Compound 48/80 : a potent hitamine liberator / by W.D.M. Paton.

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Reprinted from BRITISH JOURNAL OF PHARMACOLOGY AND CHEMOTHERAPY
September, 1951, *vol.* 6, *No.* 3, *p.* 499

LONDON
BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, W.C.1

Brit. J. Pharmacol. (1951), **6**, 499.

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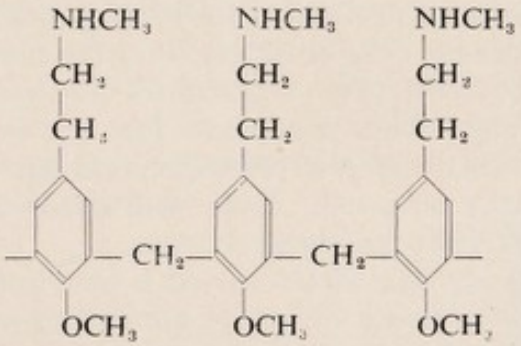
(Received May 18, 1951)

Baltzly, Buck, de Beer, and Webb (1949), analysing the depressor action of certain *iso*quinoline derivatives, found that this action was due to contamination with phenylethylamine condensation products. Several such compounds were prepared, and one of them (Compound 48/80) was found to lower the dog's blood-pressure in very small doses. Compound 48/80 was obtained from the condensation of *p*-methoxyphenethyl-methylamine with formaldehyde, and has a structure of the general type:



Compound 48/80 is probably a mixture of the dimer, trimer, and tetramer.

The present experiments deal with a pharmacological examination of this substance, and have shown that it owes its depressor activity to its ability to release histamine, and that it is the most active histamine-liberator so far investigated. Since then, Feldberg and Paton (1951) have used this substance to demonstrate the release of histamine from the cat's isolated perfused skin preparation.

METHODS

These were as described by MacIntosh and Paton (1949), and Feldberg and Paton (1951).

RESULTS

Experiments on the cat

Effects on the blood-pressure.—The injection of 0.1 mg. Compound 48/80 intravenously into a cat anaesthetized with chloralose caused a profound and rapid fall in blood-pressure, beginning 15–20 seconds after the injection. This delayed depressor response was always seen with an effective dose of the drug, whether the dose was big or small. It was usually followed immediately by partial recovery of

the blood-pressure ; but complete recovery was always slow except with very small doses.

A characteristic delayed fall in blood-pressure of 30 mm. mercury could be produced with as small a dose as 9 μg./kg. intravenously (Fig. 1). When the same dose was repeated three minutes later, a delayed depressor response was obtained again, but it was greater in magnitude and longer-lasting than after the first dose.

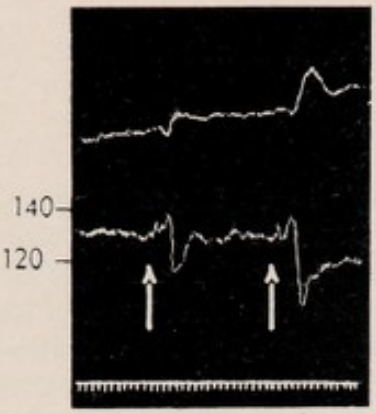


FIG. 1.—Cat, chloralose. Limb volume (upper record) and arterial blood-pressure. At arrows, 9 μg./kg. of Compound 48/80. Time 10 sec.

The depressor response was evidently due to a peripheral vasodilatation, for during the fall in blood-pressure the volume of a limb of the cat increased, waning again as the blood-pressure recovered. Fig. 1 illustrates this point for two small doses of Compound 48/80. Larger doses produced larger and more sustained effects. The heart rate was not altered until the blood-pressure had fallen considerably, when cardioacceleration usually occurred. Even with doses causing a prolonged fall in blood-pressure, the heartbeat remained vigorous.

After a dose of 50 μg./kg., which caused a long-lasting fall in blood-pressure, it was observed that the pressor action of adrenaline was reduced. Thus in one experiment, before the injection of Compound 48/80, 5 μg. adrenaline raised the pressure by 70 mm. from 125 mm. ; after the injection of 0.1 mg. Compound 48/80 the blood-pressure fell to 60 mm., but despite this reduction in blood-pressure, 5 μg. adrenaline now only caused a rise of 30 mm. ; not until 40 minutes later, when the blood-pressure had recovered to 85 mm., did the effect of this dose of adrenaline return to normal. After the injection of histamine liberators in general, and Compound 48/80 in particular, there is almost always a vigorous cardioacceleration during the later part of depressor effect, and it is highly probable that this is due partly to a release of adrenaline (or *nor*adrenaline) in response to the fall of blood pressure and to circulating histamine. Since this reaction must contribute substantially to the subsequent recovery of the blood-pressure, it is possible that the depression of the effects of such released adrenaline by Compound 48/80 contributes to its long duration of action compared to other histamine liberators.

Haematocrit.—After large doses of Compound 48/80, the proportion of red cells to plasma in the blood rose. For instance, in Exp. 2 of Table I, the haematocrit increased from an initial value of 44 per cent before the injection to a value of 51 per cent one minute after the intravenous injection of 0.7 mg./kg. of Compound 48/80. In five minutes it rose further to 58 per cent and was at the same value 24 minutes later.

Release of histamine in the cat.—The delayed depressor response, the "self-potentiation," the peripheral vasodilatation and the rise in haematocrit are characteristic of histamine liberators (MacIntosh and Paton, 1949). Experiments were therefore made to test whether histamine could be demonstrated in the plasma of animals receiving the drug. Table I gives the results from two such experiments. The plasma before the injection of Compound 48/80 had a histamine equivalent of 0.05 μg./c.c., or less. After the injection of 0.5 or 0.7 mg./kg. of Compound 48/80

TABLE I

Exp.	Dose of 48/80	Assay method	Histamine equivalents (μg./c.c.) of plasma before and after injection of 48/80				
			Time after injection of 48/80 (minutes)				
			Before	1	5	10	30
1	0.5 mg./kg.	Guinea-pig's ileum	0.05	0.5	—	0.32	0.22
2	0.7 mg./kg.	Guinea-pig's ileum	<0.05	0.9	≃0.5	—	<0.1
		Cat's B.P.	<0.1	1.0	0.3	—	<0.1

intravenously, it rose within a minute to 0.5 μg./c.c. and 1.0 μg/c.c. respectively in the two tests. That the active substance in the plasma was in fact histamine was shown by the facts that assays on cat's blood-pressure and on guinea-pig's ileum agreed quantitatively, and that small doses of mepyramine abolished its effect on guinea-pig ileum (Fig. 2*b* and *g*) and on the cat's blood-pressure (Fig. 3*b* and *h*) proportionately with the effect of an equipotent dose of histamine. Atropine did not reduce the effect of the plasma on the gut.

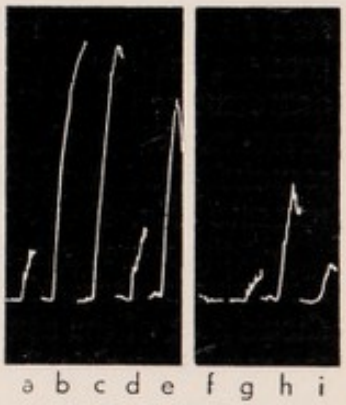


FIG. 2.—Guinea-pig's ileum. Effects of plasma from Exp. 2, Table I. (*a*), (*b*), (*c*), (*d*), 0.1 c.c. plasma withdrawn respectively before and 1 min., 5 min., and 30 min. after the intravenous injection of Compound 48/80. (*e*) 0.06 μg. histamine. Mepyramine 0.3 μg. added to bath thereafter. (*f*) 0.06 μg. histamine. (*g*), (*h*), (*i*), 0.1 c.c. of 1 min., 5 min., and 30 min. plasma respectively.

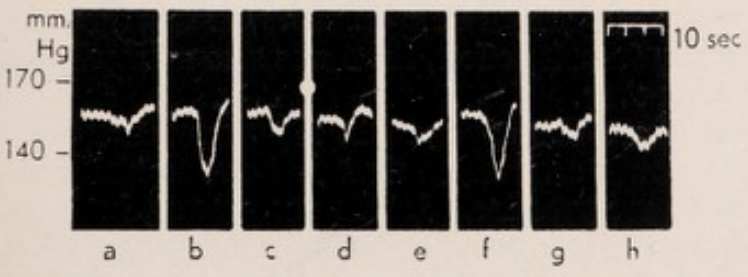


FIG. 3.—Cat, chloralose. Arterial blood-pressure. Intravenous injections. Effects of plasma from Exp. 2, Table I. (*a*), (*b*), (*c*), (*d*), 0.25 c.c. plasma withdrawn before and 1 min., 5 min., and 30 min. after intravenous injection of Compound 48/80. (*e*) 0.05 μg. histamine. (*f*) 0.3 μg. histamine. Between (*f*) and (*g*), 0.05 mg. mepyramine. (*g*) 0.3 μg. histamine. (*h*) 0.25 c.c. of 1 min. plasma.

With samples withdrawn more than one or two minutes after the injection, the determination of the histamine equivalent of the plasma was not so simple. In both experiments of Table I a substance appeared in the later samples of plasma which caused a contraction of the guinea-pig's ileum even in the presence of mepyramine. This substance was present in such large amounts in Exp. 2 of Table I as to be responsible for almost half the activity of the five minutes plasma on the gut (Fig. 2*c* and *h*). The assay on the guinea-pig's gut of the histamine equivalents of these later samples was therefore approximate, and took into account how far the response of the gut to the plasma sample was maintained after mepyramine.

The contraction produced by this unknown substance was much slower than that produced by histamine, and diminished in size rather rapidly if it was elicited at intervals of less than five minutes. This "slow-contracting substance" did not, however, depress the cat's blood-pressure relative to histamine so strongly as it affected the gut, as can be seen by comparing the effects of the one minute and five minutes sample of Exp. 2 on the gut (Fig. 2*b* and *c*) with their effects on the blood-pressure (Fig. 3*b* and *c*). In Exp. 1, this additional substance was present in considerable amounts in the plasma taken both 10 and 30 minutes after the injection of Compound 48/80; in Exp. 2, more activity was present after five minutes than at 1 minute or 30 minutes. It is possible that the persistent depressor effect of Compound 48/80 is partly due to the presence of this substance in the plasma, in addition to circulating histamine.

Experiments on the dog

In experiments with other histamine liberators it had been found that when these compounds are injected intravenously in the dog they produce a rise in portal pressure and a delay in coagulation of the blood, as well as causing a fall in blood-pressure and the appearance of histamine in the plasma. Fig. 4*a* shows that the depressor action of Compound 48/80 is likewise associated with a rise in intraportal pressure. When the same dose was repeated 60 minutes later, the animal no longer responded to it, and there was neither a fall in blood-pressure nor a rise in intraportal pressure (Fig. 4*b*).

At the same time as the depressor response to the first dose of Compound 48/80, histamine appeared in the plasma of blood withdrawn from a femoral artery. Fig. 5 shows part of the assay of this plasma on the guinea-pig's ileum, and Fig. 6 shows the results graphically. One minute after the injection, the histamine equivalent of the plasma reached its maximum of 2.2 μg./c.c., and fell thereafter fairly rapidly. At the same time, the coagulation time lengthened; but whereas the histamine reached its highest concentration in the plasma very rapidly, the prolongation of clotting time was greatest in the sample eight minutes after the injection, and then fell more slowly. One hour later, when the dose of Compound 48/80 was repeated, and failed (as noted above) to have any vascular effect, there was also no significant change in plasma histamine or clotting time.

As in the experiments with cats, there also appeared in the plasma samples a gut-contracting substance, causing a slower contraction than that due to histamine, often diphasic, which was resistant to mepyramine. It was not present, however, in great amounts, and appeared in appreciable quantities only in the sample taken 40 minutes after the first injection. It was not possible to compare accurately the

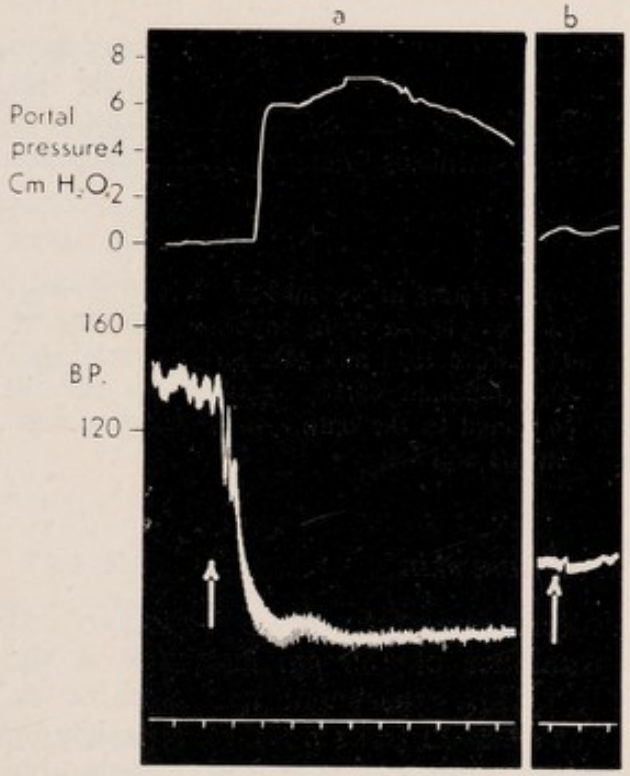


FIG. 4.—Dog, chloralose. Portal pressure (above), arterial blood-pressure (below). (*a*) At arrow, 1.0 mg./kg. Compound 48/80. (*b*) 60 min. later, the same. Time, 30 sec.

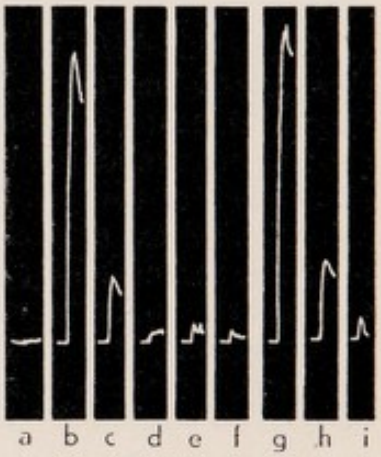


FIG. 5.—Guinea-pig's ileum. Effects of plasma from experiment of Fig. 4. (*a*), (*b*), (*c*), (*d*), (*e*), 0.1 c.c. plasma withdrawn before and 1 min., 7 min., 20 min., and 40 min. after intravenous injection of Compound 48/80. (*f*) 0.1 c.c. plasma withdrawn 2 min. after second injection of Compound 48/80 (*g*), (*h*), (*i*), 0.25 μg., 0.05 μg., 0.01 μg. histamine respectively.

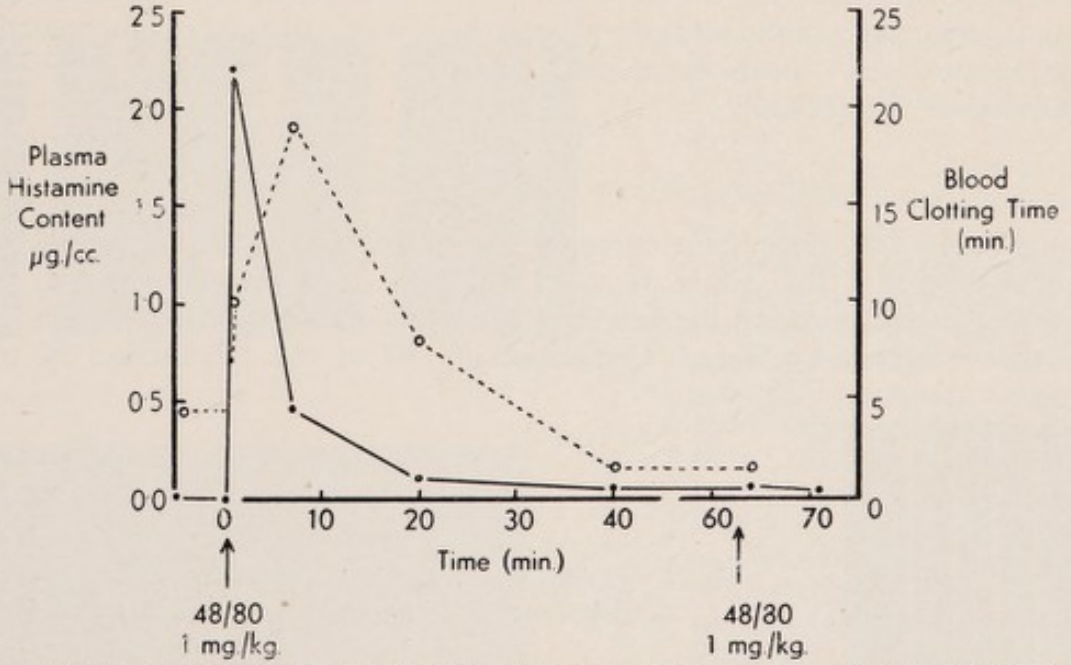


FIG. 6.—Graph of histamine concentration in plasma (μg./c.c. continuous line) and blood clotting time (minutes, dotted line) after injection of 1.0 mg./kg. of 48/80 intravenously at arrows; same experiment as Fig. 4.

amounts of this " slow-contracting substance " in different samples, for it was found that Compound 48/80 in quite small doses itself antagonized the effects of this substance. In Fig. 7 may be seen how 10 μg. Compound 48/80 reduced by two-thirds the slow contraction produced by the 40 minutes plasma in the presence of mepyramine.

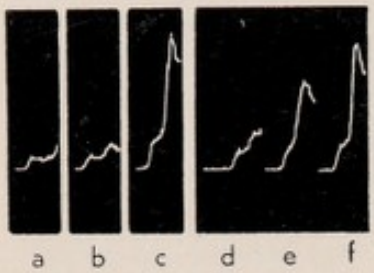


FIG. 7.—Guinea-pig's ileum, in presence of 2.5×10^{-8} mepyramine. 0.5 c.c. plasma from experiment of Fig. 4, withdrawn (*a*) before, (*b*) 1 min. after, (*c–f*) 45 min. after injection of Compound 48/80. Between (*c*) and (*d*) 10 μg. 48/80 added to the bath. 48/80 removed from bath between (*d*) and (*e*).

Release of histamine from skin

Compound 48/80, in a dose of 10 μg., injected intra-arterially into the perfused skin preparation of the cat caused the appearance of 160 μg. histamine in the venous effluent (Feldberg and Paton, 1951). This experiment was repeated on another preparation, and 65 μg. histamine was released from the skin, representing 52 per cent of the total histamine present in the portion of skin used. Detectable histamine release can be obtained with even smaller doses of Compound 48/80 ; in a third experiment 1 μg. Compound 48/80 was injected, and 7.7 μg. histamine base was collected in the venous effluent.

Compound 48/80 injected intradermally in low concentrations into human skin produces a weal and flare of a kind resembling that produced by histamine. Thus, injected intradermally into the skin of the forearm of two subjects, Compound 48/80 in a dilution of 1 : 1,000,000 caused weals of 7–9 mm. diameter surrounded by flare of 30 mm. diameter ; as the weal developed there was a distinct itchiness at the site of injection. These effects were comparable to those produced by histamine 1 : 1,000,000 or 1 : 300,000.

Experiments on the isolated guinea-pig's ileum

Compound 48/80 does not itself cause any contraction of the guinea-pig's ileum, even in a dose of 1 mg. added to a 15 c.c. bath (Fig. 8*f*). But it does possess some power of antagonizing the action of acetylcholine on this preparation. Fig. 8 shows the reduction by 50 μg. Compound 48/80 of the contraction of the gut

FIG. 8.—Guinea-pig's ileum in 15 c.c. bath. (*a*) and (*c–f*), 0.1 μg. acetylcholine; (*b*) 50 μg. and (*g*) 1 mg. Compound 48/80.

produced by 0.15 μg. acetylcholine; 10 μg. Compound 48/80 had a smaller but still distinct effect of the same sort; the pA_2 for this atropine-like action, after a two minute exposure, was estimated to be approximately 6. The effect of the drug was sometimes greatest just after it had been washed out, rather than when the response to acetylcholine was tested in its presence, and later there was also observed, during the recovery of the gut, a potentiation of the response to acetylcholine before it finally returned to normal. The response to histamine was also depressed by 50 μg. Compound 48/80, but to a smaller extent; 20 μg. had no detectable effect (Fig. 9).

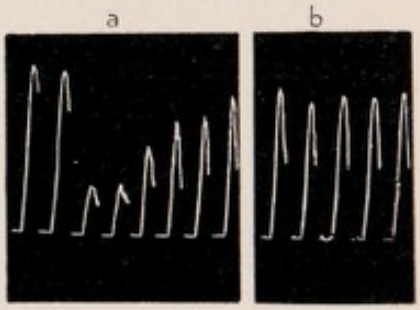


FIG. 9.—Guinea-pig's ileum in 15 c.c. bath. Contractions due to 0.1 μg. histamine. (*a*) 50 μg. Compound 48/80 added before 3rd and 4th contraction. (*b*) 20 μg. Compound 48/80 added before 3rd and 4th contraction.

A conspicuous activity of this drug on the small intestine was that of antagonizing the effects of "slow contracting substances." Fig. 7 showed the reduction by 10 μg. Compound 48/80 of the slow contraction of the gut produced in the presence of mepyramine by plasma from a dog shocked with Compound 48/80. Similar antagonisms are shown in Fig. 10, using doses of 10–100 μg. Compound 48/80 on the slow contractions produced by (*a*) substance P; (*b*) and (*c*) the "slow contracting substances" in extracts of muscularis mucosae of the dog's small intestine (this

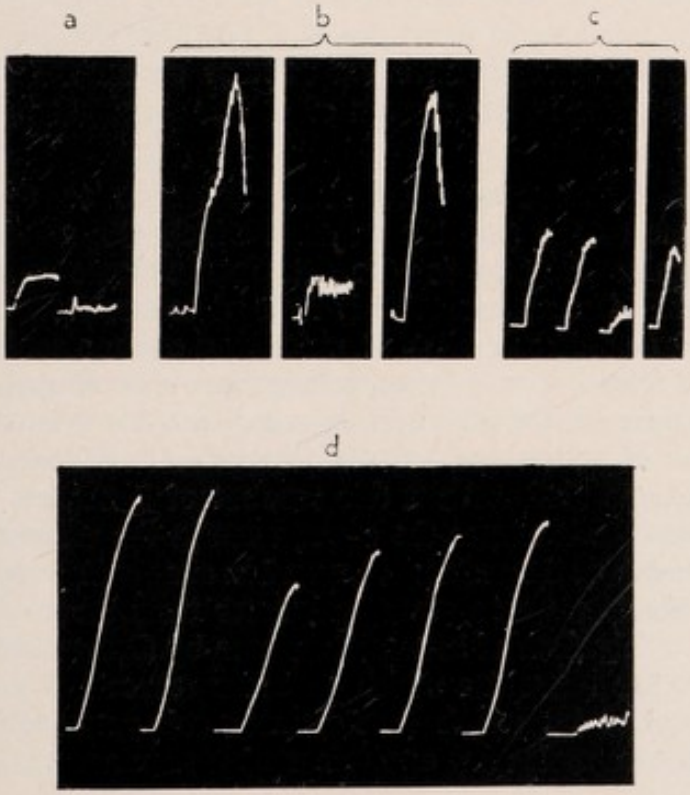


FIG. 10.—Guinea-pig's ileum in 15 c.c. bath. (*a*) Mepyramine 1.5×10^{-9}. Contractions produced by 1 mg. substance P. Before second contraction, 50 μg. Compound 48/80. (*b*) Atropine 2×10^{-9}: mepyramine 2×10^{-9}. First and second contractions produced by extract from 10 mg. muscularis mucosae of dog's small intestine, third contraction by extract from 20 mg. Before second and third contraction, 20 μg. Compound 48/80. (*c*) Atropine 1.5×10^{-9}: mepyramine 1.5×10^{-9}. Contractions produced by extract from 50 mg. of whole wall of cat's jejunum. Before third contraction, 30 μg. Compound 48/80. (*d*) Contractions produced by 200 μg. Bradykinin; before third contraction, 10 μg. Compound 48/80; before seventh contraction 100 μg. Compound 48/80.

is probably substance P ; cf. Douglas, Feldberg, Paton, and Schachter, 1951), and in an extract of the whole wall of a cat's jejunum ; (*d*) bradykinin. An extended comparison of the potency of Compound 48/80 against these various contractions was not made. But Compound 48/80 appeared to reduce all these slow contractions by about the same amount, and this antagonism was about as effective as that against acetylcholine and slightly more effective than that against histamine.

Discussion

The principal action of Compound 48/80 is that of histamine release, verified directly by plasma assay. To this are due all its vascular effects, viz., the delayed depressor response, limb vasodilatation, rise in haematocrit in the cat, and rise of portal pressure in the dog. When, as in the dog which has already received a dose of the drug (Fig. 4), no histamine release into the blood takes place, there is also no depressor action by the drug. Like the other histamine liberators already described, it also causes in the dog a delay in the clotting of blood. In all these respects Compound 48/80 resembles the other members of the group of histamine liberators. Its main interest lies in its remarkably high activity in causing the release of histamine, for this is high by any pharmacological standard. But it also possesses another property, that of causing the appearance of a slow-contracting substance in the plasma of cat and dog. A similar substance has been shown by Beraldo (1950) to appear in the plasma of dogs exposed to anaphylactic shock or injected with peptone. MacIntosh and Paton (1949), reviewing the wide range of bases for which they had obtained evidence of histamine liberation, concluded that there was probably considerable significance to be attached to the existence of so many substances capable of reproducing the salient features of anaphylactic shock. This conclusion is now further strengthened by the observations that histamine liberators may exist, not only of great diversity, but also of great potency, and that the processes of anaphylaxis and of histamine-liberation have yet another characteristic in common, that of causing the appearance in the plasma of a "slow-contracting substance."

The high potency of Compound 48/80 may have some relevance to the mechanism of histamine release. One of the possible ways in which this might occur is by competition of the histamine liberator for the sites in the tissue with which histamine is usually associated. On such a view it is hard to see how a histamine liberator could release, for each molecule injected, more than one molecule of histamine. Indeed, a lower rate of release would be expected, since it could only be rarely that every molecule of a dose of the liberator injected into an animal would be able to reach a suitable site of action and act with theoretical efficiency. But Compound 48/80 releases considerably more than this amount of histamine ; roughly, for each basic unit of its molecule it causes the appearance in the effluent from the perfused skin of 10–30 molecules of histamine. Even when simply injected intradermally into human skin, its effects were as great or greater than those of equal doses of histamine itself. The mechanism of histamine release by Compound 48/80, and presumably by all the other liberators which in other respects it so closely resembles, must therefore be one by which the liberator can cause the release of considerably more than its own molecular equivalent of histamine.

It is interesting that Compound 48/80, although itself inactive on the guinea-pig's gut, possesses the ability of antagonizing the contraction produced by the group of "slow-contracting substances," in small doses comparable to or less than those which affect the response to histamine and acetylcholine. Such an antagonistic action has not hitherto been described. The effect is hardly specific enough to be of much practical use, although it may help in defining some of the properties of these substances. It is of interest, however, in furnishing a common property between all the "slow-contracting substances" and strengthens the supposition that they are more or less closely related.

The relationship between Compound 48/80 and "slow-contracting substances" extends even further; for a substance of this type appears, after a dose of Compound 48/80, in relatively large quantity in the plasma of both cat and dog. The appearance of "slow-contracting substance" has been described before in experiments on the effect of venoms on various perfused organs (Feldberg, Holden, and Kellaway, 1938; Trethewie, 1939) and in anaphylaxis and peptone shock (Beraldo, 1950). In general it has been regarded as a side-product of the process of histamine release. But when it is found that a highly active histamine liberator not only causes the release of "slow-contracting substance" into the circulation, but bears so close a relationship to it as also to antagonize its effects, the possibility must now be considered that "slow-contracting substance" is itself directly involved in the histamine liberating reaction, although it is still too early to consider how this might come about.

The chemical structure of Compound 48/80 is unusual pharmacologically. The fact that it has proved to have such a high potency in one respect, that of histamine liberation, and that it displays a novel antagonism, that against "slow-contracting substances," suggests that a study of such basic "polymers" might be fruitful in yet other directions.

Summary

1. Compound 48/80, a condensation product of *p*-methoxyphenylethyl-methylamine and formaldehyde, injected intravenously in doses of 10 μg./kg. or more into the cat, causes a delayed depressor response. The depressor effect is associated with peripheral vasodilatation, and a rise in the haematocrit.

2. In the cat and the dog, intravenous injections of Compound 48/80 cause the release of histamine into the blood in amounts adequate to account for the depressor action of the drug. When a second dose fails to cause any further histamine release, there is also no depressor effect.

3. In the dog, Compound 48/80 causes a rise in portal pressure and a delay in the coagulation of the blood.

4. Compound 48/80 elicits a "triple response" from human skin when injected intradermally in a concentration of 1 : 1,000,000, and causes the appearance of large amounts of histamine in the venous effluent when injected into the isolated perfused skin preparation. A dose of 1 μg. Compound 48/80 caused the release of 7.7 μg. histamine base from this preparation.

5. After the injection of Compound 48/80, a "slow-contracting substance" appears in the plasma of both cat and dog. Compound 48/80 antagonizes the action of this substance on the guinea-pig's ileum, and also that of substance P,

bradykinin, and the "slow-contracting substance" present in extracts of dog and cat small intestine.

6. Compound 48/80 exerts a moderately strong antagonism to the effects of acetylcholine and histamine on the guinea-pig ileum.

I am greatly indebted to Dr. E. J. de Beer, the Wellcome Research Laboratories, Tuckahoe, N.Y., for generous supplies of Compound 48/80.

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PRINTED IN GREAT BRITAIN BY FISHER, KNIGHT & CO. LTD., GAINSBOROUGH PRESS, ST. ALBANS, HERTS.