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Angiotensin Converting Enzyme Dependent and Non-dependent Effects of a Fibrinogen-derived Pentapeptide on Microvascular Permeability in Rat Skin

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ABSTRACT

A permeability-increasing pentapeptide, termed peptide 6A, derived from plasmin-degraded human fibrinogen and known to potentiate the increase in microvascular permeability caused by bradykinin was investigated concerning its angiotensin converting enzyme (A.C.E.) related effects. When applied to a rat skin model together with a specific inhibitor of this enzyme, peptide 6A showed a potentiated effect after 30 min. but not after 5 min. The same was also true for bradykinin. These findings suggest that the degradation rate of these peptides is decreased with resulting prolongation of the period of leakage, when the action of A.C.E. is opposed. It is deduced that peptide 6A may act as a partial antagonist of this enzyme in the rat skin model. Addition of peptide 6A to a mixture of bradykinin together with inhibitors of the enzymes degrading bradykinin before application to the rat skin, significantly augmented the extravasation of ^{125}I-albumin. These findings are consistent with data indicating that peptide 6A is a prostacyclin-releaser able to induce vasodilation. This effect of peptide 6A on the microcirculation seems to be separate from its angiotensin converting enzyme-related effects.

INTRODUCTION

Degradation of fibrinogen leads to the formation of permeability-increasing peptide fragments (11). Two peptides with considerable potency to increase microvascular permeability in rat and human skin (3,10) have been isolated and synthetic counterparts have been produced (2).

Using a skin model in rats, we previously found that the two peptides in question, given the code names 6A and 6D respectively, potentiated the microvascular leakage of ^{125}I-albumin caused by bradykinin. The most potent peptide in this respect, 6A, a pentapeptide, also enhanced the permeability-increasing effect of substance P, while the effects of histamine, neurotensin and tuftsin

were not influenced by either of these peptides.

Angiotensin converting enzyme (A.C.E., Kininase II) degrades bradykinin (5), and inhibition of this enzyme leads to an increased half-life of bradykinin *in vivo*. On the basis of previous findings (7) we suggested that the potentiating effect of peptide 6A on the permeability-increasing action of bradykinin could be due to an interference with the local inactivation of bradykinin. Such an effect has actually been demonstrated *in vitro* (17). The purpose of this investigation was to further examine the relation between A.C.E. activity and the effects of peptide 6A *in vivo* in rat skin.

MATERIALS AND METHODS

Peptide 6A (Ala-Arg-Pro-Ala-Lys) with its origin in position 43-47 in the Bβ-chain in the human fibrinogen molecule (12), was synthesized (2). Bradykinin (BRS 640) was a gift from Sandoz AG, Basel, Switzerland. Substance P was purchased from Bachem Lab, Bubendorf, Switzerland and histamine from Koch-Light Ltd, England. The specific inhibitor of angiotensin converting enzyme, SQ 14,225, was a gift from the Squibb Institute, Princeton, New Jersey, USA. Arginine, an inhibitor of serum carboxypeptidase B (21), was purchased from Kebo-Grave AB, Stockholm, Sweden. Male Sprague-Dawley rats weighing 210-235 g, bought from Anticimex Farm, Stockholm, Sweden, were used for studying microvascular permeability according to a method described elsewhere (9). All experimental procedures were carried out under ether anaesthesia. In brief, the dorsal fur was shaved the day before the experiments and 24 h later the animals were given an intravenous injection of ^{125}I-labelled human serum albumin. Each test substance was dissolved in 0.02 M tris-HCl, 0.15 M NaCl, pH 8.0, and 0.1 ml was injected intracutaneously (i.c.) in at least four different places in the dorsal skin of at least four rats. Four i.c. injections of buffer in each animal served as controls. Skin circles of uniform size surrounding each injection site were cut out after 5 or 30 min. Each piece of skin was weighed and put in a gammaspectrometer, and the radioactivity per gram tissue was calculated. A permeability quotient (Q) was calculated as a numerical degree of the leakage of albumin, from the following formula:

$$Q = \frac{^{125}\text{I-albumin content in each test spot}}{\text{average } ^{125}\text{I-albumin content in buffer-injected spots in the same rat}}$$

A mean Q value ±SD for at least four simultaneously investigated rats was calculated.

The statistical significance of differences between groups was estimated

by Student's t-test.

EXPERIMENTS AND RESULTS

Properties dependent on angiotensin converting enzyme:

Five nmoles of peptide 6A were injected alone or together with 1 nmol of SQ 14,225, a dose which did not in itself increase permeability. Thirty minutes later more pronounced leakage of ^{125}I-albumin was observed in skin areas injected with the combination of the two substances ($p<0.001$, Fig. 1). After a 5-min interval, however, SQ 14,225 had not enhanced the effect of peptide 6A.

An analogous experiment was performed in which 0.05 nmol of bradykinin was injected with and without the same dosage of SQ 14,225. As shown in Fig. 1, the pattern was similar in all respects to that for the peptide 6A - SQ 14,225 combination. These results may be suggestive of a mechanism whereby determination of the biological activity of both bradykinin and peptide 6A is influenced by A.C.E.

Since peptide 6A potentiated the effect of substance P (7), it was injected together with and without SQ 14,225. The results showed (Fig. 1) that the permeability-increasing capacity of this neuropeptide over a 30-min period was augmented by the presence of an inhibitor of kininase II ($p<0.01$). In order to rule out the possibility of an unspecific potentiation of the effects of SQ 14,225 in the microcirculation, 10 nmol of histamine were injected alone or together with 1 nmol of SQ 14,225. No altered response to histamine was observed, speaking against an unspecific effect of SQ 14,225. Liberation of endogenous bradykinin mediated by the injection process as such does not seem likely in our model, since high doses of SQ 14,225 (10^3 nmol) did not increase the exudation of albumin as compared with the effect of buffer.

Properties not dependent on angiotensin converting enzyme:

To investigate whether peptide 6A had a bradykinin-potentiating ability different from that which could be explained by a competitive inhibition of A.C.E., 0.5 nmol of bradykinin and a very high dose of SQ 14,225 (to obtain a strong inhibition of A.C.E.) were injected with and without peptide 6A (Fig. 2). It was found that in spite of a strong inhibition of A.C.E. (using an inhibitor: substrate ratio of 10^3:0.5, i.e. 2000:1), peptide 6A potentiated the effect of bradykinin. Although A.C.E. is the main enzyme degrading bradykinin in the tissues (6), serum carboxypeptidase B (carboxypeptidase N, Kininase I) may contribute towards bradykinin degradation in our assay system. A dose of arginine which would completely block the activity of Kininase I (21) was therefore injected together with bradykinin and SQ 14,225 in the doses stated above, with and without peptide 6A. In spite of the inhibition of both

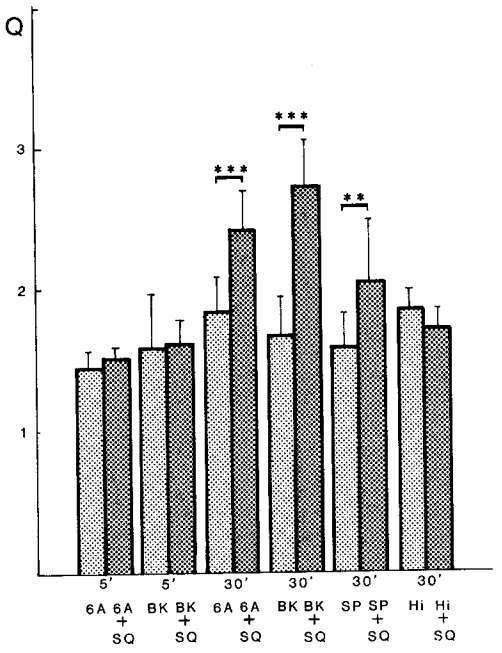

Fig. 1. Effect of SQ 14,225 (SQ; 1 nmol) on capillary leakage induced by bradykinin (Bk; 0.05 nmol) and peptide 6A; (5 nmol) at 5 and 30 min. Effects on substance P (SP; 1 nmol) and histamine (Hi;10 nmol) refer to observations at 30 min.

bradykinin-degrading pathways, peptide 6A potentiated the increase in microvascular permeability caused by bradykinin.

DISCUSSION

The mechanism whereby peptide 6A induces microvascular leakage is probably a contractile effect on the endothelium (3,11) similar to that of histamine and bradykinin (14). Peptide 6A also potentiates the effects of bradykinin on capillary permeability (7) and blood pressure (17); the latter effect, at least, is mainly dependent upon its A.C.E.-inhibiting capacity.

When peptide 6A was mixed with an inhibitor of A.C.E. (SQ 14,225) a more profound outflow of ^{125}I-albumin was observed 30 minutes after the injection compared with the effect of 6A alone, but this difference was not found at 5 minutes. The same was also true for a mixture of bradykinin and SQ 14,225 versus bradykinin during this period. These data are compatible with our previous findings in studies of peptide 6A and bradykinin over different intervals (7). The present observations thus suggest that SQ 14,225 and peptide 6A, have one common mechanism, namely A.C.E. inhibition. Put together, the data indicate that both bradykinin and peptide 6A are degraded by A.C.E. When injected in

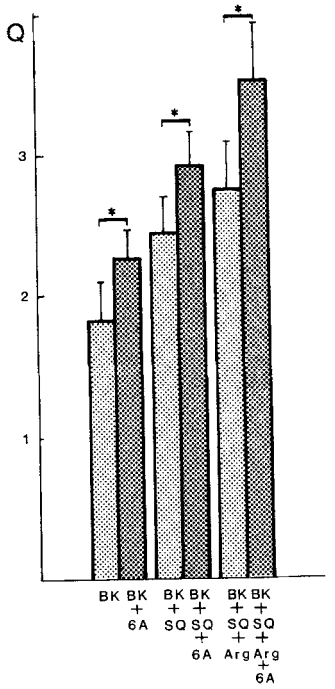

Fig. 2. Effect of fibrinogen-derived pentapeptide 6A (5 nmol) on bradykinin-induced (Bk; 0.5 nmol) permeability in the presence of inhibitors of Kininase I and II respectively; arginine (Arg: 15 nmol) and SQ 14,225 (SQ; 10^3 nmol).

combination, they seem to compete for the enzyme, and peptide 6A might act here as a partial antagonist, which would result in a reduced degradation rate of bradykinin. Inhibitors of A.C.E. seem to be responsible for the potentiation of bradykinin in several assay systems (8,13,15,18). Also the sequence Arg-Pro, found in peptide 6A, is consistent with bradykinin-potentiating capacity (13).

Since SQ 14,225 did not affect microvascular leakage, no intrinsic release of bradykinin is likely to have occurred in our assay. An unspecific action of SQ 14,225 on permeability-increasing substances can not have been responsible for the observed effects, since this compound did not affect the leakage due to histamine. The potentiation of Substance P by SQ 14,225 indicates that the activity of this peptide is influenced by A.C.E. This supports the evidence that Substance P is at least partly degraded by A.C.E. (4).

The microcirculatory mechanism responsible for the A.C.E.-non-dependent potentiation of bradykinin by peptide 6A is not yet elucidated. There are essentially two possible levels of intervention: Either the microvascular permeability as such is increased, i.e. the potentiating effect is exerted on the endothelial cell, or peptide 6A - in itself or by the effects on other active substances, induces a vasodilation, the consequences of which would be an increased net outflow of macromolecules through the interstitium (19,20). In fact, 6A has been shown to induce prostacyclin synthesis (1).

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REFERENCES

1. Andersson, R., Saldeen, K. & Saldeen, T.: A fibrin(ogen) derived pentapeptide induced vasodilation, prostacyclin release and an increase in cyclic AMP. *Thrombos. Res.* In press.
2. Belew, M., Gerdin, B., Lindeberg, G., Porath, J., Saldeen, T. & Wallin, R.: Structure-activity relationships of vasoactive peptides derived from fibrinogen degraded by plasmin. *Biochim. Biophys. Acta* 621:169-178, 1980.
3. Belew, M., Gerdin, B., Porath, J. & Saldeen, T.: Isolation of vasoactive peptides from human fibrin and fibrinogen degraded by plasma. *Thrombos. Res.* 13:983-994, 1978.
4. Cannon, D., Skrabanek, P. & Powell, D.: Substance P degradation in plasma and its partial prevention by heat inactivation and enzyme inhibitors. *Ir. J. Med. Sci.* 146:301. 1977.
5. Dorer, F.E., Kahn, J.R., Lentz, K.E., Levine, M., & Skeggs, L.T.: Hydrolysis of bradykinin by angiotensin-converting enzyme. *Circ. Res.* 34: 824-927, 1974.
6. Erdös, E.F.: The kinins. A status report. *Biochem. Pharmac.* 25:1563-1569, 1976.
7. Eriksson, M., Gerdin, B., & Saldeen, T.: Enhancement of the permeability increasing effect of bradykinin and substance P by a peptide derived from fibrinogen. *Int. J. Microcirc. Clin. & Exp.* 2:53-59, 1983.
8. Gaudreau, P., Barabé, J., St-Pierre, S. & Regoli, D.: Pharmacological studies of kinins in venous smooth muscles. *Can. J. Physiol. Pharmacol.* 59: 371-379, 1981.
9. Gerdin, B.: The use of ^{125}I-labelled human serum albumin for quantitation of microvascular permeability in rat skin. Reevaluation of an old method for studies on substances with an enhancing effect on microvascular permeability. *J. Pharmacol. Meth.* 6:167-175, 1981.
10. Gerdin, B., Juhlin, L. & Saldeen, T.: Cutaneous reactions to fibrin derived peptides. *Acta Dermatovener.* 61:558-560, 1981.
11. Gerdin, B. & Saldeen, T.: Effect of fibrin degradation products of microvascular permeability. *Thrombos. Res.* 13:995-1006, 1978.
12. Henschen, A. & Lottspeich, F.: Fibrinogen structure and fibrinolysis. *Haematologica* 65:535-541, 1980.
13. Kato, J. & Suzuki, T.: Bradykinin-potentiating peptides from the venom of Agkistridon halys blomhoffii. Isolation of 5 bradykinin potentiators and the amino acid sequences of two of them, potentiators B and C. *Biochemistry* 10:972-980, 1971.
14. Majno, G., Palade, G.E.: Studies on inflammation. I. The effect of histamine and Serotonin on vascular permeability: An electron microscopic study. *J. Biophys. Cytol.* 11:571-605, 1961.
15. Ragnarsson, U., Syvänen, A-C. & Hamberg, U.: Potentiation of bradykinin with synthetic peptides on guinea-pig ileum. *Int. J. peptide protein res.* 18:61-68, 1981.
16. Rubin, B., Laffan, R.J., Kotler, D.G., O´keefe, E., Demrio, D. & Goldberg, M.E.: SQ 14, 225 (d-3-mercapto-2-methylpropanoyl, L-proline) a novel orally active inhibitor of angiotensin I-converting enzyme. *J. Pharmacol. Exp. Ther.* 204: 271-280, 1978.

17. Saldeen, T., Ryan, J.W., &Berryer, P.: A peptide derived from fibrin(ogen) inhibits angiotensin converting enzyme and potentiates the effects of bradykinin. *Thrombos. Res.* 23:465-470, 1981.
18. Stewart, J., Ferreira, S. & Greene, L.: Bradykinin potentiating peptide PCA-Lys-Trp-Aal-Pro. An inhibitor of the pulmonary inactivation of bradykinin and conversion of Angiotensin I and II. *Biochem. Pharmacol.* 20: 1557-1567, 1971.
19. Williams, T.J., & Jose, P.J.: Mediation of increased vascular permeability after complement activation. Histamine-dependent action of rabbit C5a. *J.Exp. Med.* 153:135-153, 1981.
20. Williams, T.J., & Peck, M.J.: Role of prostaglandin mediated vasodilation in inflammation. *Nature.* 270:530-532, 1977.
21. Yang, H.Y.T. & Erdös, E.G.: Second Kininase in human blood plasma. *Nature.* 215:1402-1403, 1967.

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