PHARMACOLOGICAL ACTIONS AND FUNCTION OF BRADYKININ

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PURE bradykinin has five main actions — vasodilatation, increase in capillary permeability, pain production, accumulation and migration of leucocytes and stimulation of smooth muscle.

As a vasodilator both in animals and man bradykinin is one of the most potent substances known, and Hilton and Lewis (1957) have provided evidence which suggests that the function of bradykinin in the body is as the mediator of functional vasodilatation in glands. We have shown that during activation of salivary glands an enzyme, probably salivary kallikrein, escapes from the secretory cells into the tissue space and there acts on the plasma globulins. Thus when the gland cells are activated the enzyme escapes both into the duct to appear in the secretion and into the interstitial space of the gland around the blood vessels, to form the vasodilator polypeptide.

As kallikrein appears in many glandular secretions it seems likely that plasma kinin formation is the mechanism for producing functional vasodilatation in all these glands. Two points have been raised against this theory. Firstly Terroux *et al.* (1959) have claimed to have dissociated glandular activity and vasodilatation in the salivary glands. They measured glandular activity as oxygen uptake and showed that atropine prevents the increased uptake of oxygen in the stimulated gland but does not prevent the vasodilatation in the gland.

This is an old problem and Hilton and Lewis (1956) had in fact gone into it and had shown that after atropine, stimulation of the gland still produced an output of plasma kinin forming enzyme. Thus the dissociation of oxygen uptake and vasodilatation in the gland is not a relevant argument against the view that bradykinin is responsible for functional vasodilatation, as there is no established relationship between oxygen uptake and output of plasma kinin forming enzyme. Secondly, Schachter (1960) found that guinea-pig saliva did not act upon guinea-pig serum to form bradykinin and concluded that functional vasodilatation was not the result of plasma kinin formation. However Schachter also observed that guinea-pig serum did not form bradykinin with saliva of other species, but that guinea-pig saliva did form a plasma kinin when incubated with

the serum of other species. It seems therefore that it is not that guinea-pig saliva does not contain the plasma kinin forming enzyme but that under the conditions of this particular test the serum did not react with salivary kallikrein from any species. This may not be so difficult to understand.

Plasma or serum are known to contain a complex mixture of enzymes, inhibitors and activators and it may well be that in order to produce bradykinin from guinea-pig serum by the action of salivary kallikrein, *in vitro*, special treatment of the serum is necessary.

Neither of these points, therefore, can be held up against the view that bradykinin or at least a plasma kinin is responsible for functional vasodilatation in glands. In addition to this physiological function it has also been suggested that bradykinin may be a mediator of the inflammatory response. The only evidence for this view is that bradykinin possesses all four actions which make up the first stage of inflammation — it dilates blood vessels, increases the permeability of the vessels, it causes pain and it causes accumulation and migration of leucocytes. We wanted to see how specific bradykinin was in having this spectrum of activity, and so pure synthetic bradykinin kindly supplied by Dr. Nicolaides of Parke Davis, has been compared with other peptides which are available in pure form. We have also used a crude preparation of substance P (15 units/mg) and have assumed (on the latest estimate of Franz, Boissonnas and Stürmer (1961) on the activity of pure substance P) that the pure peptide is 2000 times more active.

Among the 5 biologically active peptides mentioned below only substance P has a spectrum of activity anything like that of bradykinin. As this preparation was far from pure, we have to wait for Dr. Boissonnas and his colleagues to complete their isolation before we can be sure of this close similarity.

I should like to discuss the actions one by one and point out some interesting facts which have come to light.

First capillary permeability — the technique employed to examine this action was to inject intravenously into guinea-pigs, a blue dye which binds to the plasma proteins. When the permeability of the vessels is increased the protein plus dye seep into the extra-vascular space and the affected area becomes blue.

Oxytocin and vasopressin had no effect on the permeability. However in addition to bradykinin and substance P, angiotensin proved to be very active — in fact it was about one tenth as active as bradykinin itself.

Substance P behaved like histamine in that, large doses cause a more diffuse area of blueing than in the case of bradykinin, but this might be due to impurities.

Angiotensin, has a flat dose-response curve like bradykinin. But the blueing is not usually as intense with angiotensin as with bradykinin.

A further striking difference is that this action of angiotensin is prevented by the antihistamine, mepyramine, which does not affect the response to bradykinin. The preparation of synthetic angiotensin used here, did not contain histamine as an impurity, nor does it release histamine. It therefore appears to be a direct action of angiotensin which is antagonised by the antihistamine.

All five peptides gave rise to some degree of pain when compared on the blister base preparation, but only substance P caused pain in doses of the same order as bradykinin. When compared on the blood flow in the cats' hind limb, again only substance P caused vasodilatation like bradykinin, in doses of the same order. Oxytocin also caused vasodilatation, but in doses 100 times greater than those of bradykinin. Both vasopressin and angiotensin caused constriction.

The test employed to examine effects on leucocytes, was to make intradermal injections into guinea-pigs and after 1 hr. to kill the animals and to fix the skin for histological examination. The test which can only be used for qualitative comparisons proved to be the least specific, as all the peptides except vasopressin caused accumulation of leucocytes on intradermal injection of concentrations of 1–10μg/ml.

This comparison shows that among the four pure peptides, bradykinin is specific, firstly in being a potent vasodilator and secondly in possessing all of the four cardinal signs of inflammation. However substance P or at least the crude sample used in these experiments also has these actions. It may be (and Dr. Gaddum has also expressed this view) that in the brain and intestine substance P takes over the role played by bradykinin in other tissues like glands and skin.

Finally the actions of the five peptides have been compared on four isolated smooth muscle preparations. On the guinea-pig ileum the pituitary hormones were the least active, oxytocin causing only relaxation; the rat uterus was sensitive to all five peptides; the large intestine, represented by the rat colon and hen rectal caecum, is particularly sensitive to substance P. When we consider the possibility of a relationship between the spectrum of activity and function, one might speculate that this high sensitivity of the large intestine to substance P reflects a function of substance P, at least, of that found in the gut, in the large intestine.

It would be interesting to know if there is an increase in the substance P content of the large intestine of patients suffering with diverticulitis or ulcerative collitis and if in such inflammatory conditions substance P again takes the role played by bradykinin in inflammation in many other tissues.

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