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Potentiation of Bradykinin by a Liver Extract[1]

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A commercial porcine liver extract, Kutapressin, was shown to potentiate *in vitro* the contraction of guinea pig ileum induced by bradykinin but not that by histamine or serotonin. The potentiation of bradykinin was not due to thiol compounds or to free amino acids and was destroyed by acid hydrolysis. It also potentiated *in vivo* bradykinin-induced capillary permeability.

Two types of compounds are known to potentiate the *in vitro* activity of bradykinin. Ferreira and Rocha e Silva (1) reported that certain thiol compounds such as glutathione, cysteine, and *p*-mercaptoacetic acid increase the bradykinin - induced contraction of guinea pig ileum. The second type of compounds are peptides or proteins. The thrombin catalyzed conversion of fibrinogen to fibrin results in the release of several peptides. Gladner *et al.* (2) and Osbahr *et al.* (3) have reported that peptide-B released from the interaction of bovine thrombin with bovine fibrinogen and the β-peptide released from the interaction of human thrombin with human fibrinogen potentiates the bradykinin - induced contraction of rat uterus. Edery (4) has described the potentiation of the action of smooth muscle by chymotrypsin, chymotrypsinogen and trypsin.

In a study of the physiological properties of Kutapressin, a commercial liver extract reported clinically efficacious for the treatment of certain skin disorders, it was noted that this extract strongly enhanced the bradykinin-induced contraction of the isolated guinea pig ileum. Kutapressin[3] is a partially purified porcine liver extract which contains numerous amino acids and peptides but no detectable protein. Recent reports indicate that individuals with acne vulgaris, herpes zoster, poison ivy, sunburn and urticarial reactions to antibiotics are benefited by its administration (5–7).

The concentration at which bradykinin shows biological activity is comparable to that of acetylcholine, adrenaline, oxytocin, and histamine. Since the physiological function of bradykinin is for the most part unknown, any substances that potentiate or inhibit the action of bradykinin are of interest. In this paper we report studies on the enhancement by Kutapressin of bradykinin induced contractions and preliminary observations on the nature and activity of the substance responsible for this potentiation.

MATERIALS AND METHODS

Guinea pig ileum assay. A 1–2 cm segment of guinea pig ileum was suspended at 36°C in a muscle bath containing 5 ml of modified Tyrode solution (8) through which a mixture of 95% O_2 and 5% CO_2 was continuously passed. The muscle contraction was recorded on a Bird kymograph with a chart speed of 2 cm per minute. The assay consisted of the following steps: (a) addition of the liver extract or other test substances to the muscle bath, (b) a 2-minute incubation period, (c) addi-

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[3] Kutapressin is a soluble extract of porcine liver manufactured by the Kremers-Urban Company, Milwaukee, Wisconsin.

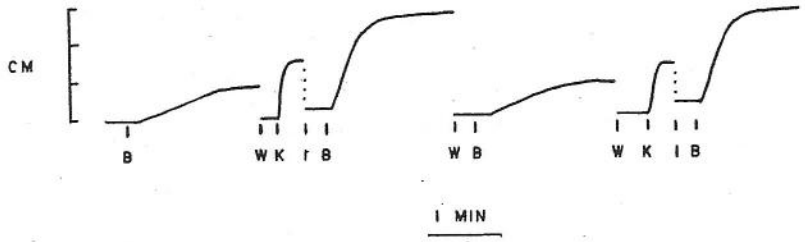

FIG. 1. The potentiation of bradykinin by Kutapressin on the isolated guinea pig ileum. (B) Addition of 0.2 ml synthetic bradykinin, 0.1 μg/ml; (W), muscle is washed; (K), addition of 0.2 ml Kutapressin; (I), 2-minute period of incubation.

tion of bradykinin to the muscle bath, (d) washing of the muscle with two portions of fresh Tyrode solution, and (e) application of bradykinin to the muscle bath. The following materials were used in the assay: Kutapressin (phenol free, Kremer's-Urban, Milwaukee, Wisconsin); synthetic bradykinin (batch No. 01312, Sandoz, Hanover, New Jersey); histamine dihydrochloride (Eastman white label, Distillation Products Co., Rochester, New York); serotonin (lot No. 5661, Calbiochem, Los Angeles, California); polycysteine [obtained from Dr. T. Richardson (9)]; glutathione (lot No. 107259, Calbiochem); cysteine hydrochloride (A grade, lot No. 503233, Calbiochem); and 2-mercaptoacetic acid (redistilled Eastman white label).

Capillary permeability studies. White guinea pigs of both sexes weighing 300–500 gm were used in these studies. The hair on both sides was removed with an electric clipper. Evans blue solution (4 mg per milliliter) was injected into an ear vein at a dosage of 2 ml per kilogram. Fifteen to 20 minutes after administration of the dye, 0.1 ml of the test solution was injected intradermally with a 26 gage needle. All solutions and dilutions were made with saline except Kutapressin, which was used as received from the manufacturer. An hour after the last intradermal injection the animal was stunned by a blow on the head, bled, and skinned. The inside of the skin was photographed.

Chemical studies. Amino acid analysis was performed on the Spinco amino acid analyzer (10). Performic acid oxidation was carried out according to Moore (11). Acid hydrolysis was carried out in 6 *N* HCl under nitrogen at 6 psi in the autoclave for 21 hours. The hydrochloric acid was removed in a rotary evaporator. The sulhydryl content of the liver extract was determined by ampiometric titration with silver nitrate according to the method of Benisch *et al.* (12).

RESULTS

Guinea pig ileum studies. The liver extract was found to have two actions on the guinea pig ileum. It caused the muscle to contract and it potentiated the action of bradykinin. Figure 1 shows the results of a typical assay. After the addition of the liver extract there was a 2-minute incubation period during which the muscle returned to a relaxed state. The bradykinin-induced contraction of the muscle was then higher in the presence of Kutapressin. Hence we conclude that this liver extract potentiates the action of bradykinin on the guinea pig ileum. After the muscle had been contracted several times and reached a steady state, the assay could be

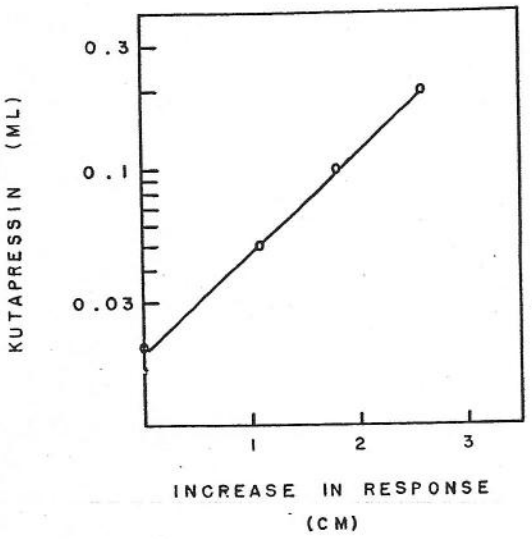

FIG. 2. Plot of the log of the dose of Kutapressin against the increase in response in the isolated guinea pig ileum of bradykinin. The increase in response is the difference between the height of contraction induced by a given amount of bradykinin in the presence and in the absence of a given amount of Kutapressin.

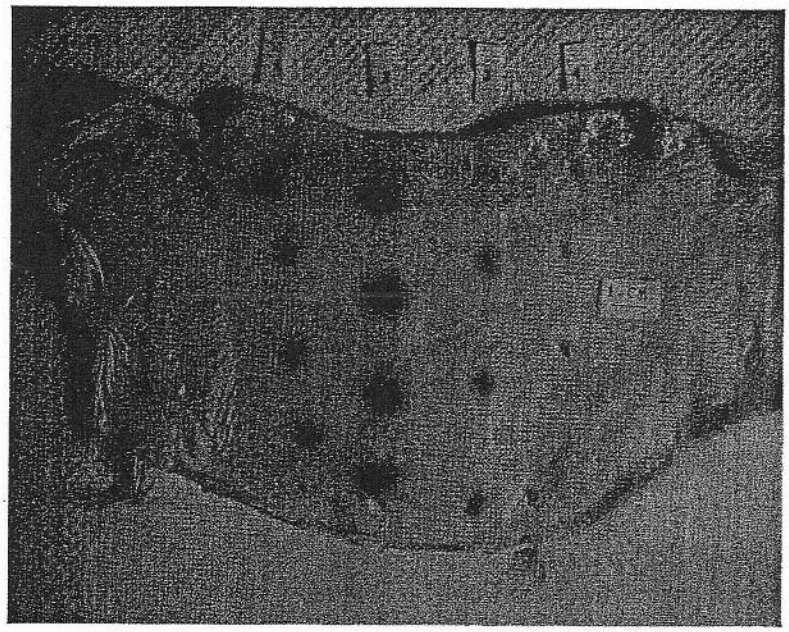

FIG. 3. Skin of guinea pig with circulating Evan's blue dye; shown are lesions produced by intradermal injections (0.1 ml) of 1, Kutapressin; 2, Kutapressin containing 0.01 μg bradykinin/ml; 3, bradykinin, 0.01 μg/ml; and 4, saline.

repeated 20–30 times with the same results. The enhancing factor in the liver extract exhibited some specificity in that it did not potentiate the effect of histamine or serotonin. Neither histamine, bradykinin, nor serotonin potentiated the contraction induced by bradykinin. There was a linear relationship between the logarithm of the dose and the resultant increase in response or height of contraction induced by bradykinin (Fig. 2). Only a limited range of doses of Kutapressin could be investigated since at higher doses the muscle would not relax from the initial contraction.

Capillary permeability studies. When a substance that causes an increase in capillary permeability is given by intradermal injection to an animal that has Evans Blue in its circulatory system, a blue spot appears at the site of injection, due to leakage of the dye from the capillaries. The size and intensity of the colored spot is proportional to the amount of active substance administered. A saline blank will cause a small spot to appear due to injury caused by the injection.

An attempt was made to see if Kutapressin would potentiate the capillary permeability action of bradykinin. To do this bradykinin was dissolved in Kutapressin and intradermal injections of the mixture were given.

TABLE I

FREE AMINO ACIDS PRESENT IN KUTAPRESSIN

Amino acid	μg/ml
Tryptophan	30.3
Lysine	91.0
Histidine	13.8
Aspartic acid	69.3
Threonine	240
Serine	158
Asparagine	92.5
Glutamic acid	347
Proline	597
Glycine	133
Alanine	1283
Valine	931
Methionine	383
Isoleucine	945
Leucine	1979
Tyrosine	243
Phenylalanine	521
Total	8027

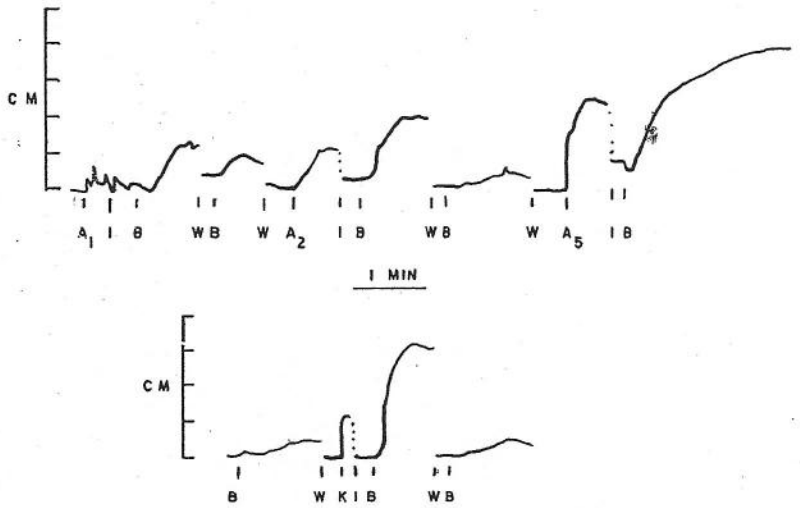

FIG. 4. Effect of the amino acids found in Kutapressin on the bradykinin-induced contraction of the isolated guinea pig ileum; (A_1) addition of 0.1 ml of the amino acid mixture; (I) 2-minute period of incubation; (B) addition of 0.1 ml synthetic bradykinin, 0.2 μg/ml; (W) muscle is washed; (A_2) addition of 0.2 ml of the amino acid mixture; (A_5) addition of 0.5 ml of the amino acid mixture; (K) addition of 0.1 ml Kutapressin.

The concentration of bradykinin was kept just below that which produced a minimal blueing. Figure 3 shows that Kutapressin contains a factor which increases capillary permeability. The blue spot produced by the mixture of Kutapressin and bradykinin was larger and more intense than the spot caused by either substance alone and was greater than expected from a simple additive action. Therefore the results indicate that this liver preparation potentiates the capillary permeability action of bradykinin.

Chemical studies: amino acid composition. Table I shows the concentrations of the free amino acids in Kutapressin. A mixture of amino acids of this composition was prepared using the DL-form of threonine, serine, alanine, and methionine and the L-form of the remainder. This amino acid mixture was found to potentiate the action of bradykinin on the guinea pig ileum (Fig. 4). However, the activity at different levels of the amino acid mixture show that the free amino acids in the liver preparation could account for only 15% of its activity. Figure 4 also shows that this higher level of the amino acid mixture also caused the muscle to contract. On a weight basis the free amino acids make up 33% of the total solid material in the liver preparation.

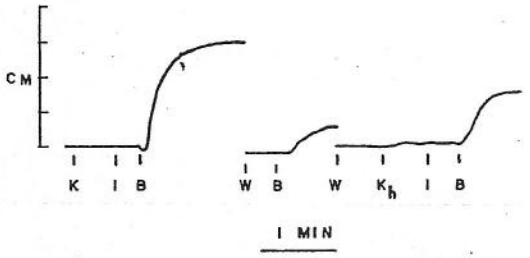

FIG. 5. Effect of acid-hydrolyzed Kutapressin on the bradykinin-induced contraction of isolated guinea pig ileum; (K) addition of 0.2 ml Kutapressin; (I) 2-minute incubation period; (B) addition of bradykinin; (W) muscle is washed; (K_h) addition of 0.2 ml acid-hydrolyzed Kutapressin.

Stability of the activity. Heating Kutapressin at 100°C at either pH 1 or 12 for 1 hour did not diminish its activity. Acid hydrolysis in 6 *N* HCl destroyed 90% of the activity (Fig. 5).

Studies of thiol compounds. The concentration of various thiol compounds needed to potentiate the bradykinin-induced contraction of the guinea pig ileum were determined in the same assay. Table II shows the results.

Analysis of Kutapressin for thiol compounds. The amino acid analysis showed that Kutapressin was 1.2×10^{-4} *M* in

TABLE II

EFFECT OF SOME THIOL COMPOUNDS ON THE BRADYKININ-INDUCED CONTRACTION OF THE ISOLATED GUINEA PIG ILEUM

Compound	Conc. (M) needed to give the activity of Kutapressin[a]
Polycysteine	1×10^{-2}
Glutathione	3×10^{-2}
Cysteine·HCl	2×10^{-1}
β-Mercaptoacetic acid	1×10^{-1}

[a] In the actual assay, 0.1 ml of the test solution was used in a 5-ml bath. Thus the bath concentration was $\frac{1}{50}$ of the indicated concentration of the test substance.

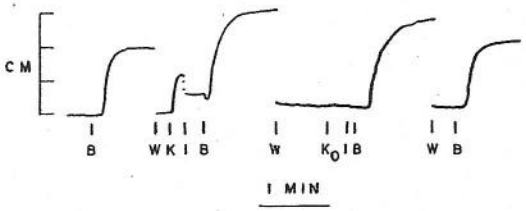

FIG. 6. Effect of performic acid oxidized Kutapressin on the bradykinin-induced contraction of the isolated guinea pig ileum; (B) addition of 0.2 ml synthetic bradykinin, 0.1 μg/ml; (W) muscle is washed; (K) addition of 0.2 ml Kutapressin; (I) 2-minute period of incubation; (Ko) addition of 0.2 ml Kutapressin that has been oxidized with performic acid.

cysteic acid. After performic acid oxidation, it was 4.5×10^{-4} M in cysteic acid, and after performic acid oxidation and acid hydrolysis it was 6.7×10^{-4} M in cysteic acid. Thus, at a maximum, this liver preparation was 2.2×10^{-4} M in peptide bound cysteine and 3.3×10^{-4} M in free cysteine. Since performic oxidized glutathione was eluted from the amino acid analyzer with cysteic acid, any glutathione present would be included in the increase of cysteic acid after performic acid oxidation. This increase was negligible in comparison with the amount of glutathione or cystine required to give the same amount of activity found in Kutapressin. Performic acid oxidation destroyed only 15% of the activity (Fig. 6). All the activity of glutathione was destroyed by oxidation. By amperiometric titration with silver nitrate Kutapressin was found to be 1.4×10^{-4} M in sulfhydryl groups.

DISCUSSION

These studies demonstrate that the commercial porcine liver extract, Kutapressin, potentiated the bradykinin-induced contraction of guinea pig ileum. This was a specific effect for bradykinin in that it did not affect the response of the ileum to histamine or serotonin. Thus the liver extract did not increase the over-all sensitivity of the ileum.

There was a linear relationship between the log of the dose and the increase in response or contraction induced by bradykinin, over the range of doses that could be investigated. It is interesting to note that this is the same dose-response relationship which is seen in substances that contract guinea pig ileum, e.g., histamine and bradykinin.

Since this liver extract is a mixture, the question arises as to what substance or substances are responsible for this potentiation of bradykinin. This can only be answered after isolation and characterization studies have been carried out. The present study shows that the bradykinin-induced contraction of guinea pig ileum was not potentiated by a prior exposure to histamine, serotonin, or bradykinin. It was also shown that the level of thiol compounds in the liver extract was 100 times lower than that level of several thiol compounds needed to give an equivalent potentiation of bradykinin. Performic acid oxidation destroys the ability of thiol compounds to potentiate the action of bradykinin but does not significantly alter the activity of the liver extract. Thus the potentiation of bradykinin by the liver extract must not be due to the thiol compounds. A mixture of free amino acids equivalent to that found in the liver extract slightly potentiated the bradykinin-induced contraction of guinea pig ileum, and could contribute only 15% of the total potentiation. Higher concentrations of this amino acid mixture gives a greater potentiation of bradykinin. Since amino acids can potentiate the action of bradykinin and as 90% of the potentiation action of the liver extract was destroyed by acid hydrolysis, it seems likely that a peptide may be responsible for most of the activity of Kutapressin. As previously noted, there are already two peptides known

that will potentiate the bradykinin-induced contraction of guinea pig ileum (2, 3).

Kutapressin also potentiated the action *in vivo* of bradykinin in increasing capillary permeability as measured by dye diffusion. The *in vivo* effect of Kutapressin on the action of bradykinin, the mechanism for the potentiation of bradykinin and the isolation and characterization of the active substance in the liver extract is currently being studied. It has been suggested that bradykinin may have a physiological significance (13). The potentiation of its action by Kutapressin might suggest a basis for the beneficial effects reported for it in conditions involving localized inflammation (5–7).

REFERENCES

1. Ferreira, S., and Rocha e Silva, M., *Biochem. Pharmacol.* **11,** 1123 (1962).
2. Gladner, J. A., Murtaugh, P. A., Folk, J. E., and Laki, K., *Ann. N. Y. Acad. Sci.* **104,** 47 (1963).
3. Osbahr, A. J., Gladner, J. A., and Laki, K., *Biochim. Biophys. Acta* **86,** 535 (1964).
4. Edery, H., *Brit. J. Pharmacol.* **22,** 371 (1964); **24,** 485 (1965).
5. Burks, J. W., and Knox, J. M., A. M. A. *Arch. Derm. Syphil.* **70,** 508 (1954).
6. Heywood, H. W., *Clin. Med.* **3,** 8 (1956).
7. Barksdale, E. E., *South. Med. J.* **50,** 1524 (1957).
8. Code, C. F., and McIntire, F. C., *in* "Methods of Biochemical Analysis" (D. Glick, ed.), Vol. III, p. 66. Wiley (Interscience), New York (1956).
9. Richardson, T., Doctoral dissertation, University of Wisconsin, Madison, Wisconsin (1959).
10. Spackmann, D. H., Stein, W. H., and Moore, S., *Anal. Chem.* **30,** 1190 (1958).
11. Moore, S., *J. Biol. Chem.* **238,** 235 (1963).
12. Benesch, R. E., Lardy, H. A., and Benesch, R. J., *J. Biol. Chem.* **216,** 663 (1955).
13. Rocha e Silva, M., *Ann. N. Y. Acad. Sci.* **104,** 190 (1963).