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THE RELEASE OF 5-HYDROXYTRYPTAMINE BY HISTAMINE LIBERATORS

BY

B. K. BHATTACHARYA AND G. P. LEWIS*

From the National Institute for Medical Research, Mill Hill, London, N.W.7

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The experiments to be described show that histamine liberators release 5-hydroxytryptamine (5-HT), at least in the rat, in addition to histamine. It is generally supposed that histamine is stored in and released from the mast cells and it seems probable that these cells are also the source of the release of 5-HT. The experiments were therefore designed to find out whether the mast cells were the source of the 5-HT released by histamine liberators. Since the experiments have been concluded Benditt, Wong, Arose, and Roeper (1955) have reported that 5-HT is a natural constituent of the mast cells of rats.

METHODS

The effect of histamine liberators was examined on rat, rabbit, cat, and dog tissues perfused with oxygenated Locke solution.

Perfusion of Rat Tissues.—The rats were anaesthetized with sodium pentobarbitone (40 mg./kg. i.p.) and the following tissues were perfused : the hindquarters, one hind limb, the gastrocnemius muscle, and the skin flaps of the hind limb.

The hindquarters of the rat were perfused, as described by Feldberg and Mongar (1954), through the abdominal aorta, and the effluent was collected from the vena cava. For perfusion of the hind limb two methods were used : in the first, the perfusion fluid was introduced directly through the femoral artery, and the limb, which was ligated as high as possible, was severed from the animal and transferred to a paraffin bath at 37° C. ; in the second, a long polythene arterial cannula was introduced through the abdominal aorta and passed into the femoral artery, the limb being perfused *in situ* and kept warm with cotton-wool pads soaked in warm paraffin. In both methods the effluent was collected from the femoral vein. In some of these experiments on the perfused limb the skin was carefully removed using a thermocautery, after ligating and cutting the main saphenous vessels.

The gastrocnemius muscle was perfused through the femoral artery and the effluent collected from the femoral vein. The muscle was prepared as described for the cat gastrocnemius by Feldberg and Paton (1951).

The skin perfused was the area supplied by the superficial epigastric artery. In the rat this artery supplies an area of skin on the upper hind leg and part of the adjacent abdomen and flank. In the first experiments the method described by Feldberg and Paton (1951) for the cat's skin flap was used. This method has the disadvantage that in the rat the skin flap when removed from the limb becomes very small owing to strong contraction. In later experiments therefore the area of skin was neither removed from the leg nor cut, but its blood circulation was separated from that of neighbouring skin areas by cauterizing the circumference of the area. The femoral and saphenous vessels were then ligated beyond the origin of the saphenous vessels in the popliteal region. For this purpose the skin was cut with a thermocautery from the popliteal region to the ankle. The perfusion fluid was introduced through a cannula in the femoral artery after all side branches between the abdominal aorta and the origin of the superficial epigastric were ligated. The venous effluent was collected from the femoral vein, all branches from the muscles being ligated. Evans blue, injected intra-arterially at the end of the perfusion, marked the area of skin that was perfused. Evans blue was also injected at the end of all other perfusion experiments of the rat tissues in order to estimate the extent of perfusion.

Perfusion of Tissues from Species Other Than the Rat.—Rabbits were anaesthetized with urethane (1.25 g./kg. i.v.), cats and dogs with sodium pentobarbitone (40 mg./kg. i.p.). In dogs the limbs, in rabbits the hindquarters, and in cats the hindquarters, the gastrocnemius muscles and skin flaps from the hind limb, were perfused. The perfusion of the hindquarters was the same as that described for rats. The skin flaps and gastrocnemius muscles in cats were perfused as described by Feldberg and Paton (1951). The hind limb of the dog was perfused through the femoral artery and the effluent collected from the femoral vein. The limb was ligated, severed from the animal, and kept warm with cotton-wool pads soaked in warm paraffin.

*Beit Memorial Research Fellow.

Assay of Perfusate.—Samples of perfusate were tested on the atropinized guinea-pig's ileum, and on the atropinized colon and uterus of rat, suspended in a 15 ml. bath. The suspension fluid for the guinea-pig's ileum was Mg free Tyrode solution and for the rat's tissues de Jalon solution. Bath temperature for the guinea-pig's ileum was 34° C., for the rat's colon 24° C., and for the rat's uterus 29–30° C. Histamine was assayed on the guinea-pig's ileum and 5-HT on the atropinized rat's colon; all values refer to base.

Chromatographic Analysis.—The 5-HT in the perfusate was identified by paper chromatography. The substance was extracted three times with acetone (5 ml.) from freeze dried perfusate (50 ml.). The extract was evaporated in a current of air to a small volume, transferred to a Whatman No. 1 filter paper and chromatographed using butanol-acetic acid-water (5:1:4). Identification was by the method of Jepson and Stevens (1953), which consists of spraying the strip with ninhydrin reagent, heating at 90–100° C. for 2 to 3 min., and examining under ultra-violet light for fluorescence. Comparison was made with a sample of 5-hydroxytryptamine creatinine sulphate.

RESULTS

Perfusion of the Rat's Hindquarters

Perfusate collected from the hindquarters of the rat after injection of compound 48/80 contracted not only the guinea-pig's ileum but also the atropinized rat's uterus which is insensitive to histamine (Fig. 1d). The contraction of the rat's uterus was not due to the presence in the perfusate of smooth muscle contracting polypeptides such as bradykinin or substance P, as shown by the following experiment: Both bradykinin and substance P are readily destroyed by chymotrypsin, whereas the activity in the perfusate remained unaffected after incubation with chymotrypsin under similar conditions. The effect of treatment with chymotrypsin on bradykinin and perfusate is illustrated in Fig. 1. On the other hand the contractions of the rat's

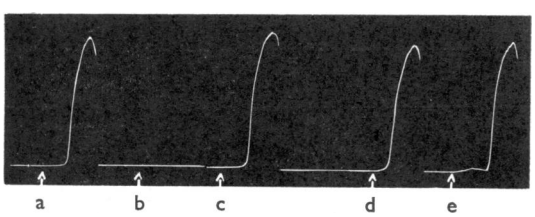

FIG. 1.—Rat uterus suspended in 15 ml. de Jalon solution; 2 μg. atropine added at each washing. At (a) and (c) response to 5 μg. bradykinin and at (b) 5 μg. bradykinin after incubation with 100 μg. chymotrypsin at 37° C. for 5 min. Responses to 0·8 ml. perfusate alone at (d) and after incubation with 100 μg. chymotrypsin at (e).

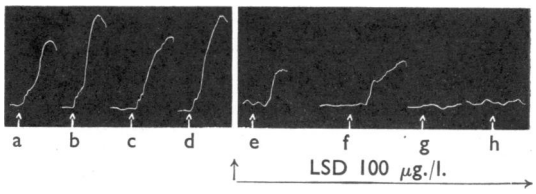

FIG. 2.—Rat colon suspended in 15 ml. of de Jalon solution at 24° C. After each washing 2 μg. atropine added. Additions to the organ bath were made at 5 min. intervals. At (a) 0·03 μg. and at (d), (e), and (g) 0·04 μg. 5-HT creatinine sulphate; at (c) 0·4 ml. and at (b), (f), and (h) 0·5 ml. of 1:10 perfusate collected after 100 μg. 48/80. During (e), (f), (g), and (h) 1·5 μg. lysergic acid diethylamide added at each washing.

uterus produced by the perfusate were antagonized by dihydroergotamine and by lysergic acid diethylamide, which are antagonists of 5-HT. In addition the perfusate collected after 48/80 also contracted the isolated rat's colon, which is insensitive to histamine but sensitive to 5-HT, and again the contractions produced by perfusate were antagonized by lysergic acid diethylamide. In the experiment of Fig. 2 the responses of the rat colon to equiactive doses of 5-HT and perfusate were reduced to the same extent, or abolished, in the presence of lysergic acid diethylamide 100 μg./l. Dihydroergotamine (100 μg./l.) had the same effect.

These results with dihydroergotamine and lysergic acid diethylamide suggested that the active substance in the perfusate was 5-HT. This was confirmed by chromatographic analysis. In these experiments the fluorescence characteristics described by Jepson and Stevens for 5-HT were fulfilled except that the activity in the perfusate flowed slightly faster than 5-HT. The R_F for the perfusate was 0.49, and for the synthetic 5-HT 0.43. This difference was not due to the synthetic drug being the creatinine sulphate and the liberated 5-HT a different salt, but to the presence of some interfering substances in the perfusate. This is shown by the following experiment: Samples of perfusate collected before (sample A) and after (sample B) injection of 100 μg. 48/80 were assayed against 5-HT. Sample A contained the equivalent of 0.1 μg. and sample B the equivalent of 5 μg. of 5-HT. Before extraction with acetone 5 μg. of synthetic 5-HT was added to sample A and both samples prepared for chromatography. In this experiment the R_F value for 5-HT in sample A was 0.50 and in sample B 0.49. One part of sample A chromatographed without adding 5-HT gave no fluorescent spot.

The biological and chemical tests thus both allow the conclusion that the substance contracting the smooth muscle tissues of the rat was 5-HT.

Comparison of the Release of Histamine and 5-HT.—The effluent collected from the rat's hindquarters before injection of 48/80 usually caused no contraction of the atropinized rat's colon when 1 ml. was added to the 15 ml. organ bath. The preparation of the colon was such that it contracted to 0.005 μg. or 0.01 μg. 5-HT. In one experiment the activity of 1 ml. perfusate corresponded to 0.01 μg. and in another to 0.02 μg. 5-HT. After the injection of 100 μg. of 48/80 the activity of 1 ml. effluent was sometimes equivalent to 0.6 μg.

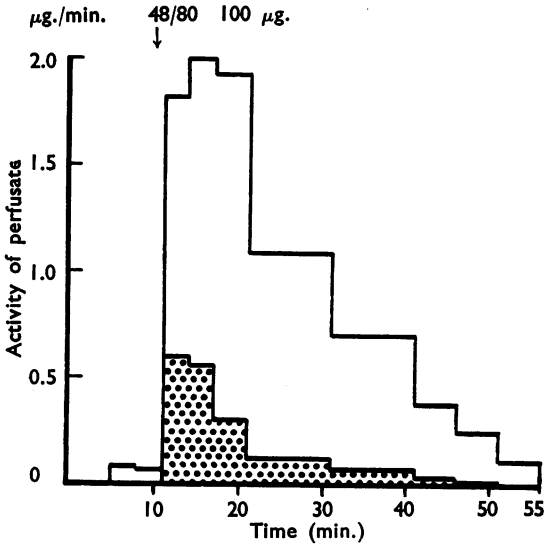

FIG. 3.—Comparison of the release of 5-HT and histamine by 100 μg. 48/80 injected intra-arterially to the perfused rat hindquarters. The open blocks represent histamine and shaded blocks represent 5-HT. The total outputs were 41 μg. histamine and 6 μg. 5-HT.

The time course of the liberation of 5-HT resembled that of histamine in that the maximum release occurred in the first samples collected after an injection of 48/80 and then declined during the following hour. This is shown in the experiment of Fig. 3, wherein the total amounts liberated were 6 μg. 5-HT and 41 μg. histamine. However, Fig. 4 shows that the release of 5-HT by 48/80 is more sudden than that of histamine. In the figure the percentage total output of both substances has been plotted against time. The results are from five experiments after the injection of 5 to 100 μg. 48/80. The scatter is large, but there is a significant difference between the values denoting 5-HT and those denoting histamine. Thus 5-HT appears to be released more suddenly than histamine since the rate of washing out from the site of release must be the same for both substances.

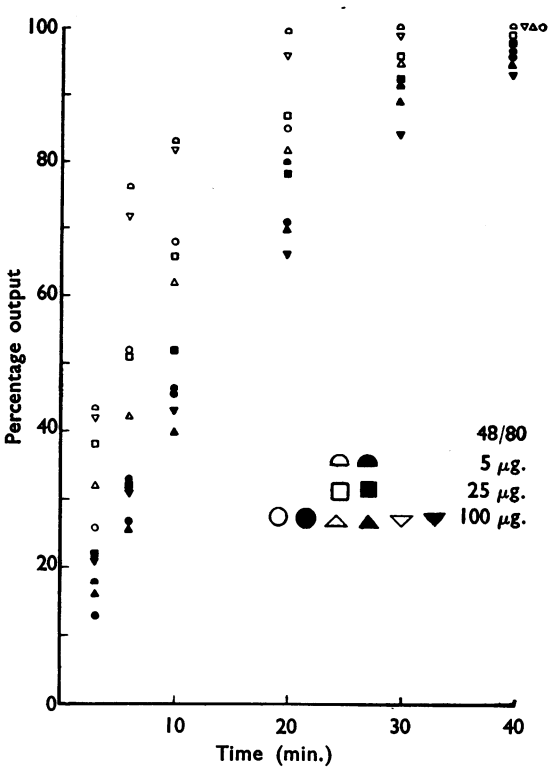

FIG. 4.—Comparison of the output of 5-HT and histamine from five perfused rat's hindquarters after injections of 5–100 μg. 48/80. The ordinates are percentages of total output of 5-HT and of histamine, the abscissae time in minutes. The open symbols (○ △ ▽ □ ⌓) represent 5-HT values and the closed symbols (● ▲ ▼ ■ ◓) histamine values.

Compound 48/80 released greater amounts of histamine than of 5-HT. This is shown in Table I. Usually the amount of 5-HT released was about 1/10 of that of histamine. With repeated injections of 48/80 the amounts of histamine released are

TABLE I

THE RELEASE OF HISTAMINE AND 5-HYDROXYTRYPTAMINE FROM THE PERFUSED HINDQUARTERS OF THE RAT

Compound Injected	Output of 5-HT (μg.)	Output of Histamine (μg.)
48/80:		
5 μg.	1·8	25
5 ,,	2·8	23
5 ,,	3·0	35
25 ,,	3·8	26
25 ,,	3·8	40
100 ,,	3·5	55
100 ,,	4·0	41
100 ,,	6·0	41
Propamidine, 5 mg.	5·0	38
Morphine sulphate, 1 mg.	2·8	1·5
5 ,,	8·1	2·2
10 ,,	10·2	3·4

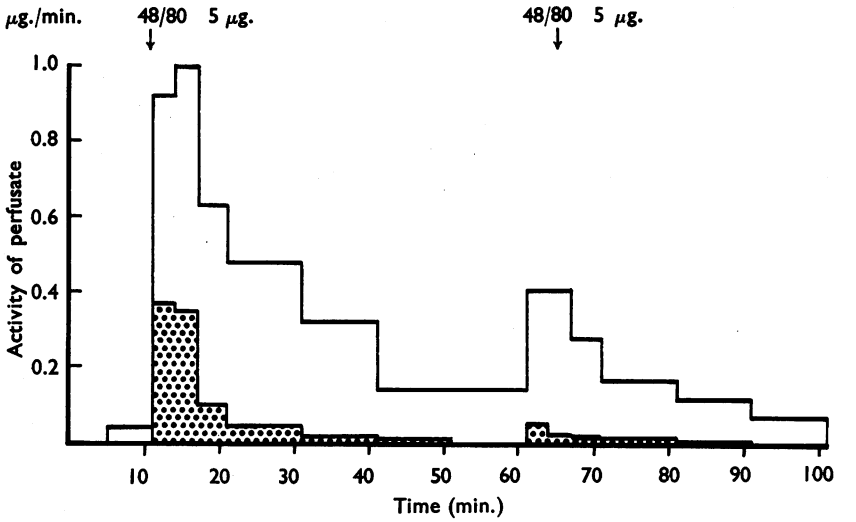

FIG. 5.—Comparison of the release of histamine and of 5-HT from the perfused rat's hindquarters after two arterial injections of 5 μg. 48/80. The open blocks represent histamine and the shaded blocks 5-HT. The total amounts liberated after the two injections were 23 μg. and 7·2 μg. of histamine and 2·8 μg. and 0·8 μg. of 5-HT.

known to decrease. This phenomenon also occurred in respect of 5-HT. In experiments in which 5 μg. of 48/80 was injected twice at an interval of 60 min. the amount of both histamine and of 5-HT released by the second injection of 48/80 was considerably less. Such an experiment is illustrated in Fig. 5. The amounts of histamine released after the two injections were 23 μg. and 7.2 μg.; the corresponding values for 5-HT were 2.8 μg. and 0.8 μg.

Other Histamine Liberators.—5-HT is released not only by 48/80 but also by propamidine and morphine. Propamidine acted like 48/80 in that the amounts of 5-HT released were smaller than those of histamine: with morphine the result was different, in each experiment the amount of 5-HT released was greater (see Table I).

Histamine.—Histamine caused no release of 5-HT; 25 μg. histamine was injected into the perfusion stream, and although the injected histamine was recovered in the venous effluent it did not contract the atropinized rat's colon and therefore did not contain 5-HT.

Rat Tissues from which 5-HT is Released

The technique of perfusion of the rat's hindquarters was such that the perfusion included the bladder, seminal vesicles and the testicles or the uterus. The 5-HT, however, did not originate from these urogenital organs, because, when both limbs were ligated as high as possible to minimize the amount of skin and muscle tissue being perfused, much smaller amounts of 5-HT, as well as histamine, were released by 100 μg. of 48/80, whereas removal of the urogenital organs before the injection of 48/80 did not reduce the release of either the 5-HT or the histamine from the perfused hindquarters. In addition, both substances were released from perfusions of single hind limbs.

A number of different experiments were performed to distinguish between skin and muscle as the source of the released 5-HT. The amounts of 5-HT released from the perfused isolated limb by 25 μg. 48/80 varied between 0.8 and 2.5 μg., and the amount of histamine between 15 and 25 μg. In one experiment in which the limb was skinned down to the ankle, but the foot—which could not be easily skinned—not tied off, the amounts of 5-HT and histamine released by 25 μg. 48/80 were of the same order as the amounts released from the unskinned limb—1.8 μg. 5-HT and 18 μg. histamine. However, when the foot was tied off from the skinned limb, in subsequent experiments, so as to obtain absolutely skin-free preparations, the amounts of 5-HT and histamine released were greatly reduced. Between 0.05 and 0.35 μg. of 5-HT was released in these experiments by 25 μg. 48/80; the amount of histamine released varied between 1 and 5 μg. These experiments show that not only is the skin the main source of the 5-HT released, but that a large proportion of the 5-HT comes from the skin of the feet.

In other experiments the gastrocnemius muscle and skin were perfused separately. The amount of 5-HT released from the perfused gastrocnemius muscle was only 0.005 μg./g. muscle, but from the perfused skin the amount calculated to be released was 0.1 μg./g. This value is probably too low, because, during the rigorous operative pro-

cedures necessary for isolating the skin, vascular damage rendered many small areas of skin inaccessible to the perfusion fluid so that in every experiment the estimated weight of tissue perfused was an overestimate. It is interesting that in these experiments the amount of histamine released from muscle, 0.75 μg./g., was almost as much as that released from skin, 0.8 μg./g.

Further evidence that the skin is the main source of the 5-HT was afforded by the fact that when a patch of skin was soaked *in vitro* in saline containing 48/80 both histamine and 5-HT were released. In one such experiment in which a patch of skin was soaked in a 1 in 1,000 48/80 solution at room temperature 2.3 μg. 5-HT and 13.3 μg. of histamine per gram tissue were released.

It is known that 48/80 releases histamine from the mast cells which abound in the skin and subcutaneous tissue, and it has been reported that the mast cells of the rat contain 5-HT as well (Benditt *et al.*, 1955). The following facts are in accord with the conclusion that the 5-HT released by 48/80 is derived from the mast cells:

(*a*) In the experiment in which the patch of skin had been soaked in 48/80 and had released large amounts of 5-HT and histamine, the underlying subcutaneous tissue when examined histologically showed that the majority of the mast cells were disrupted.

(*b*) The skin of the foot which is particularly rich in mast cells proved to be a main source of released 5-HT.

(*c*) Subcutaneous tissue which is also known to be rich in mast cells releases both histamine and 5-HT when incubated with 48/80. Table II shows the amounts of 5-HT and histamine released in these experiments during 30 min. incubation.

TABLE II

RELEASE OF 5-HYDROXYTRYPTAMINE AND HISTAMINE FROM SUBCUTANEOUS TISSUE INCUBATED WITH 48/80

48/80 (μg./ml.)	5-HT (μg./g.)	Hist. (μg./g.)
0	0·4	3
25	0·7	5
500	1·7	15

The results in Table II show that there is a relatively large spontaneous release of 5-HT and histamine from the subcutaneous tissue, probably as the result of injury during the dissection leading to disruption of some mast cells which could be seen with histological examination. The amounts released when subcutaneous tissue was incubated with 25 μg./ml. 48/80 may therefore have been due to such "spontaneous" release. The much larger amounts released by 500 μg./ml. 48/80, however, must be attributed to an action of this compound on the mast cells most of which, on histological examination, were found to be disrupted.

TABLE III

THE RELEASE OF HISTAMINE AND 5-HYDROXYTRYPTAMINE BY 48/80 FROM PERFUSED TISSUES OF CATS, DOGS, RABBITS AND RATS

Preparation	48/80 Injected (μg.)	Output of 5-HT (μg.)	Output of Histamine (μg.)
Cat hindquarters	100	0	30
	200	0	38
Cat skin	1	0	20
	5	0	26
Cat gastrocnemius muscle	25	0	6·5
Dog hind limb	500	0	10
Rabbit hindquarters	200	0	1
	500	0	0·5
Rat hindquarters	5	2·6	28
	25	3·8	33
	100	4·5	46
Rat hind limb	25	1·8	22

Perfusion of Rabbit's, Cat's and Dog's Tissues

Compound 48/80 releases histamine but no 5-HT from the perfused cat's, dog's and rabbit's tissues. This is shown in Table III. For comparison the mean amounts of 5-HT and histamine released from the perfused rat's tissues are included at the bottom of the table.

DISCUSSION

The present experiments show that, in rats, histamine is not the only smooth muscle stimulating substance released by histamine liberators, but that these liberate 5-HT as well. No evidence was obtained for the release of 5-HT by 48/80 from perfused tissues of dogs, cats or rabbits. It may therefore be that the release of 5-HT by histamine liberators is so small as not to be detected by the methods of assay used in the present experiments, or is specific for the rat, or at least not common for all laboratory species.

It is generally assumed, from the many observations which started with those of Riley and West (1952, 1953, 1955), that the mast cells are the main source of histamine in many, although not in all, tissues and that histamine liberators disrupt the mast cells and in doing so release their histamine (Riley, 1953). The recent report of Benditt *et al.* (1955) that the mast cells in the rat contain both histamine and 5-HT would suggest that the 5-HT released by histamine liberators in this species is also the result of disruption of the mast cells. The

present observation, that the skin and subcutaneous tissue, and particularly the skin of the feet, contribute to a large extent to the release of 5-HT after compound 48/80, is in accord with this conclusion, because mast cells abound in these rat tissues. If it should prove that the 5-HT and the histamine released by 48/80 and other histamine liberators originate solely from the mast cells it may be justified to use the term "mast-cell depleters" or perhaps even "mast-cell disruptors" instead of histamine liberators. The failure to obtain evidence for the release of 5-HT in cats, dogs and rabbits may be because the mast cells in these species do not contain 5-HT.

Paton (1951) has shown that in the cat 48/80 releases an unidentified slow contracting substance. No evidence for such a release was obtained in the present experiments on perfused cat's hindquarters, skin and muscles, but this does not exclude the possibility that the unidentified slow contracting substance obtained by Paton in the circulating blood of cats after intravenous injection of 48/80 was released from mast cells of other tissues in the body.

5-HT is apparently not a constituent of the mast cells of all species. Our results further indicate that in the rat the mast cells of various tissues contain histamine and 5-HT in different proportions. For instance, about the same amounts of histamine were released by 48/80 from the perfused gastrocnemius and skin of the rat—0.75 μg. and 0.8 μg./g. tissue—whereas the amounts of 5-HT released in the same experiments were 0.005 μg. and 0.1 μg./g. tissue. This suggests that the mast cells of skeletal muscle contain much less 5-HT in relation to histamine than the mast cells of the skin. Our results with morphine and 48/80 show that the proportion of histamine and 5-HT released from the same tissue by different histamine liberators varies. For instance, morphine released more 5-HT than histamine from the perfused hindquarters, whereas 48/80 released more histamine than 5-HT. Thus the two smooth muscle stimulating substances assumed to reside in the mast cells of rat tissue show a different sensitivity for their release by different histamine liberators or "mast cell depleters."

After 48/80 the maximal release of 5-HT occurred earlier than that of histamine. This suggests a somewhat different mechanism for the release of these two substances and raises the question whether the release of the one is dependent upon, or even influenced by, the release of the other.

The release of 5-HT cannot be accounted for by the action of the histamine released by injected 48/80; histamine did not liberate 5-HT. Feldberg and Smith (1953), on the other hand, found that large doses of tryptamine and 5-HT liberated histamine; they considered the possibility that the release of histamine might in some way be preceded by the release of 5-HT. In this connexion the present finding that the release of 5-HT preceded that of histamine is of interest. Nevertheless, it would be unjustified to assume from this fact alone that the release of histamine is dependent on that of 5-HT, since histamine is also released by 48/80 in those species in which there is no release of 5-HT.

It is known from the work of Jorpes (1946) that mast cells contain heparin and that in dogs their disruption releases heparin and renders the blood incoagulable. In rats the disruption of mast cells evoked by either anaphylactic or peptone shock or compound 48/80 does not change the blood coagulation time (Mota, Beraldo, and Junqueira, 1953). Riley, Shepherd, West, and Stroud (1955) have recently taken up this problem and have shown that in rats treated with 48/80 the tissues lose not only their histamine but their heparin as well, although in different proportions; a 95% loss of histamine was associated with a 53% loss of heparin, but this loss of heparin was not accompanied by any sign of its appearance in the circulating blood. It may well be, as suggested by Riley *et al.* (1955), that heparin in this species is concerned with events in the tissues rather than with the coagulability of the blood. The finding that histamine and heparin are not released to the same extent by 48/80 may be another instance in which two substances which reside in the mast cells of rat tissue show different sensitivity for their release by 48/80. As stated by Mota *et al.* (1953), the way histamine may be bound to the mast cell's cytoplasm and its relation to heparin constitutes a challenging problem.

No experiments have been performed to find out whether 48/80 depleted the rat's skin of its 5-HT as it depletes its histamine, nor do we know the exact amounts of 5-HT present in rat tissue. Feldberg and Smith (1953) stated, in their paper on the release of histamine by tryptamine and 5-HT, that acid saline extracts of rat's skin contracted the isolated rat's colon and that this activity corresponded to 10–20 μg. of tryptamine/g. tissue. It seems not unreasonable to assume that the unidentified rat colon-contracting substance of their skin extracts was 5-HT. Since 5-HT is about 50 times as active on the rat colon as tryptamine, the activity of the acid saline extracts of the rat's skin would correspond to 0.2 to 0.4 μg./g. tissue of

5-HT, amounts of the same order as those released by 48/80 from rat's skin.

Reserpine causes a reduction of 5-HT in the intestine and central nervous system (Brodie, Pletscher, and Shore, 1955; Pletscher, Shore, and Brodie, 1955). It is not known whether the 5-HT in these tissues resides in mast cells, the reserpine acting as a "mast cell depleter." The histamine in the intestinal mucosa is mainly resistant to histamine liberators and is apparently not of mast cell origin; if this also applies to the 5-HT in the intestinal wall and in the central nervous system, its release from these tissues by reserpine would be present an entirely different phenomenon.

Summary

1. The histamine liberators compound 48/80, propamidine, and morphine release from the perfused rat's tissues histamine and another smooth muscle stimulating substance which has been identified as 5-hydroxytryptamine (5-HT).

2. No evidence could be obtained for the release of 5-HT by 48/80 from the perfused tissues of cats, dogs and rabbits.

3. It is concluded that the 5-HT released by histamine liberators, like the released histamine and heparin, originates in the mast cells and is released from them.

4. It may become necessary in future to use a terminology which makes it clear that histamine liberators which act on the mast cells also release from them substances other than histamine. "Mast-cell depleters" is suggested as an appropriate term.

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