J. Physiol. (1957) 137, 51–65

SPECIES DIFFERENCES IN SUSCEPTIBILITY TO CAPILLARY PERMEABILITY FACTORS: HISTAMINE, 5-HYDROXY-TRYPTAMINE AND COMPOUND 48/80

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(*Received* 18 *January* 1957)

Rowley & Benditt (1956) recently demonstrated that the subcutaneous injection of small doses of 5-hydroxytryptamine (5-HT) into rats with a vital dye in their circulation induced a striking increase in capillary permeability as indicated by the local exudation of the dye. Moreover, 5-HT was about 200 times as effective as histamine in inducing oedema of the rat paw. Rowley & Benditt, who found no evidence in support of Feldberg & Smith's (1953) conclusion that 5-HT was a histamine-liberator, also suggested that it would be remarkable if the high permeability-increasing activity of 5-HT was 'unique to the rat'. In our own studies of permeability factors (P.F.s) in the depilated skin of guinea-pigs, rats and rabbits, by the dye-exudation technique, the potency of both histamine and the α-globulin permeability factor of guinea-pig serum (Wilhelm, Miles & Mackay, 1955) varied substantially with the species of test animal.

We have accordingly studied the P.F. potency of 5-HT in the three species: to confirm Rowley & Benditt's (1956) demonstration of the potent permeability effect of 5-HT; to determine whether their generalization to other species about this permeability effect was justified; and to find whether the 5-HT effect was mediated by histamine. Histamine and the histamine-liberator, compound 48/80 (Paton, 1951), were included in the investigation.

MATERIALS AND METHODS

Guinea-pigs. Albino guinea-pigs weighing 450–600 g were used. After depilation with a barium sulphide paste, each animal received 60 mg Pontamine Sky Blue 6X (G. T. Gurr) per kg body weight given intravenously in a 5% solution in 0·425% saline. Animals so injected are referred to as 'blued' and were used for tests without delay.

Test substances were injected intracutaneously into the depilated skin of the trunk. In the animal held moderately extended so that the spine was straight, the test area was bounded anteriorly by the plane perpendicular to the spine that passes through the base of the xiphisternum, posteriorly by a similar plane that passes through the lumbo-sacral joint, and ventrally by lines 3–4 cm from the ventral mid line. All solutions for injection were made up in 0·85% saline, which by itself induces no blueing. A standard volume of 0·1 ml. was injected with short

bevel no. 26 gauge needles, which induced small pale areas of traumatic blueing 1–4 mm in diameter. The lesions induced by test preparations were recorded 30 min later. The method of making tests in guinea-pigs is described in detail by Miles & Wilhelm (1955).

Rats. Albino rats weighing 220–320 g were used. Since even minimal anaesthesia considerably reduced the subsequent response of the skin capillaries to P.F.s, the rats were tied belly downwards by their legs for depilation, and for intravenous and intracutaneous injections. The success of this technique, without anaesthesia, largely depends on having tame, frequently handled rats. Partly tame animals struggle and become exhausted; consequently their skins react poorly and inconsistently to P.F.s.

Rats were depilated in the same manner as guinea-pigs, and kept warm until tested. Each rat received 60 mg of Pontamine Blue per kg body weight as a 2·5% solution in 0·6% saline, into an exposed vein on the inner side of the upper thigh. Intracutaneous injections, in 4 rows of 4–5 injections, were given as for guinea-pigs; within the same area of skin, the homogeneity of the response to P.F.s was satisfactory. In general, saline induced lesions less than 4 mm in diameter.

The best results were obtained with rats of a large, rapidly growing strain, which reached an optimal weight of 230–280 g before their skins became too tough. Thirty minutes after injection, the rats were killed for measurement of their lesions. Surface measurements of lesions were satisfactory, but the results in inhibition tests were checked by measuring the lesions on the under surface of the reflected skin (cf. Brocklehurst, Humphrey & Perry, 1955).

Rabbits. Non-moulting (see Whiteley, 1956) New Zealand Red Rabbits, heavier than 2·5 kg body wt., were used. The skin of the trunk was closely clipped 1–2 days previously but not depilated, and the rabbits were kept warm until tested. Rabbits received the same dose of 5% Pontamine Blue as guinea-pigs, into a marginal ear vein.

The response of rabbit skin to P.F.s was not homogeneous. The skin of the back was preferable to that of the belly. On the back the response declined considerably from the lumbosacral junction to the upper thoracic level. Each rabbit was moderately extended and a line drawn transversely across its back, just behind the posterior rib. On each side of the animal's back, 3 vertical rows each of 8 injections were made, 4 injections being above and 4 below the transverse line. To counter the heterogeneous response of the skin, each preparation was tested on both sides of each rabbit and injected so that the first injection on the left side was the mirror image of the second on the right side; e.g. on the left side, the top injection of the outside row corresponded, on the right, with the bottom injection of the inside row. The lesions were measured after 30–40 min. Wiping the test area of skin with a pad of cotton-wool soaked in liquid paraffin made it appear as clean as if depilated. Each recorded lesion-diameter is the mean of at least 4 lesions in 2 rabbits, and often of 8 lesions in 4 rabbits. Saline injections did not induce lesions greater than 2·5 mm in diameter.

Perfusion of rat hind quarters

Female rats weighing 200–250 g were anaesthetized with pentobarbitone sodium (40 mg/kg) given intraperitoneally. The alimentary canal, uterus and ovaries were ligated and excised, and the hind quarters perfused with oxygenated Locke's solution at 37° C by the method of Feldberg & Mongar (1954). The kidneys were excluded from the preparation by the abdominal ligature. Test substances were injected through the cannula in the abdominal aorta. The wt. of the hind quarters was taken as that of the whole animal minus that of the excised fore-quarters and the removed viscera.

Incubation of rat skin

From each rat, three pieces of skin approx. 10 × 15 mm were excised from behind and between the ears (see Feldberg & Miles, 1953). After removal of the loose subcutaneous tissue, each piece was weighed and then rinsed in Locke's solution. One piece of skin was placed in each of three 15 ml. flasks containing 10 ml. Locke's solution. Appropriate concentrations of 5-HT and 48/80 were added to the second and third flasks. After incubation of the preparations in a water-bath at 37° C for 15 min, the pieces of skin were removed and the fluid tested for histamine (see Schild, 1939).

Methods of assay

The perfusates and the solutions in which the pieces of skin had been incubated were assayed for histamine on the atropinized guinea-pig's ileum suspended in oxygenated Mg-free Tyrode solution in a 17 ml. bath at 34° C. Because 5-HT contracts the guinea-pig's ileum, appropriate samples were first assayed for their 5-HT content on the atropinized rat's uterus suspended in oxygenated de Jalon's solution in a 10 ml. bath at 29–30° C.

For the histamine assay, the samples containing 5-HT were tested against a standard solution of histamine to which was added the appropriate concentration of 5-HT to compensate for its action on the guinea-pig's ileum.

Test preparations

Unless stated otherwise, all substances were diluted in 0·85% saline. Histamine was used as the acid phosphate (British Drug Houses Ltd.) and 5-HT as creatinine sulphate (Upjohn Co.); amounts of each are cited as base. Compound 48/80 was obtained from the Wellcome Research Laboratories, U.S.A., and lysergic acid diethylamide (LSD) from Sandoz Products Ltd., London. Mepyramine maleate was supplied by May and Baker Ltd. Unless otherwise stated, the recorded doses of substances tested intracutaneously are the amounts in the standard injection volume of 0·1 ml.; i.e. one-tenth of the concentration per ml.

RESULTS

The permeability-increasing potency of histamine, 5-HT and 48/80

In guinea-pigs and rabbits, histamine was used as a standard P.F. preparation. In rats, a globulin fraction, G2/1R, prepared from guinea-pig serum (see Wilhelm *et al.* 1955; D. L. Wilhelm, P. J. Mill & A. A. Miles, unpublished) gave a more consistent dosage response than histamine. As with all these P.F.s, both substances induced lesions whose diameter was linear with respect to log. concentration within the range of 5–10 mm; but in the rat the slope for histamine tended to become steeper below the 5 mm level. For this reason, G2/1R was preferred as a standard P.F. in tests in rats. Its potency in terms of histamine, estimated from four satisfactory assays, was 0·78.

Fig. 1 exemplifies the tests of histamine, 5-HT and 48/80, and Table 1 summarizes the estimates of potency of 5-HT and 48/80 for each species in

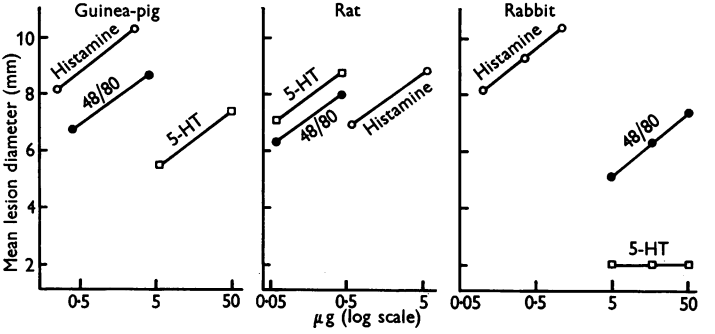

Fig. 1. The relative permeability-increasing potency of histamine, 5-hydroxytryptamine and compound 48/80 in the guinea-pig, rat and rabbit. In Figs. 1–5, the doses indicated are those in the standard intracutaneous injection volume of 0·1 ml.

terms of histamine. The outstanding features of the table are the very low potency of 5-HT in the guinea-pig and rabbit, and the very low potency of 48/80 in the rabbit.

In the rabbit, doses of 48/80 greater than 1 μg induced pale purple lesions. When the reflected skin was inspected from the under surface in daylight, the small blood vessels within the lesion were outlined in a dark blue colour. Microscopically the endothelium of many of the capillaries and venules was covered by a thin layer of dye and moderate numbers of pale blue platelet thrombi, suggesting that, although the 48/80 may have increased the porosity of the vessel wall to the extent that it clearly does in other animals, the consequent exudation of dye was prevented by the layer precipitated on the endothelium. There was no evidence of extravascular dye, and the colour of these lesions was apparently due to this intravascular precipitation. It is noteworthy that similar doses of 48/80, in guinea-pigs, cause thrombosis of the blood vessels at the centre of the lesion (Miles & Miles, 1952).

TABLE 1. The potency, relative to histamine, of various permeability factors in the guinea-pig, rat and rabbit

	Test animal		
Permeability factor	Guinea-pig	Rat	Rabbit
Histamine	1·0	(1·0)*	1·0
5-Hydroxytryptamine	0·002	11·5	<0·0005
Compound 48/80	0·11	4·8	0·0008
Guinea-pig G2/1R	—	0·78	—

* In rats, histamine is the theoretical standard and G2/1R was the actual standard; see text.

The results in Table 1 are comparable within only each species tested. For interspecies comparison, the potencies are expressed in Table 2 as Effective Blueing Doses (E.B.D.) per mg of substance. The E.B.D. (Miles & Wilhelm, 1955) is defined as the amount of substance in the standard injection volume of 0·1 ml. that on the average induces, within 30 min, a 6 mm lesion in 'blued' animals. The E.B.D. is determined from the plots of assays like that in Fig. 1, and can be used with some confidence as an expression of comparative potency, because the slopes of the dosage responses for each substance are substantially linear and parallel. Thus from the means of experiments in 104 guinea-pigs and 23 rabbits, one E.B.D. of histamine is respectively 0·031 and 0·027 μg. From the means of experiments in 116 rats, one E.B.D. of G2/1R is 0·92 μg. Since G2/1R has 0·78 times the P.F. potency of histamine, one E.B.D. of histamine in the rat is $0{\cdot}92 \times 0{\cdot}78 = 0{\cdot}72$ μg. From these results, and the data in Table 1, the number of E.B.D.'s in 1 mg of each P.F. can be calculated for each species (Table 2).

Histamine clearly is the most potent P.F. in guinea-pigs and rabbits, being approximately equipotent in the two species; it is about 25 times less potent in rats. Its relatively low potency in rats is not due to an innate unresponsive-

ness of rat skin as a test system for P.F.s in general, because the P.F. potency of 5-HT in rats is of the same order as histamine in guinea-pigs and rabbits. Despite the high P.F. potency of 5-HT in rats, it has a negligible potency in guinea-pigs and rabbits.

TABLE 2. The number of effective blueing doses in 1 mg of histamine, 5-hydroxytryptamine and 48/80 in the guinea-pig, rat and rabbit

	Test animal		
Permeability factor	Guinea-pig	Rat	Rabbit
Histamine	32,260	1,410	37,040
5-Hydroxytryptamine	60	16,240	<20
Compound 48/80	3,550	6,760	30

The mode of action of 5-HT and 48/80 as permeability factors in the rat

Tests were made in rats to determine whether the P.F. activity of 5-HT is due to the release of histamine, and to what extent the P.F. activity of 48/80 is due to the release of histamine (Feldberg & Talesnik, 1953) and of 5-HT (Bhattacharya & Lewis, 1956).

Effect of an antihistamine drug

Mepyramine maleate is a more effective antihistamine drug in guinea-pigs than in rats. In guinea-pigs 20 mg/kg given intraperitoneally 2 hr before testing totally inhibits the P.F. effect of histamine and significantly diminishes that of 48/80 (cf. Miles & Miles, 1952). In rats 50 mg/kg of mepyramine given 2 hr before testing decreased the response to histamine about 400-fold, but the response to 5-HT and 48/80 only threefold; given 45 min before testing (Brocklehurst *et al.* 1955) this dose almost abolished the response to intracutaneous histamine, but was moderately toxic.

Because mepyramine given intraperitoneally was toxic in rats at the dose level required substantially to inhibit 48/80, we tested its effect when injected locally in mixtures with the P.F. preparations. Preparations of mepyramine and of the P.F.s were mixed in equal volumes and tested after 30 min at room temperature. By this method high local doses of inhibitor, as well as control preparations alone, could be tested in the same set of animals, thus minimizing inter-animal variation.

Table 3 shows the plan of such an experiment. Preparations A, B and C represent different P.F.s, or graded concentrations of the same P.F. The mixtures were injected with tuberculin syringes 1–4 in the following order: mixture 1 with syringe 1, 2 with 2, 3 with 3, 4 with 4; then 5 with 1, 6 with 2, 7 with 3, 8 with 4; then 9 with 1 and so on. This sequence, which ensures that large doses of inhibitor are injected last, is necessary to postpone the substantial systemic inhibition that occurs owing to the absorption of part of these large doses into the general circulation. With injection according to the

vertical rows of the scheme, the results were consistently reproducible. Moreover, the control lesions induced by histamine, 5-HT and 48/80 were clearly not affected by the subsequently injected large doses of inhibitor, because they were similar in diameter and colour intensity to those in animals receiving no inhibitor.

The highest non-blueing doses of mepyramine itself were 16·2μg in guinea-pigs and 48·6μg in rats; accordingly, the respective doses of mepyramine tested did not exceed these limits.

TABLE 3. Sequence of injections in tests of the susceptibility of a permeability factor to local inhibitors, which in large intracutaneous doses also have a systemic action

		Final concentration of inhibitor (μg/ml.)				Number of syringe
Permeability factor	Saline	6	18	54	162	
Preparation						
A	1	5	9	13	17	1
B	2	6	10	14	18	2
C	3	7	11	15	19	3
Saline	4	8	12	16	20	4

Guinea-pigs. Increasing doses of mepyramine progressively inhibited histamine and 48/80. Mepyramine 5·4μg inhibited 0·083–2·25μg histamine about sixfold, and completely inhibited 0·2μg. Despite the fact that 48/80 had 1/9th the P.F. potency of histamine, 5·4μg mepyramine inhibited only 0·2μg 48/80, suggesting that 48/80 liberates P.F.s other than histamine. High doses of mepyramine near its blueing dose had a peculiar enhancing effect on 48/80. Thus the lesions from mixtures of 0·6 or 1·8μg 48/80 with 16·2μg mepyramine were larger than those from mixtures containing 5·4μg mepyramine, although 16·2μg mepyramine alone was non-blueing.

This enhancing effect of mepyramine may be due to the fact that it is a histamine liberator. Arunlakshana (1953) showed that the antihistamines diphenhydramine and antazoline liberated histamine from guinea-pig tissues, but she did not test mepyramine as a liberator. Paton & Schachter (1951) reported that the acid gastric secretion induced by 48/80 in dogs was greater after mepyramine treatment, probably because mepyramine liberated histamine. It may also be that released histamine and high doses of mepyramine have a synergic effect as a P.F.; but whatever is the correct explanation of the phenomenon it is noteworthy that Miles & Miles (1952) found that in guinea-pigs, large intravenous doses of mepyramine could not inhibit intracutaneous 48/80 more than three- to fivefold.

Rats. Histamine was much more susceptible to local mepyramine, for 1·8 and 16·2μg histamine were completely inhibited by corresponding doses of mepyramine (see Fig. 2). But only 0·2μg 48/80 was completely inhibited by the highest dose of mepyramine tested, namely 48·6μg, lesser doses giving partial inhibition. Thus 48/80, which in the rat has nearly five times the P.F.

potency of histamine, is about forty times less susceptible to mepyramine. Mepyramine did not reduce the diameter of the lesions induced by larger doses of 48/80, but considerably reduced the intensity of their colour (see Fig. 2).

Small doses of 5-HT equipotent with 48/80 were similarly susceptible to mepyramine; and, as with 48/80, mepyramine reduced the intensity of colour, but not the diameter, of the lesions induced by larger doses of 5-HT (see Fig. 3).

Effects of decrease of skin histamine in rats by treatment with 48/80

The similar susceptibility in rats of 5-HT and 48/80 to local injections of the antihistamine mepyramine maleate led us to test their potency in rats with a decreased histamine content of their skin. Accordingly, seven rats were given a course of intraperitoneal 48/80 (Feldberg & Talesnik, 1953; Brocklehurst *et al.* 1955). They received 0·25, 0·5, 1·0, 1·0, 1·0, 1·5, 2·0 and 2·5 mg/kg body wt. on the 1st, 2nd, 3rd, 5th, 6th, 8th, 9th and 10th days; 2 rats died on the 3rd day and another was killed on the 4th day. By the 11th day the four survivors had lost 10, 10, 30 and 35 g body wt. respectively, while 4 untreated rats gained 10–15 g in this period. On the 11th day the responses of each group to intracutaneous histamine, 5-HT and 48/80 were compared.

In the treated group, the response to histamine was decreased about fourfold, that to 48/80 about eightfold, but that to 5-HT was not substantially affected. The above dosage of 48/80 was lower than that used by Brocklehurst and his colleagues, which proved to be lethal to six out of eight animals from our own stock. Tested on the 11th day, the response of the two survivors of this batch to 5-HT was almost identical with that of two untreated controls, but their response to histamine was reduced about fourfold, and to 48/80 about sevenfold.

The decrease, in these animals, of the P.F. activity of histamine (see Brocklehurst *et al.* 1955) makes it difficult to assess the significance of the results for 48/80 and 5-HT. The P.F. activity of 5-HT is not substantially changed in rats treated with 48/80, and hence the lowered P.F. response to histamine probably is not due to a general non-specific effect, e.g. on peripheral blood pressure, of the 48/80 treatment.

According to Brocklehurst and his colleagues, histamine potency is restored 2 days after the cessation of subcutaneous 48/80, but is unaffected by intraperitoneal 48/80. However, in our tests, since the response to histamine is decreased fourfold, of the sevenfold reduction of the response to intracutaneous 48/80, only about twofold could be directly ascribed to the diminished histamine content of the skin. An outstanding discrepancy in the results is the insusceptibility of the 5-HT response to histamine depletion, since it will be shown that 5-HT releases histamine (p. 61). Without further analysis, it must be assumed that 48/80 and 5-HT liberate histamine from sources differing either in anatomical site or chemical nature.

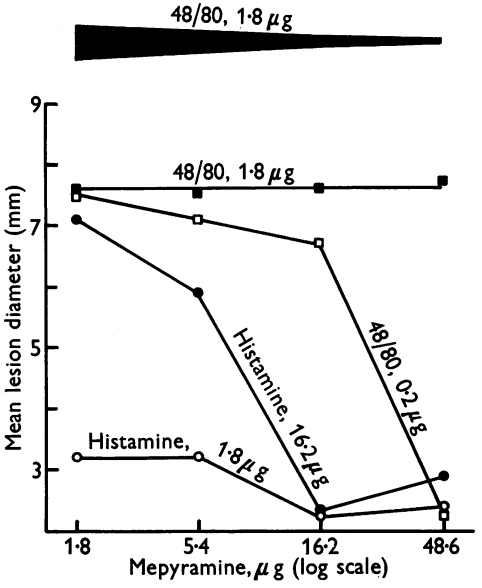

Fig. 2. The local inhibition of histamine and 48/80 by mepyramine maleate in the rat. In this and in Fig. 3, the thickness of the line at the top of the graph indicates the mean colour-intensity of lesions ranging from dark blue to very pale.

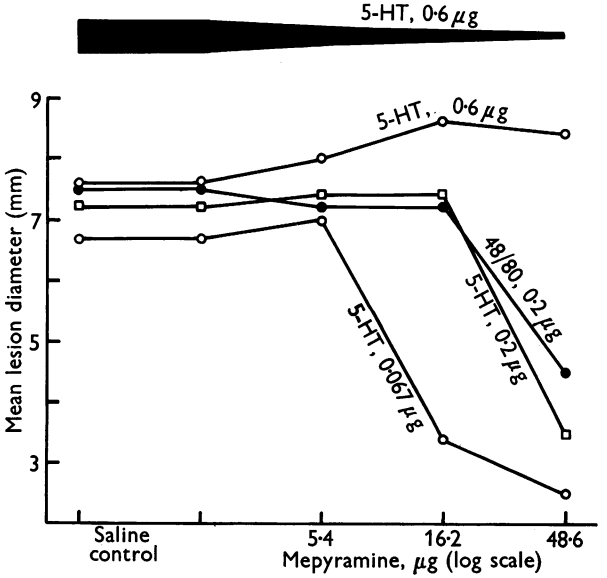

Fig. 3. The local inhibition of 5-hydroxytryptamine and 48/80 by mepyramine maleate in the rat.

Effects of LSD

Since 5-HT had no substantial P.F. potency in guinea-pigs or rabbits, the susceptibility of the various P.F.s to LSD was tested only in rats. In the rat, the L.D.50 of intravenous LSD is 16·5 mg/kg and the maximum tolerated single dose is about 3·2 mg/kg (Rothlin & Cerletti, 1956). We were unable to administer LSD intravenously because as little as 100μg induced immediate gross tremor lasting for about 20 min, and the animals became shocked. As with mepyramine, satisfactory local inhibition was obtained by the injection of mixtures of LSD and P.F. tested after 30 min at room temperature. In such tests, by the time the mixtures containing the highest concentrations of LSD were injected, some of the rats had absorbed sufficient LSD to induce occasional slight tremors.

Fig. 4 records the complete inhibition of 0·067 and 0·6μg 5-HT by 1·8 and 16·2μg LSD. In another experiment (Fig. 5) 0·2μg 5-HT was inhibited by 5·4μg LSD, but an equipotent dose of 48/80, 0·4μg, was only moderately susceptible to LSD and was not completely inhibited by 16·2μg.

Measured on the surface of the skin, the diameter and colour-intensity of the lesions induced by histamine were not affected by LSD up to 16·2μg; when the lesions were measured on the under surface of the skin, the diameter and colour-intensity of the lesions were reduced by 16·2μg LSD, but because these mixtures were last injected (see Table 3), this inhibition was possibly due to the very mild shock induced by the absorbed LSD (cf. Miles & Miles, 1952).

The moderate susceptibility of 48/80 to LSD is sufficient to account for the residual P.F. activity of 48/80 that remains after maximum possible local inhibition by mepyramine, but this explanation is inadequate, because 5-HT is as susceptible as 48/80 to local mepyramine.

The inhibition by mepyramine and LSD in normal rats and the reactions of histamine-depleted rats to the three P.F.s are summarized in Table 4.

TABLE 4. (*a*) Inhibition by locally injected mepyramine and lysergic acid, and (*b*) the effect of reduction of skin histamine, on the activity of various permeability factors in the rat

Permeability factor	Approximate number of E.B.D.s inhibited by 16·2μg	
	Mepyramine	Lysergic acid
(*a*) Histamine	22	<2
5-HT	1	9
48/80	0·5	<6*
	Approximate reduction in P.F. potency in rats treated systematically with 48/80	
(*b*) Histamine	4-fold	
5-HT	1·2-fold	
48/80	8-fold	

* Partial inhibition only.

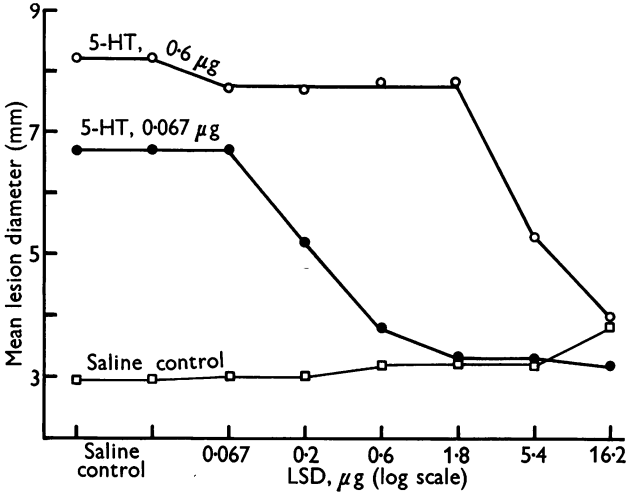

Fig. 4. The local inhibition of 5-hydroxytryptamine by lysergic acid (LSD) in the rat.

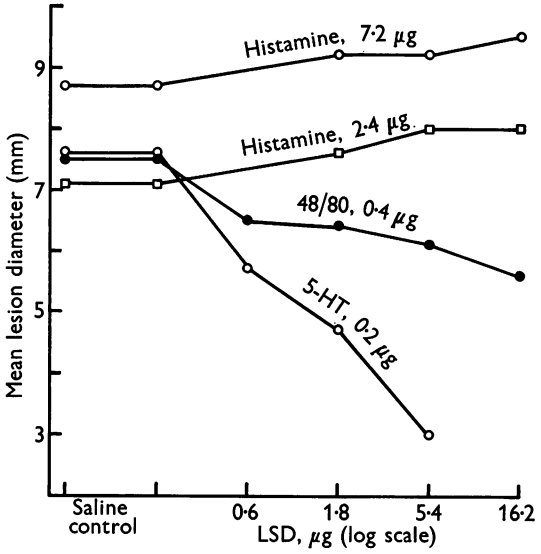

Fig. 5. The effect of LSD on histamine, 5-hydroxytryptamine and 48/80 in the rat.

Perfusion of rat hind quarters

In the perfused rat hind quarters, 5-HT is a potent vasoconstrictor, and within 1–2 min 10μg induced so much vasoconstriction that the volume of perfusate was considerably reduced for at least 10 min. The vasoconstriction produced by 2μg of 5-HT in 0·2 ml. was so small as to reduce the venous outflow only by an insignificant amount. In four experiments, 2μg of 5-HT was

followed 20 min later by 2μg of 48/80. In each, the histamine liberated by 48/80 was 6–10 times greater than that by 5-HT (Table 5 and Fig. 6). After each injection, perfusate was collected at 10 min intervals. In general, after 5-HT each successive sample contained about the same small amount of histamine, but after 48/80 there was more histamine in the second sample than in the first.

TABLE 5. Comparison of the histamine-releasing activity of 2μg of 5-hydroxytryptamine and compound 48/80 during perfusion of the hind quarters of rats

Duration of collection (min) after injection	Histamine released (μg/100 g tissue) by 2μg of		Ratio of histamine released by 5-HT and 48/80
	5-HT	48/80	
20	1·5	14·4	1:10
20	1·2	12·1	1:10
20	1·4	12·6	1:9
20	1·8	10·9	1:6
40	2·7	16·2	1:6

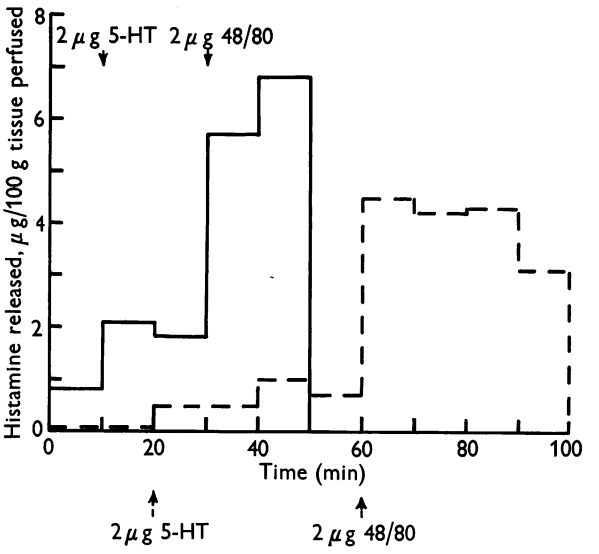

Fig. 6. The release of histamine in the perfused hind quarters of the rat by 5-hydroxytryptamine and 48/80. Two separate tests are recorded, one in solid lines, the other in broken lines.

To exclude the possibility that 5-HT was a more potent histamine liberator after longer intervals, the perfusates in one experiment were each collected for 40 min after the injection of 5-HT and 48/80 (Fig. 6). As before, the release of histamine by 5-HT was still low and steady, and the 48/80 released six times more histamine than the 5-HT.

The perfusate collected after the injection of 48/80 contained 5-HT, but the amounts were too small to be determined accurately. The small amounts of

5-HT were not residues from the prior injection of 5-HT, because in three tests the perfusate, immediately before the 48/80 was injected, did not contain 5-HT.

Incubation of rat skin

Since only low doses of 5-HT and 48/80 could be satisfactorily tested by perfusion, the effects of higher concentrations of these substances were tested by incubating pieces of rat skin at 37° C for 15 min in Locke's solution containing 5-HT or 48/80, 2–100μg/ml., as well as in Locke's solution alone.

After exposure to 5-HT 50–100μg/ml. the skin often yielded substances that induced tachyphylaxis of the guinea-pig's ileum. These were not 5-HT because the addition of equivalent amounts of 5-HT to a standard preparation of histamine did not interfere with the assays. In four satisfactory experiments, skin was incubated with 2, 2, 5 and 100μg/ml. of 5-HT and of 48/80. The amounts of histamine released had little relation to the concentration of liberator used, but more histamine was always liberated by 5-HT than by 48/80 (Table 6). After 48/80 only traces of 5-HT were found in the solutions.

TABLE 6. The release of histamine from rat skin after incubation at 37° C for 15 min in solutions of 5-HT and compound 48/80

Concn. of 5-HT and of 48/80 (μg/ml.)	Histamine released (μg/100 g skin)		
	Control	5-HT	48/80
2	—	671	—
2	—	1008	133
5	—	130	—
100	139	2740	842

Although the estimates of the histamine-liberating activity of 5-HT and 48/80 by the method of simple diffusion are not as reproducible as by the perfusion technique, they clearly indicate that more histamine is liberated by 5-HT than by 48/80. This suggests that 5-HT is more effective than 48/80 when applied directly (by diffusion) to extravascular tissues than through the capillary walls (as in perfusion), although the latter is more effective on perfusion.

DISCUSSION

When the permeability-increasing potencies of histamine, 5-hydroxytryptamine and compound 48/80 are estimated in terms of the lesions induced by these substances on injection into the depilated skin of 'blued' guinea-pigs, rats and rabbits, 5-HT is a more potent permeability factor (P.F.) than histamine in rats, but not in guinea-pigs or rabbits. This confirms the results of Rowley & Benditt (1956) who, using the induction of paw oedema as the criterion of activity, found that in rats 1μg/ml. of 5-HT produced oedema as readily as 200μg/ml. of histamine. In our intracutaneous tests on rats, 5-HT

was 11·5 times as potent a P.F. as histamine. Nevertheless, no generalization for other species is possible, either for 5-HT or any other P.F. Thus in rats histamine was about twenty-five times less potent than in guinea-pigs and rabbits, whereas 5-HT had the same order of P.F. potency that histamine had in guinea-pigs and rabbits; but 5-HT had low P.F. activity in guinea-pigs and rabbits.

Our results emphasize the danger of generalizing about permeability factors in laboratory animals from tests made in only one species. The design of our experiments permitted the dosage-responses to several P.F.s to be determined in the same group of animals without the use of anaesthesia. Since anaesthesia considerably reduces the responsiveness of the blood vessels of the skin for some hours and probably also affects the deeper capillaries, tests without anaesthesia are likely to be more reliable. The testing of pharmacologically active substances with P.F. activity, prepared from the tissues or blood of one species, in a different species, is open to criticism (see Miles & Wilhelm, 1955), but it is not as important here because the P.F.s tested are all synthetic. However, histamine and/or 5-HT occur naturally in the skin and other tissues of most mammals that have been investigated; and even with these substances, generalizations clearly cannot be made from tests in a single species. This point is also relevant to Spector's (1956) finding that a 5-HT-like substance is present in copious pleural exudates in the rat, for it does not follow that this substance is present in inflammatory exudates of other species, or that in these other species 5-HT has substantial permeability-increasing potency.

In the rat 48/80 liberates histamine (cf. Feldberg & Talesnik, 1953) and 5-HT (cf. Bhattacharya & Lewis, 1956), but the approximately equal susceptibility of 5-HT and 48/80 to both mepyramine and lysergic acid suggests that these inhibitors act by preventing the liberation of tissue histamine, rather than by antagonizing released histamine. This conclusion, however, is not supported by the insusceptibility of the 5-HT effect to the decrease of skin histamine by 48/80 in rats. Parratt & West (1956) stated that the induction of oedema in the rat required the release of both histamine and 5-HT. Rowley & Benditt (1956) also emphasized the role of 5-HT in this respect, but they considered that 5-HT does not liberate histamine in the rat; this conclusion was influenced by the failure, in their experiments, of intravenous mepyramine to antagonize the induction of paw oedema by 5-HT. Although 5-HT itself may have a direct action on capillary permeability, our perfusion and skin incubation tests clearly indicate that, in rats, 5-HT is a histamine-liberator. This was confirmed by the susceptibility of 5-HT to mepyramine, injected locally in mixtures with 5-HT. The rat thus resembles other animals; e.g. Feldberg & Smith (1953) found that perfused isolated skin flaps from the cat and dog liberated histamine on treatment with 5-HT.

The differences in the amount of histamine liberated when rat hind quarter

preparations were perfused with, or skin was incubated in, 5-HT and 48/80 draw attention to the importance of testing histamine-liberators in more than one system (see also Mongar & Schild, 1953; Feldberg & Mongar, 1954). Although the incubation of skin in solutions of histamine-liberators suffers from the disadvantage that the thickness of the skin prevents the liberator from reaching equilibrium by diffusion (Mongar & Schild, 1953), this method in our hands revealed a more abundant release of histamine by 5-HT than by 5-HT given intravascularly. Clearly the release of tissue histamine varies according to the test system, and it is as important to avoid making generalizations about histamine-liberators from tests in a single system as it is to refrain from anticipating that permeability factors have activity of the same order in all species of animals.

SUMMARY

1. The increase in capillary permeability induced by the intracutaneous injection of histamine, 5-hydroxytryptamine and compound 48/80 was compared in the skin of the back of the trunk of unanaesthetized guinea-pigs, rats and rabbits with Pontamine Blue in their circulation.

2. The responses to each substance, measured as the diameter of the blue lesions at the site of injection, were linear with respect to log. dose. The slope of the dosage-response lines was about the same in all three species of animal, making possible a direct comparison of the permeability-increasing potency of these substances.

3. The response to the three permeability factors varies widely, and independently, in the three species of test animal. Histamine is highly, and about equally, potent in guinea-pigs and rabbits, but one twenty-fifth as potent in rats. In rats, 5-hydroxytryptamine is highly potent, being eleven times as active as histamine, but in guinea-pigs and rabbits its potency is negligible. Compound 48/80 is active in guinea-pigs and rats, but has a low potency in rabbits.

4. Histamine liberation by 5-hydroxytryptamine and 48/80 from rat tissue varies quantitatively according to the test system; 5-hydroxytryptamine is the more potent on excised skin, and 48/80 on perfused hind quarters.

5. In the rat, the susceptibility of the permeability factors to inhibition by locally injected mepyramine maleate and lysergic acid, and the results of perfusing isolated rat hind quarters or treating samples of excised rat skin with the permeability factors, suggest that 5-hydroxytryptamine increases permeability, at least in part, by the liberation of histamine, and that 48/80 does so partly by the liberation of histamine and 5-hydroxytryptamine. The behaviour of the permeability factors in rats, treated with 48/80 to decrease the liberable histamine in the skin, was equivocal.

We are grateful to the Upjohn Company, Kalamazoo, Michigan, U.S.A., for 5-hydroxytryptamine creatinine sulphate; to the Wellcome Research Laboratories, Tuckahoe, U.S.A., for compound 48/80 and to Sandoz Products Limited, London, for lysergic acid diethylamide.

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