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INHIBITION OF PLASMA KININASE ACTIVITY AT SLIGHTLY ACID pH

BY

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Kinins, like bradykinin, are rapidly inactivated in blood and interstitial fluid by an enzyme called kininase. The action of this enzyme was found to be inhibited at slightly acid *p*H although the formation of kinins proceeded normally. Thus a slightly acid *p*H which is known to occur in inflamed and damaged tissue is a suitable environment to allow accumulation of kinins which are thought to be the mediators of the inflammatory response.

Acidification of plasma leads to the development of vasodepressor activity (Werle, 1934). The finding was explained by activation of kallikrein, an enzyme normally present in blood in combination with an inactivator. The kallikrein-inactivator complex was assumed to remain inactive at neutral *p*H but to dissociate below *p*H 6 and above *p*H 10, and then to release the active enzyme which causes a vasodepressor response when injected intravenously. Frey, Kraut & Werle (1950) consequently suggested that in conditions where acid metabolites are formed, such as in reactive hyperaemia, kallikrein is responsible for the accompanying vascular effects.

It is now known that kallikrein acts by forming a plasma kinin called kallidin (Werle & Berek, 1950; Werle, Kehl & Koebke, 1950) and that this peptide, and other plasma kinins, are rapidly inactivated by an enzyme in plasma called kininase (Werle, 1955; Rocha e Silva, 1955; Erdös, 1962). In the present experiments it is shown that at slightly acid *p*H's, between 6 and 7, this inactivation is inhibited. Since the *p*H does not affect the formation of plasma kinin, it is possible that a slightly acid environment might favour its accumulation by inhibition of kininase.

METHODS

Kininase activity was estimated in the following way: 3 ml. of a solution of bradykinin (500 ng/ml.) in 0.1 M phosphate buffer was incubated at 34 to 35° C with 30 mg pseudoglobulin which contains kininase at *p*H's between 7.98 and 5.65. At various times, aliquots of 0.1 ml. of the mixture were taken for estimation of bradykinin.

Plasma kinin-forming-enzyme activity was determined in the following way: 2 ml. of 0.1 M phosphate buffer containing either 50 μg crystalline trypsin (Worthington) or 0.2 ml. human saliva, which contains kallikrein, was incubated at 34 to 35° C with 10 mg pseudoglobulin at *p*H's between 7.82 and 6.02. At various times of incubation 0.2 ml. of the mixture was taken for estimation of plasma kinin.

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Estimation of bradykinin was carried out immediately after incubation, on the isolated guinea-pig ileum. The mixtures incubated below *p*H 6.50 were first neutralized with 0.1 N sodium hydroxide.

Pseudoglobulin was prepared from dog or ox plasma by the method used by Lewis (1958). One milligram of the freeze-dried pseudoglobulin was equivalent to 0.15 ml. of original plasma.

Synthetic bradykinin was obtained from Parke Davis & Co.

RESULTS

Kininase activity. Bradykinin incubated with pseudoglobulin is known to be inactivated because this fraction of the plasma proteins contains the enzyme kininase.

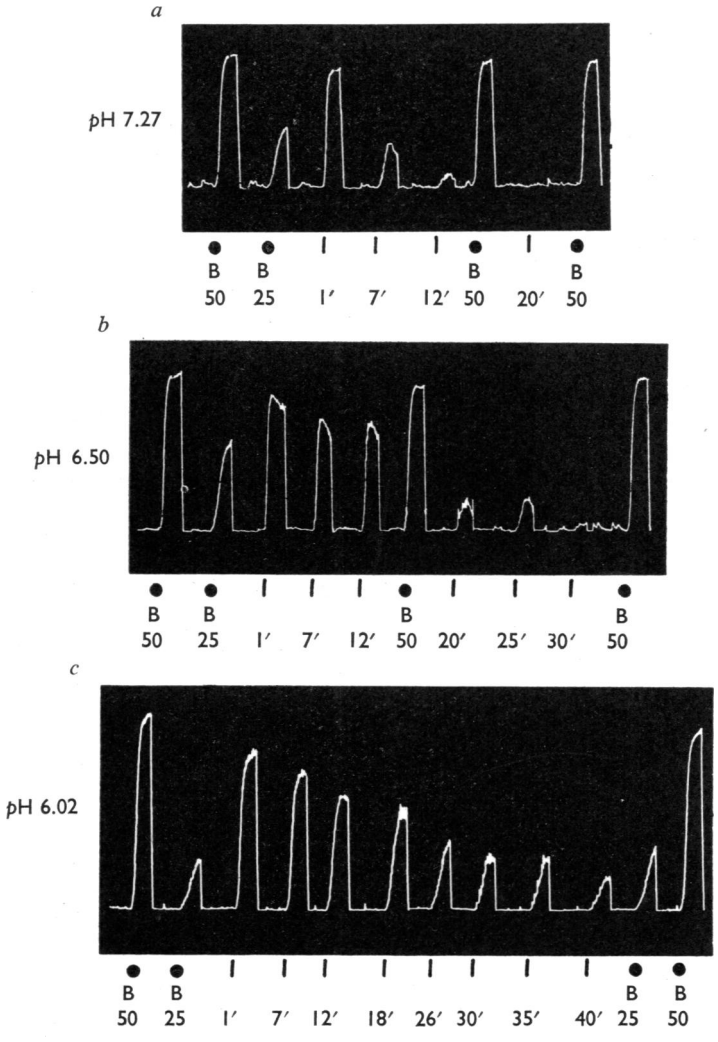

Fig. 1. Responses of the guinea-pig ileum suspended in 15 ml. Tyrode solution to bradykinin (B, doses given in ng) and to 0.1 ml. of mixtures of dog pseudoglobulin 10 mg and bradykinin 500 ng/ml. incubated for various time intervals (time given in min) at *p*H 7.27 in (*a*), *p*H 6.50 in (*b*) and *p*H 6.02 in (*c*).

The kininase activities of different batches of pseudoglobulin vary considerably, probably due to the fact that not all the enzyme activity, but only a variable part, is precipitated in the pseudoglobulin fraction. In addition, the kininase activity present in plasma from different animals may vary. Therefore when comparisons are made it is essential that the same batch of pseudoglobulin be used. This was done for the experiments illustrated in Figs. 1, 2 and 3.

In the first experiment (Fig. 1) inactivation is shown at three different *p*H's. At (*a*) incubation of the bradykinin with pseudoglobulin was at *p*H 7.27. After 7 min incubation more than 50% and after 12 min nearly all the bradykinin had been inactivated. On the other hand, at (*b*) inactivation was at *p*H 6.5 and after 12 min less than 50% of the bradykinin was inactivated. On incubation at *p*H 6.02 inactivation was further delayed, less than 50% of the bradykinin being inactivated in 26 min as shown at (*c*).

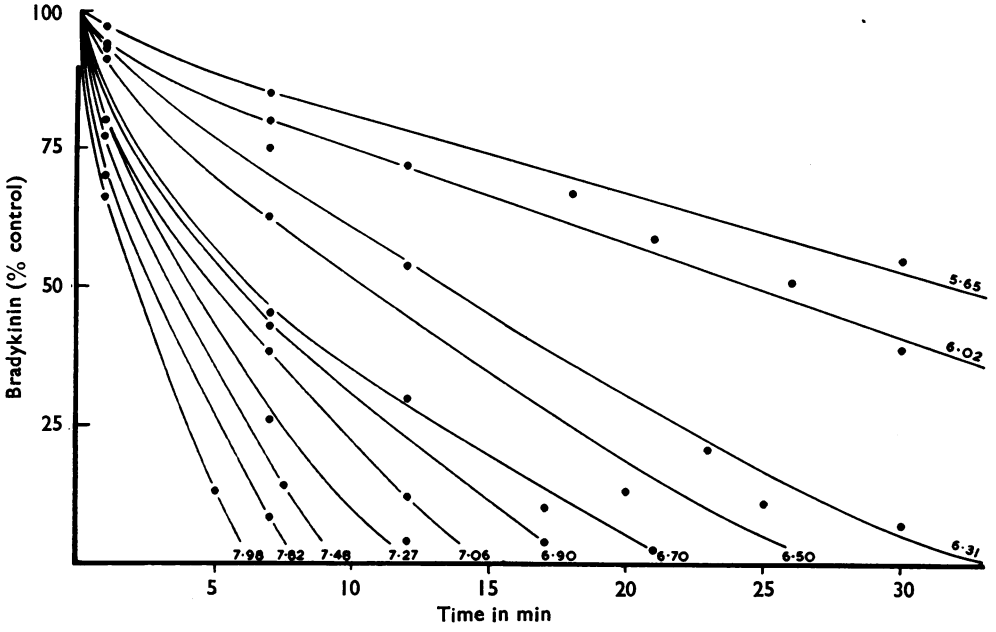

Fig. 2. Amount of bradykinin (expressed as % of control) in mixtures of dog pseudoglobulin 10 mg/ml. and bradykinin 500 ng/ml. incubated at eleven different *p*H's between 7.98 and 5.65. Each value is the mean of two incubations. The curves which represent the inactivation of bradykinin were drawn as near to the points as possible, by eye.

Fig. 2 gives the results of incubation of bradykinin with the pseudoglobulin sample at eleven different *p*H's. Each value is the mean of two incubations at the same *p*H and each curve represents inactivation at a given *p*H. It can be seen that the kininase activity gradually decreases with a lowering of the *p*H from 7.98 to 5.65 and that a change in the *p*H of less than 0.2 units is sufficient to affect the inactivation of bradykinin.

The inhibition of kininase at slightly acid *p*H is reversible, but treatment with strong acid seems to cause irreversible inhibition. This is shown in experiments in

which the solutions of pseudoglobulin used for the incubation with bradykinin were first kept for 30 min at *p*H's between 2 and 5. When kept at *p*H 3.5 or *p*H 4.5 the pseudoglobulin solutions, when neutralized and then incubated with bradykinin, caused inactivation of the peptide. However, when the pseudoglobulin solution was kept at *p*H 2, then after neutralization little or no kininase activity was found on incubation. This suggests that at *p*H 2 the enzyme is irreversibly inhibited.

Plasma kinin-forming-enzyme activity. The pseudoglobulin fraction of plasma which contains the kininase is also used as the substrate on which plasma kinin-forming-enzymes act, and, when this was incubated with human salivary kallikrein or with trypsin, a plasma kinin was formed. When incubation was continued for more than a few minutes the activity decreased because the kininase inactivated the formed plasma kinin.

As with kininase activity quantitative comparisons must be made using the same batch of pseudoglobulin, because the amount of substrate varies in different batches.

On incubation of salivary kallikrein at *p*H's between 7.82 and 6.02, approximately the same amount of bradykinin (50 to 75 ng/mg pseudoglobulin) was formed at each *p*H and the rate of formation was unaffected.

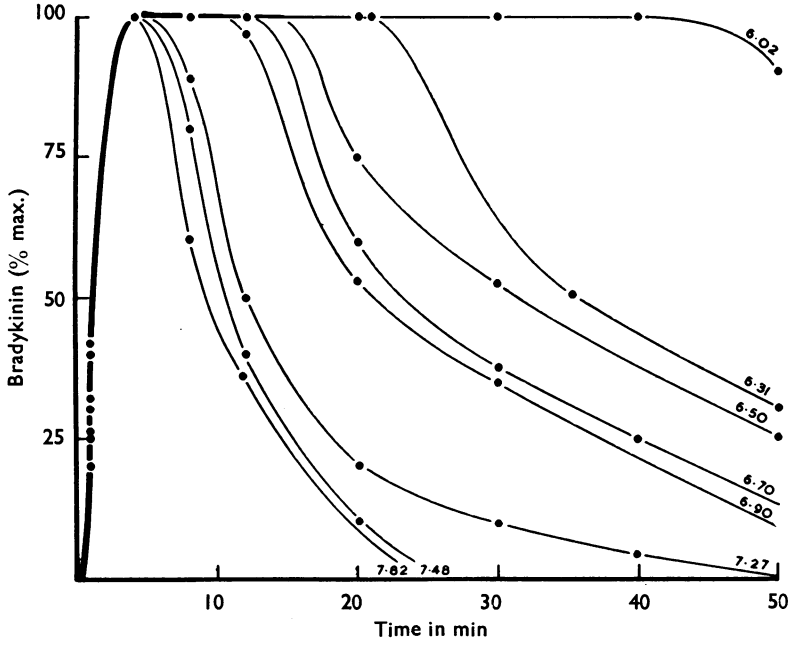

Fig. 3. Amount of bradykinin (expressed as % of the maximum formed in each experiment) in mixtures of dog pseudoglobulin 10 mg/ml. and human saliva diluted 1/10, incubated at eight different *p*H's between 7.82 and 6.02. The thick curve represents the formation of plasma kinin which was similar at all *p*H's and the other curves represent the inactivation of the formed plasma kinin.

Fig. 3 gives the results of incubation of salivary kallikrein and pseudoglobulin at eight different *p*H's. Each value is the mean of two incubations at the same *p*H. The curves represent the formation of the plasma kinin which is the same at each

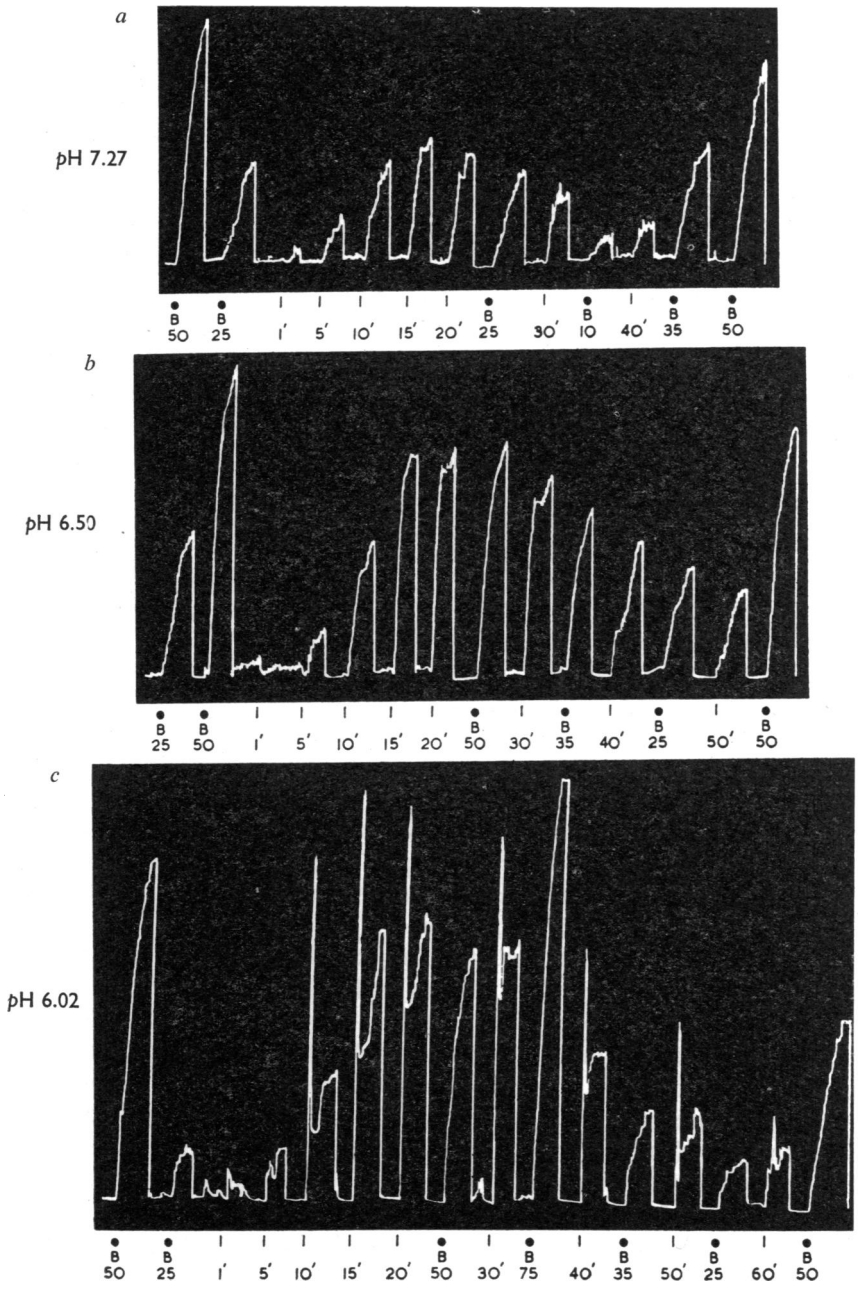

Fig. 4. Responses of the guinea-pig ileum suspended in 15 ml. Tyrode solution to bradykinin (B, doses given in ng) and to 0.1 ml. of a solution of ox pseudoglobulin 50 mg/ml. in 0.1 M phosphate buffer incubated for various intervals of time (in min) at *p*H 7.27 in (*a*), *p*H 6.50 in (*b*) and *p*H 6.02 in (*c*).

*p*H, reaching a maximum in about 4 min, and the inactivation of the formed plasma kinin which decreases as the *p*H is lowered. The inhibition of the kininase was even more pronounced in these experiments than when pseudoglobulin was incubated with bradykinin alone. At *p*H 6.02, even after 50 min incubation, the mixture retained almost full activity.

Similar results were obtained when pseudoglobulin was incubated with trypsin at *p*H's between 7.82 and 6.02.

Spontaneous formation. When the pseudoglobulin fraction was incubated with buffer solutions alone, a small amount of plasma kinin was slowly formed. At slightly acid *p*H's this spontaneous formation was enhanced. This might well represent activation of plasma kallikrein as described by Werle (1934). At acid *p*H the activity in the mixture was maintained for a longer time than at neutral *p*H and, in addition, the total amount of kinin formed was increased. Inactivation of plasma kinin, which occurs during the slow formation before it reaches a maximum, was inhibited at acid *p*H. This is illustrated in the experiments of Fig. 4*a*, *b* and *c*, in which ox pseudoglobulin 50 mg/ml. was incubated with buffer solution at *p*H 7.27, 6.50 and 6.02.

DISCUSSION

The present experiments show that relatively small changes in *p*H inhibit kininase, the enzyme in plasma which rapidly destroys plasma kinins. Werle, Götze & Keppler (1937) and Horton (1959) had observed that treatment with strong acids irreversibly destroyed the ability of plasma to inactivate its vasodepressor and smooth muscle stimulating activity. However, they did not describe the reversible inhibition of plasma kininase which occurs with small changes in *p*H, and which may be of importance for the role of bradykinin in pathological conditions in which such *p*H changes are known to occur.

The plasma kinin bradykinin is a most potent vasodilator, and in higher concentrations also reproduces the other cardinal signs of the inflammatory response—increased capillary permeability, pain and accumulation and migration of leucocytes (Elliott, Horton & Lewis, 1960; Lewis, 1962a).

Bradykinin is thought to be a mediator for the reactions of the inflammatory response (Lewis, 1962b), and the fact that an acid *p*H inhibits kininase, and thus inactivation of bradykinin, is of interest in connexion with the fact that the *p*H is slightly lowered in damaged or inflamed tissue. Menkin (1940) and Lurie (1939) found that many inflammatory exudates develop a *p*H of 6.0 to 6.5; Schade (1924) made detailed *p*H measurements of interstitial fluid with microelectrodes and showed that the *p*H of inflamed tissue might fall as low as *p*H 5.9. Inactivation of plasma kinins would be inhibited at these *p*H's so that, through this change in reaction of the inflamed tissue, a condition is brought about which would favour the accumulation of bradykinin and of other plasma kinins which may be mediators of the inflammatory response.

The question also arises whether bradykinin is involved in the vasodilatation, and sometimes pain, produced in conditions in which there is an accumulation of acid metabolites. Formation of such products is known to occur after prolonged and severe exercise and after intravenous injection of adrenaline (Lundholm, 1949;

Barcroft & Cobbold, 1956), as well as during prolonged periods of ischaemia (Stoner & Green, 1948). Although there is, as yet, no evidence of the formation of plasma kinins in these reactions, it is possible that the accompanying vasodilatation results from an accumulation of these peptides in this local acid environment.

REFERENCES

BARCROFT, H. & COBBOLD, A. F. (1956). Action of adrenaline on muscle blood flow and blood lactate in man. *J. Physiol. (Lond.)*, **132**, 372–377.

ELLIOTT, D. F., HORTON, E. W. & LEWIS, G. P. (1960). Actions of pure bradykinin. *J. Physiol. (Lond.)*, **153**, 473–480.

ERDÖS, E. (1962). *Ann. N.Y. Acad. Sci.* (In press.)

FREY, E. K., KRAUT, H. & WERLE, E. (1950). *Kallikrein Padutin.* Stuttgart: Enke.

HORTON, E. W. (1959). Estimation of urinary kallikrein. *J. Physiol. (Lond.)*, **148**, 267–282.

LEWIS, G. P. (1958). Formation of plasma kinins by plasmin. *J. Physiol. (Lond.)*, **140**, 285–300.

LEWIS, G. P. (1962a). Bradykinin—biochemistry, pharmacology and its physiological role in controlling local blood flow. *Lectures on the Scientific Basis of Medicine*, **14**, 242–258.

LEWIS, G. P. (1962b). *Annal. N.Y. Acad. Sci.* (In press.)

LUNDHOLM, L. (1949). Effect of adrenalin on the oxygen consumption of resting animals. *Acta Physiol. Scand.*, **19**, Suppl. 67.

LURIE, M. B. (1939). Studies on the mechanism of immunity in tuberculosis. *J. exp. Med.*, **69**, 579–603.

MENKIN, V. (1940). *Dynamics of Inflammation.* New York: Macmillan.

ROCHA E SILVA, M. (1955). Bradykinin: occurrence and properties. In *Polypeptides Which Stimulate Smooth Muscle*, pp. 45–57, ed. GADDUM, J. H. Edinburgh: Livingstone.

SCHADE, H. (1924). Die molekularpathologie in ihrem Verhältnis zur Zellularpathologie und zum klinischen Krankheitsbild und Beispiel der Entzündung. *Münch. med. Wschr.*, **71**, 1–4.

STONER, H. B. & GREEN, H. N. (1948). Bodily reactions to trauma. The effect of ischaemia on muscle protein. *Brit. J. exp. Path.*, **29**, 121–132.

WERLE, E. (1934). Über die Inaktivierung des Kallikreins. Zweite Mitteilung. *Biochem. Z.*, **273**, 291–305.

WERLE, E. (1955). The chemistry and pharmacology of kallikrein and kallidin. In *Polypeptides Which Stimulate Smooth Muscle*, pp. 20–27, ed. GADDUM, J. H. Edinburgh: Livingstone.

WERLE, E. & BEREK, U. (1950). Über Kallidin. *Biochem. Z.*, **320**, 136–145.

WERLE, E., GÖTZE, W. & KEPPLER, A. (1937). Über die Wirkung des Kallikreins auf den isolierten Darm und über eine neue darmkontrahierende Substanz. *Biochem. Z.*, **289**, 217–233.

WERLE, E., KEHL, R. & KOEBKE, K. (1950). Über Bradykinin, Kallidin und Hypertensin. *Biochem. Z.*, **320**, 372–383.