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Effects of FR173657, a non-peptide B_2 antagonist, on kinin-induced hypotension, visceral and peripheral oedema formation and bronchoconstriction

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1 Kinins are believed to play a key role in many inflammatory conditions. Therefore, bradykinin antagonists are being developed for potential therapeutic applications. In the present investigation we describe the pharmacology, *in vivo*, of (E)-3-(6-acetamido-3-pyridyl)-N-[N-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-N-methylaminocarbonylmethyl]acrylamide (FR173657), a novel, non-peptide bradykinin antagonist.

2 The hypotensive effects of i.v. injections of bradykinin (50 pmol) in captopril-pre-treated anaesthetized rats were significantly inhibited by 100 nmol kg^{-1} FR173657 s.c., and completely abolished by 300 nmol kg^{-1}. The full inhibitory effect developed within 60 min and remained unchanged for at least 4 h. However, the effect was reversible, since 24 h after an injection of 300 nmol kg^{-1} FR173657 no inhibitory effect could be observed.

3 The plasma protein extravasation into the pancreas and duodenum induced by an i.v. infusion of bradykinin (11 nmol kg^{-1} within 20 min) in captopril-treated anaesthetized rats was completely abolished by FR173657 at doses of 30 nmol kg^{-1} s.c. and above, given 60 min before bradykinin. FR173657 3 nmol kg^{-1} was ineffective, while a dose of 10 nmol kg^{-1} produced an intermediate effect.

4 The paw oedema induced by the subplantar injection of bradykinin (30 nmol) in anaesthetized rats was inhibited slightly by s.c. injection of FR173657 0.3 μmol kg^{-1}, whereas 1 and 3 μmol kg^{-1} produced significant inhibition of the bradykinin-induced oedema. The maximum inhibition amounted to about 50% and could not be increased even when the dose of FR173657 was increased to 30 μmol kg^{-1}. FR173657 did not effect the oedema caused by histamine or 5-hydroxytryptamine.

5 Bradykinin (20 nmol kg^{-1}, i.v.) caused increases in pulmonary inflation pressure by 300–600 Pa in anaesthetized, respirated guinea-pigs. The effect was reduced to 58 ± 9% of the initial value 60 min after the s.c. injection of FR173657 1 μmol kg^{-1}, whereas only 9 ± 7% remained after 10 μmol kg^{-1}. The bronchoconstrictor actions of histamine remained unaffected by FR173657.

6 In summary, FR173657 is a highly potent and selective bradykinin antagonist. The inhibitory action *in vivo* lasts for longer than 4 h but is fully reversible. FR173657, or similar compounds, will be a useful tool for the pharmacological investigation of pathophysiological states and may possess a therapeutic potential in diseases involving the endogenous release of kinins.

Keywords: Bradykinin antagonists; FR173657; blood pressure; hypotension; inflammation; plasma protein extravasation; paw oedema; bronchoconstriction

Introduction

It is well established that kinins are involved in the pathophysiology of many severe pathological conditions, amongst them allergic and inflammatory airway diseases (Barnes *et al*., 1988; Farmer, 1991), arthritis (Bhoola *et al*., 1992), inflammatory pain (Steranka *et al*., 1988; Dray & Perkins, 1993), neurogenic inflammation (Geppetti *et al*., 1995), endotoxic and anaphylactic shock (Sharma, 1993), acute pancreatitis (Griesbacher & Lembeck, 1996) and possibly also cerebral ischaemia (Makevnina *et al*., 1995). Therefore, the development of specific bradykinin receptor antagonists is of great importance for possible therapeutic regimens aimed at preventing the action of kinins released endogenously during the course of such conditions.

A major part of the acute pathological actions of kinins is mediated by the B_2 receptor (Bathon & Proud, 1991; Hall, 1992) while B_1 receptors seem to play important roles later during the course of subacute or chronic inflammatory states following their upregulation and *de novo* synthesis (Marceau, 1995). Current second generation antagonists for the B_2 receptor, such as icatibant (Hoe-140: D-Arg-Hyp^3-Thi^5-D-Tic^7-Oic^8-bradykinin: Lembeck *et al*., 1991; Hock *et al*., 1991; Wirth *et al*., 1991), possess a remarkable duration of action also *in vivo* despite their peptide nature. The motive for the development of non-peptide antagonists for the receptors of peptide agonists is to obtain compounds that, in case of a clinical application, can be administered orally. However, the discovery of such compounds is much more difficult than the development of peptide ligands because no model molecule is available. The possibility of the development of non-peptide antagonists for bradykinin receptors has been proven by the discovery of WIN 64338 (Salvino *et al*., 1993) which has been shown to possess good activity at bradykinin B_2 receptors not only *in vitro* (Sawutz *et al*., 1994) but also *in vivo*, at least following their intravenous administration (Hall *et al*., 1995; Sawutz *et al*., 1995).

In this investigation, the *in vivo* characteristics of FR173657 are described in experimental models having relevance to haemodynamic, inflammatory and bronchial actions of kinins.

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Methods

Rat blood pressure

Female Sprague-Dawley rats (230–280 g body wt, Forschungsinstitut für Versuchstierzucht, Himberg, Austria) were anaesthetized by i.p. injections of pentobarbitone sodium (40 mg kg^{-1}) and phenobarbitone sodium (160 mg kg^{-1}). In addition, all rats received an i.p. injection of the kininase II inhibitor, captopril (50 μmol kg^{-1}). The trachea was cannulated to allow unhindered respiration. One carotid artery was cannulated to monitor the systemic arterial pressure by a Statham pressure transducer. Bradykinin (50 pmol) was injected into one jugular vein at regular intervals of 15 min. When the ensuing shortlasting drops in blood pressure were reproducible, FR173657 (100 nmol kg^{-1} or 300 nmol kg^{-1}) was injected s.c. and the challenges with i.v. bradykinin were resumed 15 min later and continued for a period of 4 h. Control animals were injected with a corresponding volume (0.5 ml kg^{-1}) of DMSO instead of FR173657.

Visceral plasma protein extravasation

Female Sprague-Dawley rats were anaesthetized i.p. with pentobarbitone sodium (40 mg kg^{-1}) and phenobarbitone sodium (20 mg kg^{-1}). Captopril (50 μmol kg^{-1}) was injected i.p. at the same time. The azo dye, Evans blue (20 mg kg^{-1}), which stains albumin and, to a lesser extent, other plasma proteins (Allen & Orahovats, 1948) was injected into a jugular vein. Five min later, bradykinin was infused i.v. for a period of 20 min at a rate of 0.56 nmol kg^{-1} min^{-1}. In vehicle control animals this infusion was replaced by a corresponding volume (0.19 ml kg^{-1} min^{-1}) of phosphate-buffered saline. The pretreatment with FR173657 (3, 10, 30, 100 or 300 nmol kg^{-1}, s.c.) or its solvent (DMSO, 0.5 ml kg^{-1}, s.c.) was carried out 1 h before the start of the bradykinin infusion. At the end of the experiment, the animals were exsanguinated by perfusion via the ascending aorta with 40 ml of a 154 mM NaCl solution containing 5 iu ml^{-1} heparin. Pieces of the duodenum and the caudal pancreas were excised, dried for 16 h in a vacuum centrifuge and weighed to determine the dry weight of the tissues. The samples were incubated in 8 ml of formamide for 48 h at 55°C to extract the Evans blue (Gamse *et al*., 1980). The dye was then quantified on a Shimadzu spectrophotometer at a wave length of 620 nm (Saria & Lundberg, 1983). The extent of plasma protein extravasation was quantified as amount of Evans blue per dry weight of the tissues.

Rat paw oedema

Female Sprague-Dawley rats were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}) and phenobarbitone sodium (20 mg kg^{-1}). The volumes of both hind paws were measured by volume displacement with a Ugo Basile plethysmometer. Bradykinin (30 nmol), 5-hydroxytryptamine (5 nmol) or histamine (500 nmol) was given as a subplantar (s.pl.) injection into one hind paw whereas the contralateral paw was injected with a corresponding volume (50 μl) of phosphate-buffered saline. The changes in the paw volumes were then measured 5, 10, 15, 20, 25, 30, 45 and 60 min after the s.pl. injections. Changes in the paw volumes were expressed as % of the basal volume. The paw oedema caused by the agonists was quantified as net effect by subtracting the values of the contralateral vehicle control paw. FR173657 (0.3, 1, 3, and 30 μmol kg^{-1}) or its solvent, DMSO (0.5 ml kg^{-1}), was administered as s.c. injection 1 h before the s.pl. injections of bradykinin, 5-hydroxytryptamine or histamine.

Bronchoconstriction in guinea-pigs

Guinea-pigs of either sex (Forschungsinstitut für Versuchstierzucht, Himberg, Austria) were anaesthetized with urethane (1.6 g kg^{-1}, i.p.). One jugular vein was cannulated to allow i.v. injections. The trachea was cannulated and connected to a Hugo Sachs ventilation pump following the i.v. administration of pancuronium bromide (2.5 mg kg^{-1}). Artificial respiration was carried out with a stroke volume of 5 ml at a rate of 65 strokes min^{-1}. The pulmonary inflation pressure was monitored in a side-arm of the ventilation system by means of a Statham pressure transducer. Bradykinin (20 nmol kg^{-1}) or histamine (50 nmol kg^{-1}) was injected i.v. at regular intervals of 15 min. After the first 2 injections of the bronchoconstrictor agents, FR173657 was given at doses of 1.0 or 10.0 μmol kg^{-1} (s.c.); control animals were injected with a corresponding volume (0.5 ml kg^{-1}) of the vehicle, DMSO.

Substances

For the *in vivo* experiments, FR173657 ((E)-3-(6-acetamido-3-pyridyl)-N-[N-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-N-methylaminocarbonylmethyl] acrylamide) was dissolved and diluted in dimethylsulphoxide (DMSO). All solutions were prepared freshly on the day of the experiments. Stock solutions of bradykinin (Sigma Chem. Co., St. Louis, M.O., U.S.A.) were prepared in a 154 nM solution of NaCl at a concentration of 1 mM. The stock solutions were stored at −20°C and diluted as needed with phosphate-buffered saline before the experiments. Histamine and 5-hydroxytryptamine were purchased from Sigma. Pentobarbitone sodium (Nembutal) was from Sanofi Santé Animale (Libourne, France). Phenobarbitone sodium (Apoka, Vienna, Austria) was dissolved in 154 mM NaCl at concentrations of 20–80 mg ml^{-1}. Urethane was purchased from Fluka (Buchs, Switzerland) and dissolved in 154 mM NaCl at a concentration of 250 mg ml^{-1}. Pancuronium bromide (Pavulon) was obtained from Organon (Vienna). Captopril was from Squibb-von Heyden (Vienna). The composition of the phosphate-buffered saline was (in mM): NaCl 136.9, KCl 2.7, KH_2PO_4 1.5, Na_2HPO_4 7.7; the pH was 7.4 at 20°C. All salts were of analytical grade and were obtained from Merck (Darmstadt, Germany). Further substances were Evans blue (Sigma), formamide and DMSO (max. 0.03% H_2O) (Merck).

Statistical analysis

Comparisons between different treatment groups were made by non-parametric multiple comparisons for independent data: comparisons of effects after the administration of FR173657 with values obtained before this treatment at the beginning of experiments were made by non-parametric multiple comparison for dependent data (Zar, 1984). All values presented are arithmetical means with s.e.mean. Where no s.e.mean is indicated in the figures, it was smaller than the symbol depicting the mean value.

Results

Rat blood pressure

The intravenous injection of bradykinin at a dose of 50 pmol in anaesthetized rats pretreated with the kininase II inhibitor, captopril (50 μmol kg^{-1}, i.p.) resulted in a fall of the mean arterial pressure of 15–35 mmHg which lasted for only about 1 min. This effect of bradykinin could be repeated at intervals of 15 min. During the observation period of 4 h, only a slight decrease in the responsiveness to bradykinin could be seen; the effect of the last injection of bradykinin was 83±8% of the effect of bradykinin obtained at the beginning of the experiment.

The s.c. injection of FR173657 (100 or 300 nmol kg^{-1}) caused a decrease in the responsiveness which developed within a period of 60 min after the injection (Figure 1). At this time

point, the hypotensive effect of bradykinin was reduced to $40 \pm 9\%$ of its effect before the injection of 100 nmol kg^{-1} of FR173657. In animals injected with 300 nmol kg^{-1} of the antagonist, the effect of bradykinin was completely abolished. No restoration of the hypotensive action of bradykinin was observed during the remaining course of the experiment (Figure 1). The basal blood pressure (80 ± 11 mmHg) was completely unaffected even by the highest dose of FR173657 (81 ± 10 mmHg at the end of the experiment; $n = 6$).

Separate animals were injected s.c. with FR173657 (300 nmol kg^{-1}) or with its solvent, DMSO (0.5 ml kg^{-1}), 24 h before the determination of the effects of i.v. injection of bradykinin on arterial pressure. The fall in blood pressure induced by bradykinin was 16 ± 2 mmHg in the solvent controls and 17 ± 2 mmHg ($n = 5$) in the rats pretreated with FR173657.

Visceral plasma protein extravasation

The extravasation of plasma proteins into the pancreas and the duodenum was quantified as accumulation of Evans blue, injected at the beginning of the experiment, in the tissue. The i.v. infusion of phosphate-buffered saline, the vehicle for bradykinin, at a rate of 3.8 ml kg^{-1} within 20 min caused a similar degree of plasma protein extravasation in the pancreas (71 ± 10 μg Evans blue g^{-1} dry wt; Figure 2a) and in the duodenum (98 ± 10 μg Evans blue g^{-1} dry wt; Figure 2b). The i.v. infusion of bradykinin (11 nmol kg^{-1} within 20 min) caused an approximately eight-fold increase in the plasma proteins extravasation in the pancreas compared to the vehicle effect whereas the effect was much smaller in the duodenum.

The s.c. pretreatment of the rats with FR173657 at doses of 30 nmol kg^{-1} and above 60 min before the start of the infusion of bradykinin completely abolished the bradykinin-induced effect in the pancreas, while the dose of 3 nmol kg^{-1} was completely ineffective (Figure 2a). The basal Evans blue content of the pancreas in rats infused with phosphate-buffered saline instead of bradykinin and pretreated s.c. with DMSO (0.5 ml kg^{-1}, open column in Figure 2a) was the same in animals pretreated with the highest dose of FR173657 (300 nmol kg^{-1}, not shown in the figure).

A similar pattern of the effects of FR173657 was also observed in the duodenum (Figure 2b). The bradykinin-induced

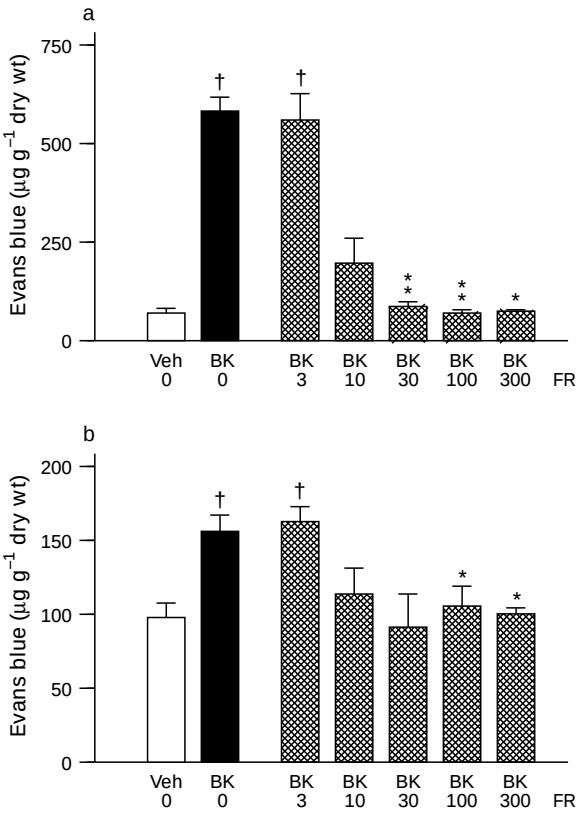

Figure 2 Effect of FR173657 on bradykinin-induced visceral plasma protein extravasation. Bradykinin (BK) was infused i.v. for a period of 20 min at a rate of 0.56 nmol kg^{-1} min^{-1} in anaesthetized rats; control rats received an infusion of the vehicle, phosphate-buffered saline (Veh; 0.19 ml kg^{-1} min^{-1}). The amount of plasma protein extravasated into the pancreas (a) and into the duodenum (b) was quantified as tissue content of the dye, Evans blue (in $\mu g\ g^{-1}$ dry wt), which was given i.v. (20 mg kg^{-1}) at the beginning of the experiment. One hour before the start of the BK infusion the animals were injected s.c. with FR173657 in the doses given below the columns (in nmol kg^{-1}) or with its vehicle (DMSO, 0.5 ml kg^{-1}). Significance of difference: $\dagger P < 0.05$ compared to vehicle alone; $*P < 0.05$, $**P < 0.01$ compared to BK alone. Columns represent mean values, vertical lines give s.e.mean; $n = 6-8$ per group.

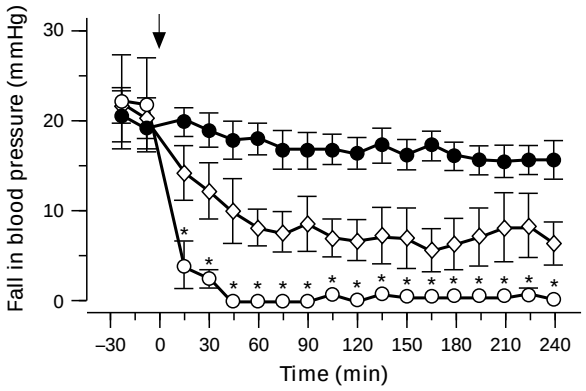

Figure 1 Effect of FR173657 on bradykinin-induced fall in rat blood pressure. Bradykinin (50 pmol) was injected i.v. at regular intervals of 15 min and the ensuing shortlasting (about 1 min) drop in mean arterial pressure was measured in a carotid artery. FR173657 (◇) 100 nmol kg^{-1} and (○) 300 nmol kg^{-1} or its vehicle, DMSO (0.5 ml kg^{-1}, ●) was injected at the time indicated by the arrow. Significance of difference from the control group: $*P < 0.01$. Symbols represent mean values of 4–5 experiments per treatment group, vertical lines show s.e.mean.

Evans blue accumulation in the duodenum was inhibited significantly by 100 and 300 nmol kg^{-1} FR173657. Pretreatment with FR173657 30 nmol kg^{-1} resulted in a mean Evans blue content of the duodenum which was similar to that observed in rats that had received an i.v. infusion of phosphate-buffered saline instead of bradykinin. As in the pancreas, the basal Evans blue accumulation in the duodenum of vehicle control rats was unaffected by pretreatment with FR173657 300 nmol kg^{-1}.

Raw paw oedema

The subplantar (s.pl.) injection of bradykinin (30 nmol) in anaesthetized rats caused oedema of the paws leading to a net increase in the paw volume of 50–60% compared to the pre-injection volume (Figure 3a). The paw oedema developed within 20–30 min, and no obvious further changes in the paw volume were observed at the end of the observation period of 60 min.

Pretreatment with FR173657 (s.c.) 60 min before the subplantar injection of bradykinin, inhibited the kinin-induced paw oedema in a dose-dependent manner (Figure 3a). The lowest dose of FR173657 (0.3 μmol kg^{-1}) caused a slight red uction of the observed values for the oedema, but the difference

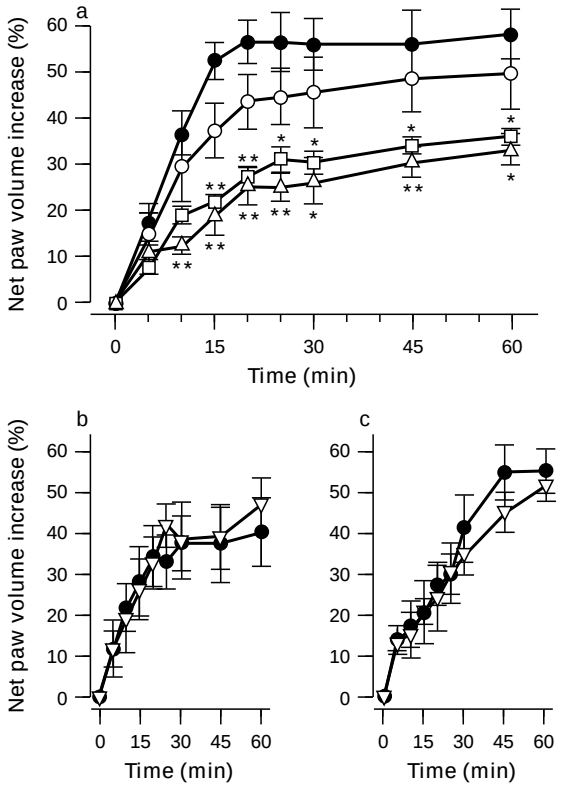

Figure 3 Effect of FR173657 on rat paw oedema. Bradykinin (30 nmol, a), histamine (500 nmol, b) or 5-hydroxytryptamine (5-HT, 5 nmol, c) was injected s.pl. into one hindpaw of anaesthetized rats while the contralateral paw was injected with phosphate-buffered saline (50 μl). The increases in the volume of the bradykinin-injected paw (minus volume changes of the contralateral paw) were measured at regular intervals by volume displacement. One hour before the injection of bradykinin, histamine or 5-HT, the rats were given a s.c. injection of FR173657 (○) 300 nmol kg^{-1}, (□) 1 μmol kg^{-1}, (△) 3 μmol kg^{-1}, (▽) 5 μmol kg^{-1} or its vehicle, DMSO (0.5 ml kg^{-1}, ●). *$P<0.05$, **$P<0.05$ compared to vehicle controls. Symbols are mean values, vertical lines show s.e.mean; $n=5-7$.

did not achieve statistical significance when all treatment groups were compared simultaneously. At higher doses (1 μmol kg^{-1} or 3 μmol kg^{-1}) the inhibition caused by FR173657 proved to be statistically significant. However, the bradykinin-induced paw oedema could not be blocked completely, but could only be reduced to about 50% of the response to bradykinin alone. Even a dose of 30 μmol kg^{-1} (not shown in the figure) was not able to produce further inhibition, consistent with the earlier finding that part of the bradykinin-induced oedema is due to a non-receptor mediated release of 5-hydroxytryptamine from skin mast cells (Griesbacher *et al*., 1996).

The s.pl. injection of 500 nmol histamine caused a paw oedema with a time course similar to that of bradykinin. Plateau values of 30–45% net paw volume increases were reached between 25 and 35 min of the injection which remained stable until the end of the 60 min observation period (Figure 3b). The s.pl. injection of 5-hydroxytryptamine (5 nmol) caused paw oedema which, when determined at 60 min, was similar to that following bradykinin (54±5% new paw volume increase). However, unlike the response to bradykinin, the oedema induced by 5-hydroxytryptamine developed continuously throughout the 60 min period of observation (Figure 3c). Neither the paw oedema induced by histamine nor that observed in response to 5-hydroxytryptamine was affected by pretreatment with FR173657 at a dose of 5 μmol kg^{-1} administered 60 min before the experiment (Figure 3b and c).

Bronchoconstriction in guinea-pigs

The i.v. injection of bradykinin 20 nmol kg^{-1} in anaesthetized, artificially ventilated guinea-pigs caused increases in pulmonary inflation pressure of 300–600 Pa. The peak of the bronchoconstriction was reached within less than 30 s. Thereafter, the inflation pressure gradually returned to values similar to those before the injection of bradykinin within a period of 2–3 min. The injections of bradykinin could be repeated at regular intervals of 15 min without apparent change in the effect. Higher doses of bradykinin could not be employed since these doses caused bronchoconstrictor effects that lasted for more than 5 min. In addition, the pulmonary inflation pressure did not fully revert to baseline values and the potency of such doses of bradykinin decreased with time. Therefore, the dose of 20 nmol kg^{-1} was used despite its small effect compared to those of histamine (see below).

When FR173657 was administered s.c., a dose-dependent inhibition of the bronchoconstrictor potency of bradykinin was observed (Figure 4a). The full inhibitory effect developed within 30–45 min. Sixty min after the administration of FR173657 (1 μmol kg^{-1}), the effect of bradykinin was reduced to 58±9% of the effect observed before the injection of the antagonist ($P<0.05$). The higher dose of FR173657, 10 μmol kg^{-1}, almost abolished the effect of bradykinin at this point (9±7% of the initial effect, $P<0.01$; note that the sig-

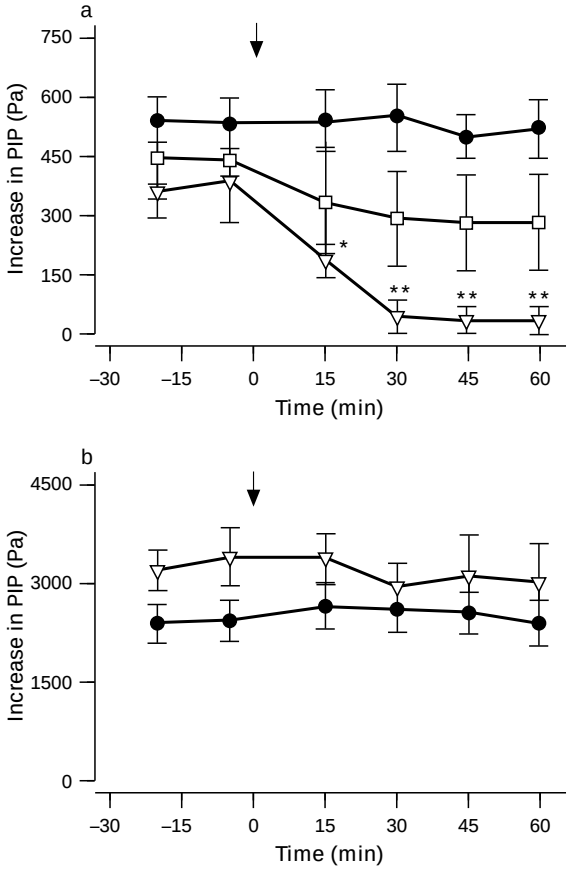

Figure 4 Effect of FR173657 on bronchoconstriction in anaesthetized guinea-pigs. Bradykinin (20 nmol kg^{-1}, a) or histamine (50 nmol kg^{-1}, b) was injected i.v. in artificially ventilated (5 ml stroke volume, 65 strokes min^{-1}) guinea pigs pretreated with pancuronium bromide (2.5 mg kg^{-1}, i.v.). Increases in pulmonary inflation pressure (PIP, in Pa) were monitored in the respiratory system. FR173657 (□) 1 μmol kg^{-1}, (▽) 10 μmol kg^{-1} or its vehicle (DMSO, 0.5 ml kg^{-1}: ●) was injected s.c at the time indicated by the arrow. Significance of difference from vehicle controls: *$P<0.05$, **$P<0.01$. Symbols represent mean values from 4–6 experiments per group, vertical lines indicate s.e.mean.

nificances shown in Figure 4a compare the different treatment groups at each individual time point). The s.c. injection of the vehicle for FR173657, DMSO (0.5 ml kg^{-1}) did not cause any change in the effect of bradykinin.

Histamine, injected i.v. at a dose of 50 nmol kg^{-1}, caused increases in pulmonary inflation pressure of 2–4 kPa. The time needed to reach the peak values (less than 10 s) was even shorter than that for bradykinin. Immediately after the peak value was reached, the respiration pressure fell rapidly, but a period of up to 5 min was needed for the full restoration of the respiratory pressure. Although histamine caused bronchoconstrictor effects much greater than those of bradykinin, histamine could be injected at intervals of 15 min without tachyphylaxis. FR173657, given s.c. at a dose of 10 μmol kg^{-1}, had no effect on the bronchoconstrictor activity of histamine (Figure 4b).

Discussion

The recently disclosed non-peptide bradykinin B_2 antagonist, FR173657, is apparently also active *in vivo* following its oral administration (Inamura *et al*., 1996) which could make the compound a good candidate for use in man provided that the high selectivity and potency and the slow reversibility which we have demonstrated *in vitro* in a separate investigation (Griesbacher *et al*., submitted) also proves to be true *in vivo*. The pharmacodynamic and/or pharmacokinetic properties of a drug are greatly influenced by its route of administration. In the present study, we have chosen the subcutaneous route of administration for the following reasons. Firstly, this procedure enables a more direct comparison with previous generations of bradykinin antagonists, especially the peptide antagonist, icatibant, but also the non-peptide antagonist, WIN 64338, which to date has only been used parenterally. Secondly, many of the potential clinical applications of bradykinin antagonists are very severe conditions requiring hospitalization with intensive care. In many of these conditions, the parenteral administration of drugs is favoured because of their faster and more secure uptake.

The determination of the time course of inhibition of a new receptor antagonist *in vivo* is important for subsequent experimental and clinical studies since pretreatment times and treatment protocols depend on these parameters. Following the subcutaneous administration of FR173657, the inhibition of intravenously applied bradykinin developed within periods of 30–60 min. Somewhat longer periods were required for the inhibition of the hypotensive effects bradykinin as compared to the bronchoconstrictor actions. Thus, a pretreatment time of 60 min was chosen for this route of administration for all further experimental studies.

The extremely slow reversibility of the inhibitory action that we have determined in our *in vitro* investigation (Griesbacher *et al*., submitted) is also a striking feature of FR173657 *in vivo*. The inhibition lasted for several hours, since no recovery of the effects of intravenously injected bradykinin could be determined until the end of the longest period of observation of 4 h. In comparison, the second generation peptide antagonist, icatibant, given by the same route of administration, loses about 50% of its inhibitory potency at the same time point when investigated in the same models (Wirth *et al*., 1991). Thus, FR173657 exhibits a decidedly longer duration of inhibition compared to previous bradykinin receptor antagonists. Since neither in the experiments monitoring the hypotensive effects of bradykinin nor in the bronchoconstriction assay a recovery of the effects of bradykinin could be observed within the period of observation, certainly the question of the reversibility of the inhibition must be addressed. The present finding that the inhibitory actions of FR173657 were completely reversed one day after its subcutaneous injection, provides evidence that the half-life of the inhibitory action of FR173657 *in vivo* is greater than 4 h but also clearly less than 24 h.

In the *in vitro* assays that we have carried out so far, the concentrations of FR173657 required for complete inhibition of the effects of submaximal concentrations of bradykinin were 100–300 nM in all intestinal, airway or vascular preparations tested, and the variations in the apparent inhibitory potencies in the different tissues or species remained within 1 log unit (Griesbacher *et al*., submitted). A comparison of the doses required for partial or complete inhibition of the effects of bradykinin *in vivo* investigated here, showed that the variations in the inhibitory potency are clearly greater than in the *in vitro* assays. While the bradykinin-induced visceral extravasation of plasma proteins was prevented completely by doses as low as 30 nmol kg^{-1}, the inhibition of the bronchoconstrictor actions of bradykinin required doses more than 100 fold higher.

Several possibilities have to be taken into account to explain such differences in potency. Differences between species are frequently the reason for such a phenomenon. Indeed, differences between the bradykinin (B_2) receptors from different species have been proposed (Regoli *et al*., 1993) but in that classification the receptors in rats and guinea-pigs would be included in the same group. Differences in the apparent affinities of previous B_2 antagonists in *in vitro* preparations from rats and guinea-pigs, as found by Hall *et al*. (1992) or Sawutz *et al*. (1995), could indicate that the B_2 receptors of the two species are different. However, the differences in the potencies of FR173657 in such *in vitro* assays (Griesbacher *et al*., submitted) are less than one log unit, so that these results are unlikely to be the result of species differences. Methodological differences in the models that were employed in this investigation should also be taken into account. Those models were FR173657 had a higher inhibitory potency used animals that were pretreated with the kininase II blocker, captopril. However, it is unlikely that captopril had any effect on the non-peptide FR173657. Furthermore, the prolonged half-life of bradykinin itself will lead to a longer presence of bradykinin at its receptors and thus also cannot explain why in these models lower doses of the antagonist were active. Hence, differences in the distribution of the antagonist in the various tissues of the body appears to be the only conceivable explanation for the differences in potency.

Comparisons of the potency of FR173657 with that of other antagonists is not easy because of frequent differences in the routes of administration used. Of particular interest is the comparison with icatibant, which is the most frequently used B_2 antagonist at present. In the experiments in which the inhibition of bradykinin-induced visceral plasma protein extravasation was studied, the dose of FR173657 producing complete inhibition (30–100 nmol kg^{-1}) was comparable to the dose of icatibant (100 nmol kg^{-1}) that inhibits the pancreatic oedema induced by bradykinin either after exogenous administration (Lembeck & Griesbacher, 1996) or following its endogenous release during inflammation (Griesbacher & Lembeck, 1992; Griesbacher *et al*., 1993). However, FR173657 seems to be somewhat less potent than icatibant in the models of peripheral plasma protein extravasation and of bronchoconstriction, where icatibant has been shown to be effective at doses of 100–300 nmol kg^{-1} (Legat *et al*., 1994) and 20 nmol kg^{-1} (Wirth *et al*., 1991), respectively.

An *in vivo* investigation can yield little information about the specificity of antagonists towards receptor subtypes. Of the two accepted subtypes for the bradykinin receptor, only the B_2 receptor is involved in the mediation of the acute effects of bradykinin investigated here, at least in the first three models. So far, the inhibitory action of FR173657 *in vivo* corresponds well with its activity *in vitro* described separately (Griesbacher *et al*., submitted). The lower potency of FR173657 in the bronchoconstriction assay may be indicative of the proposed of B_3 receptors in the guinea-pig airways, based on a low activity of different, first generation peptide antagonists (Farmer *et al*., 1989). However, the high

efficacy of FR173657 in the guinea-pig isolated trachea *in vitro* makes it unlikely that FR173657 could discriminate between B_2 and B_3 receptors, if the latter existed.

More important than selectivity towards receptor subtypes in *in vivo* investigations is the probability of unspecific inhibitory effects at other receptor systems. The magnitude of the difference in the chemical nature of FR173657 from the natural ligands for the bradykinin receptor drastically increases the likelihood of such unspecific effects, illustrated also by the ancillary pharmacology of an earlier non-peptide bradykinin antagonist, WIN 64338 (Sawutz *et al*., 1994; 1995). However, FR173657, did not affect either the effects of histamine or of those to 5-hydroxytryptamine when it was applied in doses that completely blocked the effects of bradykinin, as previously observed in the *in vitro* experiments.

In the present investigation the inhibitory effects of FR173657 on the actions of exogenously applied bradykinin were investigated. The models that were employed not only allow a pharmacological characterization of the novel antagonist, but also have relevance to the pathophysiological roles of endogenous kinins in inflammatory and allergic diseases (see Hall *et al*., 1992). Inflammatory diseases are frequently associated with severe pain which may be due to the actions of kinins released endogenously during these disease states (Dray & Perkins, 1993). Therefore, it is hoped that FR173657 will also be effective in preventing kinin-mediated pain. These actions of FR173657 are currently under investigation.

In summary, FR173657 has been shown to be a highly potent bradykinin antagonist *in vivo* whose action is extremely long-lasting, but reversible. No unspecific actions of the antagonists could be detected. Therefore, FR173657 may have a high potential for future use in disease states that involve the endogenous release of kinins.

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