Biochemical Pharmacology, Vol. 20, pp. 3179–3184. Pergamon Press, 1971. Printed in Great Britain

NEUTRALIZATION BY AN ANTIBODY OF SOME VASCULAR ACTIONS OF BRADYKININ*

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(*Received* 1 *January* 1971; *accepted* 27 *April* 1971)

Abstract—Rabbits injected with bradykinin coupled to either ovalbumen or human gamma globulin produced antibodies which are able to neutralize the actions of bradykinin on the isolated perfused vein from the rabbit ear and on the autoperfused dog hind limb. This suggests an application for these antibodies in the investigation of kinin function.

THE PEPTIDE bradykinin (BK) has a number of actions that fit it for a role as a mediator in various physiological and pathological reactions,[1] but although this peptide has been shown to occur in the body[2,3] no definite function has as yet been assigned to it. A major difficulty in investigations directed to this end is the lack of a specific antagonist. A number of compounds[4,5] are known which block at least partially, the actions of bradykinin, but these agents are not specific since they also affect the responses to other substances such as histamine and serotonin. Some specific antibodies to biologically active molecules can block the activities of these materials. This property has been found in antibodies raised against such molecules as gastrin,[6] angiotensin,[7–9] ACTH,[10] histamine,[11] 17-β estradiol and progesterone.[12] Such an immunological approach to inhibition offers a promising technique to study the role of bradykinin as a mediator in normal and abnormal states. Davis and Goodfriend[13] have demonstrated neutralization of the bronchoconstrictor action of bradykinin in the guinea-pig by both active and passive immunization but Goodfriend *et al.*[9] were unable to demonstrate the inhibition of *in vitro* effects of bradykinin with their antibody.

In this report the neutralization of the actions of bradykinin in two preparations of vascular smooth muscle by *in vitro* incubation of the peptide with antibody is described.

MATERIALS AND METHODS

Production of anti-bradykinin antibody

Bradykinin was coupled to either ovalbumen or human gamma globulin by the use of water soluble 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide HCl.[14] The bradykinin–protein complex, in distilled water, was emulsified with an equal volume of complete Freund's adjuvant and the emulsion was injected subcutaneously into rabbits at monthly intervals; each rabbit received 1 mg of kinin coupled to 4 mg of protein at each injection.

* Supported in part by a Grant-in-Aid from the National Heart Foundation of Australia.

Detection of antibodies

The method used is described in detail in a previous paper.[3] Serial dilutions of plasma were made with 0·9% saline and 0·1 ml of each was incubated for 24 hr at 4° in tubes containing 0·1 ml of 0·001 M 1,10-phenanthroline monohydrochloride, 0·1 ml of [^{125}I-Tyr^{8}]-bradykinin (6000 counts/min) dissolved in 0·9% saline and 0·5 ml of 0·1 M tris hydrochloride buffer pH 7·4, containing 0·2% gelatin, 0·1% neomycin and 0·01 M disodium ethylene diamine tetra-acetate.

After incubation, labelled peptide bound to antibody was separated from unbound by the addition of dextran-coated charcoal to adsorb the latter[15] and subsequent centrifugation. The protein-bound label in the supernatant was counted in a well-type crystal scintillation counter.

Isolation of immunoglobulins

Antibody-rich fractions were obtained from the sera of control and immune rabbits by the elution from DEAE-Sephadex A-50 with 0·02 M phosphate buffer pH 6·6 according to the method of Baumstark *et al.*[16] The globulin solutions thus obtained were dialysed against 0·02 M glycine–hydrochloric acid buffer pH 2·3, for a total of 48 hr. This step was introduced to release any endogenous bradykinin bound to the immunoglobulins. The solutions were then returned to pH 7·4 by dialysis against 0·02 M tris hydrochloride buffer.

In vitro *neutralization of the biological activity of bradykinin*

Bradykinin was incubated at room temperature with plasma or globulin solution from control or immunized rabbits. In order to inhibit the kininases present, 1,10-phenanthroline monohydrochloride[17] at a final concentration of 0·001 M was added to all incubates. Residual kinin activity following a 5-min incubation period was assayed on the isolated perfused rabbit ear vein[18] and on the autoperfused dog hind limb in which the increase in femoral artery flow was measured with a square wave electromagnetic flowmeter following the injection of these solutions distal to the flowmeter probe.

RESULTS

Rabbits injected with bradykinin coupled to either ovalbumen or human gamma globulin developed anti-bradykinin antibodies. After six injections, the dilution of the antiserum which was necessary to bind 50 per cent of added labelled peptide varied from 1/5 to 1/1000.

One of the two antisera used in this study was obtained from a rabbit injected with bradykinin–ovalbumen complex (R12) and the other was obtained from a rabbit immunized with bradykinin–human gamma globulin complex (R18). Both of these antisera could bind 50 per cent of the labelled peptide when diluted 1/1000.

The responses of the isolated perfused rabbit ear vein are shown in Fig. 1. The injection of 1 and 2 ng of bradykinin produced dose-related but sub-maximal responses. Prior incubation with a 5% solution in normal saline of plasma from rabbit R12 greatly reduced the response so that the venoconstriction to 4 ng bradykinin was little greater than that to 1 ng before incubation.

This was not due to destruction of the peptide by plasma since pre-incubation with 5% plasma from a control rabbit actually potentiated the responses to bradykinin

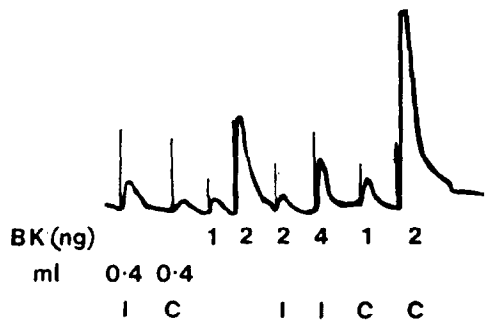

FIG. 1. Response of isolated perfused vein of rabbit ear to bradykinin (BK) and 5% solution of plasma from rabbit R12 (I.) and a control (C). Both plasmas had endogenous venoconstrictor activity. When pre-incubated with BK plasma from R12 reduced venoconstrictor responses to BK but control plasma caused potentiation.

The ability of plasma to potentiate the effects of a number of materials on vascular smooth muscle has been reported previously and appears to be due to the albumen fraction of the plasma protein.[19,20] Both immune and control plasma had intrinsic venoconstrictor activity which has previously been described[19] but the immune plasma was slightly more potent in this regard and thus does not explain the observed reduction of bradykinin response.

The study of the neutralizing ability of plasma is complicated by the presence of enzymes responsible for both the formation and destruction of bradykinin, by the potentiation of the effect of bradykinin in the vein by plasma, and by the intrinsic venoconstrictor activity of whole plasma. Globulin solutions were prepared from immune and non-immune rabbit plasma as it was thought that this step would eliminate most of these complications arising from the use of plasma. The effect of the globulin on the responses of the rabbit ear vein to bradykinin is shown in Fig. 2.

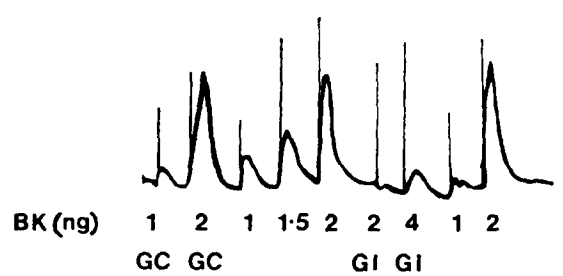

FIG. 2. Response of isolated perfused vein of rabbit ear to bradykinin (BK) and to BK pre-incubated with globulin solution from the serum of rabbit R12 (GI) and a control rabbit (GC).

Whereas globulin solution prepared from anti-bradykinin antiserum decreases the responses to bradykinin in the incubate, globulin obtained from the control rabbit plasma had no effect on the venoconstrictor action of the bradykinin.

The venoconstrictor activity of bradykinin incubated with globulin solutions did not decrease on incubation at room temperature for a further period of 1 hr following the initial assay, demonstrating that the apparent decrease in kinin content of the solution

containing globulin from the anti-bradykinin rabbit was not due to enzymatic destruction.

Neutralization was also seen on the auto-perfused dog hind limb. Figure 3 shows the markedly decreased vasodilator activity resulting from incubating bradykinin with

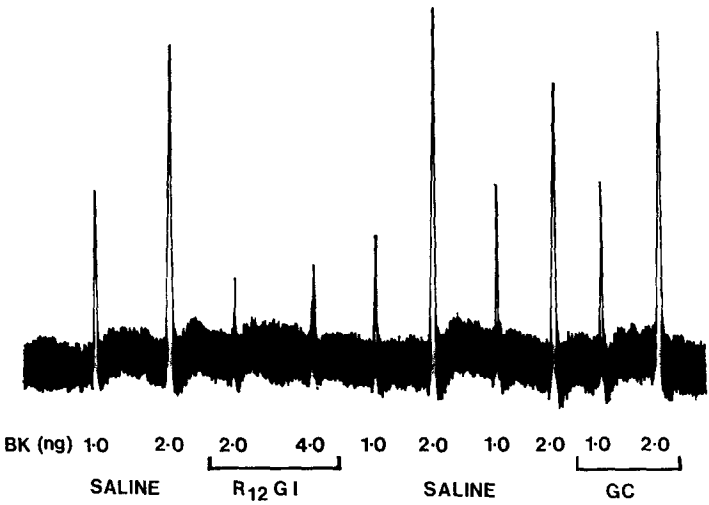

FIG. 3. Blood flow in the femoral artery of an anaesthetized dog recorded with an electromagnetic flowmeter. Injection of bradykinin (BK) into the artery caused a sharp increase in blood flow. Pre-incubation of bradykinin with globulin solution from rabbit R12 reduced the response but it was unchanged by pre-incubation with globulin from a control animal.

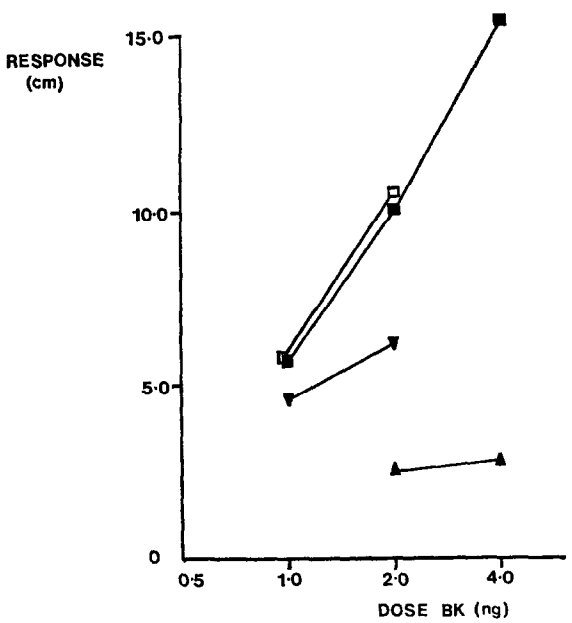

FIG. 4. Dose-response curves for increase in femoral artery blood flow in an anaesthetized dog caused by intra-arterial bradykinin (BK) in saline (■—■) and globulin solutions from control rabbit (□—□), R12 (▲—▲) and R18 (▼—▼).

immune globulin solution while control globulin is without effect on bradykinin-induced vasodilatation. Dose–response curves for the vasodilator action of bradykinin contained in saline and globulin solutions from a control rabbit, from rabbit 12 (immunized with bradykinin-ovalbumen) and rabbit 18 (immunized with bradykinin-human gamma globulin) are shown in Fig. 4. The globulin from rabbit 12 was much more effective in neutralizing bradykinin but this was not due to differences in ability to bind peptide *in vitro* since both globulins had identical titres and affinities as judged by the displacement of labelled peptide by bradykinin.

DISCUSSION

Plasma obtained from rabbits immunized with a bradykinin–protein complex have been shown to act as inhibitors of the venoconstrictor and vasodilator actions of bradykinin. The inhibitory factor is not dialysable and can be extracted from plasma by the use of a technique designed to isolate immune globulins from the other plasma proteins, suggesting that the inhibitor is in fact an anti-bradykinin antibody. The effect is not due to enzymatic destruction of the added peptide as the apparent kinin-content of the incubation solution remains constant for over an hour at room temperature.

The reason for globulin from rabbit 12 being more effective than that from rabbit 18 is not apparent from these studies. The titre and affinity of the two antibodies are not sufficiently different to explain this observation. Most probably the different neutralizing activity is due to differing sites of attachment. Other results with these two antibodies support this view. We found that R12 antibodies cannot distinguish between bradykinin, kallidin and methionyl-lysyl-bradykinin while R18 discriminates clearly between these peptides. This suggests that the specificity of R12 antibody is directed mainly to the C-terminus whereas R18 antibody binding involves regions closer to N-terminus. The C-terminus appears to be more important for biological activity[21] and this would accord well with the greater neutralizing activity of R12.

The neutralization of the activity of bradykinin on vascular smooth muscle, together with the previously demonstrated protection of guinea-pigs against the bronchial effects of intra-pleural administration of bradykinin by active and passive immunization[13] against the peptide, indicates that the use of anti-bradykinin antibodies may well be a useful tool for investigating the role of the kinin system in the body.

REFERENCES

1. G. P. Lewis, *Ann. N.Y. acad. Sci.* **104,** 236 (1963).
2. R. Zacest and M. L. Mashford, *Aust. J. Exp. Biol. Med. Sci.* **45,** 89 (1967).
3. M. L. Mashford and M. L. Roberts, *Biochem. Pharmac.* **20,** 969 (1971).
4. J. D. Horowitz and M. L. Mashford, *J. Pharm. Pharmac.* **21,** 51 (1969).
5. J. Garcia Lemme and M. Rocha e Silva, *Br. J. Pharmac.* **25,** 50 (1965).
6. B. M. Jaffe, W. T. Newton and J. E. McGuigan, *Gastroenterology* **58,** 151 (1970).
7. C. I. Johnston, J. S. Hutchinson and F. A. Mendelsohn, *Circ. Res.* **27,** Supplement 2, II–215 (1970).
8. P. R. Hedwall, *Br. J. Pharmac.* **34,** 623 (1968).
9. T. L. Goodfriend, M. E. Webster and J. S. McGuire, *J. clin. Endocr.* **30,** 565 (1970).
10. J. McGuire, J. McGill, S. Leeman and T. L. Goodfriend, *J. clin. Invest.* **44,** 10 (1965).
11. T. R. Davis and K. M. Meade, *Nature, Lond.* **226,** 360 (1970).
12. M. Ferin, A. Tempone, P. E. Zimmering and R. L. Vande Wiele, *Endocrinology* **85,** 1070 (1969).
13. T. R. Davis and T. L. Goodfriend, *Am. J. Physiol.* **217,** 73 (1969).
14. T. L. Goodfriend, L. Levine and G. D. Fasman, *Science* **144,** 1344 (1964).
15. V. Herbert, K. S. Lau, C. W. Gottlieb and S. J. Bleicher, *J. clin. Endocr.* **25,** 1375 (1965).

16. J. S. BAUMSTARK, W. A. BARDAWIL and R. J. LAFFIN, *Archs Biochem. Biophys.* **108,** 514 (1964).
17. E. G. ERDOS, A. G. RENFREW, E. M. SLOANE and J. R. WOHLER, *Ann. N.Y. acad. Sci.* **104,** 222 (1963).
18. J. D. HOROWITZ and M. L. MASHFORD, *Naunyn-Schmiedebergs Arch. Pharmak. exp. Path.* **263** 332 (1969).
19. J. D. HOROWITZ and M. L. MASHFORD, *Experientia* **24,** 1126 (1968).
20. M. WURZEL, R. C. BACON, R. B. KALT and B. W. ZWEIFACH, *Am. J. Physiol.* **206,** 929 (1964).
21. J. M. STEWART and D. W. WOOLLEY, *Nature, Lond.* **207,** 1160 (1965).