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Rapports

THE RELATION OF HISTAMINE LIBERATION TO ANAPHYLAXIS

by

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Possibly the most intriguing problem in allergy is still that of how a piece of sensitized smooth muscle comes to contract, promptly and vigorously, when exposed to the proper antigen, and I should like to discuss some of the recent work on histamine release in the light of this problem. For a description of the phenomenon, it still seems best to return to Dale's 1913 paper; and it is interesting to examine how far our recent knowledge goes to answer the questions then raised.

At the time a special interest was directed to the possibility that the union of antigen and antibody gave rise to a proteolysis, whose products brought about the physiological responses of anaphylaxis.

This paper, backed up the later one with Kellaway, made it impossible to suppose that the contraction of the smooth muscle depended on proteolytic actvity generated in constituents of the blood, since blood appeared totally unnecessary for the response. In addition, the time relationships of the contractile response to antigen, viz. its short latency, its rapid development and early maximum, comparable with those seen with "β-imidazole-ethylamine", led him to doubt that any *local* proteolytic events should be concerned. Such a doubt confronts, of course, any other time-consuming enzymatic process. Dale expressed his own preference at the time for the conception that the union of antigen with cellular fixed antibody created a disturbance of the colloid equilibrium of the limiting membrane of the smooth muscle.

The subsequent discovery of the analogies between the response of various tissues to histamine and in anaphylaxis, and of the fact that histamine is released in many of the situations of antigen-antibody union, gave a new direction to research, diverting it somewhat, away from the study of the contractile response itself, and towards an analysis of histamine, its release and its actions; so that pharmacological evidence of histamine-release, rather than the responses of smooth muscle or blood vessels came to be used extensively and profitably in the analysis of the antigen-antibody effects. But as it became clear that tissues artificially desensitized, or

already insensitive, to histamine, could still yield an anaphylactic response, the correlation of histamine-release with effective antigen-antibody union began to fail. Our position is, indeed, not that we have advanced far towards solving Dale's original problem, but that we have acquired more problems bearing perhaps different solutions. More recently a further development has taken place, the discovery that histamine is largely but not only located in mast cells, and that the process of histamine release is associated with the degranulation or disruption of these cells. Fascinating as these developments are, they carry us perhaps another step away from the anaphylactic contraction itself, as we study not the contraction, nor the hormones which may cause it, but the morphological changes associated in an obscure way with the mobilization of hormones possibly involved.

We must still ask, therefore, whether the three processes seen in anaphylaxis — mast cell change, local hormone release, muscle contraction — constitute a causal chain or no? Are they, as it were, in series, or in parallel, or both? And further, do studies with chemical releasing agents throw any light on the problem?

HISTAMINE-LIBERATORS AND PROTEOLYSIS IN ANAPHYLAXIS

It seems tolerably clear that despite some remarkable analogies there are major differences between the action of liberators and of anaphylactic antigen. Some are only to be expected; thus the incidence of effect of a liberator will depend simply on its distribution and that of releasable histamine; but in anaphylaxis, the distribution of bound antibody will also be important. But two special points are of interest. If one considers the time course of histamine release *in vivo*, or in a perfused organ, it is remarkably rapid with liberators but with antigen a little sluggish in comparison. With a liberator the depressor response in a cat occurs almost exactly at the time when blood returns rich in histamine from the tissues and reaches the systemic blood vessels again; the peak histamine concentration in the blood is within a minute or two of injection; in a perfused muscle or skin flap, vasoconstriction from the released histamine occurs promptly. But with antigen the latency is longer, the histamine release in skin is long drawn out, and reaches a slow maximum. One cannot attribute such differences simply to the time taken for protein to reach the tissues, if one recalls the rapid effect of antigen applied to the serosal surface of, say, a piece of sensitized uterus or gut. If then one wishes to exploit the analogies between histamine-liberators and anaphylaxis, by arguing for some mechanism common to the two processes, it could only be by supposing that a material resembling a histamine-liberator is formed or activated in the later stages of the anaphylactic response. The same conclusion flows from the second major difference, the fact shown by Mongar and Schild and fully discussed by them, that anaphylactic histamine release, but not release by 48/80 or octylamine, is blocked by enzyme inhibitors; and that with isolated intracellular histamine-rich particles,

antigen is now ineffective in release, but chemicals still function.

One must pause to ask, however, whether it is worth considering the possibility of liberator-formation in anaphylaxis. Although I don't think any very plausible hypothesis can yet be framed, there are some features which make one reluctant to abandon the possibility altogether. In the first place there is the high activity of many liberators;Compound 48/80, the substituted butylamine L 1935, the purified material from Ascaris obtained by Uvnäs and his colleagues, are able to mobilize the histamine *in vivo* and in perfused tissues, and to degranulate mast cells, in such low concentrations as to rank among the more active compounds at a pharmacologist's disposal. It is almost a metaphysical point, but there is abundant precedent to back one's suspicion that any synthetic compound of a high potency is a pointer, or more than a pointer, to the chemistry of the body itself. The acetyl ester of choline, and a certain phenylethylamine derivative are notable examples. We can say, too, that action of this type is not just a chemist's artefact; it is shown by products of natural origin, such as certain polypeptide antibiotics, by peptones and even by the basic amino-acids themselves. This would point to products of proteolysis as the most plausible source of anaphylactic liberator. The liberators are, in addition, for the vast majority of cases, basic compounds. We have therefore to consider a proteolysis allowing the appearance of basic polypeptides. This indeed, is not improbable. Of intracellular proteases, the cathepsin II of Bergman is said to resemble trypsin in its reactions, and trypsin specifically attacks bonds adjacent to lysine or arginine residues; so that a result of trypsin digestion is the appearance of terminal lysine or arginine residues. It is interesting that trypsin is the proteolytic enzyme *par excellence* with which anaphylactoid shock is produced—fact harnessed by Rocha e Silva to an earlier proteolytic theory, evolved before we knew much about histamine binding, that the histamine could be cleaved enzymatically from a peptide linkage. We can suggest, then, that in the anaphylactic process, intracellular cathepsin II may be activated by antigen-antibody union to from basic polypeptides.

But there are some unattractive features to such a view. First is that there is, so far as I know, no direct evidence for any such process. I have tested it by incubating sensitised guinea pig tissues with antigen in a small volume of fluid in the presence of mesenteric mast cells from an unsensitised animal (rat or guinea pig) but could demonstrate no enhanced degranulation. Proteolytic events in blood, indeed, have been demonstrated in allergic responses, but they can hardly account convincingly for anaphylactic responses in blood-free tissues. One may comment, however, that a proteolysis of the type postulated might be rather hard to detect. It is not necessary that the products are formed in large amounts, if they act in close relation to the effector organ; further they could be broken down by the enzyme which forms them—as are the kinins; and finally the tests for their presence do not, at present, have the remarkable sensitivity that is available for assaying, say, a natural transmitter. A second unattractive feature of the theory is its fundamental vagueness. The general phrase « products of proteolysis » can cover a multitude of sins. One could use it to accommodate almost any new fact. One might suppose the products to act locally as well as to diffuse to adjacent mast cells; so

that the theory is indifferent to the location of releasable histamine. One could multiply products to exert actions other than histamine release so that our knowledge that slow reacting substances are released, of a polypeptide nature, is explicable. One could postulate varying rates of formation or destruction to conform with diverse time-relationships. Clearly, one could explain anything. Not until proteolysis has been shown, and a relevant pharmacological action by its products demonstrated, can we really support such a theory. Yet, in its absence, we are left with the problem of what significance to attach to the striking property of histamine liberation displayed, specifically, by so many organic bases.

HISTAMINE-LIBERATORS AND LECITHINASE

A stimulating new approach to problems of histamine release has been made recently by Uvnäs and his colleagues. Their observation that a polysaccharide fraction isolated from hip seeds could prevent histamine-release by 48/80 and other liberators from the cat paw, and prevent disruption of rat mesentery mast cells, led them to suspect that some enzyme process was involved in the liberation process, since high molecular weight polyanions can inhibit reversibly a number of enzymes, perhaps by blocking free amino groups on the enzyme molecule. In a search for enzymes able to degranulate mast cells, they found, out of about 30 tested, only one, lecithinase A, which was significantly active. This was still effective on mast cells which, by heating to 45-50°C, has been made refractory to 48/80. They suggest that there is on the surface of the mast cell a lytic enzyme; that this is normally inactive since the active group is blocked by an inhibitor; and that when the inhibitor is removed by conjunction with 48/80 or some other suitable basic compound, the enzyme becomes actives and attacks the cell membrane. They were able further to render mast cells resistant to liberator action by treatment (in the presence of the liberator) with 1:3 diphosphoimidazole which inactivates enzymes with essential amino groups such as lecithinase A; and they could restore sensitivity by "dephosphorylation" with phosphoamidase.

There are a few obscure points in this work. Lecithinase, for instance, is inactive on the perfused cat's paw. The action of 48/80 on mast cells was found to be blocked by temperatures between 40°C and 50°C, whereas lecithinase is still active at 60°C. Lysolecithin itself was rather inactive, requiring about 500 μg/ml to have on mast cells the effect produced by Compound 48/80 at 1 μg/ml. But this new theory has the attractive features that it accounts for the action of basic liberators; that it establishes a new link with the old work on lecithin and lysolecithin, and that it could account for the appearance of non-polypeptide muscle stimulants, such as that described by Brocklehurst. It is interesting, too, in exploiting the idea that activation may be achieved by removal of an inhibitor, a notion also used by Garcia Arocha, Ashwin & Grossberg in the suggestion that liberators combine with heparin, so releasing a proteolytic enzyme previously inhibited by heparin. Another hopeful feature is that an explanation might be forthcoming for the extraordinary histamine release by large arteries, so dependent on species, (dextran and egg albumen in rat,

polyvidone and Tween 20 in the dog, horse serum in cat) whose characteristics Halpern especially has elucidated.

It was for a while possible to attribute some of this activity to an action on elements in the blood corresponding to rouleau-formation, with an aggregation of platelet and leucocytes, generally or locally, leading to histamine and 5HT release. But Halpern's observation that dextran is effective on rat skin *in vitro* renders this view untenable. If, however, an enzymic process mediates the response, space at least exists for species variations to operate.

The relation of this work to anaphylaxis is not yet clear. But il allows us to include lecithinase to intracellular proteases among the enzymes which may play a role.

THE ANAPHYLACTIC CONTRACTIONS OF GUINEA PIG ILEUM

How do these phenomena relate to anaphylaxis? I would like to start by outlining simply some of the events seen in the anaphylactic response of guinea pig ileum. Suppose one places two strips of guinea pig ileum in the same bath, one sensitized to egg albumen the other not sensitized but serving as a test for active substances released. If one then adds a large dose of the antigen (say 1 mg when an adjacent piece of gut has been shown to react well to 1 μg), two phenomena impress themselves. The first is the irreducible latency of response by the sensitized strip to antigen — of the order of 12 seconds — whereas an equiactive or less active dose of histamine acts within 1-2 seconds. The second is the extraordinary persistence of the spasm of the sensitized strip. Soon after its development, the unsensitized strip begins to contract. But if the bath is now washed out, the latter relaxes completely, and the sensitized strip is hardly changed. With further lapse of time of a few minutes, a little more activity accumulates in the bath, readly washed out again. Soon a state is reached in which the sensitized strip is in a condition of maintained spasm, yet there is no sign at all of diffusible active principles escaping from it; and this condition can persist for a period of an hour or more. If, in a similar experiment, mepyramine to a concentration of 10^{-7} is added to the bath, the sensitized strip now undergoes a somewhat slower contraction (though of little changed latency), but one which carries it to almost as intense and prolonged a spasm as normal; yet the test strip shows no activity at all.

Such an experiment recapitulates, of course, some of the classical experiments on anaphylaxis by Feldberg and Schild and their colleagues, chiefly, on lung and uterus. But it seems too, to make it hard to believe that the formation of diffusible muscle stimulants account for all the phenomena of the anaphylactic contraction. There appears to be no major barrier to diffusion in the muscle spaces; for we know that histamine added to the bath can least the outer muscle fibres within a second or two, and antigen can be only a little less rapid. Further a significant proportion of the histamine released in the anaphylactic reaction can escape into the bath some ten to twenty seconds after the contraction starts. There may be, of course, a very intense local concentration of local hormones at the start of the reaction, but there emerges no reason why such hormones should

not diffuse away quite soon, and allow the gut to relax, as it does after a massive dose of histamine or other stimulant. It is true that the relaxation after slow-reacting-substances is slower than with like histamine or acetylcholine; the recovery of the gut from their action, or indeed from any other stimulant I know, is far faster than the relaxation of the anaphylactic spasm of the ileum. Again, one might postulate a continuing localised release; but this would surely cause activity to appear in the bath fluid. Finally, one might suggest that the active substances are released, and act, within the smooth muscle cell and do not escape from it; but this is not an attractive idea, now that we know, from Castillo and Katz's work for instance, that acetylcholine acts only on the outside of a motor endplate and is devoid of action given intracellularly. The *persistence* of the spasm, then, in the absence of detectable activity in the surrounding fluid, presents a serious difficulty for any theory which attributes the spasm entirely to diffusible stimulants, whether histamine or other substances. The histamine theory could escape from some of its other difficulties, such as the ineffectiveness of antihistamines, by the entirely plausible idea of intense local concentrations; but this is hardly possible when diffusional equilibrium has been developing for an hour or more.

Such experiments make one return to Dale's conception that the anaphylactic reaction leads to a disturbance of the conditions of colloidal solution in the smooth muscle fibre, meaning by that, perhaps, some process which activates the contractile substance without activating specific chemical receptors. Mongar & Schild's work make it probable that some process resembling the enzymic is involved; the distinct latency in the anaphylactic response of the ileum, despite the great rapidity of the contraction when it appears, conforms well with such an idea. No great allowance of time is needed here; if cholinesterase can remove acetylcholine physiologically in a time of milliseconds, then even enzymes with rates or conditions of action a thousand times slower could be considered. But Mongar & Schild's work does not necessarily imply, as yet, the formation of diffusible active principles; and an analogy (*mutatis mutandis*) between anaphylaxis and, say, haemolysin action seems open.

If, however, a relatively biophysical explanation is adopted for the non-histamine part of the anaphylactic contraction, one must consider whether the breakdown of mastcells and so perhaps the histamine release itself is not also initiated by a similar disturbance of the mastcell membrane. We are, in short, still in a position not far from that of 45 years ago, confronted with enzymatic, hormonal and biophysical theories, or with combinations of them; and we still find it hard to choose between them. This is, perhaps, a matter of some practical importance; for the problem of seeking an antagonist to an enzyme or to a diffusible stimulant of muscle carries us into the familiar (though in allergy still not very successful) territory of drug antagonism, but to modify an activation of smooth muscle which may be brought about by some more direct process is a problem for which we have little guidance, save that obtained in the study of the anaphylactic contraction itself.