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THE EFFECT OF COMPOUND 48/80 ON GANGLIONIC TRANSMISSION

BY

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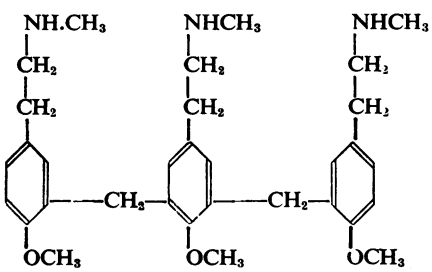
At the present time, compound 48/80 (a condensation product of *p*-methoxyphenethylmethylamine with formaldehyde), whose formula is given below (Baltzly, Buck, de Beer and Webb, 1949), is the most potent histamine liberator known (Paton, 1951 ; Feldberg and Paton, 1951). In addition, it has the ability to release heparin and a " slow contracting substance " from muscle and skin (Paton, 1951). It has now been demonstrated that 48/80 also has the property of blocking ganglionic transmission in the sympathetic ganglion of the cat. The site and mechanism of this block have been analysed.



48/80 is a mixture of dimer, trimer, and tetramer, with most of the pharmacological action residing in the trimer.

METHODS

The experiments were carried out on the perfused superior cervical ganglion of the cat anaesthetized with chloralose (80 mg./kg.). The method of ganglionic perfusion was that described by Kibjakow (1933) and subsequently modified by Feldberg and Gaddum (1934) and by MacIntosh (see Perry, 1953). Locke's solution was used as the perfusion fluid (composition : NaCl 9.2 g., KCl 0.42 g., $CaCl_2$ 0.24 g., $NaHCO_3$ 0.15 g., dextrose 2.0 g., made up to 1 litre with distilled water).

The vagosympathetic trunk was cut peripherally and stimulated by means of square waves at a frequency of 10 cycles/sec., 0.5 msec. duration, and supermaximal voltage. The vagus was tied central to the ganglion, but, in order to diminish leakage of fluid during the perfusion, no separation of the peripheral vagosympathetic trunk was usually carried out. The ganglion with its preganglionic nerve was kept immersed in warm paraffin to prevent drying, and, by suspending a light bulb above the preparation, the temperature of the paraffin could be held between 36 and 37° C. As an indication of transmission in the ganglion, the contraction of the nictitating membrane was utilized and recorded on a smoked drum by a frontal writing lever. All drugs injected into the ganglion were dissolved in Locke's solution.

In several experiments the venous effluent from the perfused ganglion was assayed for histamine or ACh. When the ACh output was determined, the perfusion fluid contained eserine sulphate 1 in 100,000.

Histamine Assay.—Histamine in the effluent was assayed on the isolated atropinized guinea-pig ileum suspended in a 15 ml. bath in Mg-free Tyrode solution at 34° C. (composition : NaCl 8.0 g., KCl 0.2 g., $CaCl_2$ 0 2 g., NaH_2PO_4 0.05 g., $NaHCO_3$ 1.0 g., dextrose 1.0 g., made up to 1 litre with distilled water). It was found in preliminary tests that 48/80 did not affect the response of the gut to histamine. If at all, there was a slight potentiation of response. All values of histamine are expressed as base.

Acetylcholine Assay.—When 48/80 and histamine were present in samples of the venous effluent, the perfusate could not be assayed for ACh on the arterial blood pressure of the cat because of the depressor action of 48/80. In preliminary tests it was found that histamine in a concentration of 10^{-6} and 48/80 in a concentration of 10^{-4} had no action on the eserinized dorsal muscle of the leech and did not influence the response of this muscle to ACh. The assay was therefore carried out on the leech muscle suspended at room temperature in a 5 ml. bath of a salt solution prepared by diluting the Locke's solution used for the ganglionic perfusion (1 litre Locke's solution to 1.4 litres with distilled water) and adding eserine sulphate 1 in 100,000. The routine adopted was to add the ACh or test solutions every 12 min. and allow the contraction to proceed for 2 min., thus

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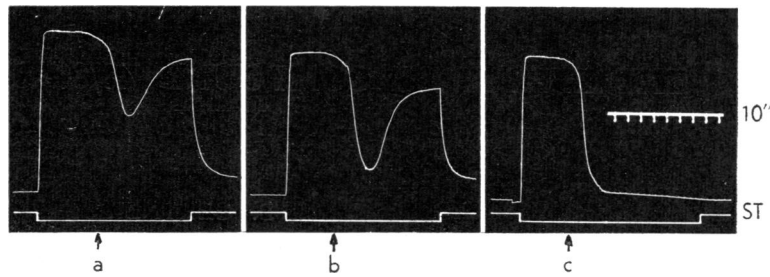

FIG. 1.—Perfused superior cervical ganglion of cat under chloralose anaesthesia. Contractions of nictitating membrane to stimulation of cervical sympathetic (ST). Injections into ganglion of 25 μg. 48/80 (at a), 50 μg. (at b), and 100 μg. (at c). Intervals of 10 min. between tracings. Time in 10 sec.

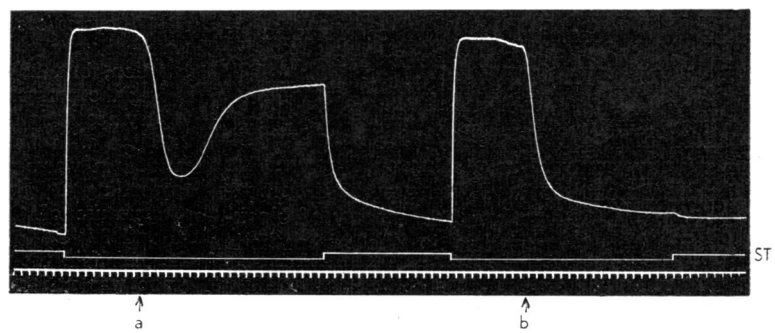

FIG. 2.—Perfused superior cervical ganglion of cat under chloralose anaesthesia. Contractions of nictitating membrane to stimulation of cervical sympathetic (ST). At a and b injection of 50 μg. 48/80 into ganglion. Time in 10 sec.

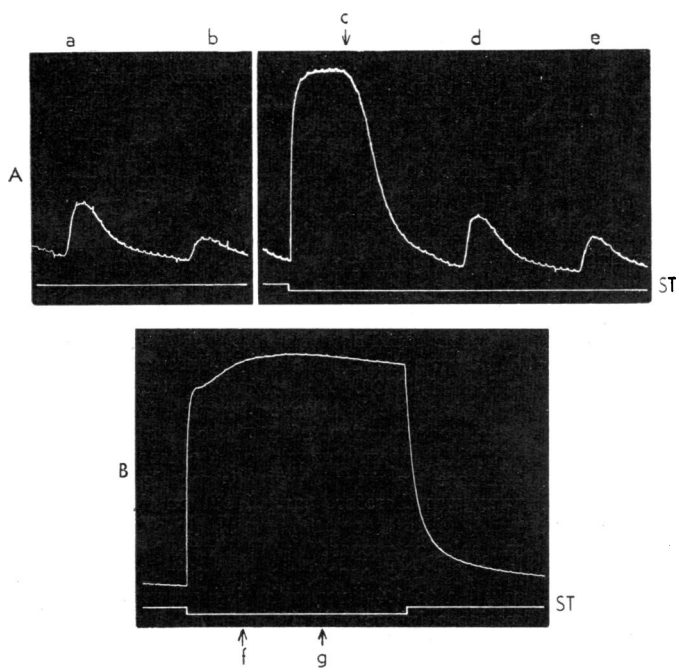

FIG. 3.—Perfused superior cervical ganglion of cat under chloralose anaesthesia. A, Contractions of nictitating membrane to intravenous administration of 15 μg. adrenaline at a, b, d, and e. At c, injection of 100 μg. 48/80 into ganglion during stimulation of cervical sympathetic (ST). B, Contraction of nictitating membrane to stimulation of postganglionic fibres (ST). Injections into ganglion of 100 μg. 48/80 (at f) and 10 mg. tetraethylammonium chloride (at g).

leaving a 10 min. interval between doses. This procedure yielded satisfactory assays, with the leech muscle remaining responsive for periods of over 6 hr.

RESULTS

Effects of 48/80 on Transmission

48/80 had no stimulating action when injected into the ganglionic circulation in a dose of 25–100 μg. When injected during continuous maximal stimulation of the preganglionic fibres, however, it regularly produced an immediate relaxation of the nictitating membrane. The dose necessary to elict this effect varied in different experiments ; in some 25 μg. gave a pronounced action, in others only a slight relaxation or none at all. Fig. 1 illustrates a typical experiment. With 25 μg. 48/80 there was some relaxation of the nictitating membrane ; with 50 μg. the effect was greater, but complete relaxation was only obtained with 100 μg. With increasing doses of 48/80 the effect not only became more pronounced but also more prolonged. In the experiment of Fig. 1 the actions of 25 and 50 μg. 48/80 were over within 2 min., whereas the complete relaxation produced by 100 μg. persisted for about 10 min.

When a dose of 48/80 which caused partial relaxation of the nictitating membrane was repeated some minutes after complete recovery, it produced a greater and longer-lasting depression than the first. Fig. 2 illustrates this point.

The relaxation of the nictitating membrane is not due to escape into the blood stream of some of the injected 48/80 acting directly on the nictitating membrane, but is the result of a true ganglionic block. This is demonstrated by the fact that during the depression the nictitating membrane contracts both to adrenaline and to postganglionic stimulation, as illustrated in the experiments of Fig. 3. In Fig. 3 A are seen the contractions of the nictitating membrane produced by intravenous administration of 15 μg. adrenaline. At a and b the drug was injected before, and at d and e after, the administration of 100 μg. 48/80 (at c), during continuous stimulation of the cervical sympathetic. This dose of 48/80 produced complete relaxation of the nictitating membrane. In Fig. 3 B the contraction of the nictitating membrane produced by postganglionic stimulation is shown not to be affected by an injection into the ganglion of either 48/80 (at f) or the known ganglion blocking drug, tetraethylammonium (at g).

Propamidine.—A ganglionic blocking effect was also obtained with propamidine isethionate, another potent histamine liberator (MacIntosh and Paton, 1949). The doses required to elicit this action were of the same order as with 48/80.

Release of Acetylcholine During Ganglionic Block Produced by 48/80

48/80 injected into the ganglionic circulation of an eserinized ganglion causes no output of ACh, nor does it affect the output of ACh during preganglionic stimulation, in spite of the occurrence of ganglionic block. This was shown by two series of experiments.

In the first, the preganglionic nerve was stimulated continuously and the perfusate collected every 2 min., the drug being injected on the second minute. During such continuous stimulation the ACh output is known to fall rapidly (Brown and Feldberg, 1936 ; Perry, 1953). This was confirmed, and is illustrated by the results of the first three experiments of Table I. The fall in ACh output was not modified by the administration of 48/80, as shown by the last three experiments of Table I. In these the injection of 100 μg. 48/80 caused pronounced block lasting for about the following 6 min. A comparison of the ACh outputs in these three experiments with the first three of Table I shows that the ganglionic block did not affect the release of ACh.

TABLE I

ACh OUTPUT (NG.) IN CONSECUTIVE 2 MIN. SAMPLES OF GANGLIONIC EFFLUENT DURING CONTINUOUS PREGANGLIONIC STIMULATION (10 CYCLES/SEC., 0.5 MSEC.)

Underlined values indicate ACh in sample collected during ganglionic block from injection of 100 μg. 48/80 into the ganglionic circulation

Expt. No.	Consecutive Periods of Collection (min.)				
	0–2	2–4	4–6	6–8	8–10
1	33	30	25	22	20
2	35	30	21	21	19
3	62	39	20	12	10
4	34	27	20	15	10
5	47	35	28	18	12
6	36	30	25	20	15

In the second series of experiments, the preganglionic trunk was stimulated every 10 min. for 2 min. and the venous effluent collected during the 2 min. period of stimulation and the following 30 sec. This procedure has the advantage of excluding any possible carry-over of ACh from one period of stimulation to the next. As seen from a comparison of the first four experiments of Table II with the first three of Table I, the fall in ACh output during each subsequent 2 min. period of stimulation is approximately the same whether a period of rest is interpolated or not. In the last four experiments of Table II, 100 μg.

48/80 was injected 30 sec. before the second period of stimulation. The injection did not influence the release of ACh but caused profound block during the subsequent period of stimulation.

Since the first period of stimulation always yielded the greatest output of ACh, in one other experiment (No. 9 of Table II) 200 μg. 48/80 was injected 30 sec. before the first stimulation period, and blocked transmission completely so that there was no retraction of the nictitating membrane. Nevertheless, the output of ACh during this period was higher than that of the subsequent periods of stimulation, when the ganglionic block had worn off.

TABLE II

ACh OUTPUT (NG.) IN GANGLIONIC EFFLUENT FROM FIVE 2 MIN. PERIODS OF PREGANGLIONIC STIMULATION (AT 10 CYCLES/SEC., 0.5 MSEC. DURATION) WITH 8 MIN. REST BETWEEN EACH PERIOD

Underlined values indicate ACh in effluent collected during ganglionic block from injection of 100 μg. 48/80 into ganglionic circulation. Amounts injected in expts. 1–8 were 100 μg.; in expt. 9, 200 μg.

Expt. No.	Collection Period				
	1	2	3	4	5
1	41	30	20	12	10
2	45	33	32	20	15
3	65	34	20	20	12
4	37	20	19	15	13
5	44	36	30	16	12
6	60	38	12	12	10
7	40	28	18	18	14
8	18	14	10	10	10
9	40	28	18	18	13

Action of Acetylcholine During the Ganglionic Block Produced by 48/80

During the ganglionic block produced by 48/80, the sensitivity of the ganglion cells to ACh is decreased. This is shown by the experiment of Fig. 4, in which the effects of 5 μg. ACh and of 10 sec. preganglionic stimulation were compared before (at a and b) and shortly after (at c and d) an injection of 75 μg. 48/80 into the ganglion. The effects of both ACh and of preganglionic stimulation were reduced approximately by half. Ten minutes later there was complete recovery for ACh as well as for preganglionic stimulation (at e and f). In other experiments of this kind complete recovery of the effect of preganglionic nerve stimulation sometimes preceded the recovery of response to injection of ACh by a few minutes.

The experiment of Fig. 5 A indicates that 48/80 not only affects the stimulating action of ACh on the ganglion but also the paralysing action, which effect has been attributed to an excess of ACh. At a, 5 μg. ACh was injected during preganglionic stimulation, producing a slight relaxation of the nictitating membrane. This relaxation suggests that the injected ACh, together with the released ACh, is sufficient to paralyse the ganglion. When the same dose of ACh was given shortly afterwards, during the block produced by 75 μg. 48/80, it had a stimulating action. As the release of ACh remains unaffected by 48/80, the sensitivity of the ganglion must have become reduced, so that a previously paralysing amount of ACh now exerted a stimulating action. The same result was obtained when the order was reversed. This is shown in Fig. 5 B, in which, instead of ACh, carbachol (carbaminoylcholine chloride) was injected during preganglionic stimulation—first during 48/80 block (at f) and later when the block had nearly worn off (at g). The paralysing effect (at g) was still preceded by a short-lasting stimulation.

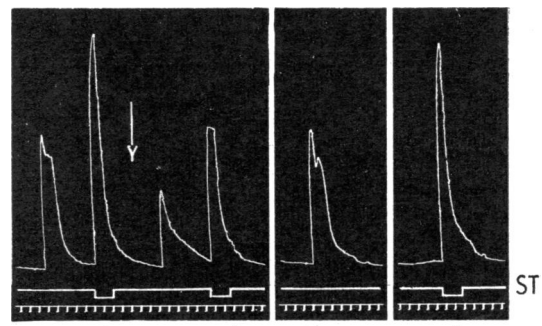

FIG. 4.—Perfused superior cervical ganglion of cat under chloralose anaesthesia. Contractions of nictitating membrane to injections into ganglion of 5 μg. ACh (at a, c, and e) and 10 sec. preganglionic stimulation (ST) at b, d, and f. At arrow, injection of 75 μg. 48/80 into ganglion. Between d and e, interval of 10 min. Time in 5 sec.

When 48/80 had completely blocked the effect of maximal preganglionic stimulation, an injection of ACh in larger doses still caused stimulation. Thus, 48/80 does not render the ganglion cells insensitive but only less sensitive to ACh.

Histamine and Synaptic Transmission in the Sympathetic Ganglion

Since 48/80 and propamidine are potent histamine liberators, the possibility had to be examined whether or not the block could be accounted for by the release of histamine. Kwiatkowski (1943) and von Euler (1949, 1950, 1951) have shown that many nerve fibres, particularly of the sympathetic system, contain rather large amounts of histamine, but it is not known if the histamine in nerves is susceptible to the action of histamine liberators.

When 48/80 was injected into the perfused ganglion, histamine appeared in the venous effluent.

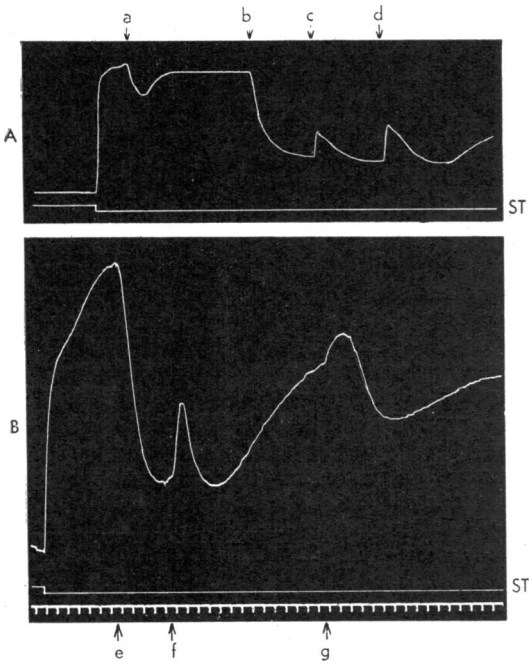

FIG. 5.—Perfused superior cervical ganglion of cat under chloralose anaesthesia. Contractions of nictitating membrane. A, Paralysing action of ACh converted by 48/80 into stimulating action. Stimulation of cervical sympathetic (ST). At a, c, and d, injection of 5 μg. ACh into ganglion; at b, 75 μg. 48/80. B, Partial conversion by 48/80 of stimulating action of carbachol into paralysing action. Stimulation of cervical sympathetic (ST). At f and g, injection of 5 μg. of carbachol; and at e, 75 μg. 48/80 into ganglion. Time in 10 sec.

The release of histamine, however, was apparently not responsible for the block because (1) histamine itself did not produce block, and (2) on repeated injections 48/80 still retained its blocking action when histamine release had ceased.

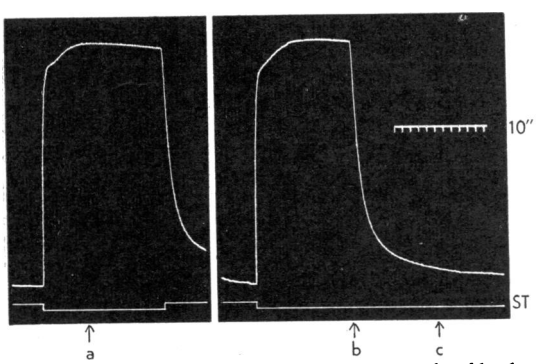

FIG. 6.—Perfused superior cervical ganglion of cat under chloralose anaesthesia. Contractions of nictitating membrane to stimulation of cervical sympathetic (ST). At a and c, injection of 100 μg. histamine into ganglion; at b, 100 μg. 48/80. Time in 10 sec.

Effect of Histamine.—The experiment of Fig. 6 shows that histamine injected during preganglionic nerve stimulation does not have any ganglionic blocking action and does not intensify the block produced by 48/80. In this experiment 100 μg. was injected at a, during maximal preganglionic stimulation, without producing any indication of even the smallest relaxation of the nictitating membrane. At c the same dose of histamine was injected during continuous preganglionic stimulation after block had been produced by 100 μg. of 48/80 (at b). The recovery from the block was not influenced by the histamine injection.

Release of Histamine.—An injection of 50 μg. 48/80 into the ganglion caused the appearance of histamine in the venous effluent; however, the amounts liberated varied greatly from animal to animal. The quantities released in seven experiments are shown in Table III, column 2; they varied between 0.05 and 0.9 μg. histamine. With repeated injections of the same dose of 48/80, the amounts of histamine liberated decreased from injection to injection until no histamine was detectable in the venous effluent with the method of assay employed. In the other experiments between 10 and 20% of the amounts of histamine released after the first injection were assayed in the perfusate collected after the second injection given 10 min. later. Once the injection of 48/80 had failed to liberate histamine, subsequent injections were likewise ineffective (see Table III, columns 3, 4, and 5). In two other experiments in which the ganglion was stimulated during the time 48/80 was injected, similar outputs of histamine to those reported in Table III were obtained. In all of the experiments reported here, mepyramine maleate could be shown to block the histamine response of the guinea-pig ileum, indicating that the active principle was in fact histamine.

TABLE III

HISTAMINE RELEASED FROM THE UNSTIMULATED PERFUSED SUPERIOR CERVICAL GANGLION BY REPEATED INJECTIONS OF 50 μG. 48/80 AT 10 MIN. INTERVALS

Expt. No.	Amount of Histamine (μg.)			
	1st Inj.	2nd Inj.	3rd Inj.	4th Inj.
1	0·05	0·005	0·005	—
2	0·12	—	—	—
3	0·07	0·03	—	—
4	0·20	0·04	0·01	0·005
5	0·09	0·005	—	—
6	0·90	0·02	0·005	—
7	0·08	—	—	—

Discussion

The histamine liberators, 48/80 and propamidine, have at least one other action in common which has hitherto not been described—that of producing ganglionic block in the perfused sympathetic ganglion. Analysis of the block produced by 48/80 has shown that the release of ACh from the preganglionic endings is not interfered with, but that the sensitivity of the ganglion cells to the action of ACh is reduced. There was no evidence that the block could be explained by persistent depolarization, since neither 48/80 nor propamidine excited the ganglion cells to discharge before the onset of block. Furthermore, it was possible to overcome the block, at least partially, by injecting a large dose of ACh into the perfused ganglion.

Histamine is a normal constituent of nervous tissue. It was therefore natural to consider the possibility that the histamine liberators produce the block by the release of histamine from the ganglion, particularly when it was found that 48/80 did release histamine from the perfused ganglion. Nevertheless, this possibility could be excluded because of the following three facts: (1) Injections of histamine into the ganglion do not impair ganglionic transmission. A similar observation was made by Feldberg and Vartiainen (1934). (2) The block produced by 48/80 is not enhanced by a subsequent injection of histamine. (3) The block produced by 48/80 bears no relation to the amounts of histamine released. These decrease with repeated injections of the same dose of 48/80, whereas the block becomes more pronounced with each injection. After a few injections, when histamine release can no longer be detected, 48/80 exerts an even stronger blocking action than after the first injection.

Nevertheless, we must be cautious at the present stage of our knowledge in excluding any connexion between histamine liberating and ganglion blocking properties of 48/80 and propamidine. We do not know if there are histamine liberators which lack the ability to produce ganglionic block. Certainly one other known, but less potent, histamine liberator is also a ganglion blocking substance—namely, (+)-tubocurarine. On the other hand, there are many ganglion blocking drugs which are not histamine liberators.

That 48/80 and propamidine both block the transmission of impulses across the sympathetic ganglion is of particular interest in relation to some of those systemic effects of histamine liberators which are not readily explicable by an action of released histamine. For example, Nasmyth (1955) has recently shown that, in rats depleted of their tissue histamine by repeated injections of 48/80, this compound still produces a fall in arterial blood pressure, although the depressor effect is much milder than in normal rats. He discusses the problem of whether this reduced effect is due to release of small residual amounts of tissue histamine or to an unspecific action of 48/80. The ganglion blocking effect of 48/80 could easily explain this finding.

It was found that in the perfused ganglion histamine was ineffective not only in impairing ganglionic transmission but also in stimulating the ganglion. Trendelenburg (1954) has observed a ganglion stimulating action with histamine, but his experiments were performed in a ganglion with its normal blood circulation intact. It is thus possible that any ganglion stimulating action of histamine is lost when the ganglion is perfused with artificial salt solution.

48/80, a secondary amine, and propamidine, a diguanidine, do not bear any great chemical similarity to onium compounds, such as hexamethonium and the thiophanium drugs, which possess such strong ganglion blocking actions. From a purely structural comparison, there seems to be little basis for explaining why propamidine and 48/80 block ganglionic transmission, whereas histamine, another amine derivative, exerts no such action at all. If the ganglionic blocking effects were purely a non-specific action of amines or guanidines in general, one would expect them all, including histamine, to exert ganglionic actions.

48/80 is chemically very similar to the sympathomimetic amines, some of which—adrenaline and ephedrine, for example—have been shown to depress ganglionic transmission (Bülbring and Burn, 1942; Marrazzi, 1939). As yet no typical sympathomimetic actions have been described for 48/80, possibly because of the histamine release obscuring any other pharmacological actions. If such effects, however weak, are reported in the future, one would then have to consider the possibility that 48/80 owed its ganglionic blocking effect to its structural resemblance to sympathomimetic amines.

Summary

1. 48/80 and propamidine cause ganglionic block in the perfused superior cervical ganglion of the cat. This block has been analysed for 48/80.

2. 48/80 does not produce block by decreasing the amount of acetylcholine released from the preganglionic endings, but by rendering the ganglion cells less sensitive to the released ACh.

3. 48/80 releases histamine from the perfused ganglion.

4. The release of histamine is not the cause of block produced by 48/80, because: (*a*) histamine does not impair ganglionic transmission; (*b*) the block produced by 48/80 is not enhanced by histamine; (*c*) the amounts of histamine released decrease, whereas the block becomes stronger with each injection of 48/80. When 48/80 no longer releases histamine, it still exerts its blocking action.

I am most indebted to Dr. W. Feldberg for his valuable advice during the course of this investigation.

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