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THE EFFECTS OF RESERPINE AND COMPOUND 48/80 ON THE RELEASE OF AMINES FROM THE MAST CELLS OF RATS

BY

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Recently we have found that histamine liberators like compound 48/80, morphine and propamidine release not only histamine but 5-hydroxytryptamine (5HT) as well, from the perfused hindquarters of rats (Bhattacharya and Lewis, 1956). The mast cells of the skin and subcutaneous tissue were thought to be the source of both the histamine and the 5HT since, in the rat at least, they contain both amines (Riley and West, 1953; Benditt, Wong, Arose, and Roeper, 1955; Parratt and West, 1956). Further, Pletscher, Shore, and Brodie (1956) have shown that reserpine has the property of depleting a number of organs such as intestinal tract, brain and platelets of their 5HT, which in these organs is not associated with mast cells. In the present experiments, therefore, we have examined the effect of reserpine on the 5HT content of mast cells in skin and subcutaneous tissue.

Reserpine was either injected into perfused hindquarters of rats to ascertain if, like 48/80, it released 5HT or histamine or both; or rats were treated with intraperitoneal injections of reserpine and then 48/80 was injected into their perfused hindquarters and the amounts of histamine and 5HT released were compared with those released from the hindquarters of untreated rats and of rats previously treated with intraperitoneal injections of 48/80 or morphine. In addition, the effect of reserpine on mast cells in the mesentery and subcutaneous tissue was studied histologically and compared with that of 48/80.

METHODS

Rats weighing 130 to 160 g. were anaesthetized with intraperitoneal injections of 40 mg./kg. pentobarbitone sodium. The hindquarters were perfused with oxygenated Locke solution through the abdominal aorta and the venous effluent was collected from the vena cava. The venous effluent was tested for histamine on the atropinized guinea-pig ileum and for 5HT on the atropinized rat's colon, as described by Feldberg and Toh (1953). All values of 5HT and histamine refer to base. Details of the perfusion and assay have been given elsewhere (Bhattacharya and Lewis, 1956). All substances injected arterially were given in a volume of 0.5 ml. The standard dose of 48/80 used in studying the release of 5HT and histamine was 100 μg. Reserpine (Light & Co.) was dissolved, after adding one drop of glacial acetic acid, in a mixture of propylene glycol, alcohol, and water in the ratio of 1:1:6 to give a concentration of 5 mg./ml., and was diluted with distilled water when injected in lower concentrations. Morphine was used as sulphate; doses injected refer to the salt.

Studies of Mast Cells of Subcutaneous Tissue and Mesentery.—Spreads were made of both tissues within a few minutes after death. The subcutaneous tissue was taken from the loose areolar tissue of the interscapular region. The spreads were fixed and stained with toluidine blue according to the procedure described by Riley (1953).

RESULTS

The previous finding, that 48/80 releases 5HT as well as histamine from the perfused hindquarters of the rat, has been confirmed. In Table I the results of four experiments are given in which 100 μg. 48/80 was injected intra-arterially into the perfused hindquarters. The amounts of histamine released varied between 85.3 and 102 μg. (mean 91.3 μg.), those of 5HT between 4.3 and 8 μg. (mean 5.8 μg.).

Intra-arterial injections of 0.5 to 2 mg. of reserpine released neither histamine nor 5HT from the perfused tissues.

Release of Histamine and of 5HT by 100 μg. 48/80 from the Perfused Hindquarters of Rats Previously Treated with Intraperitoneal Injections of 48/80, Morphine, or Reserpine

Previous Treatment with 48/80.—After repeated intraperitoneal injections of 48/80 the amounts of histamine, as well as of 5HT, released from the perfused hindquarters by an intra-arterial injec-

TABLE I

RELEASE OF HISTAMINE AND 5-HYDROXYTRYPTAMINE BY INTRA-ARTERIAL INJECTION OF 100 μG. 48/80 FROM PERFUSED HINDQUARTERS OF RAT AFTER REPEATED INTRAPERITONEAL INJECTIONS OF 48/80

No. of Days during which 48/80 was Injected	Output in μg. of	
	5HT	Histamine
0	8·0	86
0	5·5	85
0	4·3	92
0	5·2	100
1	1·1	8·5
1	1·3	37
1	1·8	32
2	0·4	2·7
3	0·7	5·3
4	3·0	7·4
7	0·8	4·7
8	0·8	8·7
9	1·0	14
10	0·5	9·7

tion of 100 μg. 48/80 were much smaller than those released from the perfused hindquarters of normal rats. This is shown in Table I. The rats received daily two intraperitoneal injections of 48/80, one in the morning and one in the evening. The first dose was 100 μg., and was increased with each injection by 50 μg. up to 500 μg., so that after the fourth day the rats were given 500 μg. twice daily. The perfusion was carried out in the morning following the last injection. The results of Table I show that the intraperitoneal treatment with 48/80 for 1 day is sufficient to reduce greatly the amounts of histamine and 5HT released by the intra-arterial injection of 100 μg. 48/80 into the perfused hindquarters. The mean amounts released in the three experiments given in Table I were about a quarter to a third of those released from untreated rats. There was further reduction in the amounts of histamine and 5HT released from rats which had been treated for two days with intraperitoneal injections of 48/80. In the experiment given in Table I the amount of histamine released was about 3% and that of 5HT about 7% of the mean amounts released from untreated rats. Further treatment with intraperitoneal injections of 48/80 for longer periods and with higher doses, however, did not cause a further reduction.

Feldberg and Talesnik (1953) have shown that the histamine content of skeletal muscle and skin of rats, when reduced by repeated intraperitoneal injections of 48/80, remains low for several days and then rises gradually in the following weeks. In the present experiments the effect of intra-arterial injections of 48/80 was studied on the histamine and 5HT output from the perfused hindquarters of rats which were first treated for 3 or 10 days with repeated intraperitoneal injections of 48/80 and were then allowed to recover. The results are shown in Table II. The first column gives the days of treatment, the second the number of days between the last intraperitoneal injection and the perfusion, and the third and fourth columns the amounts of 5HT and histamine released by the intra-arterial injection of 100 μg. 48/80 into the perfused hindquarters. The amounts are given in μg. and are also expressed as % of the average amounts released from the perfused hindquarters of untreated rats, i.e., the first four experiments of Table I.

There is a definite increase in the amounts of histamine and 5HT released from the perfused hindquarters when 11 days or more have elapsed between the last intraperitoneal injection and the perfusion. However, even when the perfusion is carried out 24 or 33 days after the last intraperitoneal injection, the amounts of histamine and 5HT released are only half or less than the amounts released from the hindquarters of untreated rats.

Previous Treatment with Morphine.—The results obtained from four rats which were given daily two intraperitoneal injections of morphine are

TABLE II

RELEASE OF HISTAMINE AND 5HT BY INTRA-ARTERIAL INJECTION OF 100 μG. 48/80 FROM PERFUSED HINDQUARTERS OF RATS PREVIOUSLY TREATED WITH INTRAPERITONEAL INJECTIONS OF 48/80

Days of Treatment with 48/80	Days between last 48/80 Injection and Perfusion	Output of 5HT		Output of Histamine	
		(In μg.)	(As % of Normal)	(In μg.)	(As % of Normal)
0	—	5·8	100	91	100
3	1	0·7	12	5·3	5·7
3	11	1·1	19	26	29
3	17	1·6	28	57	61
10	1	0·5	9	9·7	11
10	12	1·2	21	25	28
10	18	2·3	40	27	29
10	24	3·3	57	41	44
10	33	2·2	38	37	39

TABLE III

RELEASE OF HISTAMINE AND 5HT BY INTRA-ARTERIAL INJECTION OF 100 μG. 48/80 FROM PERFUSED HINDQUARTERS OF RATS PREVIOUSLY INJECTED INTRAPERITONEALLY WITH MORPHINE

Pretreatment with Morphine		Output in μg. of	
No. of Days Treated	Total Morphine Injected (mg./kg.)	5HT	Histamine
1	20	4·5	110
2	50	2·4	101
3	80	2·2	48
3	80	2·8	67

shown in Table III. On the first day 10 mg./kg. was injected each time, subsequently the dose was increased to 15 mg./kg.

The release of 5HT and histamine by the arterial injection of 100 μg. 48/80 into the perfused hindquarters of one of these rats, which had been treated with morphine for one day only, was of the same order as that observed in control rats. In the rats treated with morphine for 3 days there was a definite reduction in the amounts of 5HT and histamine released from the perfused hindquarters by 48/80.

Previous Treatment with Reserpine.—Rats were injected intraperitoneally with 5 mg./kg. reserpine and intra-arterial injections of 100 μg. 48/80 were made into their perfused hindquarters 3, 6 or 18 hr. later. The amounts of histamine released were normal, and in the experiments at 3 and 6 hr. 48/80 also released the usual amounts of 5HT. However, in the experiment in which perfusion was carried out 18 hr. after the reserpine injection, the release of 5HT by 48/80 was reduced (Table IV).

TABLE IV

RELEASE OF HISTAMINE AND 5HT BY INTRA-ARTERIAL INJECTION OF 100 μG. 48/80 FROM PERFUSED HINDQUARTERS OF RATS PREVIOUSLY INJECTED INTRAPERITONEALLY WITH RESERPINE

Pretreatment with Reserpine		Interval in Hr. between Reserpine Injection and Injection of 48/80	Output in μg. of	
mg./kg./ Injection	No. of Injections		5HT	Histamine
5	1	3	5·9	107
5	1	6	4·4	110
5	1	18	2·0	117
3	2*	18	0·6	106
3	3*	18	0·5	86
3	3*	18	1·0	89

* One injection daily.

In a second series of experiments three rats were given intraperitoneal injections of 3 mg./kg. reserpine on two or three successive days and the hindquarters perfused 18 hr. after the last injection. As shown in Table IV the 48/80 again released the usual amounts of histamine but only about 10 to 20% of the amounts of 5HT released from the hindquarters of untreated rats. Compound 48/80 released the usual amounts of both histamine and 5HT from rats treated with 1 ml./kg. of the reserpine solvent.

Effect of Intraperitoneal Injections of 48/80, Morphine, and Reserpine on Mast Cells in Subcutaneous Tissue and in the Mesentery

Compound 48/80.—The intraperitoneal injections of 48/80 affected the mast cells of the mesentery more readily than those of the subcutaneous tissue. After two intraperitoneal injections of 100 and 150 μg. 48/80 on the same day less than half of the mast cells of the subcutaneous tissue were disrupted (Fig. 1b), whereas in the mesentery practically all were totally disrupted. This difference might well be due to the high concentration of 48/80 in the peritoneal cavity. After two intraperitoneal injections of 48/80 each day for three days, doses being increased from 100 μg. by 50 μg. with each injection, the subcutaneous tissue also contained only about 5–10% of the normal number of mast cells and they were partly disrupted (Fig. 1c). In rats in which intraperitoneal injections of 48/80 were continued for 10 days by increasing the dose given per injection up to 500 μg., no further damage occurred.

It required several days after cessation of the injections of 48/80 before the reappearance of the mast cells was observed. Three rats had been treated with intraperitoneal injections of 48/80 for three days and were allowed to survive for 3, 5 and 11 days. The recovery of the mast cells in the subcutaneous tissue and mesentery was the same; after three days there was no recovery, after 5 days some, and after 11 days there was nearly complete recovery (Fig. 1); the number of mast cells was nearly normal, but some were not heavily granulated. In rats which had been treated for 10 days with intraperitoneal injections of 48/80 recovery of the mast cells in the mesentery was more delayed than that in the subcutaneous tissue. In the latter the mast cells had fully recovered 12 days after the last injection; in fact the number present seemed to be greater than usually found in untreated rats; on the other hand there were no mast cells, or only a few, in the mesentery even 18 days after the last injection.

Morphine.—The mast cells of the mesentery were more affected by intraperitoneal injections of morphine than the mast cells of the subcutaneous tissue. After 1 to 3 days' treatment with morphine injections twice daily in a dose of 10 mg./kg./injection on the first day and 15 mg./kg. subsequently, the mast cells of the subcutaneous tissue showed a normal appearance and their number had not decreased. In the mesentery, however, many disrupted mast cells were encountered even after only 1 day's treatment with morphine, and after 2 and 3 days' treatment the number of mast cells was greatly reduced and those present were disrupted.

Reserpine.—The mast cells of the rats treated with intraperitoneal injections of reserpine and used for the experiments concerned with the release of 5HT and histamine showed no disrup-

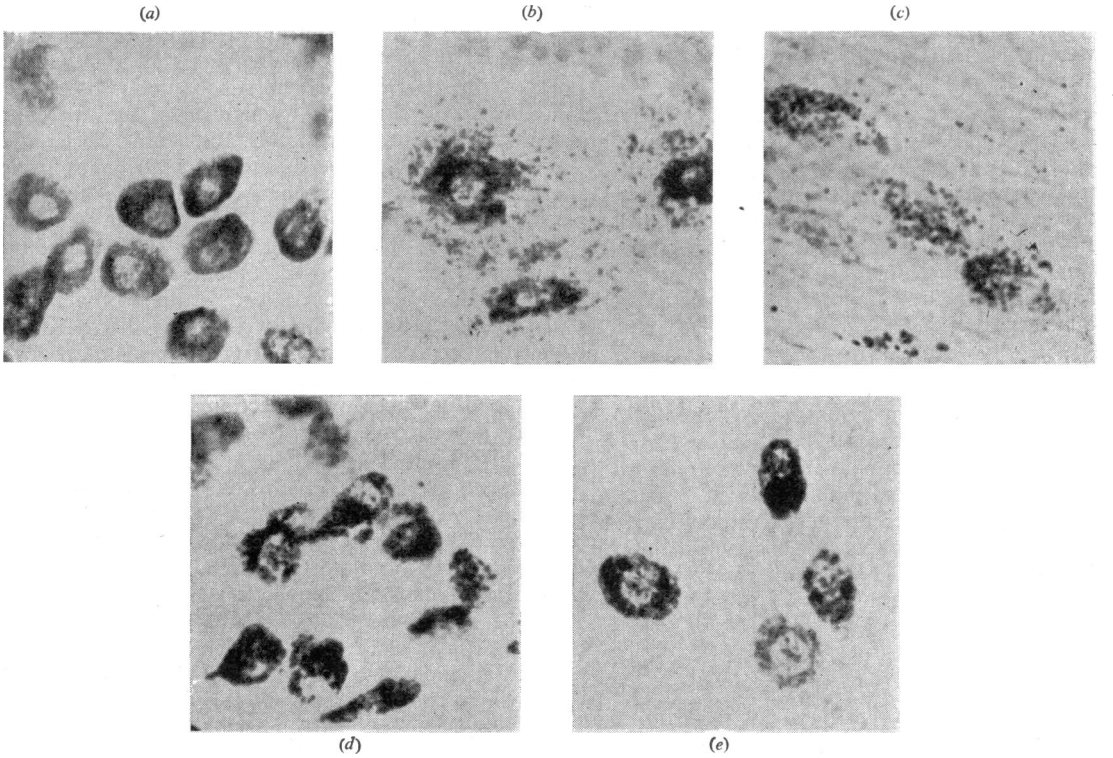

FIG. 1.—Subcutaneous tissue spreads from untreated rats in (*a*), and from rats treated with intraperitoneal injections of 48/80 for 1 day in (*b*), and for 3 days in (*c*). In (*d*) and (*e*), 5 and 11 days respectively had elapsed since the treatment with intraperitoneal injections of 48/80 for 3 days. The spreads were stained with toluidine blue. Magnification, × 680.

tion either in the subcutaneous tissue (Fig. 2) or in the mesentery, and the number of mast cells present in both these tissues was normal.

However, as seen in Fig. 2, there is a slight change in the appearance of the mast cells in rats treated with intraperitoneal injections of reserpine for 3 days as compared with those in normal rats. The reserpine treatment causes some swelling and degranulation of the mast cells, changes observed by Riley and West (1955) when they examined the effect on mast cells of small doses of 48/80.

DISCUSSION

The results of the present experiments show that both 48/80 and reserpine act on the mast cells of rats but that they act in a different way. 48/80 disrupts the mast cells and in doing so releases the histamine and 5HT they contain. This action of 48/80 is an immediate one and has been termed explosive. Reserpine does not disrupt the mast cells, nor does it release their histamine, but it depletes them of their 5HT as it depletes other tissues like brain, intestine, and platelets of this amine. Further, the action of reserpine on the mast cells differs from that of 48/80 in that it requires several hours before a depletion of 5HT occurs.

These conclusions were derived not from experiments in which the 5HT and histamine content of the tissues was assayed, but by determining the amounts of histamine and 5HT released by 48/80 from the perfused hindquarters of normal rats and of rats previously treated with intraperitoneal injections of either 48/80 or reserpine. Injections of 48/80 into the artery of the perfused hindquarters of normal rats led to the appearance in the venous effluent of relatively large amounts of histamine and 5HT, whereas reserpine similarly injected had no effect of this kind. In these acute experiments there was therefore no indication of an effect of reserpine on the amine content of the mast cells On the other hand it was possible with intraperitoneal injections of reserpine to reduce the amount of 5HT released from the mast cells by 48/80 provided sufficient time was allowed for the reserpine to act. This effect of reserpine, which was brought about without disruption of

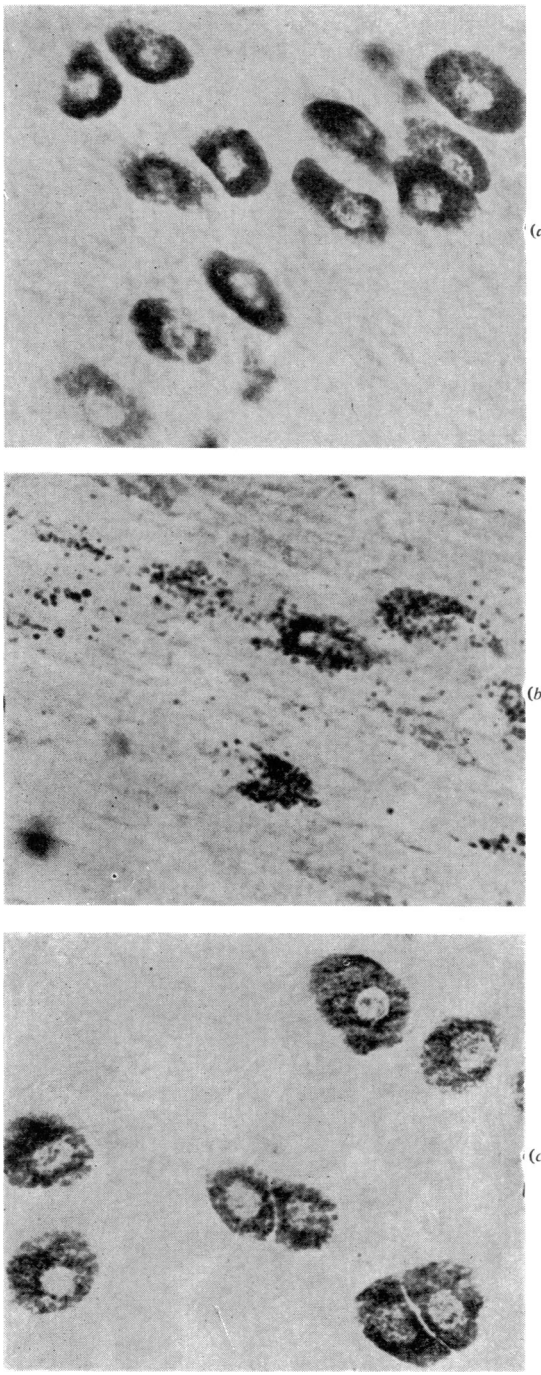

FIG. 2.—Subcutaneous tissue spreads from untreated rats in (*a*), and from rats treated for three days with intraperiotneal injections of 48/80 (doses as in text) (*b*), and reserpine 3 mg./kg. daily in (*c*). The spreads were stained with toluidine blue. Magnification, × 680.

the mast cells and without concomitant release of histamine, was shown when 48/80 was injected into the perfused hindquarters of rats treated with intraperitoneal injections of reserpine. The 48/80 released normal amounts of histamine but traces only of 5HT, and the mast cells were shown not to be disrupted. Intraperitoneal injection of reserpine thus acts entirely differently from that of 48/80 which was shown to disrupt the mast cells and release both their histamine and their 5HT.

The finding that reserpine requires several hours to affect the 5HT content of the mast cells is in agreement with the action of reserpine in depleting other tissues of their 5HT. According to Pletscher *et al.* (1956) there is also a latency of hours before the 5HT content of brain or intestinal tract is reduced by an injection of reserpine.

Experiments on the recovery of mast cells after disruption by 48/80 show that, when they have reappeared, 48/80 does not release the normal amounts of histamine and 5HT from them despite the fact that the histological picture is normal. Twelve days after prolonged treatment with intraperitoneal injections of 48/80, the mast cells were again present in the subcutaneous tissue, normal in number and appearance. Nevertheless at this stage of recovery, only small amounts of histamine and 5HT were released by 48/80 from the perfused hindquarters. This may mean that the mast cells in their early stages of recovery are either more resistant to the action of 48/80 or that they do not yet contain their full complement of amines.

The finding, also reported by Riley and West (1955), that, after prolonged treatment with intraperioneal injections of 48/80, when the mast cells have practically disappeared from the subcutaneous tissue and mesentery, they return in the subcutaneous tissue much earlier than in the mesentery, is difficult to explain. The fact that 48/80 is injected into the peritoneal cavity may mean that it can act on the mesentery in a much higher concentration than on the subcutaneous tissue, which it reaches through the blood stream, and that the high concentration exerted for long periods in the mesentery not only disrupts mast cells but affects their precursors as well.

SUMMARY

1. Reserpine released neither histamine nor 5-hydroxytryptamine (5HT) when injected into the perfused rat hindquarters under conditions in which 48/80 released large amounts of both amines.

2. 48/80 released only small amounts of both histamine and 5HT from the perfused hindquarters

of rats previously treated with intraperitoneal injections of 48/80. In these rats the mast cells of the mesentery and subcutaneous tissue were disrupted and had disappeared.

3. 48/80 released large amounts of histamine but only small amounts of 5HT from the perfused hindquarters of rats previously treated with intraperitoneal injections of reserpine. In these rats the appearance of the mast cells was slightly different from those in normal rats, but they were not disrupted and were present in normal number.

4. Whereas 48/80 acts on mast cells, disrupting them, thereby releasing the histamine and 5HT they contain, reserpine does not disrupt them and specifically depletes them of 5HT, as it depletes other tissues of this amine.

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