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The nonpeptide B_2 receptor antagonist FR173657: inhibition of effects of bradykinin related to its role in nociception

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1 The nonpeptide bradykinin B_2 receptor antagonist, FR173657 ((E)-3-(6-acetamido-3-pyridyl)-N-[N-(2, 4-dichloro-3-[(2-methyl-8-quinolinyl) oxymethyl] phenyl]-N-methylaminocarbonylmethyl] acrylamide), was tested in models involving bradykinin-induced activation of primary afferent neurones *in vitro* and *in vivo*.

2 Bradykinin-induced contractions of the rabbit isolated iris sphincter muscle mediated by tachykinin release from trigeminal afferent neurones were inhibited in a non-competitive manner by FR173657. A pK_B value of 7.9 was calculated. Effects of substance P were unaffected by FR173657.

3 Nociceptive behavioural responses following intraplantar injection of bradykinin in unanaesthetized rats were reduced by 0.3 μmol kg^{-1} FR173657 s.c. ($P<0.05$), and completely abolished by 3 μmol kg^{-1} ($P<0.05$). Peroral administration of 5 μmol kg^{-1} FR173657 abolished the bradykinin effects ($P<0.05$); lower doses had no significant effect.

4 Shortening by intraplantar injection of bradykinin of the paw withdrawal latency in response to radiant heat was abolished by 3 μmol kg^{-1} FR173657 s.c. ($P<0.05$), while 300 nmol kg^{-1} had an intermediate effect. Hyperalgesia induced by prostaglandin E_2 remained unaffected by FR173657.

5 Blood pressure reflexes following i.p. instillation of bradykinin in anaesthetized rats were inhibited by FR173657 s.c. with an ID_{50} of 1.1 μmol kg^{-1}, while the peptidic B_2 antagonist icatibant (Hoe-140; D-Arg^0-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) caused inhibition at significantly lower doses (ID_{50} 8.5 nmol kg^{-1} $P<0.001$). Responses to hydrochloric acid i.p. remained unaffected by FR173657.

6 FR173657 or similar nonpeptide compounds may be useful for the development of drugs for diseases involving pain induced by the release of endogenous kinins, i.e. especially in acute inflammatory conditions.

Keywords: Bradykinin antagonists; FR173657; icatibant (Hoe-140); nociception; hyperalgesia

Introduction

The potent effects of kinins on blood vessels leading to hyperaemia, hypotension and oedema formation have firmly established the view of an involvement of kinin release in pathophysiological conditions, especially in states of acute inflammation (Marceau *et al*., 1983; Proud & Kaplan, 1988). Kinins are potent stimulators of afferent nerve fibres and as such are probably the most potent endogenous algesic substances (Juan & Lembeck, 1974). Since most of the acute inflammatory effects of kinins including pain are induced via activation of B_2 receptors (Bathon & Proud, 1991; Hall, 1992), the development of bradykinin B_2 receptor antagonists is hoped to lead to compounds that in the end might be useful for the treatment of inflammatory diseases.

All bradykinin antagonists that were available until recently were peptide analogues of bradykinin. Although the so-called 'second generation' antagonists such as D-Arg^0-Hyp^3-Thi^5-D-Tic^7-Oic^8-bradykinin (icatibant or Hoe-140: Lembeck *et al*., 1991; Hock *et al*., 1991; Wirth *et al*. 1991) still were of peptide nature, their improved duration of action *in vivo* has already lead to initial clinical trials of this compound (see Wirth *et al*., 1995). Recently, the identification of an orally active, nonpeptide ligand for the B_2 receptor, FR173657 (Aramori *et al*., 1997; Asano *et al*., 1997), promises to be a major advance for the development of drugs targeting the effects of kinins released during inflammatory diseases. FR173657 is highly potent and selective in intestinal, uterine, bronchial and vascular smooth muscles *in vitro* (Griesbacher *et al*., 1997; Rizzi *et al*., 1997) and exhibits a marked duration of action *in vivo* (Griesbacher & Legat, 1997). Vascular effects of endogenous kinins are also potently inhibited in experimental models of pleuritis (Majima *et al*., 1997), pancreatitis and cystitis (Griesbacher, 1997).

The potent algesic actions of kinins further highlight the importance of bradykinin antagonists for a possible clinical use against inflammatory pain (Steranka *et al*., 1988; Dray & Perkins, 1993). FR173657 has as yet not been tested in any pain-related model. The present investigation aims at defining the ability of FR173657 to inhibit actions of bradykinin that are directed at afferent nerves (C-fibres) that are involved in the signalling of pain.

Methods

Isolated iris sphincter muscle of the rabbit

Rabbits of either sex (3–5 kg, Dept. Animal Biology) were killed by i.v. injection of an overdose of pentobarbitone sodium. Both eyes were enucleated immediately and the iris sphincter muscle of each eye was prepared according to Kern (1970). The muscle was suspended in an organ bath in Tyrode solution (37°C, gassed with 95% O_2 and 5% CO_2) under a resting tension of 1.5 mN. After an equilibrium period of 30 min, cumulative concentration-response curves to bradykinin (1 nM–2 μM) or substance P (3–900 nM) were obtained

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according to Ueda *et al*. (1984) using an isometric K30 force transducer (Hugo Sachs, March-Hugstetten, Germany). Only one concentration-response curve was established in each individual preparation. FR173657 (30 or 300 nM) was added to the organ bath 5 min before the start of the cumulative additions of bradykinin or substance P, whereas the vehicle (DMSO, final bath concentration 0.03% v/v) was employed for control tissues.

Nociceptive behavioural responses

In order to observe nociceptive behavioural responses to intraplantar injections of bradykinin, female Sprague-Dawley rats were placed in an observation chamber (40×40 cm), placed in a darkened environment, 10 min before the test. Bradykinin (50 nmol in 50 μl phosphate-buffered saline) was given as a intraplantar injection into one hind paw and the animal was returned to the observation chamber. The ensuing behaviour was observed through the glass floor of the chamber using a mirror for a period of 30 min after the injection. The behaviour was rated in a blind manner with scores modified from the formalin test (Cohen *et al*., 1984) as described previously (Legat *et al*., 1994). The times (T_S in s) which the animal spent within each of the 3 scores (S) of non-normal behaviour (1: favouring the uninjected paw; 2: elevating the injected paw off the ground; 3: licking or biting the injected paw except when part of grooming behaviour) were recorded using a personal computer. As in previous investigations (e.g. Griesbacher *et al*., 1994) the nociceptive activity during each one-min period was expressed as a mean score value S* calculated using the formula

$$S* = \frac{\sum_{S=1}^{3}(S \cdot T_S)}{60}$$

For this model, both the s.c. and the p.o. route of administration were chosen for FR173657. The antagonist (300 nmol kg^{-1} or 3 μmol kg^{-1}) was injected s.c. 60 min before the experiment, while control animals received an injection of an appropriate volume (0.5 ml kg^{-1}) of the vehicle, DMSO. For the p.o. administration, rats were fasted overnight and given FR173657 (0.5 – 5.0 μmol kg^{-1}) in 0.5% (w/v) methylcellulose (1 ml kg^{-1}).

Thermal hyperalgesia

Thermosensitivity was determined according to Hargreaves *et al*. (1988) using a Plantar Test apparatus (Ugo Basile, Varese, Italy). Animals were placed in plastic cages ($22 \times 17 \times 14$ cm) with a glass floor. After a 10 min habituation period, the plantar surface of one hind paw was exposed to a beam of radiant heat applied from below. The radiant heat source consisted of an infrared bulb (Osram halogen-bellaphot bulb 8V, 50W). A photoelectric cell detected light reflected from the paw and turned off the lamp automatically when movement of the paw interrupted the reflected light. The paw withdrawal latency was measured to the nearest 0.1 s; the cut-off time was 23 s. Five minutes after the last of 3 control measurements (performed at 10 min intervals) intraplantar injections were made in volumes of 50 μl under brief nitrous oxide analgesia. One hindpaw was injected with bradykinin (0.5 nmol) whereas the contralateral paw was injected with the vehicle (phosphate-buffered saline containing bestatin, thiorphan and captopril; 50 pmol each). The peptidase inhibitors were added in order improve the separation of the kinin effect from the solvent effect (Schuligoi *et al*., 1994) Ten minutes after the treatment, the paw withdrawal latencies were determined for the treated and contralateral paw; measurements were repeated at 10 min intervals for 30 min. As a measure of the nociceptive threshold, the mean of the paw withdrawal latencies determined at 10 and 20 min after the injection of bradykinin was used. In control experiments prostaglandin (PG) E_2 was injected into the paw at a dose of 0.9 nmol which causes a lowering of the nociceptive threshold similar to that of bradykinin. The vehicle for PGE_2 was ethanol (final concentration 0.2%) which does not have an effect of its own (Schuligoi *et al*., 1994). FR173657 (0.3 or 3 μmol kg^{-1}) was administered s.c. 60 min before the experiment, whereas control animals were injected with DMSO (0.5 ml kg^{-1}).

Visceral nociceptive blood pressure reflexes

Female Sprague-Dawley rats (200 – 300 g) were anaesthetized with pentobarbitone sodium and phenobarbitone sodium (40 mg kg^{-1}, i.p.). The trachea was cannulated to allow unhindered respiration. One carotid artery was cannulated and connected to a Statham pressure transducer to monitor systemic arterial pressure. Similar to the method of Holzer-Petsche (1992), the abdomen was opened by a midline incision and a polyethylene cannula was placed into a fold of the mesentery, avoiding direct contact of the tip of the cannula with the stomach or intestine. Bradykinin (100 pmol) was instilled via this cannula at intervals of 15 min and the ensuing shortlasting hypotensive response was taken as an index for the activation of visceral nociceptive afferent nerve fibres (Holzer-Petsche, 1992). For each challenge with bradykinin the i.p. cannula was placed in a new position, in order to avoid sensitization or desensitization of the visceral nociceptors. Immediately after the third challenge with bradykinin, FR173657 (0.3 – 10 μmol kg^{-1}), icatibant (3 – 100 nmol kg^{-1}) or their vehicles (DMSO or 154 mM NaCl, respectively; 1 ml kg^{-1}) was administered s.c. and the regular i.p. injections of bradykinin were continued for a further period of 90 min. In order to ascertain that the inhibition caused by FR173657 was specific, the antagonist was tested at the highest dose (10 μmol kg^{-1}) against blood pressure reflexes elicited by 100 mM hydrochloric acid applied in samples of 100 μl, as described above for bradykinin.

Animal experiments followed the NIH Principles of Laboratory Animal Care and the Austrian Law on Experiments in Living Animals, and were granted permission by the Commission for Animal Experiments of the Austrian Ministry for Science.

Substances

(E) - 3 - (6 - acetamido - 3 - pyridyl) - N - [N - [2,4 - dichloro-3-[(2-methyl- 8-quinolinyl) oxymethyl] phenyl]-N-methylaminocarbonylmethyl] acrylamide (FR173657, a gift from Fujisawa Pharmaceutical Co., Osaka, Japan) was dissolved in DMSO; further dilutions were made with 154 mM NaCl solution for the *in vitro* application and with DMSO for the *in vivo* experiments using s.c. injections. For the p.o. administration, FR173657 was suspended in 0.5% (w/v) methylcellulose (Fluka, Buchs, Switzerland). All solutions were prepared freshly on the day of the experiments. Bradykinin (Sigma Chem. Co., St. Louis, M.O., U.S.A.) was dissolved in a 154 mM solution of NaCl at a concentration of 1 mM. Substance P (Sigma) was dissolved in 0.01 M acetic acid, further dilutions were made in 154 mM NaCl. The stock solutions were stored at -20°C and diluted as needed with phosphate-buffered saline before the experiments. Prostaglandin E_2 (Sigma) was dissolved (10 mM) in ethanol and diluted

with saline. Bestatin and thiorphan were obtained from Sigma, captopril was from Heyden (Regensburg, Germany). Phosphate-buffered saline (mM): NaCl 136.9, KCl 2.7, KH_2PO_4 1.5, Na_2HPO_4 7.7; pH was 7.4 at 20°C. Tyrode solution (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.2, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, D-glucose 5.6; pH 7.4 at 37°C when gassed with 5% CO_2 in O_2. All salts were of analytical grade and were from Merck (Darmstadt, Germany). DMSO (dimethylsulphoxide, dried) was obtained from Merck. Pentobarbitone sodium (Nembutal) was from Sanofi Santé Animale (Libourne, France), phenobarbitone sodium was obtained from Apoka (Vienna, Austria).

Data analysis

Comparisons between different treatment groups were made using non-parametric multiple comparisons for independent data (Zar, 1984). The effects of FR173657 on the concentration-response curves to bradykinin in the isolated iris sphincter muscle of the rabbit were analysed according to Kenakin (1993), in order to obtain an estimate for the apparent affinity of the antagonist in this tissue. Equieffective concentrations of bradykinin in the absence ([A]) and presence ([A′]) of a concentration ([B]) of FR173657 were calculated by linear regression analysis after logit-log transformation for linearization of the curves. The slope (b) of a plot of 1/[A] versus 1/[A′] was used to calculate pK_B by the equation $pK_B = -\log_{10}([B]/(b-1))$. Values of [A] and [A′] had to be calculated in separate groups of tissues so that pK_B could only be determined as one estimate with 95% confidence interval. For the calculation of ID_{50} values for FR173657 and icatibant in the visceral nociception test, the bradykinin-induced reflex fall in blood pressure determined at 90 min after the administration of the bradykinin antagonist was expressed as % of the bradykinin-induced effect at the beginning of the experiment and was plotted against the dose of the antagonist. Following logit-log transformation of the inhibition curve of each antagonist, linear regression analysis (Zar, 1984) was used to determine the ID_{50}. For the comparison of the potency of the two antagonists, the 2 inhibition curves were tested for deviation from parallelity and the horizontal distance in log units was calculated (Geigy, 1982). All other values presented are arithmetical means with s.e.mean.

Results

Isolated iris sphincter muscle of the rabbit

Bradykinin produced concentration-dependent contractions of the iris sphincter muscle when applied in a cumulative manner at concentrations between 1 nM and 2 μM. The force of the contractions induced by the highest bradykinin concentrations was 6.4 ± 0.8 mN. The pD_2 value for the contractile effects of bradykinin was determined as 7.3 ± 0.1 ($n=8$). The contractions induced by bradykinin were inhibited significantly by FR173657 (Figure 1a). The inhibition consisted mainly in a pronounced, concentration-dependent reduction in the maximum effect ($P<0.01$), indicating a non-competitive mode of action of FR173657. However, at the same time a certain rightward shift of the concentration-response curve was observed, inasmuch as the $-\log EC_{50}$ for bradykinin in the presence of 300 nM FR173657 was only 6.7 ± 0.1 ($n=4$), which is significantly ($P<0.01$) different from the pD_2 value of bradykinin determined under control conditions (see above). The pK_B value calculated according to Kenakin (1993), as an estimate of the apparent affinity of FR173657, was determined as 7.9 (95% confidence interval 7.1–8.8). However, the contractile effects of substance P remained completely unaffected by FR173657 (300 nM (Figure 1b).

Nociceptive behavioural responses

For the observation of nociceptive behavioural responses bradykinin was given as an intraplantar injection into one hindpaw of unanaesthetized rats. The injection procedure itself, when carried out with the solvent, phosphate-buffered saline, alone did not produce any behavioural signs indicative of a nociceptive effect. When bradykinin (50 nmol) was injected intraplantar, nociceptive behavioural responses were observed within less than 1 min of the injection (Figure 2). The responses consisted mainly of leaning towards the uninjected paw (score 1) and lifting the injected paw off the ground (score 2). This kind of behaviour was observed during the first 20 min following the injection. However, also phases of normal behaviour where the animal applied its full weight onto the injected paw (score 0) was visible during this period. More severe signs of effects unpleasant to the animal, i.e. licking or biting the injected paw (score 3) unrelated to grooming behaviour, were observed only during short periods (total less than 1 min) during the first 10–15 min after the injection of bradykinin. After at least 20 min the rats showed no signs of nociception until the end of the observation period of 30 min,

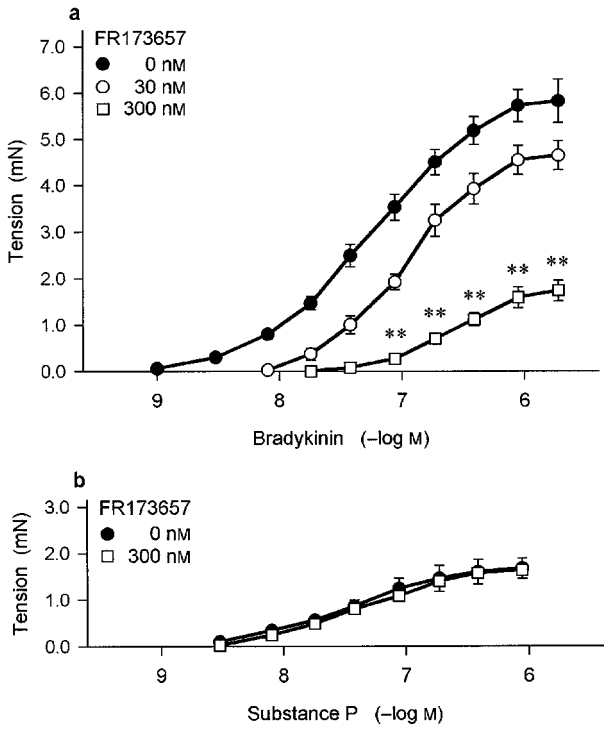

Figure 1 Effect of FR173657 on isometric contractions of the isolated iris sphincter muscle of the rabbit in response to bradykinin (a) and substance P (b): the peptides were applied to the tissue in a cumulative manner and one concentration-response curve was established in each individual preparation. FR173657 was added to the organ bath 5 min before the first addition of the agonist in concentrations of 30 nM or 300 nM whereas control tissues were treated with the solvent of FR173657 (DMSO, final concentration 0.03% v/v). Significance of difference from solvent controls: $^{**}P<0.01$. Symbols represent mean values, vertical lines show s.e.mean; where no s.e.mean is indicated it was smaller than the symbol. $n=4-8$ per group.

but rather explored their environment or exhibited grooming behaviour, as did normal rats.

The s.c. injection of FR173657 (Figure 2a), given 60 min before the experiment, resulted in a dose-dependent inhibition of the nociceptive behavioural responses to bradykinin. The lower dose of 300 nmol kg^{-1} caused a partial, significant ($P<0.05$) inhibition of the effect. No licking or biting of the paws (score 3) occurred in these animals ($n=6$), whereas control animals exhibited this behaviour for 39 ± 11 s ($n=6$, $P<0.05$). Furthermore, the duration of the responses rated as score 1 or 2 was shortened from a total of 846 ± 53 s ($n=6$) during the first 20 min of observation in the controls to only 486 ± 64 s ($n=6$, $P<0.05$) observed only during the initial 10 min of the observation period. The higher dose of 3 μmol kg^{-1} abolished the nociceptive responses altogether.

Following oral administration of FR173657 (Figure 2b), the nociceptive behavioural effects of bradykinin were almost abolished ($P<0.05$) when the antagonist was used at a dose of 5 μmol kg^{-1}. Pretreatment with 1.5 5 μmol kg^{-1} strongly reduced the effects in 4 out of 8 animals, whereas in the remaining 4 rats virtually no effect was noted; hence the reduction in the mean values shown in Figure 2b for this dose did not achieve statistical significance. The lowest dose of 0.5 μmol kg^{-1} was completely ineffective (not shown in the figure).

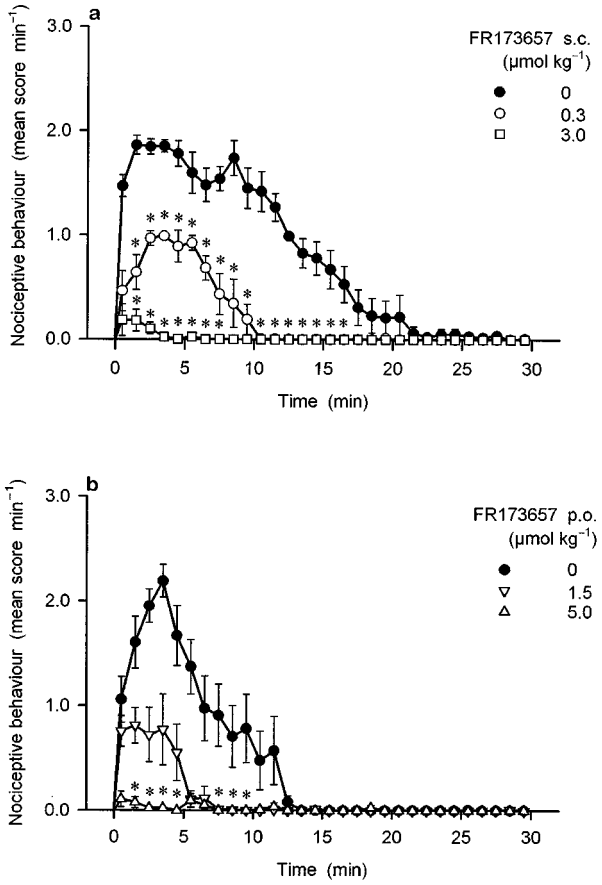

Figure 2 Effect of FR173657 on bradykinin-induced nociceptive behavioural responses in rats. Bradykinin (50 nmol) was injected intraplantar into one hindpaw and the ensuing behaviour was monitored and rated with scores (see Methods). For each one min period a mean score value was calculated from the times that the animals spent in each of the scores during that period. One hour before the experiments, the rats were pretreated with FR173657 either s.c. (0.3 or 3 μmol kg^{-1}, a) or p.o. (0.5 or 1.5 μmol kg^{-1}, b); control rats received a corresponding volume of the solvent (0.5 ml kg^{-1} DMSO, s.c. or 1 ml kg^{-1} 0.5% methyl cellulose, p.o.). Symbols represent mean values, vertical lines show s.e.mean; where no s.e.mean is indicated it was smaller than the symbol. $n=4-8$ per group.

Thermal hyperalgesia

The radiant heat stimulus of the infrared light applied to the plantar surface of the hindpaws of rats elicited a paw withdrawal reaction after a latency of about 8–10 s in all animals. The response was stable when the challenge with the heat stimulus was applied at 10 min intervals. The intraplantar injection of a low dose of bradykinin (0.5 nmol) together with a cocktail of peptidase inhibitors (bestatin, thiorphan and captopril) shortened the paw withdrawal latency by about 30% when determined 10 and 20 min after the intraplantar injection. However, due to the low dose of bradykinin, the lowering of the thermal nociceptive threshold in some animals was confined to one of the two measurements so that the mean of the two values was found to be the most suitable parameter to assess the hyperalgesic effect of bradykinin consistently.

In animals pretreated with FR173657 (3 μmol kg^{-1}, s.c.) 60 min before the beginning of the experiment, the bradykinin-induced shortening of the paw withdrawal latency in response to radiant heat was completely prevented ($P<0.05$) (Figure 3). The effect of bradykinin seemed to be only partially reduced by the lower dose (300 nmol kg^{-1}) of FR173657. However, this effect failed to reach statistical significance. The intraplantar injection of prostaglandin E_2 (0.9 nmol) lowered the thermal nociceptive threshold to an extent similar to that seen after bradykinin (Figure 3). However, this effect remained completely unaffected by FR173657 even when applied at a dose (3 μmol kg^{-1}) that completely blocked the effects of bradykinin (see above).

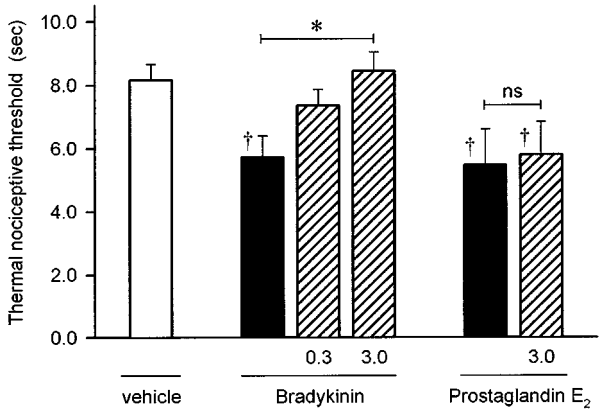

Figure 3 Effect of FR173657 on bradykinin-induced thermal hyperalgesia in rats: bradykinin (0.5 nmol) was injected into one hindpaw in combination with bestatin, thiorphan and captopril (50 pmol each) while controls received an equivalent volume (50 μl) of the vehicle (phosphate-buffered saline with the peptidase inhibitors). Separate groups of rats were injected with prostaglandin E_2 (0.9 nmol) instead of bradykinin. The thermal nociceptive threshold was determined as the mean paw withdrawal latency (in s) in response to infrared irradiation of the plantar surface of the paw determined at 10 and 20 min after the intraplantar injection. FR173657 was given s.c. 60 min before the experiment in the doses given below the columns (in μmol kg^{-1}). Significance of difference from vehicle controls; † $P<0.05$; significance of effect of FR173657 as indicated by the horizontal lines: *$P<0.05$. Columns are mean values and vertical lines show s.e.mean; $n=6-12$.

Visceral nociceptive blood pressure reflexes

The instillation of bradykinin (100 pmol) into folds of the mesentery caused shortlasting (1 min) hypotensive reflex responses (−10 to −25 mmHg) which could be repeated at regular intervals of 15 min without an apparent decrease or increase in the size of the response. Following the s.c. administration of FR173657, these reflex response were inhibited in a dose-dependent manner (Figure 4a). While the lowest dose that was used (300 nmol kg^{-1}) did not have an effect, all higher doses (1–3 μmol kg^{-1}) caused a decrease in the responses to bradykinin. For all doses, the effect developed over a period of at least 60 min and was significant ($P<0.05$) when compared to the reflex responses obtained in control animals treated with the solvent, DMSO (1 ml kg^{-1}). An ID_{50} value of 1.1 μmol kg^{-1} (95% confidence interval 0.2–6.0 μmol kg^{-1}) was calculated for FR173657.

Nociceptive hypotensive reflex responses elicited by instillation of 100 μl samples of 100 mM hydrochloric acid instead of bradykinin amounted to -26 ± 6 mmHg under basal conditions. This effect was unchanged 90 min after the administration of 10 μmol kg^{-1} FR173657 (-31 ± 7; $n=5$) or its vehicle DMSO (-26 ± 7; $n=5$).

The peptidic bradykinin antagonist icatibant also caused dose-dependent inhibitions of the effects of bradykinin in this model (Figure 4b). However, the inhibition had already developed within 30 min and also occurred at lower doses. The ID_{50} value for this antagonist was determined as 8.5 nmol kg^{-1} (95% confidence interval 0.7–93 μmol kg^{-1}) which is significantly ($P<0.001$) smaller than the value determined for FR173657.

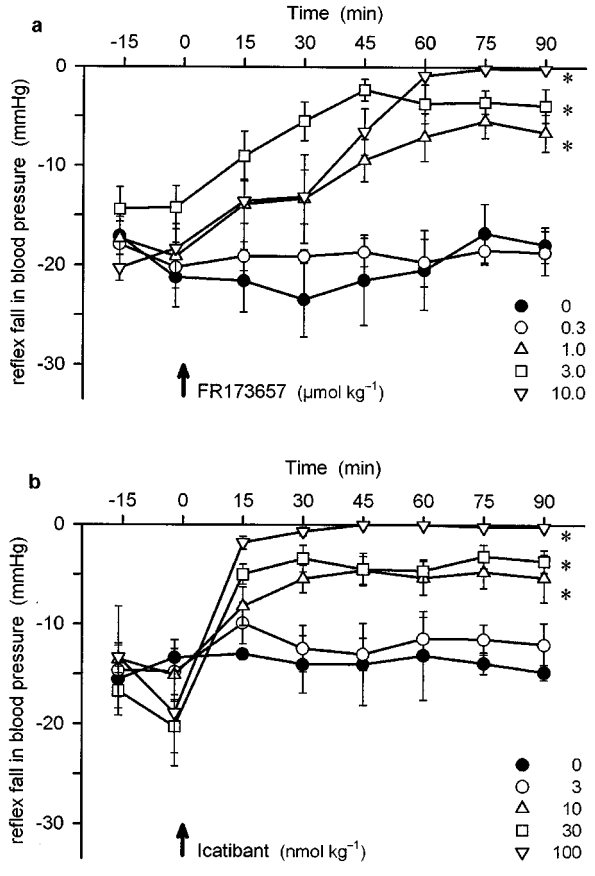

Figure 4 Effect of B_2 receptor antagonists on nociceptive blood pressure reflexes elicited by i.p. instillation of bradykinin (100 pmol): bradykinin was applied at regular intervals of 15 min and the shortlasting (1 min) hypotensive reflex responses were monitored in a carotid artery. At the time indicated by the arrow either FR173657 (0.3–10 μmol kg^{-1}, a) or icatibant (3–100 nmol kg^{-1}, b) was given as s.c. injection. Control animals were injected with the respective vehicle (DMSO or 154 mM NaCl; 1 ml kg^{-1}). Symbols represent mean values and vertical lines show s.e.mean; $n=5-8$.

Discussion

Many severe pathological conditions, especially inflammatory disorders involve the endogenous release of kinins (see Hall, 1992). Many of these conditions are also characterized by severe symptoms of pain. Since kinins are probably the most potent endogenous algogens known (Juan & Lembeck, 1974), the inflammatory pain is likely to be due to release of kinins (Dray & Perkins, 1993). Hence, bradykinin receptor antagonists might not only be used for the inhibition of the vascular effects of kinins, but also may have a potential as analgesic drugs. Most of the acute nociceptive effects of kinins have been attributed to the B_2 receptor subtype although in the subacute stages B_1 receptors might also be induced (Perkins *et al*., 1993; Dray & Perkins, 1997). Antagonists available to date have been amino acid sequence analogues to bradykinin and thus were subject to enzymatic degradation. The peptide nature of these compounds also precluded any oral administration. FR173657 is the first nonpeptide bradykinin B_2 antagonist for which an oral bioavailability has been demonstrated (Asano *et al*., 1997; Majima *et al*., 1997).

Iris sphincter muscle contractions

The nociceptive actions of kinins involve the activation of thin, unmyelinated nerve fibres (C-and Aδ-fibres) which are characterized pharmacologically by their sensitivity to the neurotoxin, capsaicin (Fitzgerald, 1983). Activation of these primary afferent fibres not only leads to propagation of the nociceptive signal towards the central nervous system, but also induces the release of neuropeptides from the peripheral nerve terminals. This mechanism can also be suitably studied in the isolated iris sphincter muscle of the rabbit *in vitro*. Bradykinin elicits contractions of this preparation not via a direct action on the smooth muscle fibres of the sphincter muscle but by the release of tachykinins (substance P and/or neurokinin A) from the peripheral terminals of primary afferent trigeminal nerve fibres (Ueda *et al*., 1984) following the activation of B_2 receptors (Griesbacher & Lembeck, 1987). Such trigeminal afferents may be involved in the mediation of pain in a number of ocular diseases (Hitchings, 1980; Yanofsky, 1988), especially in inflammatory conditions.

The inhibitory potency of FR173657, estimated as a pK_B value of 7.9 in the present study, is about 1 log unit lower than the affinity estimates that have been determined previously for this antagonist in intestinal, tracheal and uterine smooth muscles (8.7–9.3; Asano *et al*., 1997; Griesbacher *et al*., 1997; Rizzi *et al*., 1997). Compared to the first nonpeptide compound that was published as B_2 antagonist, WIN 64338 (Sawutz *et al*., 1994), FR173657 seems to be clearly more potent in the iris sphincter preparation, since a pK_B of only 6.6 for WIN 64338 was found by Hall *et al*. (1995). While WIN 64338 reportedly acts competitively in the iris sphincter preparation (Hall *et al*., 1995), the apparent mode of antagonism of FR173657 is not competitive since the maximum bradykinin-induced effect was significantly reduced. This behaviour has already been observed with FR173657 in

some, but not all, smooth muscle preparations involving direct actions of bradykinin (Griesbacher *et al*., 1997).

Nociceptive behaviour and thermal hyperalgesia

Two kinds of effects of bradykinin related to its nociceptive action in the skin have been investigated in the rat paw, namely nociceptive behavioural responses following the intraplantar injection of a high dose of bradykinin (which also induces a pronounced paw oedema) and thermal hyperalgesia induced by a small bradykinin dose together with peptidase inhibitors. Both effects involve the B_2 receptor-mediated activation of afferent neurones. However, while the former effect is a direct action of bradykinin itself, the latter effect is an indirect one relying on the secondary, local release of prostaglandins from the tissue (Schuligoi *et al*., 1994).

Both effects were inhibited in a dose-dependent manner by FR173657. Despite the fact that the doses of bradykinin are largely different in the two models, the effective doses of FR173657 were identical in both tests. It is interesting to note that the antagonistic potency of the antagonist (ID_{50} of about 300 nmol kg^{-1}) is equal to that found previously for the inhibition of the vascular effects of bradykinin in the rat paw (see Griesbacher & Legat, 1997). Hence, nociceptive and vascular B_2 receptors in the rat skin are equally accessible for inhibition by this antagonist.

Visceral nociceptive reflexes

The i.p. instillation of bradykinin in anaesthetized rats activates capsaicin-sensitive afferent neurones and leads to reflex falls in blood pressure which allows the extent of stimulation to be quantified (Holzer-Petsche, 1992). These hypotensive reflex responses belong to a class of cardiovascular reflexes typical of nociceptive 'pseudo-affective' responses following acute somatic or visceral stimulation (Ness & Gebhart, 1990).

While the finding that this effect was inhibited by FR173657 is not surprising in the light of the aforementioned data, the effective dose range requires some consideration. The ID_{50} of 1.1 μmol kg^{-1} might seem to be similar to that needed to block the nociceptive actions of bradykinin in the skin (see above), the doses are extremely high compared to those required to inhibit the bradykinin-induced increases in visceral vascular permeability (Griesbacher & Legat, 1997). There, FR173657 is active at very low doses (ID_{50} about 10 nmol kg^{-1}). Hence, the difference in the potency of FR173657 to antagonize visceral vascular and visceral nociceptive effects is in the range of 2 log units, whereas no such difference has been found in the skin (see above). Certain 'tissue-dependent' differences have also been found in other *in vivo* models not related to nociception (Griesbacher & Legat, 1997). Compared to FR173657 the peptide antagonist icatibant is more potent by about 2 orders of magnitude (ID_{50} 8.5 nmol kg^{-1}). However, the effective doses of icatibant in this test correspond well to those doses that have been found previously for most other *in vivo* models. Icatibant also exhibits a different time course of inhibition. While FR173657 required at least 60–75 min to develop its full inhibitory effect following s.c. administration, the respective time period was only 15–30 min for icatibant. The data obtained here have thus to be taken into account when planning studies with FR173657.

All the effects of FR173657 described here can be regarded as specific actions of the antagonist at bradykinin B_2 receptors, since the actions of substance P, prostaglandin E_2 or hydrochloric acid on afferent neurones remained completely unaffected by FR173657.

In summary, we have shown that FR173657 is a potent inhibitor of pain-related actions of bradykinin both *in vitro* and *in vivo*. The oral bioavailability of this nonpeptide B_2 receptor antagonist which has been confirmed in the present study may open new therapeutic strategies aiming at a more causal intervention in painful diseases involving the endogenous release of kinins, i.e. especially in inflammatory pain.

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