximum inhibition produced was 60% and 70% respectively.

4 At doses up to 1 mg kg^{-1}, Hoe 140 was completely ineffective against the abdominal constriction response induced by zymosan. In contrast, morphine, ibuprofen and indomethacin had similar potencies against zymosan, kaolin and acetic acid-induced abdominal constriction.

5 Although zymosan, acetic acid and kaolin all produce qualitatively similar responses, it is appears that they achieve this by different mechanisms. The extent to which BK is involved as a mediator differs between the various types of abdominal constriction assay.

Keywords: Analgesia; bradykinin antagonist; BK_2 receptor; abdominal constriction; indomethacin; ibuprofen

Introduction

Injection of bradykinin (BK) or its application to a blister base causes pain in human volunteers (Whalley *et al.*, 1987). Moreover, significant levels of BK are found at sites of inflammation (Melmon *et al.*, 1967). This has led to speculation that BK antagonists may be useful for the relief of inflammatory pain. However, the extent to which BK contributes to the pain associated with pathological conditions remains to be determined. Although there are significant amounts of BK in inflammatory exudates, other mediators such as prostaglandin E_2 (PGE_2) and PGI_2 are also present. The relative contribution of these mediators to pain and hyperalgesia remains largely unknown.

In animal models it had been established that BK is involved in the pain and hyperalgesia caused by a variety of irritant substances. The hyperalgesia which follows the injection of sodium urate crystals into the rat paw is attenuated by the BK_2 receptor antagonists D-Arg-[Hyp^3,D-Phe^7]BK (NPC 567) and D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]BK (NPC 349). These same compounds have also been reported to reduce the abdominal constriction response induced by intraperitoneal injection of 0.6% acetic acid (Steranka *et al.*, 1987). However, the low potency of these antagonists, and their susceptibility to peptidases has severely limited further investigation of their antinociceptive efficacy.

Recently a much more potent and stable BK_2 receptor antagonist has become available. In radiogland binding studies D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]BK (Hoe 140) was reported to displace BK with a K_i value of 8×10^{-10} M (Hock *et al.*, 1991). In isolated tissue assays, Hoe 140 antagonized the action of BK at the BK_2-receptor with pA_2 values ranging from 9.3 in the rat uterus to 8.1 in the guinea-pig ileum (Lembeck *et al.*, 1991; Hock *et al.*, 1991). The compound was reported to be devoid of activity at the BK_1 receptor (Hock *et al.*, 1991). When administered to rats, Hoe 140 inhibited the hypotensive response to intravenous BK for up to 5 h (Wirth *et al.*, 1991). This compound therefore presents the first opportunity to evaluate in detail the involvement of BK in animal models of antinociceptive activity.

The aim of this study was to determine the antinociceptive activity of Hoe 140 in the abdominal constriction model. A range of stimuli were employed. The BK-induced response was used to determine the dose of Hoe 140 required to antagonize BK receptors *in vivo*. Other studies employed kaolin, which is reported to activate the kallikrein-kinin system (Fujiyoshi *et al.*, 1989b,c), and zymosan which activates the complement cascade and induces PGI_2 synthesis (Rampart *et al.*, 1981; Doherty *et al.*, 1987). The precise mechanism by which dilute acetic acid induces the abdominal constriction response has yet to be elucidated. However, in common with zymosan and kaolin, intraperitoneal injection of acetic acid induces the synthesis of cyclo-oxygenase products, and the resulting abdominal constriction response is attenuated by cyclo-oxygenase inhibitors (Doherty *et al.*, 1987; Fujiyoshi *et al.*, 1989a; Deraedt *et al.*, 1980). A preliminary account of this work has already been published (Heapy *et al.*, 1991).

Methods

All studies were conducted on groups of 10 female mice of the Alderley Park strain weighing between 18 and 25 g. Each experiment was repeated on at least 3 separate occasions. Drugs were dissolved in saline, to which a small amount of $NaHCO_3$ was added in the case of indomethacin and ibuprofen (final pH 7.0–7.4). All compounds were administered subcutaneously in a volume of 10 ml kg^{-1} 30 min before challenge with the constriction-inducing agent.

The abdominal constriction response was induced by injecting 0.4 ml of either acetic acid (0.25% v/v), kaolin (7.5 mg ml^{-1}), zymosan (2.4 mg ml^{-1}) or bradykinin (25 μg ml^{-1}). When BK was used in combination with PGE_2, mice were injected with 0.2 ml PGE_2 (0.5 μg ml^{-1}) 15 min prior to

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a 0.2 ml challenge with BK (5 μg ml^{-1}).

With the exception of groups challenged with BK, the responses were counted over a 15 min period by a trained observer who pressed the appropriate key on a microcomputer each time a response occurred. The computer recorded the time at which each response was logged, allowing subsequent analysis of the time course of the response. ED_{50} values (dose to reduce the number of responses to 50% of control) were calculated by linear regression from the data accumulated over the full 15 min observation period.

Because the response to BK was transient, the BK-treated groups were observed for 30 s following challenge, and the number of mice responding during this period was recorded. ED_{50} values (dose which protected 50% of animals) were calculated by the method of Litchfield & Wilcoxon (1949).

Materials

D-Arg-[Hyp^3,Thi^5,D-Tic^{7}Oic^8]BK (Hoe 140) was synthesized by Dr R. Cotton (ICI Pharmaceuticals). Other materials were obtained from commercial sources: morphine from Macfarlan-Smith, Edinburgh; zymosan, PGE_2, bradykinin, indomethacin and ibuprofen from Sigma, Poole, England.

Results

Antagonism of bradykinin by Hoe 140

To determine the doses of Hoe 140 required to antagonize the actions of BK *in vivo*, the ability of the compound to inhibit BK-induced abdominal constriction responses was determined. Two protocols were employed. In the first, responses were induced by the intraperitoneal injection of 10 μg BK. In the second, mice were pretreated with PGE_2 (100 ng, i.p.) 15 min prior to BK injection. The effect of the PGE_2 was to sensitize the mice, thus allowing the dose of BK to be reduced ten fold to 1 μg.

Animals given PGE_2 (100 ng, i.p.) alone did not exhibit the abdominal constriction response (data not shown). In both assays Hoe 140 produced a dose-related inhibition and completely abolished the response at a dose of 30 μg kg^{-1} (Figure 1a, Table 1).

Antinociceptive tests

Antinociceptive activity was measured in three abdominal constriction models employing acetic acid, zymosan and kaolin as stimuli. Preliminary experiments were conducted to select concentrations of the three algesic agents which produced similar levels of response. At the chosen concentrations, the mean number of responses per 15 min observation period was 15.6 ± 0.8 for zymosan, 18.5 ± 0.9 for acetic acid and 18.3 ± 0.7 for kaolin. Further confirmation that the three agents produce stimuli of similar intensity is provided by the finding that morphine had similar potency in each assay (Figure 1).

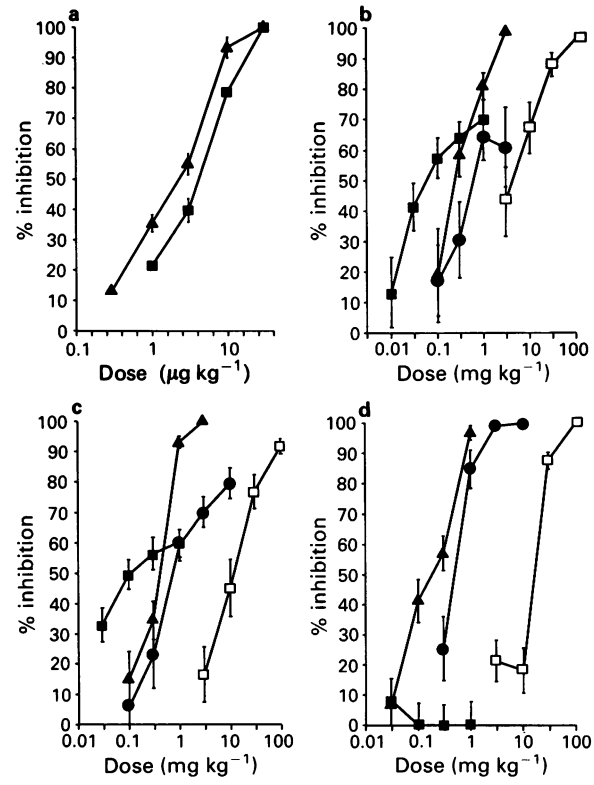

Figure 1 Dose-response curves: (a) Inhibition by Hoe 140 of the percentage of mice exhibiting an abdominal constriction response after 10 μg bradykinin (BK)/mouse (▲), or 1 μg BK/mouse in mice pretreated 15 min earlier with 100 ng PGE_2 (■). (b, c, d) Inhibition by Hoe 140 (■), indomethacin (●), ibuprofen (□) and morphine (▲) of the number of abdominal constriction responses induced by (b) 0.25% acetic acid. (c) kaolin and (d) zymosan. All compounds were injected subcutaneously 30 min before challenge. Data points represent the mean of 3 experiments, each of which employed groups of 10 mice; vertical bars show s.e.mean.

Antinociceptive activity of Hoe 140

Hoe 140 produced potent inhibition of the acetic acid induced-abdominal constriction response (Figure 1b). However, the bradykinin antagonist was unable to inhibit the response completely. The highest dose tested (1 mg kg^{-1}) produced a maximum inhibition of 60–70%. In contrast, morphine was able to abolish the response. An unexpected finding was the difference between the two cyclo-oxygenase

Table 1 Potencies of Hoe 140 and standard antinociceptive agents in abdominal constriction models

	ED_{50} (mg kg^{-1}, s.c.) *and 95% confidence interval*			
	Hoe 140	*Morphine*	*Indomethacin*	*Ibuprofen*
Zymosan	NA	0.17 (0.12–0.25)	0.52 (0.31–0.78)	13.4 (6.3–22.4)
Acetic acid	0.016[a] (0.008–0.024)	0.33 (0.21–0.53)	0.47 (0.18–2.51)	3.8 (1.1–7.9)
Kaolin	0.0027[a] (0.002–0.004)	0.27 (0.20–0.35)	0.86 (0.49–1.66)	12.3 (6.9–20.8)
Bradykinin	0.0019 (0.001–0.004)	–	–	–
Bradykinin + PGE_2	0.0037 (0.003–0.005)	–	–	–

[a]ED_{25} values quoted.

inhibitors. Whilst ibuprofen was able to abolish the response, indomethacin appeared capable of inhibiting it by only 70%.

Similar findings were obtained when kaolin was used as the stimulus (Figure 1c). Only a portion of the response (60%) was sensitive to Hoe 140, whilst both ibuprofen and morphine produced complete inhibition. As in the acetic acid assay, a small portion of the response appeared to be insensitive to indomethacin.

In marked contrast to the other assays, Hoe 140 was completely without effect against zymosan-induced abdominal constriction (Figure 1d). Morphine, indomethacin and ibuprofen all abolished the response at doses similar to those which were effective in the other assays.

Time course studies

In addition to measuring the total number of responses during the 15 min observation period, the time at which each response occurred was also recorded. The time courses following injection of acetic acid (Figure 2), kaolin (Figure 3) and zymosan (not shown) were broadly similar. The response to each agent peaked 4 to 5 min after injection, and slowly declined thereafter. The rate at which the acetic acid response decayed was somewhat slower than with the other two agents, and the response appeared to be biphasic, with an early peak occurring 4 min after injection followed by a less intense but more sustained response between 8 and 13 min.

Hoe 140 reduced both the early and late parts of the acetic acid response, but even at the highest dose the compound was unable to suppress completely either component (Figure 2a). In contrast, when kaolin was used to induce the response

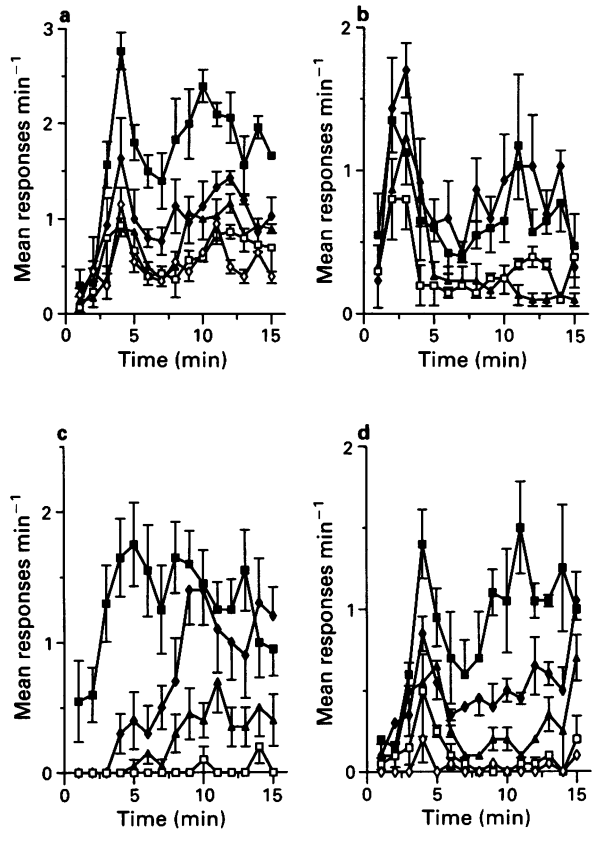

Figure 2 Time course for the inhibition of acetic acid-induced abdominal constriction response by (a) Hoe 140, (b) indomethacin, (c) morphine and (d) ibuprofen. (a) (■) Vehicle; (◆) Hoe 140 0.03 mg kg^{-1}, (▲) 0.1 mg kg^{-1}, (□) 0.3 mg kg^{-1} and (◇) 1 mg kg^{-1}. (b) (■) Vehicle; (◆) indomethacin 0.1 mg kg^{-1}, (▲) 1 mg kg^{-1} and (□) 3 mg kg^{-1}. (c) (■) Vehicle; (◆) morphine 0.3 mg kg^{-1}, (▲) 1 mg kg^{-1} and (□) 3 mg kg^{-1}. (d) (■) Vehicle; (◆) ibuprofen 3 mg kg^{-1}, (▲) 10 mg kg^{-1}, (□) 30 mg kg^{-1}, (◇) 100 mg kg^{-1}.

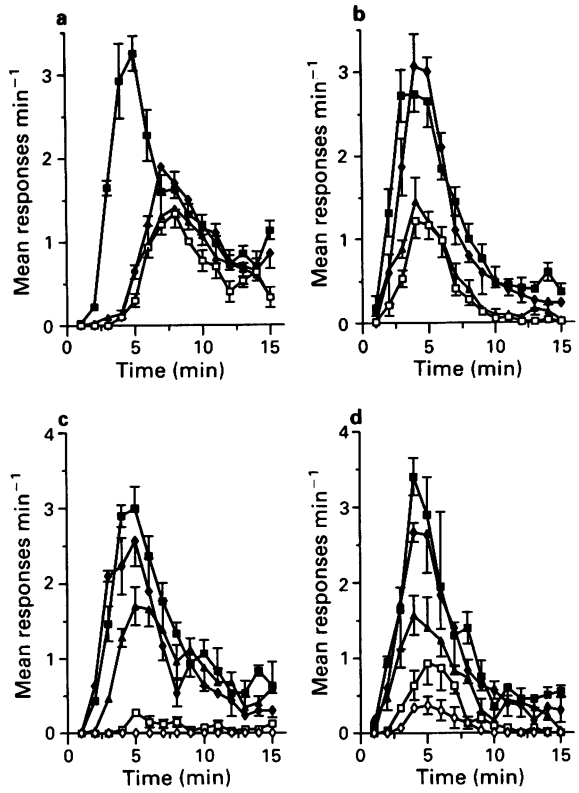

Figure 3 Time course for the inhibition of kaolin-induced abdominal constriction response by (a) Hoe 140, (b) indomethacin, (c) morphine and (d) ibuprofen. (a) Vehicle (■); (◆) Hoe 140 0.01 mg kg^{-1}, (▲) 0.1 mg kg^{-1}, (□) 1 mg kg^{-1}. (b) (■) Vehicle; (◆) indomethacin 0.1 mg kg^{-1}, (▲) 1 mg kg^{-1} and (□) 3 mg kg^{-1}. (c) (■) Vehicle; (◆) morphine 0.1 mg kg^{-1}, (▲) 0.3 mg kg^{-1}, (□) 1 mg kg^{-1}. (d) (■) Vehicle; (◆) ibuprofen 3 mg kg^{-1}, (▲) 10 mg kg^{-1}, (□) 30 mg kg^{-1}, and (◇) 100 mg kg^{-1}.

(Figure 3a), Hoe 140 abolished the effect at early time points, but was without any significant effect from 7 min onwards.

The difference in efficacy between indomethacin and ibuprofen is also apparent in the time course data. In both the acetic acid and the kaolin assays the cyclo-oxygenase inhibitors were less effective against the early part of the response than against the later component. However, whilst the early response was inhibited by higher doses of ibuprofen, a significant proportion of the response appeared to be completely resistant to indomethacin.

Discussion

The first part of this study clearly demonstrates that Hoe 140 is a potent antagonist of bradykinin following subcutaneous administration to the mouse. This confirms and extends earlier findings in the dog, guinea-pig and rat (Wirth *et al.*, 1991; Lembeck *et al.*, 1991, Bao *et al.*, 1991). Hoe 140 therefore represents a valuable tool with which to investigate the role of bradykinin in other *in vivo* assays.

Earlier studies using BK antagonists such as D-Arg-[Hyp^3,D-Phe^7]BK and D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]BK have revealed that BK antagonists display antinociceptive activity against BK-induced and urate-induced hyperalgesia of the rat paw (Steranka *et al.*, 1988) and in an acetic acid-induced abdominal constriction assay (Steranka *et al.*, 1987). In the latter study, these compounds failed to inhibit totally the response to acetic acid. Given the relatively low potency and short duration of action of these early antagonists, it was impossible to determine whether this remaining response was due to mediators other than BK, or due to the poor phar-

macokinetics of the antagonists used. The present study clarifies this point. At doses thirty times greater than were required to abolish BK-induced responses, Hoe 140 produced only 70% inhibition of the acetic acid-induced response. This clearly demonstrates that BK is a major component, but not the only mediator of the acetic acid response. However, there is little evidence from the time course study of any temporal separation between the components of the acetic acid response.

The effects seen against kaolin were qualitatively similar to those obtained with acetic acid. The proportion of the response that was sensitive to Hoe 140 was similar to both assays. It has previously been reported that the kaolin-induced abdominal constriction response is abolished by soyabean trypsin inhibitor, a non-specific kallikrein inhibitor (Fujiyoshi *et al.*, 1989c). This would imply that the response is almost entirely due to BK. The present finding that a significant proportion of the response is insensitive to a potent antagonist at the BK_2 receptor could be interpreted as suggesting the action of BK involves both BK_1 and BK_2 receptors. Hoe 140 is not an antagonist at the BK_1 site (Hock *et al.*, 1991). Alternatively, additional mediators could contribute to the response. Data from the time course study, which show that Hoe 140 abolished the kaolin response only at early time points (<6 min), confirm that this response consists of more than one component.

Of particular interest is the finding that Hoe 140 had no effect against the zymosan-induced response. Nevertheless, in all three assays the response was inhibited by cyclo-oxygenase inhibitors. The most obvious interpretation is that a cyclo-oxygenase product is the final common mediator produced by all stimuli, but in the kaolin and acetic acid models, prostanoid synthesis is, at least in part, secondary to kinin generation. The prostanoid synthesis induced by zymosan is independent of the kinin system. This hypothesis is consistent with earlier observations that acetic acid, kaolin and zymosan all induce the release of prostanoids into the peritoneal cavity (Deraedt *et al.*, 1980; Doherty *et al.*, 1987; Fujiyoshi *et al.*, 1989a). It has also been shown that kinin levels in the peritoneal cavity increase following injection of kaolin (Fujiyoshi *et al.*, 1990), but do not increase in response to zymosan (Damas *et al.*, 1990). Furthermore, Ferreira *et al.* (1973) have demonstrated that BK is a potent stimulus for prostaglandin release.

An interesting finding was the apparent difference in antinociceptive efficacy between two cyclo-oxygenase inhibitors, ibuprofen and indomethacin. This finding is in accord with an earlier study by Luttinger (1985) in which ibuprofen was shown to increase the reaction time of mice in response to dipping their tails in water at 45°C, whereas indomethacin was without effect. Since both indomethacin and ibuprofen are assumed to share a common mode of action, there is no simple explanation for these findings.

The results of this study demonstrate that kinins play a major role in the abdominal constriction response induced by acetic acid and kaolin, but are minimally involved in the response to zymosan. Since cyclo-oxygenase inhibitors were effective in all three assays, one interpretation of these findings is that BK antagonists will prove to be less effective in the treatment of pain than the non-steroidal anti-inflammatory agents. On the other hand, it is known that kinins are present in synovial fluid from patients with rheumatoid arthritis. Moreover, the concentrations of BK found in patients are comparable to those which have been shown to activate nociceptors (Dunn & Rang, 1990). On this basis it could reasonably be argued that kinins must at least contribute to arthritic pain. Thus a BK_2 receptor antagonist may prove to be an effective treatment for inflammatory pain, and the predictive value of the zymosan abdominal constriction assay would be called into question. The data available at present do not allow either of these possibilities to be dismissed.

Given the wide range of mediators produced at inflammatory sites, it is difficult to know whether removing the action of just one of these, BK, will provide a significant therapeutic benefit. It is likely that this question will only be answered when a BK antagonist is evaluated in man.

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