Short communications

Action of bradykinin potentiating factor (BPF) and dimercaprol (BAL) on the responses to bradykinin of isolated preparations of rat intestines

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BPF and BAL inhibited kininase activity of homogenates of rat intestine. However, BFP potentiated and BAL inhibited the contractions induced by bradykinin on rat isolated duodenum (low calcium solution) and terminal ileum (normal calcium solution). Neither BPF nor BAL affects the relaxation induced by bradykinin of rat duodenum bathed in normal Tyrode. These results suggest that two different types of pharmacological receptor are involved in the action of bradykinin on rat intestine, and that other factors besides the inhibition of agonist destruction participate in the mechanism of potentiation of kinin action by BPF.

The effects of bradykinin on several isolated preparations are potentiated either by bradykinin potentiating factor (BPF) extracted from the venom of the *Bothrops jararaca* (Ferreira, 1965; Ferreira & Rocha e Silva, 1965) or by thiol compounds (Picarelli, Henriques & Oliveira, 1962; Ferreira & Rocha e Silva, 1962; Sherman & Gautieri, 1969). The potentiation was thought to be due to inhibition of kinin destruction by the tissue, for bradykinin potentiators are strong inhibitors of kininase activity in plasma or tissue homogenates. However, this interpretation has been questioned because (a) the concentration of an agent which enhances the effect of bradykinin on isolated preparations is smaller than that required to inhibit kininase activity of tissue homogenates (Auerswald & Doleschel, 1967) and (b) the slow inactivation of kinin incubated with guinea-pig ileum segments contrasts with the rapid and intense potentiation of the smooth muscle contraction of this preparation (Cirstea, 1965).

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Bradykinin relaxes rat duodenum and contracts rat terminal ileum preparations (Gaddum & Horton, 1959). When the bathing fluid has a low calcium concentration, the relaxation of the rat duodenum is short-lasting and is followed by a contraction proportional to the concentration of the bradykinin (Antonio, 1968). This biphasic response provides a means of testing the relevance of kininase inhibition for the potentiation of bradykinin effects. If the potentiation of the effects of kinin was due only to an increase in the quantity of the agonist as a result of a reduction of its inactivation, a kininase inhibitor would be expected to produce a similar potentiation of both the contraction and the relaxation.

Methods.—Inhibition of kininase activity by BPF and BAL was tested by incubating bradykinin with homogenates of duodenum and ileum of the rat. Segments of gut (3–4 cm long) were washed in Tyrode solution (NaCl, 8 g; KCl, 0·2 g; $CaCl_2$, 0·2 g; $MgCl_2$ 0·1 g; $NaHCO_3$, 1·0 g, NaH_2PO_4, 0·5 g and 1 g of dextrose in a litre of solution) and the mucosa of each segment carefully removed. The smooth muscle was chopped into small pieces and homogenized in Tyrode solution. After the removal of the heavier particles by low speed centrifugation, the supernatant was dialysed (against distilled water, 24 h, 4° C) and lyophilized. Protein was measured by the Folin-Ciocalteau procedure. The test mixture consisted of the following: duodenum or ileum extract, 10–20 mg protein/ml; bradykinin, 1 μg/ml; 0·05 M Tris buffer, pH 7·5, 0·5 ml for 2 ml samples; with or without BPF or BAL (10–50 μg/ml). The enzyme preparation was omitted from the standard mixtures as a control. All mixtures were incubated at 37° C.

The activity of kininase was assayed with or without inhibitor by measuring the rate of destruction of added bradykinin. Aliquots taken from the incubation mixtures were assayed directly upon the guinea-pig ileum. The potentiation of bradykinin effects upon isolated preparations was determined from the responses of duodenum and ileum of the rat suspended in aerated Tyrode solution containing atropine (0·1 μg/ml), at 37° C. The calcium composition of the Tyrode was sometimes reduced to 0·01 g $CaCl_2$/litre. BPF or BAL was added to the bath

fluid 5–10 s before the agonist: this gives a maximum effect (Stewart, Ferreira & Greene, 1971). BPF was extracted from the *Bothrops jararaca* venom as previously described (Ferreira, 1965). The synthetic pentapeptide, Pyroglutamyl-Lys-Trp-Ala-Pro (BPP_{5a}), which potentiates bradykinin and corresponds to one of the nine peptides responsible for the pharmacological actions of BPF (Ferreira, Bartelt & Greene, 1970) was used in some experiments. Synthetic bradykinin was obtained from Cyclo Chemical Corporation, USA; BAL (2,3-dimercaptopropanol) from Mann Research Laboratories, USA; acetylcholine chloride from Roche, Brazil; eledoisin (Eld 940) from Sandoz, Switzerland; atropine sulphate, Merck, Darmstadt, Germany.

Results.—BPF or BAL (50 μg/ml) completely inhibited the kininase activity of an incubate containing homogenates of rat duodenum or ileum which, without inhibitor, inactivated 50% of added bradykinin in 12 minutes. Partial inhibition of kininase activity of these homogenates was observed with concentrations of BPF or BAL of 10–20 μg/ml.

On the rat isolated duodenum bathed in normal Tyrode solution BPF (20 μg) did not change the relaxation induced by bradykinin (1–10 μg/ml). In low calcium Tyrode solution the effects of bradykinin (10–100 ng/ml) were biphasic. When BPF (10–20 μg/ml) was added together with bradykinin, there was a selective potentiation of the contractor response (five experiments). BPP_{5a} also potentiated bradykinin contractions but was one-half to one-third as effective as BPF. BAL (0·1–20 μg/ml, five assays) had no consistent action on the relaxation induced by bradykinin on the rat duodenum. However, when this preparation was bathed in low calcium Tyrode solution BAL (10–20 μg/ml) caused a slight potentiation of the relaxation in three out of five assays. In all these experiments BAL prevented the contractor response usually induced by bradykinin.

The rat terminal ileum contracted to bradykinin (40–100 ng/ml), in normal Tyrode solution. The actions of BPF and BAL on these preparations were similar to their actions on the duodenum bathed in low calcium Tyrode solution; BPF (5–20 μg/ml) potentiated and BAL (5–20 μg/ml) inhibited the contractions (nine assays). Smaller concentrations of BAL (0·1–1 μg) did not change the effects of bradykinin. The contractions produced by eledoisin and acetylcholine on the rat duodenum and terminal ileum were unaffected either by BPF or by BAL (10–20 μg/ml).

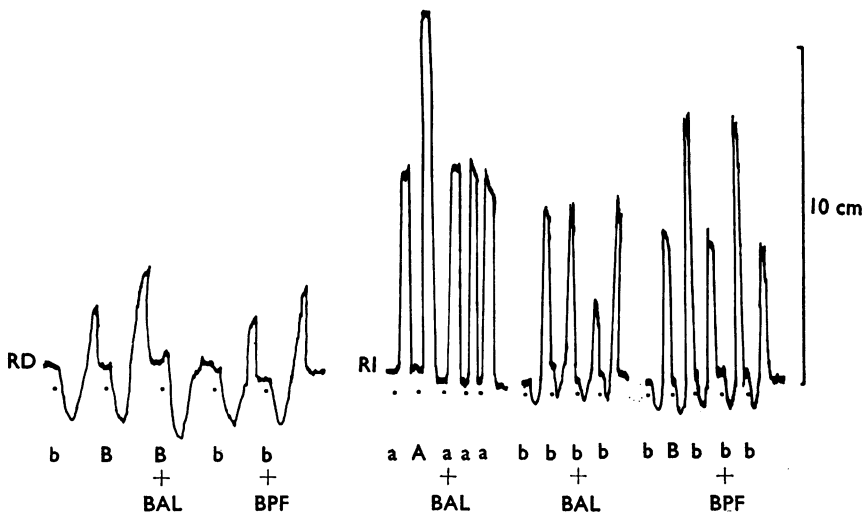

FIG. 1. Action of BPF and BAL upon the effects of bradykinin on the rat duodenum (RD) bathed in low calcium Tyrode solution and on the rat terminal ileum (RI). The final concentration of the agonist in the organ bath was as follows: Rat duodenum assay, bradykinin; b=15 and B=30 ng/ml. Rat terminal ileum assay: acetylcholine, a=15 and A=25 ng/ml; bradykinin b=50 and B=70 ng/ml. BPF (20 μg/ml) and BAL (20 μg/ml) were added 5–10 s before the agonists.

The presence of atropine in the bathing fluid did not modify the action of BAL or BPF in these preparations. The action of BAL and BPF on the responses to bradykinin of the rat duodenum bathed in low calcium Tyrode solution (RD) and on the terminal ileum (RI) is shown in Fig. 1. BAL in a concentration which effectively inhibited bradykinin contractor effects on the rat terminal ileum, did not modify the responses to acetylcholine.

Discussion.—These experiments show that enzymes which inactivate bradykinin are present in duodenum and ileum of rat; furthermore these enzymes are inhibited by both BPF and BAL. However, the enzyme inhibitors did not have the expected effects on the contraction and relaxation of the smooth muscle preparations. Whereas BPF potentiated, BAL inhibited the contractions induced by bradykinin on the isolated duodenum (bathed in low calcium Tyrode) and on the terminal ileum of the rat. Furthermore, BPF had no effect on the kinin-induced relaxation of the duodenum. Consequently the BPF potentiating action cannot be simply explained by the inhibition of tissue kininase or by any mechanism which implies an effective increase of the agonist in the vicinity of the pharmacological receptor. As BAL specifically inhibited the contracting but not the relaxing effect of bradykinin, there may be two types of receptor for bradykinin. The receptor related to the contraction of the rat gut is antagonized by BAL. It may also be sensitized by BPF, possibly by promoting an increased affinity of the receptor for bradykinin.

A possible mechanism for such a sensitization would be allosteric transition (Monod, Changeux & Jacob, 1963) of the pharmacological receptor (Goldstein, Aronow & Kalman, 1968). This mechanism appears to be important in isolated preparations. However, *in vivo* inhibition of kininase activity of blood and tissues (especially lungs), has been shown to play a decisive role on the fate of circulating bradykinin (Ferreira & Vane, 1967; Ryan, Roblero & Stewart, 1968), so receptor sensitization may play a secondary role under these conditions.

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