Br. J. Pharmac. (1987), **92**, 851–855

New synthetic antagonists of bradykinin

[1]M. Schachter, Y. Uchida, D.J. Longridge, T.Labedz, [†]E.T. Whalley, *R.J. Vavrek & *J.M. Stewart

Department of Physiology, University of Alberta, Edmonton, Canada, T6 2H7; *Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado, U.S.A. 80262 and [†]Department of Pharmacology, Materia Medica and Therapeutics, University of Manchester, Oxford Road, Manchester M13 9PT

1 A new synthetic bradykinin analogue was found to be an antagonist of bradykinin-induced vascular permeability in rabbit skin. It was effective in equimolar concentrations.

2 These analogues also antagonized the action of bradykinin in contracting the guinea-pig isolated ileum. The mean pA_2 values of five different antagonists ranged from 5.3–6.4 respectively, on this preparation.

3 Our observations, together with those of others suggest that these antagonists act on the same receptor types, viz., B_2, in rabbit blood vessels and in smooth muscle of guinea-pig ileum.

4 Our results support the view that the way is now promising for the synthesis of potent specific antagonists of bradykinin for experimental and therapeutic use.

Introduction

Bradykinin (BK) and related kinins are potent enhancers of vascular permeability (Holdstock *et al.*, 1957; Bhoola *et al.*, 1960; Bissett & Lewis, 1962; Uchida *et al.*, 1983). However, definite evidence of their involvement in inflammation has been uncertain, since effective antagonists have not been available. A number of BK analogues have recently been synthesized which are specific antagonists of BK on the guinea-pig isolated ileum. rat uterus and blood pressure (Table 1), (Vavrek & Stewart, 1985). Our findings support the observations of Vavrek & Stewart (1985) and, in addition, show that one of them, compound B-3824, is also an effective antagonist of BK-induced vascular permeability in rabbit skin. We selected it for our study since we found it more effective than compound B-3832, which in turn was more effective than compound B-3820 in the rabbit permeability test.

At least two receptor types have been proposed for kinins by Regoli & Barabe (1980) which they designated as B_1 and B_2. The B_1 receptor is thought to be formed by *de novo* synthesis in vascular smooth muscle *ex situ* in particular, notably in the rabbit isolated

Table 1 Primary structures of bradykinin (BK), BK antagonists and their pA_2 values on guinea-pig isolated ileum

Compound	*Structure*									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	pA_2
BK	Arg–	Pro–	Pro–	Gly–	Phe–	Ser–	Pro–	Phe–	Arg	—
B-3820	Arg–	Pro–	Hyp–	Gly–	Thi–	Ser–	DPhe–	Thi–	Arg.TFA	5.66
B-3824	DArg–	Arg–	Pro–	Hyp–	Gly–	Thi–	Ser–	DPhe–	Thi–	Arg.TFA	6.38
B-3832	DArg–	Arg–	Hyp–	Hyp–	Gly–	Thi–	Ser–	DPhe–	Thi–	Arg.TFA	5.95
B-3878	Lys–	Lys–	Arg–	Pro–	Pro–	Gly–	Thi–	Ser–	DPhe–	Thi–	Arg.TFA	5.62
B-3880	Arg–	Pro–	Pro–	Gly–	Thi–	Ser–	DPhe–	Thi–	Arg.HOAc	5.27

Thi, β-(2-thienyl)-L-alanine; Hyp, L-4-hydroxyproline; TFA, trifluoroacetic acid salt.

[1]Author for correspondence at present address: Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ.

aorta. The B_1 receptor is characterized by its response to the selective competitive antagonist des-Arg^9-[Leu^8]BK (Regoli *et al.*, 1978; Regoli & Barabe, 1980). The B_2 receptor is thought to mediate contraction of the rat uterus, cat and guinea-pig ileum (Barabe *et al.*, 1977). Subsequently Marceau *et al.* (1981) described B_2 receptors for the increased vascular permeability caused by kinins in rabbit skin. Our results are consistent with the conclusion of Marceau *et al.* (1981) that this action of bradykinin is on receptors of the B_2 type.

Some of these results have already been presented in a preliminary form (Longridge *et al.*, 1986; Stewart *et al.*, 1986).

Methods

Hartley guinea-pigs (450–600 g) and New Zealand white male rabbits (3–4 kg) were anaesthetized with sodium pentobarbitone (Somnotol, MTC Pharmaceuticals, Hamilton, Canada) (30 mg kg^{-1}) intraperitoneally (guinea-pigs) or intravenously (rabbits). In one set of experiments the rabbits were injected with a 5% solution of pontamine sky blue 6BX (BDH Chemicals Ltd, Poole) in 0.9% NaCl (1.0 ml kg^{-1}) via the marginal ear vein. The abdominal skin was shaved and depilated with 'Neet'. Solutions (agonists alone, or in combination with antagonist) were injected intradermally in a volume of 0.1 ml 5 min after the injection of the pontamine sky blue. The rabbits were killed 40 min later by exsanguination, the appropriate areas of skin removed, and the dye extracted from each blue injection site and measured by the spectrophotometric method of Katayama *et al.* (1978). In another set of similar experiments on vascular permeability, the rabbits were unanaesthetized, pretreated with captopril (Squibb) (1 mg kg^{-1} s.c.) to enhance the effect of bradykinin, injected with Evans blue dye (Sigma) (10 mg kg^{-1} i.v.), and measurements of cutaneous dye extravasation were made from the mean diameter of the lesion after animals had been killed by an overdose of sodium pentobarbitone, given intravenously.

Contractions of the guinea-pig isolated ileum bathed in Tyrode solution (containing atropine sulphate and mepyramine maleate, 10^{-6} M) were recorded isometrically with a force displacement transducer. The assay of various BK analogues for antagonism of BK-induced contractions was performed by incubation of the ileum with the analogue for 30 s before administering a dose of BK. Analogues were assayed according to the method of Schild (1947) with antagonist potencies expressed as pA_2 values, the latter derived from 6 determinations on 6 different ileal strips with each antagonist. Probability values (P) were calculated by the Student's paired t test.

Bradykinin (BK), lysyl-bradykinin (L-BK) (Sigma, St Louis, MO) and all BK analogues (2×10^{-4} M) (Table 1; Vavrek & Stewart, 1985) were dissolved in sterile Tyrode solution and subsequent dilutions made in Tyrode solution before use. Angiotensin II (AII, Sigma), 5-hydroxytryptamine (5-HT, Sigma), substance P (SP, Sigma) and des-Arg^9-[Leu^8] BK (Peninsula Lab. Inc., San Carlos, CA) were similarly dissolved.

Results

The primary structures of the new BK antagonists and their relative potencies in antagonizing the effect of bradykinin in contracting the guinea-pig isolated ileum are shown in Table 1. Their mean pA_2 values ranged from 5.3–6.4, compound B-3824 being the most potent with a mean pA_2 value of 6.4. This BK analogue has amino acid replacements at positions proline-3, phenylalanine-5, proline-7 and phenylalanine-8, with L-4-hydroxyproline (Hyp), β-2-thienyl-L-alanine (Thi), D-phenylalanine (D-Phe) and β-2-thienyl-L-alanine (Thi) respectively. In addition to these replacements, D-arginine is added to the N-terminal residue (Table 1).

Figures 1–3 show the effects of BK and of the BK antagonist B-3824 on vascular permeability in rabbit skin in our experiments using anaesthesia, pontamine blue dye, and measuring the dye extracted from the skin lesions.

Figure 1 shows the dose-response curves of BK, L-BK and histamine in rabbit skin. Those for BK and L-BK are identical in the dose range used (0.01–10 nmol 0.1 ml^{-1}). The curve for histamine (1.0–1000 nmol 0.1 ml^{-1}), however, is shifted well to the right indicat-

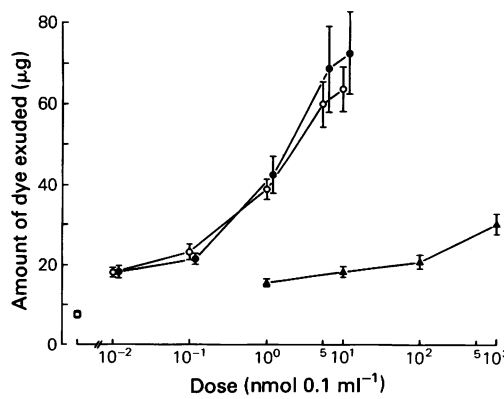

Figure 1 The effect of bradykinin (BK, ○), lysyl-BK (●), histamine (▲) and Tyrode solution (□) on rabbit skin. Each point represents the mean of 3–7 experiments; vertical lines indicate s.e.mean.

ing a much lower potency. AII, 5-HT, SP and des-Arg^9 BK, were even less effective than histamine.

Figure 2 shows the result of a typical experiment in which BK (0.01–10 nmol injected intradermally in 0.1 ml) increased vascular permeability in rabbit skin. It also shows the effect of a range of concentrations of the antagonist B-3824 when injected together with 1.0 mol BK. Similar results were obtained in five rabbits. It was apparent that this antagonist in a concentration of 1 nmol 0.1 ml^{-1} reduced the increase in vascular permeability caused by 1.0 nmol BK to that of 0.1 nmol. Even with as little as 0.1 nmol antagonist, a reduction in the effect of 1.0 nmol BK was seen. Tyrode solution alone was without effect. Higher concentrations of the antagonist B-3824, (e.g. 100 nmol) showed an agonist effect (Figure 2).

Figure 3 was derived from results on 2 rabbits in which randomized injections of BK alone, or with antagonist B-3824, were made intradermally (Figure 2). The responses to 5 doses of BK (0.01–10 nmol 0.1 ml^{-1}) were obtained and the effect of 5 different doses of the BK antagonist B-3824 (0.01–100 nmol 0.1 ml^{-1}) on the response to a standard dose of BK (1.0 nmol 0.1 ml^{-1}) determined. The intradermal injection of as little as 0.1 nmol B-3824 significantly reduced the increase in permeability induced by 1 nmol BK ($P < 0.05$). Also, 1 nmol B-3824 reduced the effect of 1 nmol BK to that induced by 0.1 nmol (see also Figure 2). Figure 3 also shows that 10 nmol B-3824 had no agonist activity, but agonist activity appeared at higher doses, e.g. 100 nmol.

In 2 rabbits, and using the above technique, the selective B_1 antagonist des-Arg^9-[Leu^8] BK (Regoli & Barabe, 1980) (0.1–100 nmol 0.1 ml^{-1}) was found not to reduce the effect of an intradermal injection of BK (1 nmol 0.1 ml^{-1}).

Similar results were obtained with the BK antagonist B-3824 in unanaesthetized rabbits (see Methods) as those in the anaesthetized rabbits. In these experiments, in which conditions were somewhat different, and in which the diameter of the extravasated dye around the injection site was simply measured in mm, 10 nmol B-3824 completely abolished the response to 0.1 nmol BK. The mean lesion diameter of 11.3 ± 0.4 mm was reduced to zero, or immeasurable size at these doses of BK and antagonist. These values are the results of 12 measurements each for BK alone and for BK plus B-3824 in 4 unanaesthetized rabbits. Unlike the anaesthetized rabbits, no agonist activity was observed after an injection of 100 nmol B-3824; this may be due to the different conditions, particularly in the use of a different dye (Evans blue) to detect the increased vascular permeability.

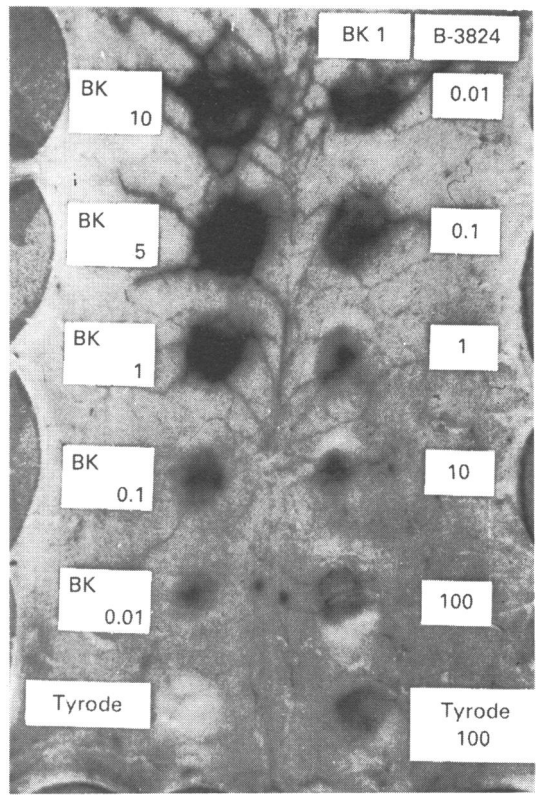

Figure 2 Rabbit skin (reverse side) showing increased vascular permeability to circulating dye after intradermal injections of 0.1 ml test solutions. Left: various doses of bradykinin (BK; 0.01–10 nmol 0.1 ml^{-1}). Right: standard dose of BK (1 nmol 0.1 ml^{-1}) plus various doses of antagonist B-3824 (0.01–100 nmol 0.1 ml^{-1}).

Discussion

Our results show that the BK antagonist B-3824 is not only an antagonist of BK-induced contraction of the guinea-pig isolated ileum but is also effective against BK-induced vascular permeability in blood vessels of rabbit skin. If B-3824 proves to be as effective in human skin as it is in that of rabbit, and particularly should it prove effective against the pain-producing effects of BK in man (Armstrong *et al.*, 1957; Holdstock *et al.*, 1957; Schachter, 1960), it could be a useful anti-inflammatory or analgesic agent. We did not test the specificity of these antagonists against other peptides since AII and SP proved to be ineffective in increasing vascular permeability in the rabbit. However, it has been shown by Vavrek & Stewart (1985) that although B-3824 is a potent inhibitor of BK on the rat uterus and guinea-pig ileum preparations, it is ineffective against AII and SP on these

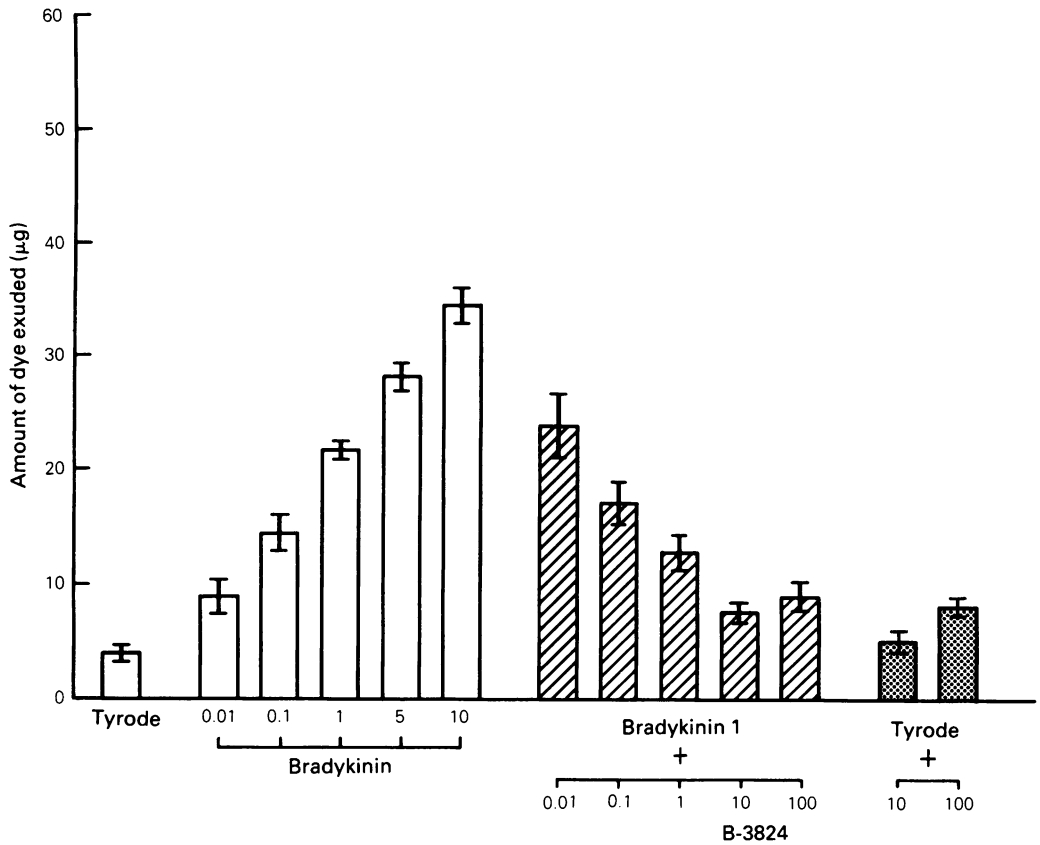

Figure 3 Effects of the bradykinin antagonist B-3824 on bradykinin (BK)-induced vascular permeability in rabbit skin. Numbers indicate dose (nmol 0.1 ml^{-1}) of BK or of antagonist B-3824. Columns are mean values and vertical lines show s.e.mean. Open columns: increase in permeability induced by various doses of BK. Hatched columns: effect of various doses of antagonist B-3824 on vascular permeability induced by 1 nmol BK. Stippled columns: effect of antagonist B-3824 in high concentrations; 100 nmol increased vascular permeability more than the control solution of Tyrode. The results were derived from 2 rabbits. In one rabbit each dose was repeated 4 times, in the other 3 times; making a total of 7 tests for each dose from which the means were derived. Effect of injections of Tyrode solution alone, or plus B-3824, is the mean effect of 4 injections, 2 in each rabbit.

preparations. It would appear therefore that these BK analogues are specific inhibitors of BK.

Although the classification of BK receptor types is still unsettled (Whalley *et al.*, 1984; Boschov *et al.*, 1984), our results are consistent with the view of Barabe *et al.* (1979) and of Marceau *et al.* (1981) that the BK receptor subclass B_2 mediates vascular permeability in rabbit skin and contraction of the guinea-pig ileum. Thus we confirmed the result of Regoli & Barabe (1980) that the specific B_1 antagonist des-Arg^9-[Leu^8]BK failed to reduce the vascular permeability induced by BK in the rabbit. Our screening experiments indicated the same descending order of potency of analogues B-3824, B-3832 and B-3820, respectively, in antagonizing the effects of BK on vascular permeability in rabbit skin and on contraction of the guinea-pig ileum. These observations are consistent with the view that the same or a similar BK receptor exists in both these systems. Also, further evidence of the competitive nature of the antagonism (Vavrek & Stewart, 1985) was obtained from the data from which the pA_2 values were derived (Table 1); tests on the guinea-pig ileum indicated parallel shifts in the dose-dependent inhibitory effects of the different antagonists.

The potency of the antagonist B-3824 on the vascular permeability effects of BK in rabbit skin is evident from its effectiveness in equimolecular concentrations against BK (Figure 3). It showed no agonist actions of its own at this concentration, and even 10 times this concentration, 10 nmol 0.1 ml^{-1}, had no agonist activity However, at a much higher dose

(100 nmol) some agonist activity was noted. Since only 4 pairs of matched controls were carried out at this concentration, the formal P value of < 0.1 is of limited significance. Indeed, complete inhibition of the increased permeability caused by BK was observed without agonist activity when similar experiments were carried out under different conditions. In the latter instance tests were made in rabbit skin but without anaesthesia, using a different dye (Evans blue), and measuring the diameter of exuded dye rather than the amount of dye extracted.

Finally, our results support the veiw of Vavrek & Stewart (1985) that the development of effective bradykinin antagonists has begun. The available evidence indicates that this inhibition is competitive and specific and that new compounds will be synthesized. They should prove to be valuable tools for the investigation of the roles of kinins in physiology and pathology as well as for their possible therapeutic use in man.

This work was supported by the Medical Research Council of Canada and the Alberta Heart foundation. D.J.L. held a Studentship of the Alberta Heritage foundation for Medical Research and Y.U. a Heritage Foundation Fellowship.

References

ARMSTRONG, D., JEPSON, J.B., KEELE, C.A. & STEWART, J.W. (1957). Pain-producing substance in human inflammatory exudates and plasma. *J. Physiol.*, **135**, 350–370.

BARABE, J., DROULIN, J-N., REGOLI, D. & PARK, W.K. (1977). Receptors for bradykinin in intestinal and uterine smooth muscle. *Can. J. Physiol. Pharmacol.*, **55**, 1270–1285.

BARABE, J., MARCEAU, F., THERIAULT, B., DROUIN, J-N. & REGOLI, D. (1979). Cardio-vascular actions of kinins in the rabbit. *Can. J. Physiol. Pharmacol.*, **57**, 78–91.

BHOOLA, K.D., CALLE, J.D. & SCHACHTER, M. (1960). The effect of bradykinin, serum kallikrein and other endogenous substances on capillary permeability in the guinea-pig. *J. Physiol.*, **152**, 75–86.

BISSETT, B.W. & LEWIS, G.P. (1962). A spectrum of pharmacological activity in some biologically active peptides. *Br. J. Pharmacol.*, **19**, 168–182.

BOSCHOV, P., PAIVA, A.C.M., PAIVA, T.B. & SHIMUTA, S.T. (1984). Further evidence for the existence of two receptor sites for bradykinin responsible for the diphasic effect in the rat isolated duodenum. *Br. J. Pharmacol.*, **83**, 591–600.

HOLDSTOCK, D.J., MATHIAS, A.P. & SCHACHTER, M. (1957). A comparative study of kinin, kallidin and bradykinin. *Br. J. Pharmacol.*, **12**, 149–158.

KATAYAMA, S., SHIONOYA, H. & OHTAKE, S. (1978). A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. *Mircobiol. Immunol.*, **22**, 89–101.

LONGRIDGE, D.J., SCHACHTER, M., STEWART, J.M., UCHIDA, Y. & VAVREK, R.J. (1986). Synthetic competitive antagonists of bradykinin on vascular permeability in the rabbit. *J. Physiol.*, **372**, 73P.

MARCEAU, F., KNAP, M. & REGOLI, D. (1981). Pharmacological characterization of the vascular permeability effects of kinins in the rabbit skin. *Can. J. Physiol. Pharmacol.*, **59**, 921–926.

REGOLI, D. & BARABE, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32**, 1–46.

REGOLI, D., MARCEAU, F. & BARABE, J. (1978). *De novo* formation of vascular receptors for bradykinin. *Can. J. Physiol. Pharmacol.*, **56**, 674–677.

SCHACHTER, M. (1960). Some properties of kallikrein, bradykinin and wasp venom kinin. In *Polypeptides Which Affect Smooth Muscles and Blood Vessels*. ed. Schachter, M. pp. 232–236. London: Pergamon.

SCHILD, H.O. (1947). pA, A new scale for the measurement of drug antagonism. *Br. J. Pharmacol.*, **2**, 189–206.

STEWART, J.M., VAVREK, R.J. & WHALLEY, E.T. (1986). Antagonism of the vascular permeability actions of bradykinin in the rabbit. *Br. J. Pharmacol. Proc. Suppl.*, **87**, 211P.

UCHIDA, Y., TANAKA, K., HARADA, Y. UENO, A. & KATORI, M. (1983). Activation of plasma kallikrein-kinin system and its significant role in pleural fluid accumulation of rat carrageenin-induced pleurisy. *Inflammation*, **7**, 121–131.

VAVREK, J.R. & STEWART, J.M. (1985). Competitive antagonists of bradykinin. *Peptides*, **6**, 161–164.

WHALLEY, E.T., SAMPAIO, C.A.M., FRITZ, H. & EDWARDS, G. (1984). The effect of kinins on paw oedema and uterus in rats. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 202–206.

(Received May 7, 1987.
Accepted July 24, 1987.)