

COMPARISON OF HISTAMINE RELEASE BY COMPOUND 48/80 AND OCTYLAMINE IN PERFUSED TISSUES

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It has recently been shown that a series of simple mono-amines are histamine releasers. The histamine release was studied in incubated tissue particles of guinea-pig lung, and octylamine was found to be several times more active than 48/80. When, however, the activity of these compounds in producing the triple response on the human skin *in vivo* was measured, the result was different. The mono-amines were active in about the same concentrations as on the tissue particles, but 48/80 was nearly a thousand times more active than octylamine. This discrepancy raised the question as to whether the activity of a histamine releaser varies when examined by different methods (Mongar and Schild, 1953). In the present experiments, the activity of 48/80 and octylamine was therefore compared by another method widely used in the past for the study of histamine releasers—namely, the perfusion of isolated tissues.

METHODS

The tissues perfused were the skin of the hind legs and gastrocnemius muscle of the cat, the hindquarters of rats and guinea-pigs, and lungs of all three species. The cats were anaesthetized with chloralose intravenously, and the rats and guinea-pigs with pentobarbitone sodium intraperitoneally. The skin flaps of the hind legs and of gastrocnemius muscles of the cat were perfused by the method described by Feldberg and Paton (1951) and Feldberg and Schachter (1952). For the perfusion of the hindquarters a cannula was inserted into the lower abdominal aorta and the venous outflow collected from a cannula in the vena cava. A mass ligature was firmly tied round the body proximal to the cannulation sites and the upper half of the animal cut off. For perfusion of the lungs the pulmonary artery was cannulated. In the experiments on rats and guinea-pigs the lungs were removed from the body and placed in a jacketed funnel at 37° C., the trachea was cannulated, the lungs were inflated with air and the venous effluent allowed to flow out into the funnel through an incision in the left auricle. In the experiments on cats the lungs were

kept *in situ* and rhythmically inflated by an artificial respiration pump; both the pulmonary artery and vein were cannulated and the venous outflow collected through the venous cannula. The perfusion fluid was warm, aerated Locke or Tyrode solution.

The histamine in the venous samples was assayed on the atropinized guinea-pig's ileum. All values refer to the base.

Octylamine was obtained as the free base from Light & Co. The hydrochloride was prepared by suspending the oily liquid in about 10 times the volume of distilled water and titrating with conc. HCl till neutral. This solution of hydrochloride is stable. Compound 48/80 was obtained through the kindness of the late Dr. C. H. Kellaway of the Wellcome Research Institution. Both octylamine and 48/80 were injected, if not otherwise stated, in a volume of 0.5 ml.

RESULTS

Perfusion of Cat Tissues

Skin Flaps.—Weight for weight, the histamine-releasing activity of 48/80 in the perfused cat's skin preparation is about 200 times as strong as that of octylamine.

In one experiment, 50 μ g. octylamine released 18.3 μ g., and a subsequent injection of 0.5 μ g. 48/80, 22 μ g. of histamine. Since a second injection produces on the same skin flap a reduced release of histamine, 48/80 is certainly more than 100 times as active as octylamine. In two experiments the substances were compared by injecting one into a skin flap from the right, and the other into that from the left hind leg of the same cat. In the one experiment, 5 μ g. octylamine released 0.58 μ g., and 0.5 μ g. 48/80 released 30.6 μ g. histamine; in the other experiment, 50 μ g. octylamine released 10.1 μ g. from the right, and 0.2 μ g. 48/80 released 4.7 μ g. histamine from the left, skin flap. This experiment is shown in Fig. 1, which also illustrates that successive injections of histamine liberator produce diminishing effects. From the three experiments it can be concluded that 48/80

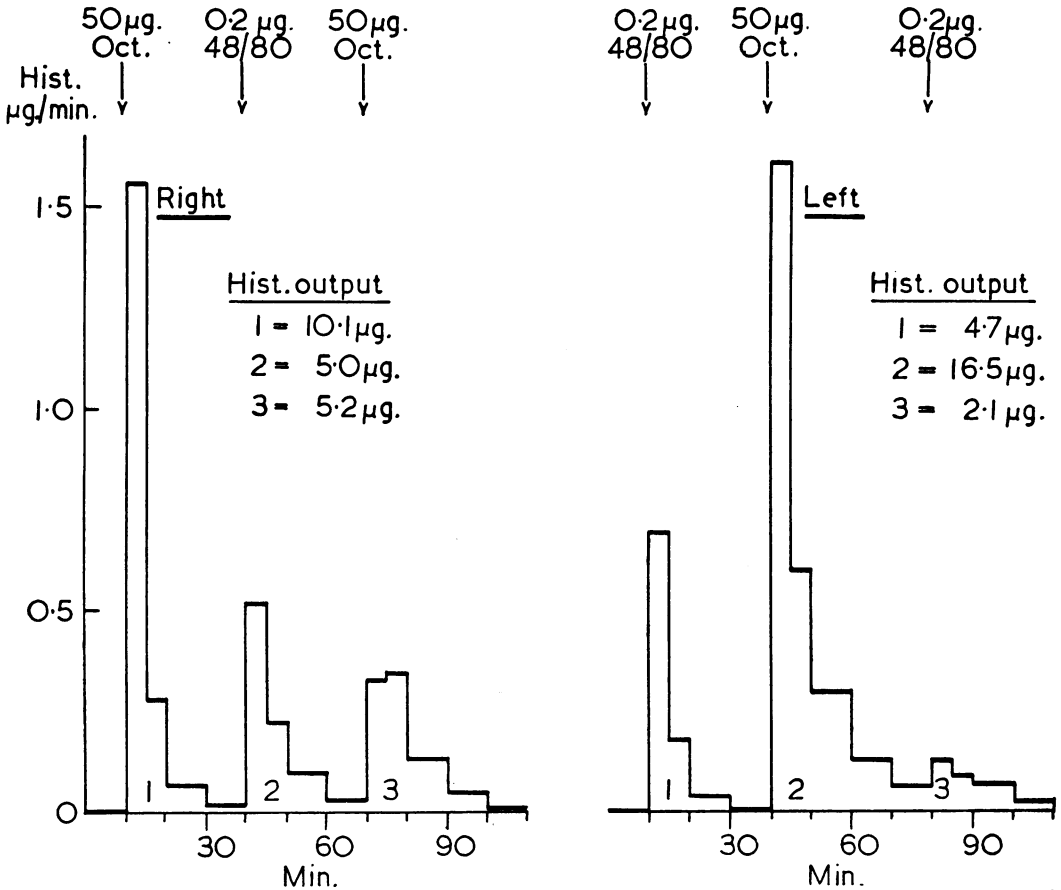


FIG. 1.—Comparison of histamine release by arterial injections of octylamine and 48/80 into perfused skin flaps from the two hind limbs of a cat. 50 µg. octylamine releases more histamine than 0.2 µg. 48/80.

is considerably more than 100 and rather less than 250 times as active as octylamine.

In order to find out if a different result would be obtained when octylamine and 48/80 were left in contact with the skin for a longer period than that attained on single arterial injection, the substances were given by infusion. In one experiment, one skin flap was infused with 100 µg./ml. octylamine, the other with 1 µg./ml. 48/80. The amounts of histamine released during 100 min. infusion were 137 and 210 µg. respectively. In another experiment the concentrations used for infusion were 20 µg./ml. octylamine and 0.1 µg./ml. 48/80. This experiment is illustrated in Fig. 2. The histamine output during 100 min. infusion was 39 and 51 µg. respectively, showing that, weight for weight, 48/80 is again about 200 times as active as octylamine. In Fig. 3 the amounts of histamine collected in the successive samples are plotted, for both infusion experiments, as percentages of the

total release. When the results are expressed in this way, it becomes clear that the initial rate of output is greater with 48/80 than with octylamine. For instance, after 25 min. infusion, the percentages of the total histamine collected in the two experiments were 52 and 62% with 48/80 and 38 and 34% with octylamine.

TABLE I
OUTPUT OF HISTAMINE FROM PERFUSED GASTROCNEMIUS MUSCLES OF THE SAME CAT BY ALTERNATE INJECTIONS OF 48/80 AND OCTYLAMINE

Order of Injection	Right Gastrocnemius		Left Gastrocnemius	
	Injection	Output of Histamine (µg.)	Injection	Output of Histamine (µg.)
1st	5 µg. 48/80	3.7	1,000 µg. octylamine	3.6
2nd	500 µg. octylamine	0.2	5 µg. 48/80	2.1
3rd	5 µg. 48/80	0.3	1,000 µg. octylamine	0.4

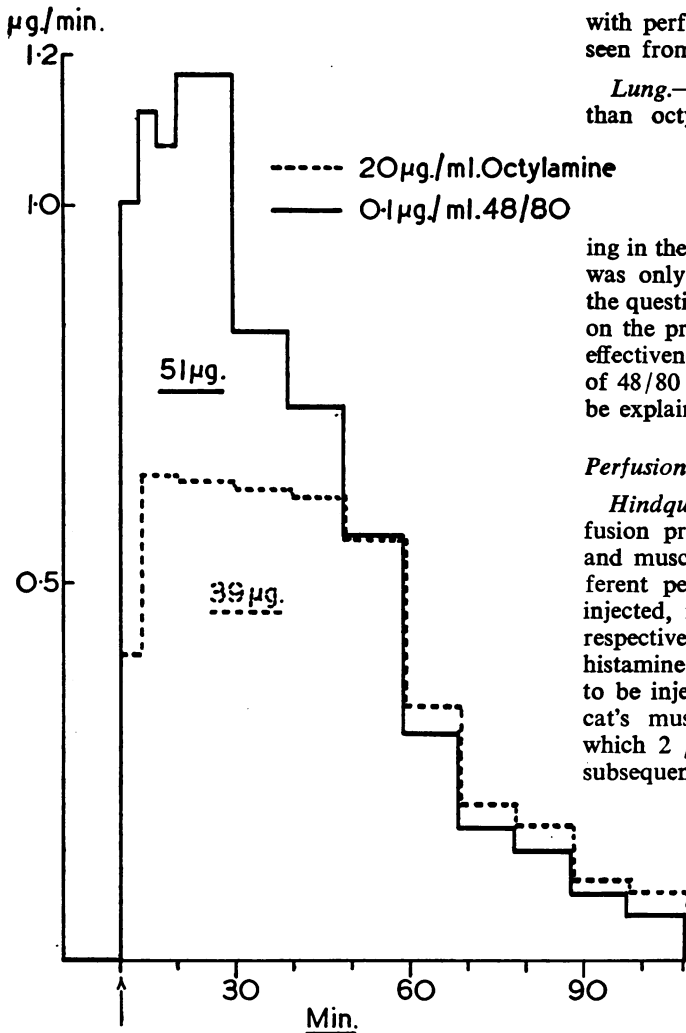


FIG. 2.—Comparison of histamine release by arterial infusion of octylamine and 48/80 into perfused skin flaps from the two hind limbs of a cat. Infusion of 20 µg./ml. octylamine releases less histamine than does 0.1 µg./ml. 48/80.

Gastrocnemius Muscle.—The relative activity of 48/80 to octylamine in releasing histamine from the perfused gastrocnemius muscle is the same as

with perfused skin flaps—i.e., about 200. This is seen from the experiment summarized in Table I.

Lung.—Compound 48/80 was more effective than octylamine in releasing histamine in this tissue, but was not as active as on skin and muscle preparations. This is illustrated by the results of the two experiments given in Table II. The finding in the second experiment that 2 mg. octylamine was only effective when given after 48/80 raises the question of how far this release was dependent on the previous action of 48/80. Further, the ineffectiveness in both experiments of a second dose of 48/80 which had been previously active cannot be explained by depletion of the tissue histamine.

Perfusion of Rat Tissues

Hindquarters (Muscle and Skin).—In this perfusion preparation, as in the perfused cat's skin and muscle, 48/80 is highly active. In three different perfusions, 1, 2, and 10 µg. 48/80 were injected, releasing 2.8, 4.2, and 28 µg. histamine respectively. In order to produce a release of histamine with octylamine, much larger doses had to be injected, as in the perfusion experiments of cat's muscle and skin. In the experiment in which 2 µg. 48/80 released 4.2 µg. histamine, a subsequent injection of 200 µg. octylamine released 1.9 µg. histamine; in the experiment in which 1 µg. 48/80 released 2.8 µg. histamine, a previous injection of 500 µg. octylamine had only released 0.3 µg. histamine. It was not possible to obtain a large histamine release by increasing the dose of octylamine to 5 mg.; this dose, which caused intense reduction in venous outflow and oedema, produced an output of only 2 µg. histamine. From these results it appears that the difference of activity of octylamine and 48/80 in this preparation is greater than on perfused cat

TABLE III

TABLE II
OUTPUT OF HISTAMINE FROM PERFUSED CAT'S LUNGS AFTER ALTERNATE INJECTIONS OF OCTYLAMINE AND 48/80

1st Experiment		2nd Experiment	
Injection	Output of Histamine (µg.)	Injection	Output of Histamine (µg.)
1 mg. octylamine	Nil	2 mg. octylamine	Nil
1 " 48/80	21.3	0.1 " 48/80	11.5
5 " octylamine	2.8	2 " octylamine	2.5
1 " 48/80	Nil	0.1 " 48/80	Nil

TABLE III
OUTPUT OF HISTAMINE FROM PERFUSED RAT LUNGS AFTER ALTERNATE INJECTIONS OF OCTYLAMINE AND 48/80

1st Experiment		2nd Experiment	
Injection	Output of Histamine (µg.)	Injection	Output of Histamine (µg.)
1 mg. octylamine	5.9	1 mg. octylamine	1.2
0.5 " 48/80	3.8	0.5 " 48/80	2.6
1 " octylamine	1.4	1 " octylamine	0.5
0.5 " 48/80	1.8	0.25 " 48/80	0.3
1 " octylamine	0.9	1 " octylamine	0.4

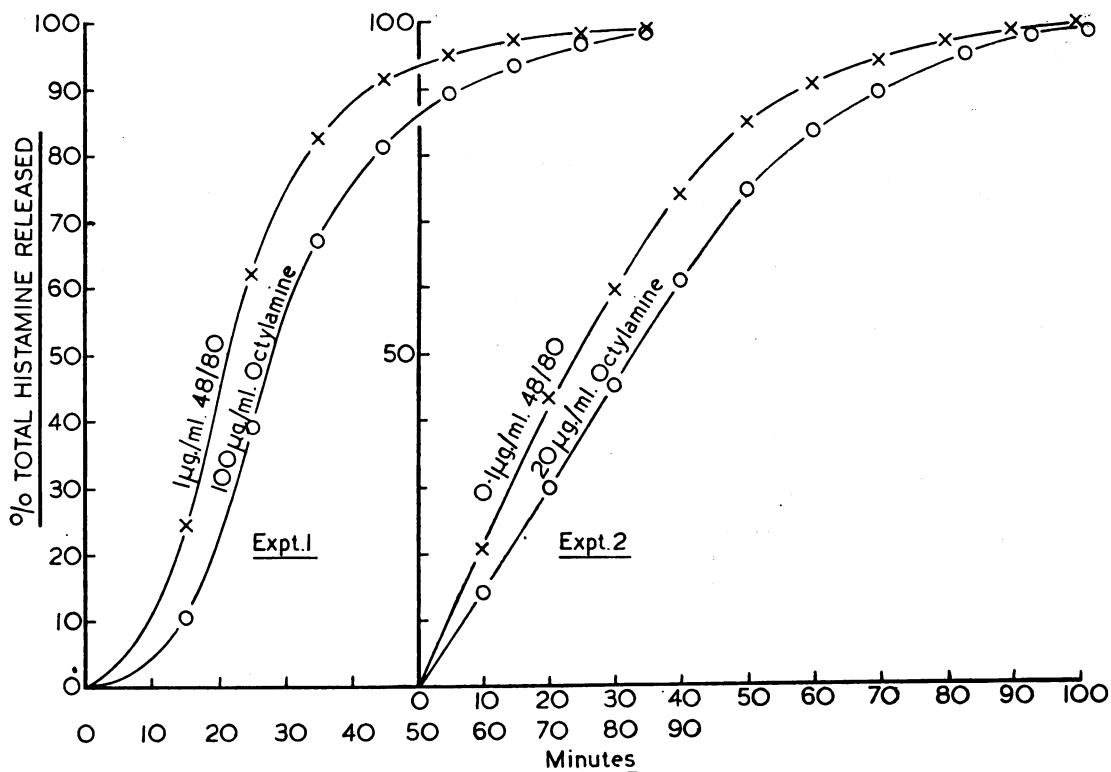


FIG. 3.—Comparison of histamine release curves obtained by infusion of octylamine and 48/80. The initial rate of release is faster with 48/80 than with octylamine. Each comparison was made on perfused skin flaps from the two hind limbs of a cat. Expt. 2, same as that of Fig. 2.

tissue, and resembles more that observed on the human skin by Mongar and Schild (1953).

It is worth mentioning that the development of oedema was much more pronounced after large doses of octylamine than after doses of 48/80 which gave larger releases of histamine.

Lung.—On this perfused tissue the difference in the potency of 48/80 and octylamine was not pronounced. Compound 48/80 was about two to four times as active as octylamine, as seen from the results in Table III.

Perfusion of Guinea-pig Tissues

Hindquarters (Muscle and Skin).—On this preparation 48/80 is not as active as on the rat's perfused hindquarters. The threshold amount of 48/80 was about 20 µg., and even 50 µg. usually released less than 1 µg. histamine. For instance, in one experiment three successive doses of 50 µg. 48/80, injected at 10-min. intervals, released 0.28, 0.43, and 0.32 µg. histamine, and a subsequent

fourth injection of 500 µg. 48/80 released 3.4 µg.

When compared with octylamine, it was found that 48/80 was about 60 times more active in releasing histamine. This is illustrated by the results of two perfusion experiments in which the effect of 2 mg. octylamine was compared in one with 50 µg., and in the other with 25 µg., 48/80. The output of histamine in µg. was 1.1, 0.3, 1.3 in the first experiment with the higher dose of 48/80, and 0.2, 0.7, 0.1 in the second experiment. The underlined values refer to the histamine released by the 2 mg. octylamine.

Lung.—With doses of 0.5 to 1 mg. of either 48/80 or octylamine, usually only a fraction of a µg. (between 0.02 and 0.8 µg.) was released from this perfused tissue. Only in one out of eight experiments was the release of histamine by both octylamine and 48/80, when injected in doses of 1 mg., greater than 1 µg. The ability to release histamine appeared to be of the same order with the two substances. Thus in eight experiments, octylamine gave a greater release in three, and

48/80 in two; in the remaining three there was no appreciable difference when the two releasers were given alternately to the same preparation.

DISCUSSION

A comparison of the effectiveness of 48/80 and octylamine in releasing histamine gives a different result when it is made on perfused skin and muscle preparations from that when it is made on perfused lungs. On skin and muscle 48/80 is many times more active than octylamine—at least 1,000 times in the rat, about 200 in the cat, and about 60 in the guinea-pig—and a release can be obtained with a few $\mu\text{g.}$ and, on some preparations, such as the cat's skin, with a fraction of a $\mu\text{g.}$ of 48/80. On the perfused lungs, however, 48/80 as well as octylamine had usually to be injected in doses of the order of 1 mg. to release measurable amounts of histamine, though cat lung appeared to release more readily to 48/80 than did rat and guinea-pig lung. The activities relative to octylamine are about 20, 3, and 1 respectively. Thus, a comparison of the activity of two releasers depends to a great extent on the species and tissue used for perfusion.

Apart from the species and tissue differences seen when comparing the activity of histamine releasers, the results will also be greatly influenced by the method used for their assay. If, instead of perfusion experiments, the histamine releasers are allowed to act in incubation experiments on tissue particles, the relative activities are different. For instance, Mongar and Schild (1953) found that with this method octylamine was more active than 48/80, when allowed to act on guinea-pig's lung particles. It is not clear why different methods give such different results, but the unique property of 48/80 in releasing histamine, when tested in very low concentrations, either in perfusion experiments or on the intact human skin,

is no longer shown when tested in incubation experiments on skin particles (Mongar and Schild, unpublished experiments).

It is evident from our results that when the activities of two histamine releasers are compared, different ratios can be obtained according to the species, tissues, and methods used. This has to be kept in mind in the assay of histamine releasers. The problem is even more complicated, since the relative ability to release histamine in different species and tissues, and with different methods, may be different for each compound, so that the results obtained using one compound as a standard may have no relation to those obtained when a different reference compound is used.

SUMMARY

1. The histamine-releasing activities of compound 48/80 and octylamine were compared on perfused skin, muscle, and lung of the cat, rat, and guinea-pig.
2. On skin flaps and gastrocnemius muscle of the cat, 48/80 was about 200 times more active than octylamine. On the perfused hindquarters preparation of the rat 48/80 was over 1,000 times, and on that of the guinea-pig about 60 times, more active than octylamine.
3. On perfused lungs, 48/80 was 1 to 20 times more active than octylamine, depending on the species.
4. The importance of tissue, species, and method specificity in assaying histamine releasers is discussed in the light of these results.

REFERENCES

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