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THE DIFFERENCE IN BEHAVIOUR OF BASOPHIL LEUCOCYTES AND MAST CELLS TOWARDS COMPOUND 48/80

BY

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The basophil leucocytes were first observed by Ehrlich (1891). Because of their resemblance to tissue mast cells, which he had previously described, he called them " blood mast cells ". Considerable controversy arose over the relationship between these two types of cells. Eventually Ehrlich's conviction that the blood mast cell was of myeloid origin was amply substantiated by later workers (Maximow, 1910). In various species there is a certain reciprocal relationship in numbers between these two types of cells—for example, in the rabbit blood mast cells predominate, whereas in rats and mice " tissue mast cells " are more numerous (Riley, 1959). Like the mast cell, the circulating basophil leucocyte contains histamine. The basophil leucocyte is the principal carrier of histamine in normal human blood (Valentine, Lawrence, Pearce & Beck, 1955; Code & Mitchell, 1957; Rorsman & Rosengren, 1958). Furthermore, this cell is able to synthesize histamine as well as to store it (Lindell, Rorsman & Westling, 1961; Hartman, Clark & Cyr, 1961). The histamine liberator, compound 48/80, releases from the mast cells not only their histamine but also the granules both *in vivo* and *in vitro* (Paton, 1951; Fawcett, 1954; Salvato, 1961; Mota, 1963; Horsfield, 1965). It has been claimed that basophil leucocytes are also degranulated by compound 48/80 (Hunt & Hunt, 1958; Marks, Sorgen & Ginsburg, 1959; Shelley & Juhlin, 1962), but Levi & Meneghini (1959) were unable to confirm this. The present investigation was carried out in an attempt to clarify this problem.

METHODS

Direct prick test on human subjects

This was carried out with compound 48/80 in a concentration of 1:1000 in physiological saline. Three healthy subjects and eight patients suffering from chronic non-allergic urticaria were investigated.

Direct basophil leucocyte test

The technique used was that described by Haye, Cruickshank & Cooper (unpublished). Blood was collected from the same healthy subjects and patients mentioned above, also from four sandy lop rabbits.

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Collection and concentration of rat mast cells

Adult inbred hooded rats were anaesthetized with ether, bled by cardiac puncture and then killed by air embolism. Mast cells were obtained by washing out the peritoneal cavity with 20 ml. of Hanks balanced salt solution at 37° C without any added anticoagulant. The fluid was centrifuged at 578 g (using a swing-out head), the supernatant discarded and the remaining concentrated cell suspension used.

Histamine assay

This was carried out on guinea-pig isolated ileum at 37° C in a 2 ml. hand-operated bath containing Tyrode solution. The solution was gassed with a mixture of 95% oxygen and 5% carbon dioxide. In the control experiments either 0.1 ml. of resuspended mast cells in Hanks fluid were added to the bath or 0.45 ml. of a basophil-rich cell suspension in plasma. Either suspension was left in contact with the ileum for 15 sec. In the tests the cell suspensions were incubated for 10 min with 0.05 ml. of a 10^{-4} solution of compound 48/80. One series of experiments was carried out at room temperature using E.D.T.A. as the anticoagulant, another at 37° C using heparin. A 1 min cycle was used throughout. All histamine values were expressed as base. The antihistamine, mepyramine maleate, was added to the organ bath in a concentration of 0.1 μg/ml. and left in contact with the tissue for 1¼ min before the test.

RESULTS

Direct prick test on human subjects

Positive weal and flare reactions were recorded in all the 11 subjects tested with compound 48/80. Physiological saline, which was used as a control, did not produce this response. Dermographia could not be elicited in any of the subjects.

Direct basophil leucocyte test

Human. Table 1 shows the effect *in vitro* of compound 48/80 on human basophil leucocytes from the same 11 subjects investigated by the prick test. In 10 there was

TABLE 1

THE EFFECT *IN VITRO* OF COMPOUND 48/80 ON HUMAN BASOPHILS

Patient no.	Diagnosis	"%" Basophil degranulation using the following: Control	48/80 - 1/1000 (dil.)
1	Normal	12	31
2	Normal	12	10
3	Normal	16	14
4	Chronic urticaria	10	16
5	Chronic urticaria	21	22
6	Chronic urticaria	6	8
7	Chronic urticaria	4	8
8	Chronic urticaria	12	13
9	Chronic urticaria	20	25
10	Chronic urticaria	32	28
11	Chronic urticaria	25	28
Mean value±S.E.		15±3	18±4

no marked difference in the percentage degranulation between the test and the saline control. In one patient (No. 1) the test slide showed a rise of 19% degranulation compared with the control. This value, however, was within the normal limits of 0 to 30% accepted by Shelley (1962) and Haye (1965).

Rabbit. There was no difference in the percentage basophil degranulation between tests and controls in all the four rabbits tested (mean percentage degranulation, control = 19%, tests = 22%).

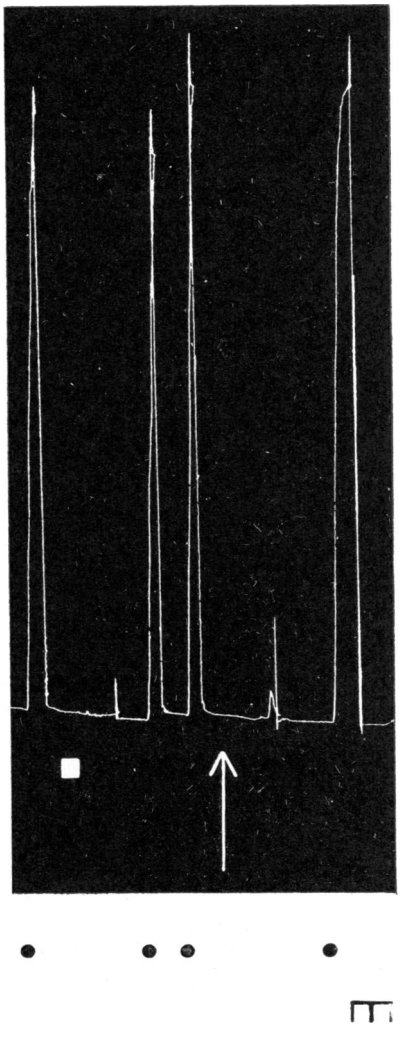

Fig. 1. Guinea-pig isolated ileum in Tyrode solution. Bath volume 2.0 ml. Temp. 37° C. At each ● 50 ng of histamine were added to the bath. At □ 0.45 ml. of a basophil rich cell suspension from a healthy volunteer was added to the bath and at the arrow the same amount of cell suspension incubated for 10 min with 0.05 ml. of a 10^{-4} solution of compound 48/80. Time marks 10 sec.

Histamine assay

Human. Compound 48/80 in a final concentration of 2.5 μg/ml. produced no contraction. Five specimens of basophil-rich cell suspensions were incubated with compound 48/80. Three of these were obtained from the patients on whom the prick test had been performed, two from different patients. Three of the five tests were performed at room temperature using E.D.T.A. as the anticoagulant. In order to exclude the possible temperature dependence of histamine liberation as well as interference by the individual anticoagulant used, a further two experiments were performed at 37° C using heparin as the anticoagulant. In no case could any release of histamine be detected. The sensitivity of the isolated ileum preparation varied, but was at times as great as 2 ng/ml.

Rabbit. Four similar experiments were carried out with a basophil-rich cell suspension in plasma from rabbit blood. Two experiments were performed at room temperature using E.D.T.A. as the anticoagulant and two at 37° C using heparin. Again no release of heparin could be detected. The basophil-rich cell suspension from heparinized blood on its own, however, caused a slow contraction of the isolated ileum, but incubation at 37° C with compound 48/80 for 10 min caused no further change of the spasmogenic effect. Neither of these contractions were abolished by mepyramine maleate, but they were reduced in height. Figure 2 illustrates such an experiment.

Rat mast cells. In one experiment a rat mast cell suspension on its own caused a contraction of the guinea-pig isolated ileum. This was abolished by mepyramine maleate. On a second, less sensitive, preparation a similar specimen from a different rat caused

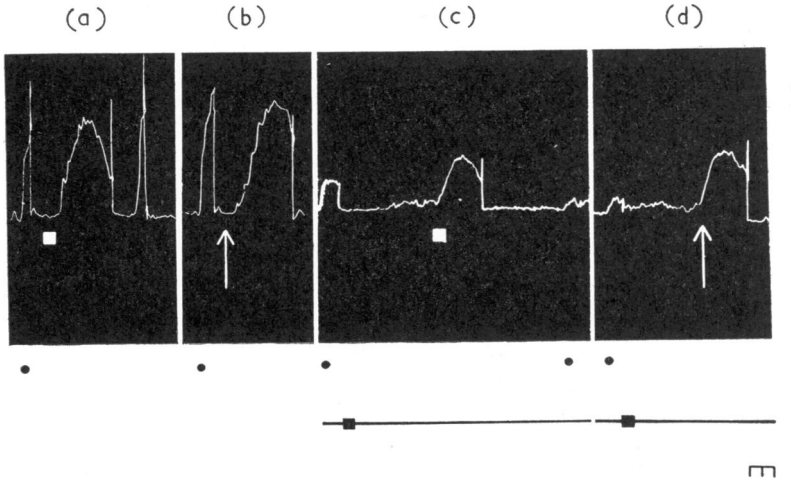

Fig. 2. Recording as Fig. 1. At each ● 10 ng of histamine were added to the bath. Panel (a): at □ 0.45 ml. of a basophil-rich cell suspension in plasma from a rabbit was added. Panel (b) as panel (a): at the arrow the same amount of cell suspension was added, but after incubation for 10 min with 0.05 ml. of a 10^{-4} solution of compound 48/80. Panel (c) repeat of experiment (b) in the presence of mepyramine. In experiments (c) and (d) there was a residual effect of mepyramine from previous experiments which was reinforced by adding further 200 ng at ■. These were left in the bath without washing (as denoted by the black line).

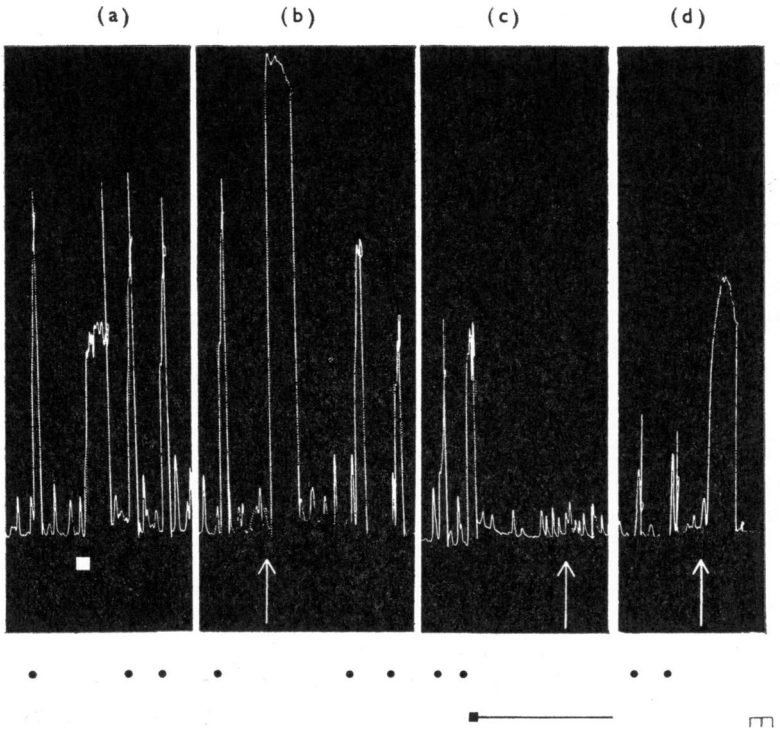

Fig. 3. Recording as Fig. 1. At each ● 2 ng of histamine were added to the bath. Panel (a): at □ 0.1 ml. of a suspension of mast cells from a rat were added. Panel (b) as panel (a): at the arrow the same amount of mast cell suspension was added, but after incubation for 10 min with 0.05 ml. of a 10^{-4} solution of compound 48/80. Panel (c) as panel (b), but at ■ 200 ng of mepyramine were added to the bath and left without washing (denoted by the black line). Panel (d) as panel (b), but the amount of histamine added at ● was 5 ng.

no such contraction. Nevertheless, incubation of the mast cell suspension with compound 48/80 at 37° C for 10 min released a spasmogenic agent on both occasions. This again could be abolished after pretreatment of the gut with mepyramine maleate. Figure 3 illustrates such an experiment.

DISCUSSION

Tissue mast cells release their granules and histamine in response to compound 48/80 (Paton, 1951; Fawcett, 1954; Salvato, 1961; Mota, 1963; and Horsfield, 1965). Histamine release from rat mast cells in the presence of compound 48/80 has been confirmed in the present investigation (see Fig. 3). A histamine-like cutaneous response was obtained in all of 11 subjects in whom compound 48/80 was introduced. Juhlin & Rune (1962) demonstrated with a quantitative iontophoretic technique a typical triple response to compound 48/80 in normal healthy subjects and in patients with urticaria and eczema.

The effect of compound 48/80 on basophil leucocytes is less certain. Degranulation of these cells has been studied after exposure both *in vivo* and *in vitro* to compound

48/80. Thus Hunt & Hunt (1958) as well as Marks *et al.* (1959) claim to have shown a fall in circulating basophil leucocytes in rabbits and cockerels after systemic administration of the compound. On the other hand Levi & Meneghini (1959), were unable to find such a fall in 10 patients after intravenous administration of compound 48/80. Shelley & Juhlin (1962) reported degranulation of normal human basophil leucocytes in the presence of the compound, but we have been unable to confirm this with basophil leucocytes from eight patients with non-allergic urticaria and from three healthy control subjects. Marks *et al.* (1959) were unable to demonstrate such *in vitro* degranulation in rabbits and cockerels in contrast to the positive *in vivo* results mentioned above. In the present investigation we have confirmed this negative *in vitro* finding in rabbits. There remained the possibility that compound 48/80 might liberate histamine from basophil leucocytes in the absence of degranulation. Smith (1958) has shown that the two processes in mast cells are not necessarily linked. However, neither human (see Fig. 1) nor rabbit basophils (see Fig. 2) liberated any detectable amounts of histamine on incubation with compound 48/80. In order to exclude the possible temperature dependence of histamine liberation or interference by the individual anticoagulant used, further experiments were carried out at 37° C and changing the anticoagulant from the original E.D.T.A. to heparin, again with negative results. The basophil-rich cell suspension from rabbit blood itself has a spasmogenic effect. This was only partly due to histamine, because mepyramine slightly reduced but did not abolish it (see Fig. 2).

SUMMARY

1. The release *in vitro* of histamine from rat mast cells by compound 48/80 was confirmed.

2. No evidence of degranulation *in vitro* of human or rabbit basophil leucocytes by compound 48/80 was found.

3. There was no evidence of histamine release *in vitro* by compound 48/80 from human or rabbit basophil leucocytes.

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