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# Effect of bradykinin antagonists on bradykinin-induced plasma extravasation, venoconstriction, prostaglandin $E_2$ release, nociceptor stimulation and contraction of the iris sphincter muscle in the rabbit

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**1** The inhibition of the bradykinin-induced plasma extravasation by six bradykinin (Bk) antagonists was tested on rabbit skin. All of them showed inhibitory effects without an agonistic action in the doses used. B4310 (Lys-Lys-3-Hyp-5,8-Thi-7-DPhe-Bk) was the most active antagonist and was therefore used in the subsequent experiments.

**2** B4310 (5–500 nM) antagonized the bradykinin-induced reduction of the venous outflow from the rabbit isolated ear in a dose-dependent manner without affecting the arterial vasoconstriction induced by angiotensin II.

**3** The bradykinin-induced release of prostaglandin $E_2$ ($PGE_2$) from the perfused rabbit ear was reduced by 63% when B4310 (800 nM) was infused before, during and after the bradykinin injection.

**4** Bradykinin was injected into the ear artery of anaesthetized rabbits and the reflex hypotensive response was used as indicator of the nociception. The response was antagonized by a local infusion of B4310 (50 and 500 nM). The antagonism was dose-dependent and reversible. The parallel shift of the dose-response curve to bradykinin suggests a competitive inhibition. However, B4310 did not antagonize acetylcholine-induced nociceptor stimulation.

**5** B4310 inhibited bradykinin-induced stimulation of the trigeminal nerve which results in a substance P-mediated contraction of the iris sphincter muscle. A $pA_2$ of 7.59 was calculated. B4310 did not inhibit capsaicin-induced contractions.

**6** It is concluded that B4310 inhibits specifically five different actions of bradykinin which are related to its possible pathophysiological role.

## Introduction

Two decades of frustrating search for antagonists of bradykinin ended with the discovery of several sequence-related peptides with an antagonistic activity above a $pA_2$ of 6.0 (Stewart & Vavrek, 1987; Vavrek & Stewart, 1987). Their antagonistic potencies had been tested against bradykinin effects on the rat uterus (Vavrek & Stewart, 1987), on the guinea-pig ileum (Vavrek & Stewart, 1985; Longridge *et al.*, 1986; Vavrek & Stewart, 1987), on arteries and veins and on the dog urinary bladder (Regoli *et al.*, 1986) and on human lung fibroblasts (Crecelius *et al.*, 1986). Stewart *et al.* (1986) found that these peptides were also able to antagonize the bradykinin-induced increase in vascular permeability in the rabbit skin.

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The aim of the present investigations was to assess the effect of bradykinin antagonists on those actions of bradykinin which are related to its possible role in pathophysiology. Firstly, the ability of six different compounds to inhibit the plasma extravasation caused by bradykinin was tested. The most suitable of them was then used to measure its inhibitory activity on bradykinin-induced venoconstriction (Guth *et al.*, 1966; Goldberg *et al.*, 1976) and prostaglandin $E_2$ ($PGE_2$) release (Lembeck *et al.*, 1976; Juan, 1977), two actions which contribute to the plasma extravasation caused by bradykinin. In further experiments the specific stimulation of nociceptive sensory neurones by bradykinin (Juan & Lembeck, 1974) was used to investigate the specificity, potency and reversibility of the action of this bradykinin antagonist. Ueda *et al.*

(1984) showed, that bradykinin acts directly on the trigeminal substance P-ergic nerve and causes a release of substance P which contracts the isolated iris sphincter. This preparation was used to study the effect of B4310 on bradykinin receptors on substance P-ergic afferent neurones.

Two types of bradykinin receptor have been defined, $B_1$ and $B_2$ receptors (Regoli & Barabé, 1980; Marceau *et al.*, 1981). Plasma extravasation has been defined as a $B_2$ effect. However, the receptors involved in the other types of response described here have not been classified.

## Methods

*Plasma extravasation in the rabbit skin*

Six groups of female rabbits (3.5–4.5 kg) that had their back fur clipped, were pretreated i.p. with mepyramine and cimetidine (3 mg $kg^{-1}$ of each). Thirty min later they were anaesthetized with pentobarbitone (30 mg $kg^{-1}$, i.v.) and given an i.v. injection of a solution of Evans blue (10 mg $ml^{-1}$, 20 mg $kg^{-1}$). Each rabbit received 18 intracutaneous injections into the back skin. They consisted of bradykinin and bradykinin antagonists, either alone or in combination, and also of the drug vehicle (phosphate buffered 0.9% NaCl solution, pH 7.4) in volumes of 0.1 ml similar to the procedure described by Miles & Miles (1952). Twenty min later the animals were killed by a blow on the head and bled. The dorsal skin was removed, the blue areas around the injection sites were punched out (diameter 18 mm) and incubated in 4 ml formamide at 60°C for 24 h (Gamse *et al.*, 1980). The amount of Evans blue in the solution was measured photometrically at 620 nm. For each bradykinin antagonist a different group of 3–4 rabbits was used.

*Venoconstriction in the isolated perfused ear of the rabbit*

Male rabbits (2.5–3.5 kg) were anaesthetized with pentobarbitone (30–35 mg $kg^{-1}$, i.v.). Both ears were isolated and perfused via their central artery with Tyrode solution (37°C, gassed with 95% $O_2$ and 5% $CO_2$) (Juan, 1977). The Tyrode solution was supplied from an open reservoir which was adjusted in height so that the initial arterial inflow was 5 ml $min^{-1}$. Injections of bradykinin and angiotensin II were made at intervals of one hour into the arterial inflow cannula. Infusion of B4310 in concentrations of 0.5 nM to 500 nM was started five min before these injections. The venous outflow was recorded by an electronic device which transformed drop intervals to ordinates.

*Prostaglandin $E_2$ release from the isolated perfused ear of the rabbit*

Male rabbits (2.5–3.5 kg) were anaesthetized with pentobarbitone and the ears were isolated as described above. The arterial inflow was adjusted to a constant flow rate of 3 ml $min^{-1}$ using a roller pump (Juan, 1977). Four hours after the beginning of the perfusion 1.6 nmol bradykinin was injected into the arterial inflow cannula. In one ear B4310 in a final dilution of 800 nM was added to the infusion medium ten min before the bradykinin injection. The contralateral ear served as control. The $PGE_2$ content of the venous outflow, collected in five min fractions, was measured by radioimmunoassay (Juan, 1977). The first fraction was collected before the addition of B4310, the second before and another three following the injection of bradykinin. The total amount of the bradykinin-induced release of $PGE_2$ over 15 min was calculated by subtracting three times the amount of $PGE_2$ in fraction one from the sum of $PGE_2$ in fraction 3 + 4 + 5.

*Nociceptor stimulation*

Male rabbits (3–4 kg) were anaesthetized with pentobarbitone (30–35 mg $kg^{-1}$, i.v.). A carotid artery was exposed and cannulated for blood pressure registration. A jugular vein was cannulated for additional injections of pentobarbitone whenever the blood pressure became unstable. One ear was separated from the head with the exception of the auricular nerve which remained connected to the body. The ear was perfused via the central artery with Tyrode solution to which $PGE_1$ was added (final concentration 10 μM) to augment the vascular responses to bradykinin and acetylcholine (Juan & Lembeck, 1974). The infusion rate was 5 ml $min^{-1}$. Injections of various doses of bradykinin and acetylcholine were made into the arterial inflow cannula before, during and after the infusion of B4310 in final concentrations of 50 or 500 nM. Bradykinin was injected at intervals of 15 min.

*Bradykinin-induced substance P-ergic response of the iris sphincter muscle of the rabbit*

Rabbits of either sex (2.5–4.0 kg) were killed by i.v. injection of an overdose of pentobarbitone. Both eyes were enucleated immediately after death and the iris sphincter muscle was prepared according to the method of Kern (1970). Cumulative concentration-response curves for bradykinin and capsaicin were obtained according to Ueda *et al.* (1984). B4310 was added to the organ bath (Krebs-Ringer solution, gassed with 95% $O_2$ and 5% $CO_2$, 37°C) 5 min before the cumulative addition of bradykinin. The preparation of the contralateral eye served as control without addition of B4310. For each concentration of B4310

(25 to 200 nM) a different group of 6 animals was used. The values of the responses to bradykinin and capsaicin from six experiments each, measured as % of the maximum response ($p$), were transformed to logits ($l$) using the equation

$$l = \log_e \frac{p}{100 - p}$$

and were plotted against the logarithm of the bradykinin concentrations in nM. A regression line was fitted using the least squares method to calculate the $EC_{50}$ of bradykinin with and without the antagonist. Values for $pA_2$ and $pA_{10}$ were determined according to Schild (1957).

*Substances*

The following substances were used (Bk = bradykinin, Thi = β-(2-thienyl)-L-alanine, Hyp = L-4-hydroxyproline): B4144 (5,8-Thi-7-DPhe-Bk), B4146 (3-Hyp-5,8-Thi-7-DPhe-Bk), B4148 (Lys-Lys-2-Hyp-5,8-Thi-7-DPhe-Bk), B4162 (DArg-3-Hyp-5,8-Thi-7-DPhe-Bk), B4308 (Lys-Lys-2,3-Hyp-5,8-Thi-7-DPhe-Bk), B4310 (Lys-Lys-3-Hyp-5,8-Thi-7-DPhe-Bk) (Dr J.M. Stewart & Dr R.J. Vavrek, Denver, U.S.A); bradykinin (Serva Feinbiochemie, F.R.G.). Stock solutions of all peptides were prepared in a 0.15 M solution of NaCl containing 1 g $l^{-1}$ gelatine and 25 mg $l^{-1}$ cialit (sodium 2-(ethylmercuri-thio)-benzoxazole-5-carboxylate, Asid-Institut GesmbH., F.R.G.) to prevent adsorption to glass and bacterial growth. Mepyramine (Smith Kline & French Labs, Ltd, U.K.), cimetidine (Kwizda, Austria), pentobarbitone (Ceva GesmbH., F.R.G.), Evans blue (Sigma Chemical Company, U.S.A.), angiotensin II (Ciba, Switzerland), acetylcholine (Becker, Austria), $PGE_1$ (Upjohn, U.S.A.), [$^3$H]-$PGE_2$ (Radiochemical Center Amersham, U.K.), $PGE_2$-antiserum (Sigma Chemical Company, U.S.A.). Capsaicin (Sigma Chemical Company, U.S.A.) was dissolved in a 0.15 M solution of NaCl (saline) which contained ethanol (10%) and Tween 80 (10%) and diluted with saline. The composition of the Tyrode solution was (in mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, $NaH_2PO_4$ 0.4, glucose 5.6. The composition of the Krebs-Ringer solution was (in mM): NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 15.5, $KH_2PO_4$ 1.2, glucose 11.5.

*Statistical analysis*

All values presented represent means ± s.e.mean. One way analysis of variance and the Scheffé-test were used except in the experiments on nociceptor stimulation where multiple comparisons were made according to Wilcoxon and Wilcox. The dose-response curves of the iris sphincter muscle contractions were tested for non-parallelism and non-identity by $t$ tests.

## Results

*Plasma extravasation in rabbit skin*

The doses of peptides chosen were 1.6 nmol for bradykinin and 20 and 60 nmol for the bradykinin analogues. When B4310 was tested in combination with bradykinin an additional dose of 7 nmol was used. The results of the experiments are shown in Figure 1. The bradykinin-induced plasma protein extravasation was 2 to 4 fold higher than the values obtained with injection of the vehicle (phosphate buffered saline) alone ($P < 0.01$) and quite different in the different groups of rabbits. Two of the bradykinin analogues (B4162, B4148) showed a slight, but not significant agonistic activity at higher doses. The highest antagonistic effects were observed with B4310

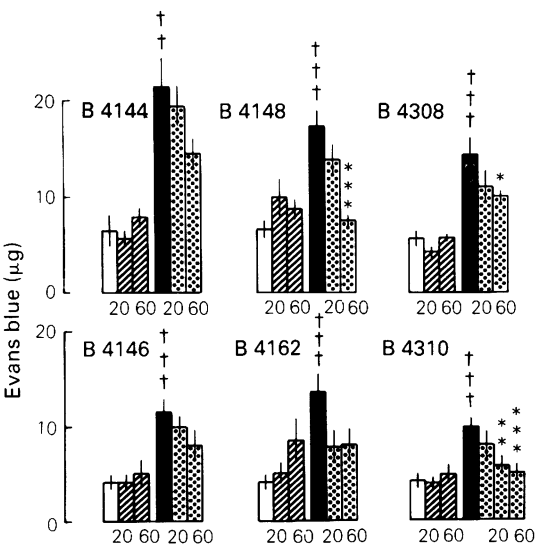


**Figure 1** Plasma extravasation in rabbit skin. Intracutaneous injections of 1.6 nmol bradykinin (Bk, solid columns) and bradykinin antagonist alone (hatched columns) or in combination with Bk (stippled columns). Open columns: drug vehicle (phosphate buffered 0.9% NaCl solution, pH 7.4). Each column represents µg Evans blue dye extravasated into the skin (see methods). Doses of the antagonists are given below the columns in nmol. ††$P < 0.01$, †††$P < 0.001$; Significantly different from vehicle; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Significantly different from Bk: Columns represent mean values, $n = 8$–13 (for B4144: $n = 4$); vertical lines show s.e.mean.

(20 and 60 nmol) and B4148 (60 nmol) ($P<0.01$). Significant antagonistic results ($P<0.05$) were also obtained with B4162 (20 and 60 nmol) and B4308 (60 nmol). Since B4310 showed no agonist activity and reduced the effect of bradykinin in a dose-dependent manner, only this peptide was used in further experiments.

*Venoconstriction in the isolated perfused ear of the rabbit*

Infusion of B4310 in concentrations of 0.5 to 500 nM showed a dose-dependent inhibition of the effect of 1.6 nmol bradykinin (Figure 2). No significant effect could be shown with a concentration of 0.5 nM. Significant results were obtained with 5 nM B4310 ($P<0.05$), and 50 and 500 nM B4310 caused highly significant reductions of the effect of bradykinin ($P<0.001$). However, infusion of 500 nM B4310 did not affect the reduction in venous out-flow induced by 97 and 290 nmol angiotensin II. Although bradykinin-induced vasoconstriction was also observed in the experiments investigating $PGE_2$ release, from the rabbit isolated ear, with the constant flow method (mentioned below), only perfusion under constant pressure gave suitable results for the quantitative estimation of the potency of B4310.

*$PGE_2$ release from the isolated perfused ear of the rabbit*

The basal release of $PGE_2$ into the venous effluent from the ear remained unchanged during an infusion of B4310 (800 nM) (Figure 3). An injection of 1.6 nmol bradykinin induced the release of $15.0 \pm 1.9$ ng $PGE_2$ from the control ears during the following 15 min, but only of $4.4 \pm 1.0$ ng $PGE_2$ when B4310 (800 nM) was infused before, during and after the bradykinin injection. This amounted to a reduction in $PGE_2$ release by $63 \pm 9\%$ ($P<0.01$).

The venous outflow during the 15 min period after the injection of bradykinin was reduced by $9.4 \pm 0.7$ ml in the controls, but only by $1.7 \pm 0.4$ ml when it was injected during the infusion of B4310, i.e. an inhibition of $81 \pm 3\%$ ($P<0.001$, not shown in Figure 3).

*Nociceptor stimulation*

The injection of substances like bradykinin or acetylcholine, into the circulation of the separated ear (remains in contact with the head only by the intact auricular nerve) elicits a reflex fall of the systemic blood pressure. This can be used as a quantitative index of the intensity of nociceptor stimulation in the

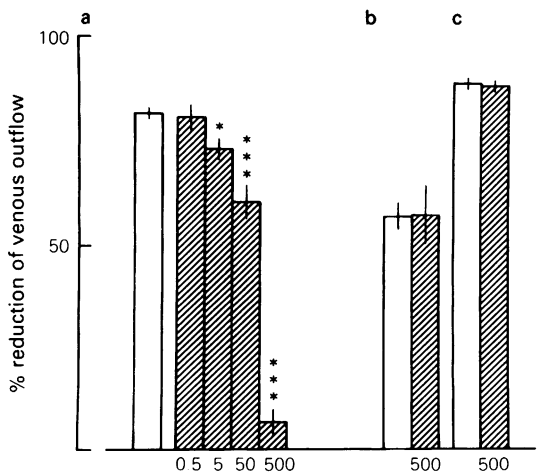


**Figure 2** Rabbit isolated perfused ear % reduction of venous out-flow induced by injection of (a) bradykinin (1.6 nmol) and (b and c) angiotensin II (97 (b) and 290 (c) nmol) before (open columns) and during an infusion of B4310 (hatched columns). The concentrations given below the columns are in nM. $*P<0.05$, $***P<0.001$, significantly different from controls. Columns represent mean values, $n = 6-13$; vertical lines show s.e.mean.

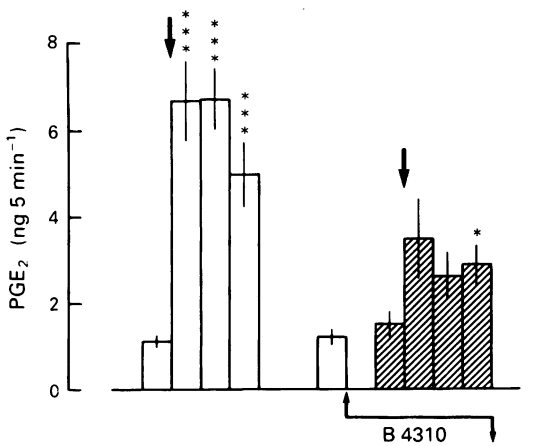


**Figure 3** Rabbit isolated perfused ear: amount of $PGE_2$ (in ng) in the venous effluent in fractions collected for 5 min before and after an injection (⬇) of 1.6 nmol bradykinin (Bk) in controls (open columns) and during an infusion of B4310 in a final concentration of 800 nM (hatched columns). $*P<0.05$, $***P<0.001$; Significantly different from values before Bk injection. The difference in the Bk stimulated release of $PGE_2$ during the 15 min periods after the injection of Bk was significant, $P<0.01$. Columns represent mean values, $n = 3-6$; vertical lines show s.e.mean.

ear. The reflex fall in blood pressure following arterial injection of various doses of bradykinin was measured before, during and after an infusion of B4310 (Figure 4). During an infusion of B4310 the dose-response curve for bradykinin was shifted to the right. The extent of the shift depended on the dose of the antagonist. To compare the dose-response curves with and without antagonist, the doses of bradykinin producing a reflex fall in blood pressure by 20 mmHg were calculated. Shifts by factor of 8.7 ± 3.5 and 26.4 ± 4.0 were seen when 50 nM and 500 nM B4310 were infused respectively. When bradykinin was injected 15 to 45 min after the end of the infusion of B4310, the dose-response curves were shifted back to the left. Injection of acetylcholine also caused dose-dependent falls in blood pressure. However, this effect was unaffected by B4310 (Table 1).

*Bradykinin-induced substance P-ergic response of the iris sphincter muscle of the rabbit*

Both bradykinin and capsaicin cause concentration-dependent contractions of the iris sphincter muscle by releasing substance P from trigeminal neurones (Ueda, 1974). The maximum responses to bradykinin in the presence of B4310 showed no significant differences from those observed under control conditions. The dose-response curves to bradykinin with and without the antagonist were tested for non-parallelism: no significant differences in the slope of the regression lines could be found. B4310 (25–200 nM) shifted the dose-response curve to bradykinin to the right in a dose-dependent manner. When the log of the dose-ratio was plotted against the negative log of the antagonist concentration, a regression line could be fitted by use of the least squares method (Figure 5). The slope of this regression line was close to −1. A $pA_2$ of 7.59 and a $pA_{10}$ of 6.65 were determined. B4310 in a concentration of 200 nM failed to inhibit contractions of the iris sphincter muscle induced by capsaicin (results not shown). To test whether B4310 had an agonistic effect of its own, it was applied in concentrations up to 1600 nM. Only in one of seven experiments did the antagonist in a concentration of 800 nM induce a contraction of the preparation. This contraction was about one third of the maximum response obtained on subsequent addition of bradykinin.

**Table 1** Nociceptor stimulation: reflex fall in carotid blood pressure (in mmHg) after an injection of acetylcholine into the perfusion fluid of the rabbit isolated ear before, during and after an infusion of 50 or 500 nM B4310

| *Acetylcholine (nmol)* | *Before* | *During an infusion of B4310* | *After* |
|---|---|---|---|
| | | *50 nM* | |
| 30 | 21 ± 1 | 22 ± 1 | 20 ± 1 |
| 60 | 24 ± 1 | 26 ± 1 | 25 ± 1 |
| | | *500 nM* | |
| *30* | 20 ± 2 | 22 ± 2 | 22 ± 3 |
| 60 | 27 ± 2 | 25 ± 2 | 23 ± 0 |

Data shown are means ± s.e.mean; $n = 3-7$.
None of the values during the infusion of B4310 proved to be significantly different from those before and after the infusion.

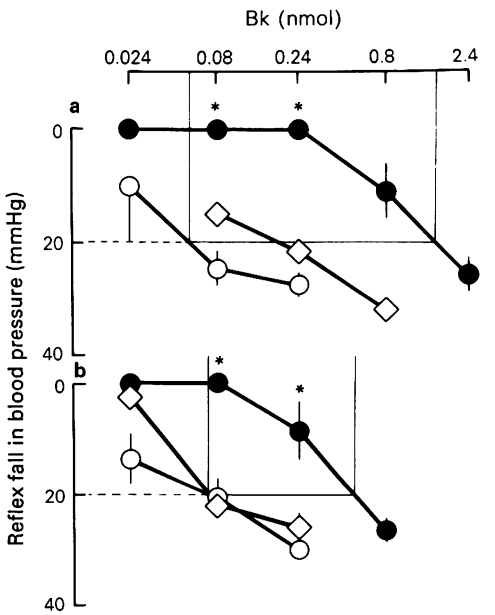


**Figure 4** Nociceptor stimulation: reflex fall in carotid blood pressure following an injection of bradykinin (Bk) into the auricular artery, in doses shown before (○), during (●) and after the end (◇) of an infusion of B4310 (a) 500 nM or (b) 50 nM. Each point represents mean and vertical lines s.e.mean, $n = 3-4$. Where no s.e.mean is indicated it was smaller than the symbol. $^*P < 0.05$, significantly different from values before infusion of B4310 (Wilcoxon & Wilcox).

## Discussion

*Plasma extravasation*

Stewart *et al.* (1986) have observed that bradykinin antagonists reduced the area of the plasma extravasation around an intracutaneous injection of bradykinin. In this investigation only the size of the blue area

a

| Bk | B4310 | | DR |
|---|---|---|---|
| $y = -3.0 + 1.9x$ | $y = 3.7 + 2.0x$ | (25 nM) | 1.78 |
| $y = -2.8 + 1.7x$ | $y = 3.8 + 1.7x$ | (50 nM) | 3.93 |
| $y = -2.6 + 1.8x$ | $y = 4.1 + 1.9x$ | (100 nM) | 4.48 |
| $y = -2.8 + 2.1x$ | $y = 4.4 + 1.9x$ | (200 nM) | 8.66 |

b

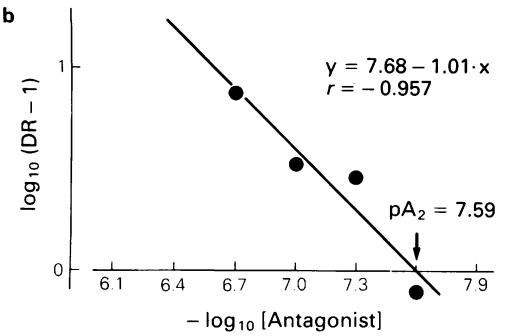


**Figure 5** Iris sphincter muscle: (a) logit/log-regression lines for the responses to bradykinin under control conditions (Bk) and in the presence of the antagonist (B4310) in the concentrations given in parentheses. The dose-ratio (DR) for each antagonist concentration was obtained in a group of six separate experiments. The significance of difference in each pair of regression lines was $P < 0.001$. (b) Log (DR – 1) plotted against negative log of the concentration of B4310 according to Schild (1957). The regression lines were calculated using the least squares method.

was measured. It seemed appropriate to re-investigate the effect of bradykinin antagonists on the plasma extravasation by measuring the total amount of Evans blue released into a defined area around the injection site and also to test the antagonists alone in order to detect a possible agonistic effect. Pretreatment with antihistamine drugs excluded a possible influence of histamine released by bradykinin (Stern *et al.*, 1962) or by the bradykinin antagonist.

Only the antagonists B4148 and B4162 caused a slight, but not significant, increase in plasma extravasation compared to that produced by the vehicle alone. However, the possibility that doses higher than those tested might cause significant plasma extravasation cannot be excluded. The injection of 1.6 nmol bradykinin increased the plasma extravasation 2 to 4 fold ($P < 0.01$), depending on the group of rabbits tested. The injection of any one of the six antagonists (20 or 60 nmol) together with bradykinin reduced the plasma extravasation. As the antagonist B4310 had a strong antagonistic effect without agonistic tendencies it was selected for all further experiments.

*Venoconstriction*

The reduction of venous outflow from the rabbit isolated ear results from venoconstriction (Guth *et al.*, 1966; Goldberg *et al.*, 1976). During an infusion of B4310 the effect of bradykinin was inhibited, depending on the dose. In contrast, the reduction of the venous outflow induced by angiotensin II, which predominantly constricts arteries (Folkow *et al.*, 1961), remained completely unchanged. These results stress the specificity of B4310 on the effect of bradykinin.

*Release of prostaglandin $E_2$*

Bradykinin has been shown to induce an immediate release of $PGE_2$ from the rabbit isolated ear (Lembeck *et al.*, 1976; Juan, 1977). The infusion of B4310 alone did not influence the basal release of $PGE_2$. Although B4310 antagonized the reduction of the volume of the venous outflow normally caused by bradykinin, it reduced the bradykinin-induced release of $PGE_2$ by $63 \pm 9\%$ ($P < 0.01$) during the 15 min following the injection of bradykinin. Thus it can be assumed that the change in the release of $PGE_2$ did not result from a change in the rate of the vascular flow, but is due to a direct inhibition of the bradykinin-induced release of $PGE_2$

*Nociceptor stimulation*

Among numerous endogenous substances, bradykinin had by far the highest potency, followed by acetylcholine (Juan & Lembeck, 1974). In the preparation used here, the reflex fall in systemic blood pressure depends on the dose of the algogene injected and allows a quantification of the intensity of nociceptor stimulation (Juan & Lembeck, 1974). In the present experiments responses to effective doses of bradykinin (0.024–0.24 nmol) were blocked when injected during an infusion of B4310 (50 or 500 nM). Only much higher doses of bradykinin elicited a fall in blood pressure. As the antagonist caused a near parallel shift in the dose-response curves to bradykinin, a competitive inhibition at the receptor site may be assumed. Recent experiments with the antagonist B4144, not included here, confirmed also that the inhibitory action of bradykinin antagonists on nociceptors is concentration-dependent. The effect of the bradykinin antagonist B4310 was reversible: 15 min after the end of its infusion the dose-response curve to bradykinin returned to the original position. The specificity of B4310 as a bradykinin antagonist on nociceptors was tested by its possible action on nociceptor stimulation by acetylcholine: the response to acetylcholine remained unchanged during an infusion of B4310.

*Iris sphincter muscle*

Lembeck & Holzer (1979) showed that inflammatory responses following antidromic stimulation of sensory nerves are initiated by peripheral release of substance P from chemosensitive pain fibres. The ocular hypertensive and miotic responses to intracameral administration of bradykinin and the contraction of the isolated iris sphincter muscle of the rabbit, induced by bradykinin or electrical transmural stimulation, are mediated by substance P which is released from peripheral terminals of substance P-ergic trigeminal neurones (Butler & Hammond, 1980; Ueda *et al.*, 1984). The contraction of the iris sphincter muscle induced by bradykinin was inhibited by B4310 in a dose-dependent manner whereas the response to capsaicin was unaffected by the antagonist. Ueda *et al.* (1984) convincingly showed that capsaicin contracts the muscle exclusively by release of substance P and we therefore regarded an additional test of B4310 on the contraction induced by substance P as not necessary. If B4310 could antagonize substance P, it should result in an inhibition of the action of capsaicin. Regoli *et al.* (1986) showed that B4144 did not inhibit the response to substance P on guinea-pig ileum, rabbit jugular vein and rabbit aorta. These findings indicate that the antagonist B4310 interacts with bradykinin on its receptors on substance P-ergic primary afferent neurones. It suggests that B4310 is able to inhibit the bradykinin-induced neurogenic inflammation.

In summary, the bradykinin antagonist B4310 was shown to inhibit bradykinin-induced venoconstriction and $PGE_2$ release, two mechanisms that are involved in the plasma extravasation caused by bradykinin (Rowley, 1964; Chahl, 1976). No significant agonistic effect of any of the antagonists tested was seen in the doses used. Dose-dependency of the effect of B4310 on bradykinin-induced venoconstriction could be demonstrated as well as specificity. The inhibition of the nociceptor stimulation by bradykinin was especially important for the assessment of bradykinin antagonists, since it was dose-dependent, reversible and specific. In the iris sphincter muscle experiments, the parallelism of the dose-response curves to bradykinin with and without the antagonist, the constancy of the maximum responses to bradykinin and the slope of the regression line in the Schild plot point to a possibly competitive agonist-antagonist interaction on bradykinin receptors.

Bradykinin is a potent endogenous stimulator of nociceptors (Juan & Lembeck, 1974). It is involved in various inflammatory reactions (Lewis, 1970) and causes a variety of effects on vascular and other smooth muscles as well as the release of prostaglandins. Many of these effects contribute to the signs of inflammation. The availability of a specific antagonist may enable us to determine the role of bradykinin in different inflammatory reactions and could provide a new possible form of analgesic treatment.

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