Effects of the bradykinin antagonist B4310 on smooth muscles and blood pressure in the rat, and its enzymatic degradation

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1 Six competitive bradykinin (Bk) antagonists were tested for their agonistic properties on the rat uterus. Five of these peptides showed agonistic effects only at concentrations at least two orders of magnitude higher than those of bradykinin.

2 The antagonistic potency of Lys-Lys-3-Hyp-5,8-Thi-7-DPhe-Bk (B4310) in the rat uterus ($pA_2 = 7.24$) and in the rat duodenum ($pA_2 = 7.31$) was very similar to that determined in an earlier study for the antagonism of the bradykinin-induced stimulation of the trigeminal nerve in the rabbit iris sphincter muscle preparation ($pA_2 = 7.59$).

3 The fall in mean arterial blood pressure induced by i.a. injections of bradykinin was greatly reduced during an i.a. infusion of B4310, but not 10 min thereafter, which indicates a rapid inactivation of B4310 *in vivo*. Bacitracin possibly interferes with the enzymatic cleavage of B4310 but seems to have no effect on the degradation of bradykinin.

4 An i.a. infusion of captopril greatly enhanced the potency of bradykinin in inducing a fall in arterial blood pressure, confirming the important role of angiotensin converting enzyme in the cleavage of bradykinin. However, the design of this experiment did not allow conclusions about the effect of captopril on the degradation of B4310.

5 B4310 incubated with rat lung tissue disappeared from the incubation medium within a few minutes, i.e. as fast as bradykinin, which explains its short duration of action *in vivo*. Captopril partially inhibited the cleavage of both bradykinin and B4310.

6 The present results show that the bradykinin antagonists available at present are useful tools for the investigation of the biological role of bradykinin. However, the susceptibility to enzymatic degradation may limit their usefulness in animal experiments or in clinical studies.

Introduction

Actions of bradykinin have been shown to be inhibited by recently introduced competitive antagonists (Stewart & Vavrek, 1987) in a variety of biological systems such as the rat uterus (Stewart & Vavrek, 1987), the guinea-pig ileum (Stewart & Vavrek, 1987), arteriolar dilatation (Ellis *et al.*, 1987; Barton *et al.*, 1987), venous constriction (Griesbacher & Lembeck, 1987), plasma extravasation (Longridge *et al.*, 1986; Griesbacher & Lembeck, 1987), sensory nerve stimulation (Griesbacher & Lembeck, 1987;

Laneuville & Couture, 1987; Steranka *et al.*, 1987; Whalley *et al.*, 1987) and prostaglandin release (Crecelius *et al.*, 1986; Griesbacher & Lembeck, 1987).

In order to obtain additional information about the pharmacological properties of these antagonists we used (1) the rat uterus to quantify not only their antagonistic activities but also their agonistic effects at higher concentrations, (2) the rat duodenum to test the antagonistic action on a smooth muscle preparation which responds to bradykinin with a relaxation and (3) the rat blood pressure to show antagonism against bradykinin in an *in vivo* preparation.

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During passage through the pulmonary circulation, bradykinin is inactivated by several peptidases (Ferreira & Vane, 1967b; Alabaster & Bakhle, 1972; Orawski *et al.*, 1987). Agonistic analogues of bradykinin also have been shown to be broken down in the lung (Stewart & Vavrek, 1987). Angiotensin converting enzyme and carboxypeptidases seem to be major cleaving enzymes responsible for the inactivation of bradykinin (Sheikh & Kaplan, 1986a,b). It has been concluded that the blood pressure lowering effect of angiotensin converting enzyme inhibitors may partly be the result of the protection of circulating bradykinin from enzymatic degradation (Benetos *et al.*, 1986a,b). However, the degradation of bradykinin in plasma or isolated arteries seems to be the result of the action of peptidases different from angiotensin converting enzyme (Marceau *et al.*, 1981; Whalley, 1987).

Earlier experiments indicated that the bradykinin antagonist B4310 has a short duration of action (Griesbacher & Lembeck, 1987). To obtain further information on the inactivation of B4310 *in vivo* we studied the effect of infusions of the peptidase inhibitors bacitracin and captopril on the activity of this bradykinin antagonist. Additionally, the degradation of B4310 by rat lung tissue was determined by bioassay on the rat uterus, and by high performance liquid chromatography (h.p.l.c.).

A preliminary account of the work described in this paper has been presented to the British Pharmacological Society (Lembeck & Griesbacher, 1987).

Methods

Rat uterus

Female Sprague-Dawley rats (180–240 g) were pretreated with diethylstilboestrol ($100 \mu\text{g kg}^{-1}$, s.c.) 40–44 h before the experiment and their oestrous cycle followed by use of vaginal lavage cytology as described by Traurig *et al.* (1984) and the criteria devised by Long & Evans (1922). Animals in oestrous were killed by cervical dislocation and subsequent exsanguination. The distal parts (1 cm) of the uterine horns were mounted in an organ bath in De Jalón solution (32°C , gassed with 95% O_2 and 5% CO_2) containing $2.8 \mu\text{M}$ indomethacin and $0.35 \mu\text{M}$ atropine. A resting tension of 0.5 g was applied to the tissue and contractions recorded isotonicity on a pen recorder.

Rat duodenum

Male Sprague-Dawley rats (170–270 g) were killed as above and the proximal part (1.5 cm) of the duodenum was suspended in De Jalón solution contain-

ing $0.35 \mu\text{M}$ atropine and $0.35 \mu\text{M}$ mepyramine. The resting tension was 1 g and relaxations were recorded isotonicity.

Bradykinin was applied to both tissues at intervals of 5 min, the contact time was 1 min. B4310 was added to the organ bath 4 min before the addition of bradykinin. For the determination of pD_2 values the antagonists were applied to the rat uterus in the same way as bradykinin. The values of the responses to the peptides, measured as % of the maximum response to bradykinin, were transformed to logits and were plotted against the logarithm of the concentrations in nM. A regression line was fitted by the least squares method to calculate EC_{50} or pD_2 values. Values for pA_2 and pA_{10} were determined according to Schild (1957).

Rat blood pressure

Female Sprague-Dawley rats (180–280 g) were fasted overnight and pretreated with hyoscine butylbromide (40 mg kg^{-1} , s.c.) 30 min before anaesthesia with pentobarbitone sodium (50 mg kg^{-1} , i.p.). The animals were injected with pancuronium bromide ($3 \mu\text{g kg}^{-1}$, i.v.) and respired artificially. Blood pressure was recorded in a carotid artery with a Statham transducer. The contralateral carotid artery was cannulated for retrograde infusions of B4310 (15 nmol min^{-1} , 0.15 ml min^{-1}) and i.a. injections of bradykinin (100 or 25 pmol in a volume of 0.1 ml). After every injection the tubing was rinsed with 0.2 ml of 0.9% NaCl. Infusions of bacitracin (0.2 mg min^{-1} , 0.1 ml min^{-1}) or captopril ($5 \mu\text{g min}^{-1}$, 0.05 ml min^{-1}) were made into a jugular vein.

Degradation of B4310 by rat lung

Sprague-Dawley rats of either sex (190–280 g) were killed by cervical dislocation. After exsanguination the lung was removed immediately and chopped into slices ($30 \mu\text{M}$) with a McIlwain tissue chopper: 2 ml of a $10 \mu\text{M}$ solution of B4310 in Tris buffer was incubated for 15 min with 10 mg of the chopped lung or with the same amount of tissue which had been heated to 96°C for 4 min prior to incubation. In addition, lung slices in Tris buffer alone and B4310 alone were incubated in the same way. After incubation, all preparations were heated to 96°C for 4 min, centrifuged for 15 min at $20\,000 \text{ ms}^{-2}$ at 4°C and the supernatant tested for antagonistic potency against bradykinin on the rat uterus.

For h.p.l.c. analysis B4310 and bradykinin were incubated at a concentration of $10 \mu\text{M}$ in a volume of 1 ml with 50 mg of chopped lung tissue either alone or in combination with captopril ($5 \mu\text{M}$). Each incubation procedure was repeated three times.

High performance liquid chromatography

H.p.l.c. analyses were performed in a similar manner to that described by Sheikh & Kaplan (1986a,b) with a Beckman gradient system and a Kontron LCD725 UV detector. Samples were applied to a reverse phase column (Waters μ Bondapak C₁₈, 3.9 × 300 mm), and the separations were carried out with a linear gradient of solvent B (0.05% (v/v) trifluoroacetic acid in acetonitrile) from 10 to 50% over a period of 10 min. Solvent A was 0.05% trifluoroacetic acid in deionized and glass-distilled water. The flow rate was 1.5 ml min⁻¹ at room temperature. Products detected at 214 nm were identified by standards of bradykinin and B4310, respectively. Quantification was performed by comparing the peak heights of known amounts of standards with the peak heights of the co-eluting peaks in the incubation samples. The retention times were 8.0 min for bradykinin and 8.4 min for B4310. The sensitivity limit was 100 ng per injected sample (500 μ l) for both peptides at a signal-to-noise ratio of 3:1.

Substances

The following substances were used (Thi = β -(2-thienyl)-L-alanine, Hyp = L-4-hydroxyproline): bradykinin (Bk), 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.). 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-(ethylmercurithio)-benzoxazole-5-carboxylate, Asid-Institut GmbH, F.R.G.) to prevent adsorption to glass and bacterial growth. Atropine (Merck Sharp & Dohme, U.S.A.), mepyramine (Smith Kline & French, U.S.A.), diethylstilboestrol (Serva Feinbiochemie, F.R.G.), indomethacin (Merck Sharp & Dohme, U.S.A.), pentobarbitone sodium (Nembutal, Ceva GmbH, F.R.G.), hyoscine butylbromide (Buscopan, Boehringer Ingelheim, F.R.G.), pancuronium bromide (Pavulon, Organon, Netherlands). Bacitracin (Sandoz GmbH, Austria) and captopril (Squibb Pharma—von Heyden GmbH, Austria) were dissolved and diluted in a 0.15 M solution of NaCl. The composition of the De Jalón solution was (in mM): NaCl 153.9, KCl 5.6, CaCl₂ 0.7, MgCl₂ 0.1, NaHCO₃ 5.0, glucose 3.0. Tris buffer: 0.1 M Tris/HCl in 0.15 M NaCl, pH 7.4.

Statistical analysis

All values represent means $\pm$ s.e.mean. Student's *t* test for paired or unpaired comparisons was used as

appropriate. Concentration-response curves to bradykinin and bradykinin analogues in the smooth muscle experiments were tested for non-parallelism and non-identity by *t*-tests. The results from the degradation experiments with rat lung were evaluated using the Friedman-test and the Wilcoxon-Wilcox-test.

Results

Agonistic and antagonistic effects in the rat isolated uterus

To determine the agonistic potency of the six bradykinin analogues, concentration-response curves to these peptides were established on the rat uterus. The highest concentration used was 3 μ M. All the peptides, except B4308 and B4162, showed agonistic activity (Table 1) with maximum responses equal to those induced by bradykinin. pD₂ values were calculated for these peptides and are shown in Table 1. B4308 and B4162 induced contractions of the rat uterus in the high dose range of 600 to 3000 nM only in 5 and 6 preparations, respectively, of a total of 15 preparations. The highest concentrations produced not more than approximately two thirds (B4308) and one half (B4162) of the maximum contraction induced by bradykinin. However, preparations, in which B4308 and B4162 had no agonistic activity, responded to bradykinin in a concentration-dependent manner. Therefore, the pD₂ values shown in Table 1 for these bradykinin analogues are given in parentheses.

For evaluation of the antagonistic activity of B4310, concentration-response curves to bradykinin were established in the presence of the antagonist in concentrations of 25 to 200 nM. B4310 shifted the

Table 1 Rat uterus: calculated pD₂ values $\pm$ 95% confidence intervals and slope of the regression lines for the concentration-response curves for bradykinin and the bradykinin analogues

Peptide	pD ₂	Slope
Bradykinin	8.54 $\pm$ 0.12	5.05
B4144	7.26 $\pm$ 0.26	6.26***
B4146	6.17 $\pm$ 0.08	4.92
B4148	6.41 $\pm$ 0.11	3.57***
B4162	(5.53 $\pm$ 0.75)	(2.74***)
B4308	(5.76 $\pm$ 3.52)	(2.90***)
B4310	5.83 $\pm$ 0.08	4.36

n = 5–8. For B4162 and B4308 these parameters were calculated only from those preparations which responded to these peptides (see text), and the results are given in parentheses. Significance of difference in slope compared to bradykinin: ****P* < 0.001.

Figure 1 Rat uterus: logarithm of (dose-ratio (DR) minus 1) plotted against negative logarithm of the concentration of B4310 according to Schild (1957). The regression line was calculated by the least squares method. Each point represents mean values, $n = 5$; vertical lines show s.e.mean. $y = 5.4 - 0.71 \times x$; $r = -0.609$.

concentration-response curves to bradykinin to the right in a concentration-dependent manner. No significant differences in the slope of the regression lines or the maximum responses to bradykinin could be found. The ratios of the EC_{50} values for bradykinin in the presence of the antagonist and under control conditions, calculated separately from each of five experiments, were plotted against the negative logarithm of the antagonist concentration and a regression line was fitted by the least squares method (Figure 1). The slope of this regression line was -0.71 which is not significantly different from the theoretical value of -1 for competitive antagonism

Figure 2 Rat duodenum: Schild-plot for the inhibition of the bradykinin-induced relaxation by B4310. Each point represents mean values, $n = 5$; vertical lines show s.e.mean. $y = 7.46 - 1.02 \times x$; $r = -0.89$.

Figure 3 Rat blood pressure: fall in mean arterial pressure induced by 100 pmol bradykinin (Bk) injected i.a. at intervals of 10 min (indicated by arrows). I.a. infusion of B4310 (15 nmol min^{-1} , $\bullet$) or 0.15 M NaCl (0.15 ml min^{-1} , $\circ$) began 4 min before the third injection of Bk and lasted for 5 min. Mean values with s.e.mean shown by vertical lines, $n = 6$. Significance of difference between B4310 and saline: $**P < 0.01$.

($\hat{t} = 1.8$, d.f. = 18). A pA_2 of 7.24 and a pA_{10} of 5.90 were calculated for B4310.

Antagonism of the bradykinin-induced relaxations of the rat duodenum

In the experiments on the rat duodenum, B4310 was used in concentrations of 100 to 800 nM. Maximum responses to bradykinin and the slopes of the concentration-response curves to bradykinin were not altered by the antagonist. The shift of the concentration-response curves in the presence of B4310 was evaluated as mentioned above. The Schild-plot is shown in Figure 2. The slope of the regression line was close to unity and the values calculated for pA_2 and pA_{10} were 7.31 and 6.38, respectively.

Inhibition of the bradykinin-induced fall in rat blood pressure

Injections of 0.1 nmol bradykinin into a carotid artery resulted in a fall in mean arterial blood pressure of 30 to 35 mmHg. Repeated injections at intervals of 10 min produced consistent reductions in blood pressure with no desensitization evident. B4310 (15 nmol min^{-1}) was infused i.a. 4 min before the third injection of bradykinin for 5 min (Figure 3).

Control animals received an infusion of a 0.15M sodium chloride solution. B4310 reduced the bradykinin-induced fall in blood pressure from 35 ± 5 mmHg to 9 ± 1 mmHg, whereas in the saline-infused control animals the fall in blood pressure remained as before. Nine minutes after the end of the infusion the effect of bradykinin was the same in both groups (35 ± 6 mmHg and 33 ± 4 mmHg, respectively).

Effect of bacitracin The effect of the i.a. infusion of B4310 on the fall in arterial blood pressure by 100 pmol bradykinin i.a. was tested during continuous i.v. infusion of bacitracin (0.2 mg min^{-1}) and compared with control animals receiving an i.v. infusion of saline. The inhibition of the effect of bradykinin was more pronounced in the bacitracin-group (4 ± 1 mmHg) than in the saline-group (13 ± 1 mmHg) ($P < 0.01$; Figure 4; 3rd arrow). The next i.a. injection of bradykinin during continuous infusion of bacitracin or saline but without infusion of B4310 resulted in an equal fall in blood pressure in both groups (Figure 4; 4th arrow). The effect of bradykinin was therefore not influenced by bacitracin.

Effect of captopril Infusion of captopril ($5 \mu\text{g min}^{-1}$) greatly increased the fall in blood pressure induced by the small dose of 25 pmol bradykinin i.a. from 14 ± 1 mmHg to 55 ± 2 mmHg ($P < 0.001$; Figure

Figure 4 Rat blood pressure: fall in mean arterial pressure induced by 100 pmol bradykinin (Bk) injected i.a. at intervals of 10 min (arrows). Infusion of bacitracin (0.2 mg min^{-1} , i.v., ●) or 0.15M NaCl (0.1 ml min^{-1} , ○) began 1 min after the second injection of Bk and lasted for 20 min. B4310 was infused in both groups for time indicated. Mean values with s.e.mean shown by vertical lines, $n = 8$. Significance of difference between bacitracin and saline: $***P < 0.01$. Where no s.e.mean is indicated it was smaller than the symbol.

Figure 5 Rat blood pressure: fall in mean arterial pressure induced by i.a. injections of 25 pmol bradykinin (Bk) (arrows). Infusion of captopril ($5 \mu\text{g min}^{-1}$, i.v.) began 1 min after the second injection of Bk and lasted throughout the experiment (both groups). I.a. infusion of B4310 (15 nmol min^{-1} , ●) or 0.15M NaCl (0.15 ml min^{-1} , ○) began 4 min before the third injection of Bk and lasted for 5 min. Significance of difference between B4310 and saline: $**P < 0.01$. Mean values with s.e.mean shown by vertical lines, $n = 5$.

5; 3rd arrow). Additional infusion of B4310 reduced the bradykinin-induced fall in blood pressure to 23 ± 2 mmHg ($P < 0.01$; Figure 5; 4th arrow). During control infusions of a 0.15M solution of sodium chloride instead of B4310, the effect of bradykinin remained as before.

Degradation of B4310 by rat lung

Determination by bioassay In each rat uterus preparation bradykinin was applied to the tissue in a concentration that produced a contraction of approximately 70% of the maximum effect. B4310, incubated with or without rat lung tissue, was applied to the rat uterus in a volume corresponding to a concentration of 200 nM of unincubated peptide. Incubation with native lung tissue greatly reduced the potency of the antagonist solution to inhibit the action of bradykinin on the rat uterus as compared to incubation of the antagonist without lung tissue ($P < 0.01$, Wilcoxon-Wilcox-test). Inactivation of lung enzymes by heat treatment prevented the degradation of B4310 during the incubation (Table 2). Incubates of native lung tissue alone had no contractile activity on the rat uterus.

Determination by h.p.l.c. Bradykinin and B4310 were incubated for 5, 15 and 45 min with chopped rat lung in the absence or presence of $5 \mu\text{M}$ captopril (Table 3). Both peptides were destroyed to a large extent within the first 5 min. After 15 min of incubation the two peptides were not detectable any

Table 2 Degradation of B4310 by rat lung: % inhibition of bradykinin-induced contractions of rat uterus by B4310 in a volume corresponding to a concentration of 200 nM of unincubated antagonist

Incubation of B4310	% inhibition of bradykinin
Without tissue	73 ± 8
With heat-inactivated lung	68 ± 7]*
With native lung	37 ± 9]**

B4310 was incubated for 15 min at 37°C either without tissue, with heat-inactivated lung slices or with native lung tissue. Significance of difference: * $P < 0.05$, ** $P < 0.01$ (Wilcoxon-Wilcoxon-test). Means ± s.e.mean; $n = 8$.

more in the incubates. Captopril inhibited the disappearance of both peptides markedly, but not completely, during the first 15 min of incubation. Thereafter no further degradation could be seen.

Discussion

The discovery of competitive peptide antagonists to bradykinin (Vavrek & Stewart, 1985; Stewart & Vavrek, 1986; 1987; 1988) and the recent development of apparently organ-specific bradykinin antagonists (Stewart, personal communication) prompted further investigation of the pharmacological properties of these peptides (1) to quantify their agonistic compared with their antagonistic potencies, (2) to show antagonism on smooth muscles which respond to bradykinin in different ways, (3) to use an *in vivo* method for the assessment of their activity and duration of action, and (4) to obtain information on enzymatic degradation of the antagonists *in vitro* and *in vivo*. In extension of earlier work (Griesbacher & Lembeck, 1987) only the antagonist B4310 was used, except in the experiments on the rat uterus.

Table 3 Amount of bradykinin (Bk) or B4310 remaining after incubation with chopped rat lung tissue in the presence or absence of 5 µM captopril

	5 min	15 min	45 min
Bk	27 ± 8	nd	nd
Bk + captopril	64 ± 10	13 ± 5	19 ± 4
B4310	12 ± 3	nd	nd
B4310 + captopril	41 ± 3	8 ± 7	10 ± 1

Determination of bradykinin and B4310 in the incubation medium by h.p.l.c. Means ± s.e.mean; $n = 3$.
nd = not detectable.

Agonistic and antagonistic properties

To assess the agonistic properties of the six bradykinin antagonists we used the rat uterus since this organ is very sensitive to bradykinin (Table 1). B4144 had agonistic effects at concentrations about 20 fold higher than those effective for bradykinin. The rest of the analogues tested in this experiment showed agonistic effects only at concentrations two to three orders of magnitude higher than bradykinin. In the case of B4162 and B4308 some preparations did not react to the peptides at all and B4310 which was used in all other experiments was approximately 500 times less potent in inducing contractions of the rat uterus than bradykinin. It is therefore unlikely that under usual experimental conditions the agonist activity of the bradykinin antagonists would interfere with their antagonistic action.

To assess the antagonistic potency of B4310 in smooth muscle preparations we used the rat uterus and the rat duodenum, two organs which respond to bradykinin in opposite ways: the rat uterus is contracted by bradykinin whereas the rat duodenum responds to bradykinin with a relaxation. The pA_2 values for B4310 determined in these preparations (7.24 and 7.31, respectively) are very close to that determined earlier for the antagonism of the bradykinin-induced stimulation of the trigeminal nerve in the rabbit iris sphincter muscle preparation ($pA_2 = 7.59$; Griesbacher & Lembeck, 1987). An organ-selective specificity of B4310 is therefore rather unlikely.

Degradation of B4310 in vivo and in vitro

Stewart & Vavrek (1986) showed that bradykinin antagonists inhibited the blood pressure lowering effect of bradykinin. We used retrograde injections of bradykinin into the carotid artery to assess its vasodilator effect by measuring the rapid and shortlasting fall in arterial blood pressure. It was possible to apply bradykinin repeatedly at a dose of 100 pmol in intervals of 10 min showing that there is no development of tolerance to this dose. The effect only lasted for approximately 1 min, confirming the short half-life of bradykinin in blood, calculated to be less than 30 s in man (Saameli & Eskes, 1962), in the dog (McCarthy *et al.*, 1965) and in the cat (Ferreira & Vane, 1967b). An i.a. infusion of B4310 (15 nmol min⁻¹) almost abolished the effect of the i.a. injection of bradykinin. The full restoration of the action of bradykinin 10 min after the end of the antagonist infusion demonstrated the short duration of the action of B4310.

As it was likely that the antagonistic peptides are cleared in an enzymatically similar manner to bradykinin, the influence of the peptidase inhibitors bac-

tracin and captopril were studied. Bacitracin has been shown to inhibit the cleavage of several different peptides such as TRH and LH-RH (McKelvy *et al.*, 1976), β -endorphin (Patthy *et al.*, 1970) or substance P (Lembeck *et al.*, 1978). However, bacitracin (0.2 mg min^{-1} , i.a.) did not augment the fall in blood pressure induced by bradykinin, thus suggesting that the cleavage of bradykinin is not an effect of bacitracin-sensitive peptidases. The antagonistic potency of B4310 on the other hand appeared to be enhanced, but not prolonged, by bacitracin (Figure 4, 3rd arrow). A possible effect of bacitracin on the enzymatic degradation of B4310 would, however, need more detailed investigation. Bradykinin is largely eliminated from the blood by a single passage through the pulmonary circulation (Ferreira & Vane, 1967a,b; Ryan *et al.*, 1970). Angiotensin converting enzyme seems to play a critical role in the inactivation of bradykinin (Dorer *et al.*, 1974a,b), although other peptidases such as aminopeptidase P may be involved additionally in the lung (Orawski *et al.*, 1987) and in the periphery (Marceau *et al.*, 1981; Whalley, 1987). As the angiotensin converting enzyme inhibitor captopril (Rubin *et al.*, 1978) did not attenuate the breakdown of bradykinin in plasma (Marceau *et al.*, 1981) and isolated arteries (Whalley, 1987) it had been assumed that angiotensin converting enzyme would not contribute to the inactivation of bradykinin in the periphery. In our experiments, however, captopril ($5 \mu\text{g min}^{-1}$, i.a.) increased the fall in arterial blood pressure induced by an i.a. injection of 25 pmol bradykinin to a size almost three times that of 100 pmol bradykinin in the control experiment (compare 3rd arrow in Figure 5 with 1st and 2nd arrows in Figure 4). This seems to indicate that bradykinin is destroyed very fast in the periphery by an enzyme which is sensitive to captopril, as the action of intra-arterially injected bradykinin is regarded as an immediate action on the arteries before the passage through the lungs (Haddy *et al.*, 1970). Angiotensin converting enzyme is present in vascular endothelial cells (Caldwell *et al.*, 1976; Ryan *et al.*, 1976) and its low potency for inactivation of bradykinin in isolated arteries (Whalley, 1987) might be different from its effect *in vivo*.

tivation of bradykinin in isolated arteries (Whalley, 1987) might be different from its effect *in vivo*.

B4310 also reduced the effect of bradykinin during the infusion of captopril (Figure 5) as it did in the control experiments (Figure 3), but the design of the experiment did not allow conclusions about the role of angiotensin converting enzyme in the cleavage of B4310. The enzymatic degradation of B4310 was therefore tested *in vitro*. B4310 was incubated with chopped lung tissue and its antagonistic potency was then tested on the bradykinin-induced contractions of the rat uterus. The reduced antagonistic activity of B4310 after incubation with native lung tissue and the inhibition of this inactivation by heat-treatment of the tissue confirms the enzymatic cleavage of B4310 by lung tissue, but does not allow further conclusions (Table 2). Determination by h.p.l.c. of the amount of B4310 and of bradykinin persisting after incubation with chopped lung tissue demonstrated the high degree of degradation of both peptides within the first 5 min of incubation. The cleavage of both peptides was greatly, but not completely, inhibited by captopril, which confirms the contribution of further enzymes in the lung. The fast cleavage of B4310 *in vitro* explains the finding that antagonism of bradykinin actions *in vivo* could only be seen during an infusion of B4310 and the rapid recovery of the bradykinin response after cessation of the infusion.

Our experiments show that the bradykinin antagonists available at present are useful tools for the investigation of the biological role of bradykinin. However, the susceptibility to enzymatic degradation may limit their use in animal experiments or in clinical studies. Therefore long-acting and stable bradykinin antagonists remain to be discovered.

The authors thank Dr J.M. Stewart and Dr R.J. Vavrek for the generous gifts of bradykinin antagonists. The investigation was supported by the Pain Research Commission of the Austrian Academy of Sciences, the Austrian National Bank (grant No. 2811) and the Austrian Funds for Scientific Research (grant No. P5616).

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(Received April 27, 1988

Revised September 12, 1988

Accepted October 19, 1988)