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ACTION OF COMPOUND 48/80 ON THE MAST CELLS AND HISTAMINE CONTENT OF GUINEA-PIG TISSUES

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Feldberg and Paton (1951) and Paton (1951) showed that 48/80 is a very potent histamine liberator in dogs and cats. Riley and West (1953) demonstrated a positive correlation between the histamine content and the mast cell population in a variety of tissues, and Riley (1953) showed in experiments on rats that the histamine liberators stilbamidine and (+)-tubocurarine disrupt the mast cells. Mota (1953) and Mota, Beraldo, and Junqueira (1953) observed the disruptive action of 48/80 on rat mast cells both *in vivo* and *in vitro*, and Fawcett (1954, 1955) added further evidence. Norton (1954) observed that the *in vitro* action of 48/80 on rat mast cells is dependent on the concentration of the drug, and developed a method for its assay. These facts support the hypothesis that 48/80 liberates histamine from mast cells.

Not all tissues or species, however, are susceptible to the action of 48/80. Thus Feldberg and Talesnik (1953) have shown that repeated intraperitoneal injections of compound 48/80, which practically deplete the skin and striated muscle histamine in rats, do not reduce the histamine content of the liver, stomach, and duodenum. Later Mota, Beraldo, Ferri, and Junqueira (1954) showed that repeated injections of compound 48/80 in rats depleted the histamine only from those organs in which the histamine is probably of mast cell origin.

The disruptive action of 48/80 on the mast cells was studied by Junqueira and Beiguelman (1955), who suggested that this phenomenon depends upon certain enzymatic systems. Recently, Feinberg and Sternberger (1955) observed that 48/80 failed to produce asthma or emphysema in guinea-pigs, such as usually occurs in anaphylactic or histamine shock, and that the repeated administration of 48/80 caused no desensitization of the animals. They also showed that antihistamines had no protective effect against the drug. In view of these differences between the effects of this drug on the guinea-pig and on the other species (cat, dog, and rat) it was decided to investigate the effect of 48/80 on the histamine and mast cell contents of the guinea-pig.

METHODS

Guinea-pigs of both sexes, 200–500 g., were used. For microscopical observation of the mast cells the tissues were fixed with lead subacetate in acidified alcohol and stained with toluidine blue as previously described (Mota *et al.*, 1954). Since guinea-pig mast cells are rather difficult to preserve the fixative was always injected through the vessels. Lung was fixed by intratracheal or intrabronchial injection. The skin of the lateral region of the head (near the inferior eyelid and snout) was chosen for its richness in mast cells and because it can be easily fixed by injecting the fixative into the carotid artery. Only mesentery was fixed by immersion, stained, and examined as a whole.

Anaesthesia.—When anaesthesia was used the guinea-pigs were injected intraperitoneally with 0.6 ml./100 g. of a mixture containing 10% pentobarbitone sodium and 20% urethane in distilled water.

Histamine Extraction and Assay.—When skin samples were taken the skin was first shaved. The tissues were dried between filter papers, weighed, cut into N-HCl in very small pieces, boiled for 2 or 3 min., neutralized, and assayed on the atropinized guinea-pig ileum by the method of Feldberg and Talesnik (1953). All histamine values are given as base.

RESULTS

Effect of Compound 48/80 on the Lung Mast Cells and Histamine.—Eleven guinea-pigs were used in experiments on the histamine content of the lung. After anaesthesia a cannula was tied into the trachea and then connected to a respiration pump. The thorax was opened and lung samples were collected before, and 2 to 15 minutes after, the intracardiac injection of 0.5 mg. 48/80, in 1 ml. saline. Lung samples were taken alternately from the right and left inferior pulmonary lobes after ligature of the pedicle and then extracted. In another group of three animals the lung samples were fixed and stained for mast cells. Seven out

of the fourteen animals showed respiratory disturbances as shown by diminution in the amplitude of the respiratory movements (four cases), and respiratory stoppage with collapsed lungs (three cases). In the other seven cases there were no observable

TABLE I

HISTAMINE CONTENT (μg./g.) OF GUINEA-PIG LUNG BEFORE AND AFTER THE INJECTION OF COMPOUND 48/80

Guinea-pig	Before	After
1	37·7	43·9
2	36·5	42·8
3	43·5	40·2
4	30·8	28·6
5	59·2	63·4
6	45·0	44·7
7	54·2	56·0
8	32·1	32·2
9	17·5	27·6
10	32·1	30·1
11	28·9	24·1

alterations in the respiratory movements. All the lungs became collapsed and cyanotic. The results of the histamine estimates in these experiments are shown in Table I. No difference could be seen either in the mast cell population or in the histamine content of the lung after the injection of 48/80. This is in contrast to the decrease in the histamine and mast cell content of the sensitized guinea-pig lung after anaphylactic shock (Mota and Vugman, 1956).

Effect on the Blood Histamine.—Twelve guinea-pigs were given intracardiac injections of 0.5 mg. 48/80. Heparinized blood samples were collected by cardiac puncture before and immediately after the death of the animal. Plasma was immediately obtained by centrifugation and assayed for histamine. The results of this experiment are shown in Table II. Although the histamine content of the plasma remained practically unchanged all the injected animals died. Similar experiments with

TABLE II

HISTAMINE CONTENT (μg./ml.) OF GUINEA-PIG PLASMA BEFORE AND AFTER THE INJECTION OF COMPOUND 48/80

Guinea-pig	Before	After
1	0	0·28
2	0·10	0
3	0	0
4	0	0·05
5	0·05	0·05
6	0	0
7	0·22	0·25
8	0·10	0·06
9	0·25	0·25
10	0·10	0·25
11	0·20	0·20
12	0·05	0·05

three rats showed an increase of the plasma histamine ranging from 0.0–0.1 up to 8 to 10 μg./ml.

Effect of Repeated Intraperitoneal Injections of 48/80.—Six guinea-pigs were injected intraperitoneally daily with increasing doses of 48/80 according to the following schedule: 100, 200, 200, 300, 300, 400, 400, 500, 500, 600, 800, 1,000, 1,000, 1,500, and 1,500 μg. No change could be observed in the behaviour of the animals until the dose reached 500 μg. From then on they became uneasy, pawing of the nose and intense scratching being the most frequent signs. Half an hour after the injection of the first 1,500 μg. of 48/80 the animals remained in lateral decubitus, with paralysis of the hindlegs, tremors, and apparent respiratory disturbances. Circulatory changes characterized by ischaemia alternating with hyperaemia were frequently observed in the vessels of the ear. All the signs subsided after three hours. After the second injection of 1,500 μg. 48/80 the animals presented exactly the same signs; one of them died half an

TABLE III

HISTAMINE CONTENT (μg./g.) OF SKIN AND MESENTERY OF CONTROL GUINEA-PIGS, AND AFTER REPEATED INJECTIONS OF COMPOUND 48/80

Guinea-pig	Foreleg Skin		Head Skin		Mesentery	
	Control	Injected	Control	Injected	Control	Injected
1	10·3	7·6	9·2	14·1	10·0	8·7
2	5·7	7·6	11·9	10·4	15·1	16·0
3	4·3	7·0	8·6	11·4	7·7	15·0
4	4·5	8·2	9·5	10·8	16·6	14·2
5	8·7	4·3	9·8	12·1	10·4	22·0
6	8·7	8·7	12·8	11·7	20·0	13·0

hour later, four in 45 min., and the last one in two hours. In all the animals the lungs were collapsed and cyanotic, and five of them had haemorrhagic ulcerations in the gastric mucosa. Six other animals were injected simultaneously with the same volume of saline and were stunned by a blow on the head and bled from the jugular vein.

From both the injected animals and the controls, samples of mesentery and skin (from symmetric sites in the foreleg, paw, and the lateral region of the head) were taken for histamine assay, and samples of mesentery, lung, and skin were fixed for mast cell observation. The results of this experiment are shown in Table III. In spite of repeated injections of large doses of 48/80, no difference could be detected between the histamine and mast cell content of the control and injected animals.

In vitro *Effect of 48/80.*—Since 48/80 has a strong disruptive action on rat mast cells it was decided to test its action directly on the guinea-pig mast

cells. Two guinea-pigs were lightly anaesthetized with ether and bled from the jugular vein. Pieces of mesentery were carefully dissected out and placed in cold isotonic sucrose. This procedure required about five minutes. Two or three pieces were then placed into solutions of 48/80 in isotonic sucrose containing 100, 500, and 1,000 μg./ml. during 15 min. at 37° C., removed, fixed, stained, and examined. In spite of the high concentration of 48/80, and its relatively long and direct contact with the tissues, no modification in the morphology of the mast cells was observed.

Action of Antihistamines.—Twenty guinea-pigs were given intracardiac injections of 2.5 mg./kg. 48/80, ten of them having received intraperitoneally 5 mg. chlorpheniramine hydrogen maleate one hour before. Five of these animals survived, of which three had received the antihistamine. All the other animals displayed spasmodic contractions of the extremities and respiratory embarrassment, and died in 2 to 3 min. At necropsy generalized cyanosis and collapsed lungs were found. The histological examination of the lungs showed atelectasis and mild hyperaemia.

Discussion

Compound 48/80 is an effective histamine liberator in the dog (Paton, 1951; Feldberg and Talesnik, 1953), cat (Paton, 1951; Feldberg and Paton, 1951; and Smith, 1953), rat (Feldberg and Talesnik, 1953; Mota *et al.*, 1954; Riley and West, 1955), and man (Feinberg, Feinberg, Rebhun, and Malkiel, 1954). Our results show that compound 48/80 is not an effective histamine liberator in the guinea-pig, and has no action on its mast cells. These results corroborate those of Feinberg and Sternberger (1955) showing that the release of histamine is not the major toxic action of this drug in the guinea-pig, and those of Feldberg and Mongar (1954), who found 48/80 a poor histamine liberator when guinea-pig lung was perfused with this drug. Although the injection of this drug caused no lung emphysema and no diminution in the lung histamine content, the presence of respiratory disturbances and atelectasis in the lungs suggests that compound 48/80 has some action on the respiratory mechanism. Results of the plasma histamine assays showed that histamine is not liberated in sufficient quantity to raise the plasma histamine level, as it does in the rat (this work) and in the cat and dog (Paton, 1951). Furthermore, the facts that repeated intraperitoneal injections of compound 48/80 do not deplete the skin or mesentery of the guinea-pig of histamine or of mast cells and that the animals do not become refractory to it also suggest that this compound does not have much histamine-releasing action in the guinea-pig. This conclusion is supported by the fact that repeated intraperitoneal injections of compound 48/80 (0.5 mg./kg.) in the guinea-pig before and during the period of sensitization had no effect on anaphylactic shock (Norton, personal communication). In contrast with the rat, the guinea-pig's mast cells and histamine are very resistant to the action of compound 48/80. The dog seems to behave like the rat, since the injection of compound 48/80 promotes a decrease in the liver histamine and mast cells (Mota, unpublished data), and a decrease in the number of mast cells in the tongue (Arvy and Quivy, 1955).

Feldberg and Miles (1953) injected compound 48/80 intravenously in guinea-pigs previously injected with pontamine blue, and observed that different areas of the skin were blued in proportion to their histamine content. Miles and Miles (1952) using the same stain showed intensified blueing of the cutaneous area injected with compound 48/80. This phenomenon was considered to be an effect of the released histamine since it was antagonized by mepyramine. From our own results we think that this histamine-releasing action of compound 48/80 must be very small and localized. However, the intense scratching and the gastric ulcers which we observed in some animals after the injection of this compound do suggest a release of histamine, although we could not detect any decrease in the skin histamine of the animals injected with compound 48/80 compared with the controls. The same thing seems to happen with the mesentery histamine, even when the compound is applied directly. However, a small decrease in the histamine content would be masked by the large individual variations in the results. The fact that in guinea-pig tissues, *in vitro*, compound 48/80 releases as much histamine as the antigen, as observed by Mongar and Schild (1952), might be explained by the dependence of the histamine-releasing mechanism on the experimental conditions (Paton, 1955). In any case it is clear that the main effect of compound 48/80 on the guinea-pig does not depend on the release of histamine or on the disruption of mast cells such as occurs in the rat and dog.

Summary

1. Lethal doses of the histamine liberator 48/80, intravenously administered to guinea-pigs, killed the animals without producing lung emphysema. Antihistamines were ineffective in protecting the animals against this action.

2. Plasma histamine determinations before and after the injection of the compound showed that the histamine level remained practically unchanged. No difference could be detected between the skin and mesentery histamine content of the control and injected animals. Likewise no changes were observed in the mast cells from the lungs, skin, and mesentery. No effect on the mast cells of the mesentery was found when they were directly exposed to the drug.

3. The effect of 48/80 on the guinea-pig is thus not mediated through histamine liberation, and the mast cells and the tissue histamine in this species behave quite differently from those of the rat and dog, being very resistant to the action of compound 48/80.

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