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RELEASE OF HISTAMINE BY A SUBSTITUTED BUTYLAMINE (L 1935): COMPARISON WITH COMPOUND 48/80

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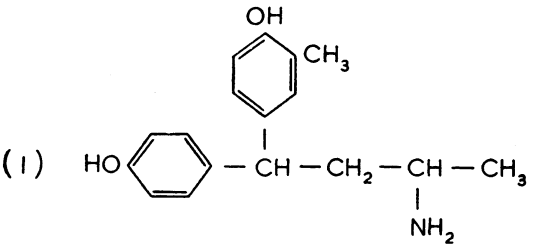
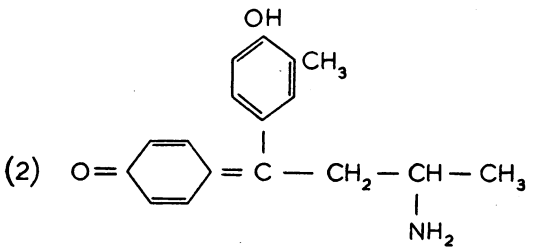
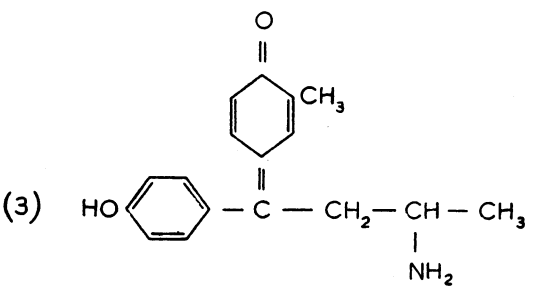
Charlier and his co-workers (Charlier, 1954; Charlier, Dallemagne and Philippot, 1954; Charlier and Vandersmissen, 1954) recently described the pharmacological properties of a substituted butylamine or methylpropylamine which is an equimolecular mixture of 3-4′-hydroxyphenyl-3-4″-hydroxy-3″-methylphenyl-1-methylpropylamine and its dehydro- form. The structural formula is given below. The substance consists of (1) with a tautomeric mixture of (2) and (3).







This substance, referred to in brief as L 1935, when injected intravenously into cats or dogs in doses of 0.25–1 mg./kg., caused a long-lasting fall of arterial blood pressure. Since the effect was prevented by promethazine, the authors suggested that it was accounted for, or at least partly, by release of histamine and that L 1935 was a histamine liberator. In man, an intravenous injection of 0.5 mg./kg. led to erythema and an urticarial rash with intense itching and some pain sensation, oedema of the face, and a fall in arterial blood pressure. With smaller doses (0.1 mg./kg.) erythema and mild itching only occurred (Lecomte, 1955). These findings also suggested a strong histamine-releasing property of L 1935. So far, however, no direct evidence for its histamine-releasing property has been brought forward.

The present experiments were undertaken to obtain this direct evidence by determining the release of histamine from perfused tissues and to compare the effect of L 1935 with that of the potent histamine liberator 48/80. In addition, a few experiments were performed on the arterial blood pressure of the cat in order to see if the depressor action of L 1935 showed the latency characteristic of histamine liberators. Finally, the effect of L 1935 was studied on the guinea-pig's ileum and compared with that of 48/80.

METHODS

The histamine-releasing property was tested on perfused skin flaps of the cat's hind legs and on perfused hind quarters of the rat. The cats were anaesthetized with chloralose intravenously and the skin flaps perfused from the saphenous artery with Locke solution and the venous outflow collected from the saphenous vein, according to the method described by Feldberg and Paton (1951) and Feldberg and Schachter (1952). The rats were anaesthetized with pentobarbitone sodium intraperitoneally. The hind quarters were perfused from the lower abdominal aorta and the venous outflow collected from the vena cava, as described by Feldberg and Mongar (1954). In both

series of perfusion experiments the Locke solution contained 2 mg./ml. glucose. The venous effluent was assayed for histamine on the atropinized guinea-pig's ileum preparation. All values of histamine refer to the base.

For comparison of the histamine-releasing potency of L 1935 with that of 48/80 three injections were given in each preparation. The interval between injections was about 30 min. Either an injection of L 1935 was bracketed between two injections of 48/80, or an injection of 48/80 was bracketed between two injections of L 1935. The doses of either L 1935 or 48/80 used for the first and third bracketing injection were the same in each given preparation. This procedure was adopted because the amounts released by the same dose of a histamine liberator usually decrease with successive injections except when the amounts released are very small—for example, about 1 μg. histamine or less. For the assay it is best to adjust the dose used for injection so that it releases not more than a few micrograms of histamine, and that the output practically comes to an end within 30 min. In the experiments on the cat's skin, the flap from the left leg was always perfused first and the flap from one side was used to bracket L 1935, that from the other side to bracket 48/80. This made it possible to compare the results obtained from the two sides, particularly when the doses injected into both skin flaps were the same.

The cats whose skin flaps were perfused were kept under chloralose anaesthesia after the flaps had been removed, and were later used for comparing the effects of L 1935 and of 48/80 on the arterial blood pressure. For this purpose the left carotid artery was cannulated for recording the arterial blood pressure, and the right jugular vein for injections. The vagi were cut in the neck.

For the experiments on the isolated guinea-pig's ileum preparation a piece of ileum about 4 cm. long was suspended in 15 ml. magnesium-free Tyrode solution at 33° C.

RESULTS

Perfused Skin Flaps of Cat's Hind Legs

L 1935 was found to be a potent histamine liberator when injected into the saphenous artery of the perfused skin flap of the cat's hind leg.

TABLE I

HISTAMINE RELEASE BY L 1935 FROM PERFUSED SKIN FLAPS OF THE CAT'S HIND LEGS

L 1935 (μg. Injected)	Output of Histamine (μg.)
0·5*	4·03
1·0	8·50
2·0	2·34
2·0*	3·52
2·4*	12·3
2·5	14·9
2·5*	1·74
2·5*	9·29

* Injected 30 min. after an injection of 48/80.

TABLE II

COMPARISON OF THE HISTAMINE OUTPUT BY L 1935 AND COMPOUND 48/80 FROM PERFUSED SKIN FLAPS OF THE CAT'S HIND LEGS

Expt. No.	Left Side: Injections of 48/80 or L 1935 (μg.)	Left Side: Output of Histamine (μg.)	Right Side: Injections of 48/80 or L 1935 (μg.)	Right Side: Output of Histamine (μg.)
1	0·5 48/80	11·6	1 L 1935	8·5
	0·5 L 1935	4·03	0·5 48/80	11·3
	0·5 48/80	4·99		
2	2·5 L 1935	15·0	0·5 48/80	32·0
	0·5 48/80	17·9	2·5 L 1935	9·29
	2·5 L 1935	3·08	0·5 48/80	6·01
3			0·125 48/80	0·93
			2·5 L 1935	1·74
			0·125 48/80	0·68
4	0·125 48/80	0·17	2 L 1935	2·34
	2·0 L 1935	3·52	0·25 48/80	3·63
	0·125 48/80	0·75	2·0 L 1935	0·31
5	0·2 48/80	13·9	2·4 L 1935	—*
	2·4 L 1935	12·3	0·2 48/80	14·5
	0·2 48/80	4·14	2·4 L 1935	4·29

* Injection spoiled.

As seen from the results of Table I, the injection of 0.5–2.5 μg. resulted in the release of several μg. of histamine. When the histamine-releasing power of L 1935 was compared with that of 48/80, it was found that 48/80 was more potent. This is evident from the results of the five experiments given in Table II. From the results of experiments Nos. 1, 2, and 3 it is evident that 0.5 μg. 48/80 releases more histamine than do 0.5 μg., 1 μg., and 2.5 μg. of L 1935; that 2.5 μg. of L 1935, however, releases more histamine than does 0.125 μg. 48/80. Thus, 48/80 is more than five and less than twenty times as active as L 1935; the experiment

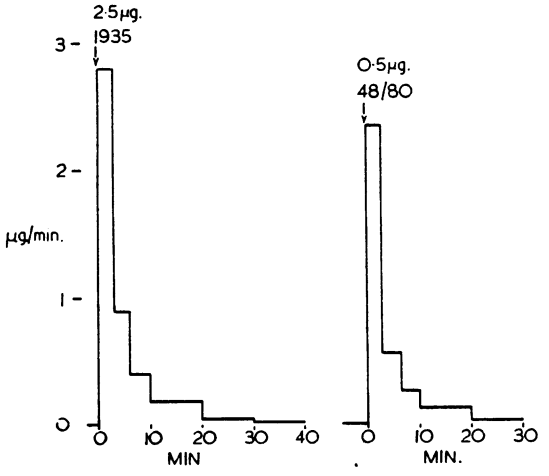


FIG. 1.—Comparison of the rate of output of histamine from perfused skin flaps of cat's hind legs by L 1935 and 48/80. The effect of L 1935 and of 48/80 was obtained from different cats. Ordinates, output of histamine in mg./min. Abscissae: time in min. after injection.

No. 4 shows that 48/80 is more than eight and less than sixteen times as active, and experiment 5 that it is about twelve times as active as L 1935.

The output of histamine is as rapid after L 1935 as after 48/80, and is more gradual with both substances after the second or third injection. In order to compare the output of histamine by 48/80 and L 1935, it is best to use experiments in which the total release of histamine is of the same order. For instance, in experiment No. 1 a first injection of 0.5 μg. 48/80 released 11.6 μg. histamine, and in experiment No. 2 a first injection of 2.5 μg. L 1935 released 15.0 μg. In Fig. 1 the output of histamine in μg./min. for these experiments is plotted against the time of perfusion after the injections in minutes. The samples were collected at successive intervals of 3, 3, 4, 10, and 10 minutes.

As with 48/80, the output of histamine after successive injections of the same dose of L 1935 decreased so much that it was not deemed advisable to give more than three injections to each preparation.

As with 48/80, injections of L 1935 in doses which caused a release of several μg. of histamine were followed by the quick development of oedema which was particularly striking in the loose subcutaneous tissue.

Perfused Hind Quarters of the Rat

L 1935 causes a release of histamine from the perfused hind quarters of the rat, but the doses required are higher than for the cat's skin flaps. Table III shows the increasing output of histamine in six perfusion experiments after increasing doses

TABLE III

HISTAMINE RELEASE BY L 1935 FROM PERFUSED HIND QUARTERS OF THE RAT

L 1935 Injected (μg.)	Output of Histamine (μg.)
10	1·38
50	3·19
50	3·69
50	4·15
100	7·18
200	9·36

of L 1935. When compared with 48/80 it was found, as in the corresponding experiments on the cat's skin flap, that 48/80 was more active in releasing histamine than L 1935 (Table IV). From experiments 1 and 2 in Table IV it is seen that 50 μg. L 1935 is weaker than 10 μg. 48/80 but stronger than 2.5 μg. Thus, 48/80 is more than five times and less than twenty times as active as L 1935. In the succeeding four experiments the

TABLE IV

COMPARISON OF THE HISTAMINE OUTPUT BY L 1935 AND 48/80 FROM PERFUSED HIND QUARTERS OF THE RAT

Experiment No.	Injections of 48/80 or L 1935 (μg.)		Output of Histamine (μg.)
1	50	L 1935	3·19
	10	48/80	9·81
	50	L 1935	0·97
2	50	L 1935	3·69
	2·5	48/80	1·73
	50	L 1935	1·47
	10	48/80	1·87
3	10	L 1935	1·38
	1	48/80	0·92
	10	L 1935	0·53
4	50	L 1935	4·15
	5	48/80	4·11
	50	L 1935	1·05
5	5	48/80	9·17
	50	L 1935	2·6
	5	48/80	1·49
6	100	L 1935	7·18
	10	48/80	6·94
	100	L 1935	4·00
7	200	L 1935	9·36
	20	48/80	3·10
	200	L 1935	0·28

ratio between L 1935 and 48/80 was kept at 10:1, but the substances were tested in increasing amounts. Taking into account the fact that the preparations became less sensitive with successive injections, it is evident that, weight for weight, 48/80 is about 10 times as active as L 1935, or perhaps even a little more.

Effect on Cat's Blood Pressure

Like compound 48/80, L 1935 caused a fall in blood pressure with the characteristic latency of 20–40 sec. In the experiment of Fig. 2, the latency after the injection of 100 μg. L 1935 was about 40 sec. When the effects of the two substances were compared, it was found that L 1935, in a dose 10 times greater than 48/80, produced a somewhat smaller depressor effect, as illustrated in Fig. 2. A more exact comparison was difficult to obtain in the few experiments carried out

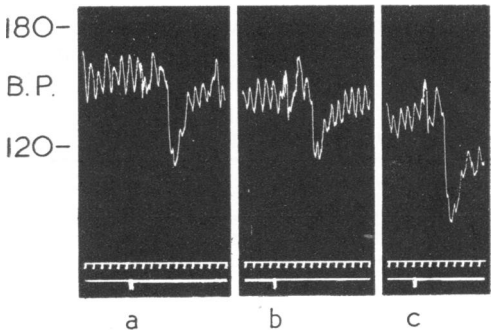


FIG. 2.—Arterial blood pressure of 3.8 kg. cat under chloralose anaesthesia. At *a* and *c* 10 μg. 48/80, at *b* 100 μg. L 1935 intravenously. Time in 10 sec.

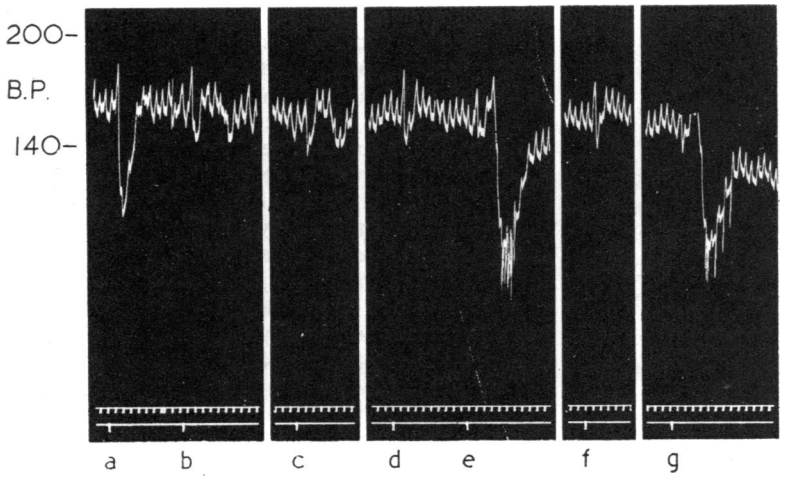


FIG. 3.—Arterial blood pressure of a 3.4 kg. cat under chloralose anaesthesia. Intravenous injections of 0.2 μg. histamine (at *a*), of 15 μg. 48/80 at *b* and *e*, of 150 μg. L 1935 at *c* and *f*, and of 250 μg. L 1935 at *g*; at *d* 2 ml. saline which produced an immediate fall of pressure. Time in 10 sec.

because a second and third injection of the same dose of 48/80 sometimes caused increasing effects, whereas with further injections the blood pressure of the cat became less sensitive to the depressor action of both substances. In the experiment of Fig. 3 the first injection of 15 μg. of 48/80 and the subsequent injection of 150 μg. of L 1935 were subthreshold, but when 15 μg. of 48/80 was injected a second time after the 150 μg. of L 1935 there resulted a sharp fall in arterial blood pressure. A second injection of 150 μg. of L 1935, however, remained subthreshold, whereas 250 μg. caused a fall in blood pressure. This kind of result was obtained in other experiments as well, and it appeared that on repeated alternate injections of the two substances, 48/80 was first about 10 to 12 times, but later about 15 times, as active as L 1925. There was some indication that a first or second injection of a small dose of L 1935, which produced a certain degree of tachyphylaxis to L 1935 itself, increased the sensitivity of the cat to 48/80.

Effect on the Guinea-pig's Ileum Preparation

Compound 48/80 is known to have several effects when tested in relatively high concentrations on the isolated guinea-pig's ileum preparation. It produces a strong transient contraction followed, after the 48/80 has been washed out, by a period of increased motor activity and decreased sensitivity to histamine and acetylcholine. The decreased sensitivity may occur whilst the motor activity develops (Mongar and Schild, 1952 ; Dews, Wnuck, Fanelli, Light, Tornaben, Norton, Ellis and de Beer, 1953 ; Feldberg and Smith, 1954).

When 1 mg. of L 1935 was added to the bath the gut showed, after 90 sec., a strong contraction in two out of six preparations. With successive administration of the same dose of L 1935 the contractions became smaller and disappeared. Fig. 4 shows the contraction produced by the first administration of 1 mg. L 1935 to one of these preparations and illustrates the development of strong motor activity after washing out the L 1935. Motor activity developed also when there was no direct stimulating effect of L 1935 on the ileum preparation. In Fig. 5, which illustrates the depressing action of L 1935, the development of increased motor activity after the washing out is visible, although there was no direct stimulation.

After L 1935 is washed out the preparation becomes less sensitive not only to histamine but also to acetylcholine. When the depression is pronounced, full recovery seldom occurs.

When the depressing effect of L 1935 was compared with that of 48/80 the two compounds were

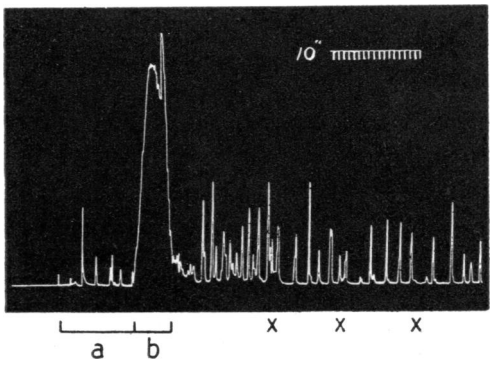


FIG. 4.—Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution; 0.02 μg. atropine sulphate throughout. At *a* 500 μg. and at *b* 1 mg. of L1935 kept in the bath for 90 sec. Time in 10 sec. At each x the Tyrode solution of the bath was renewed.

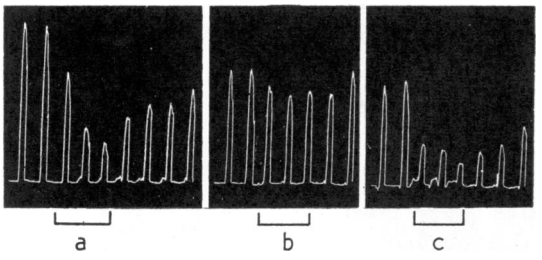


FIG. 5.—Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution; 0.02 μg. atropine throughout. Contractions due to 0.06 μg. histamine added to the bath for 15 sec. at 1 min. intervals. At *a* 1 mg. of L 1935, at *b* 200 μg. and at *c* 1 mg. of 48/80 were added to the bath. The additions were made 30 sec. before three successive histamine injections, as indicated at the bottom of the figure.

found to be about equally active, weight for weight. This is illustrated in Fig. 5, which shows that 200 μg. of 48/80 produces a much weaker depression than 1 mg. of L 1935, but that the depressions produced by 1 mg. of 48/80 and by 1 mg. of L 1935 are comparable.

As these actions of L 1935 were obtained with much larger doses than those present in the amounts of venous effluent used when testing for histamine, they did not interfere with the assay.

DISCUSSION

L 1935 was found to be a potent histamine liberator when injected into perfused flaps of the cat's skin or into the perfused hind quarters of the rat. The time course of the release of histamine was found to be about the same for both substances; therefore the release after L 1935 is probably as "explosive" as that after 48/80.

On the perfused skin preparations, doses of L 1935 as small as 1 or even 0.5 μg. caused the release of detectable amounts of histamine. Although L 1935 is not as potent as compound 48/80, it is more potent on these perfused tissues than any other of the known histamine liberators. For instance, the potent histamine releasers propamidine and octylamine are 100–200 times less active on the perfused cat's skin flap than 48/80, whereas L 1935 was found to be only about 10 times less active. Feldberg and Mongar (1954) have emphasized that when a comparison is made between two histamine liberators different ratios can be obtained according to the species, tissues, and methods used. This fact has to be kept in mind when stating that compound 48/80 is about 10 times as active as L 1935 in releasing histamine.

The depressor action we observed on the cat's arterial blood pressure after the intravenous injections of relatively small doses of L 1935 seems to be fully accounted for by released histamine. The fact that the blood pressure did not fall immediately after the injection but only after a latency of 20–40 sec. speaks against a direct depressor action of L 1935 in these experiments. Further, L 1935 was 10 to 15 times less active in causing a fall of blood pressure than 48/80; this has to be compared with the ratio of about 1:10 for the histamine-releasing activity of the two compounds in the perfusion experiments and strongly suggests that release of histamine is the sole cause of the depressor action we observed. The conclusion need not necessarily apply to the depressor effect of larger doses of either 48/80 or L 1935.

The progressive diminution in the depressor action of L 1935 when injected repeatedly into the cat cannot be explained by a gradual depletion of the histamine stores of the tissues, because it occurred after relatively small doses. These can have caused the release of only a very small fraction of the available histamine. The decreased sensitivity is thus a tachyphylaxis which concerns the mechanism of release. There was some indication that this tachyphylaxis developed more rapidly after the intravenous injections of L 1935 than after those of 48/80, but more experiments would be needed to make certain about this difference.

One of the problems presented by histamine liberators in general is how far release of histamine accounts for all the observed effects. It seems safe to conclude that this is so with regard to the itching, the erythema, the urticaria, the facial oedema, and the fall in arterial blood pressure observed in man after intravenous injections of L 1935. However, large doses of L 1935 injected into animals may well give effects which are not accounted for by histamine release. On the guinea-pig's intestine such effects certainly occur after L 1935, and it is interesting to note that strikingly similar effects are obtained with other histamine liberators as well. The potency of L 1935 in depressing the sensitivity of the guinea-pig's ileum was found to be about as great as that of 48/80, in contrast to the ratio of 1:10 obtained for the histamine-releasing property of the two compounds when tested on perfused tissues of the cat and the rat. Their histamine releasing property has not been compared on the guinea-pig's ileum, and on this tissue the ratio may be different. There is, however, no necessity to assume that the depression observed with both substances is related to their potency in releasing histamine. Histamine-releasing substances seem to have other properties in common which are not related to their potency in releasing histamine. For instance,

Gertner (1955) recently observed that 48/80 as well as propamidine caused block of ganglionic transmission; but the doses required to produce this effect appeared to bear no relation to the potency of the two compounds in releasing histamine.

SUMMARY

1. L 1935 causes a release of histamine from perfused skin flaps of the cat's hind legs and from perfused hind quarters of the rat. Weight for weight it is 10–12 times less active than compound 48/80.

2. L 1935, when injected intravenously into cats, causes a fall in arterial blood pressure after a latency of about 20–40 sec. Weight for weight it is 10 to 15 times less active than 48/80. On repeated injections the cat becomes progressively less sensitive to the depressor action of L 1935.

3. L 1935 produces on the isolated guinea-pig's ileum, when added to the bath in relatively high concentration, transient contraction followed, after washing out, by a period of increased motor activity and decreased sensitivity to histamine and acetylcholine. Similar effects are produced by 48/80. Weight for weight the two substances are about equally active on the guinea-pig's ileum.

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