Brit. J. Pharmacol. (1963), **21**, 306–322.

THE SITE OF THE 5-HYDROXYTRYPTAMINE RECEPTOR ON THE INTRAMURAL NERVOUS PLEXUS OF THE GUINEA-PIG ISOLATED ILEUM

BY

G. BROWNLEE AND E. S. JOHNSON

From the Department of Pharmacology, King's College, Strand, London, W.C.2

(*Received May* 18, 1963)

Dose/response measurements were made on the guinea-pig isolated ileum with six agonists, acetylcholine, 5-hydroxytryptamine, nicotine, dimethylphenylpiperazinium, choline phenyl ether and histamine. The dose effects were repeated in the presence of each of twelve antagonists and one anticholinesterase. Acetylcholine and histamine were chosen because of their direct mode of action on smooth muscle, nicotine, dimethylphenylpiperazinium and choline phenyl ether were used as examples of drugs that act at the ganglionic acetylcholine receptor. 5-Hydroxytryptamine was the drug investigated. Hyoscine blocked the contractions caused by acetylcholine, 5-hydroxytryptamine and the ganglion-stimulants but left the responses to histamine unchanged. The anticholinesterase *N,N'*-diisopropylphosphorodiamidic fluoride (mipafox) potentiated all the agonists except histamine. The strength of potentiation decreased in the order 5-hydroxytryptamine, nicotine, dimethylphenylpiperazinium and choline phenyl ether, and acetylcholine. The local anaesthetic procaine inhibited to the same extent contractions elicited by 5-hydroxytryptamine, nicotine, dimethylphenylpiperazinium and choline phenyl ether. These results showed that 5-hydroxytryptamine, like nicotine, choline phenyl ether and dimethylphenylpiperazinium, mediated its response through the nervous plexus. Of those tested 5-hydroxytryptamine was the only specific antagonist to 5-hydroxytryptamine; lysergic acid derivatives produced spasm and prolonged changes in tone; phenoxybenzamine caused non-specific block. The diverse modes of action of a number of ganglion-blocking agents were selectively used. Thus hexamethonium, pentolinium, and nicotine in its competitive phase, blocked contractions due to nicotine, dimethylphenylpiperazinium and choline phenyl ether and left those due to 5-hydroxytryptamine, acetylcholine and histamine unchanged. The depolarizing ganglion-blocking agents, dimethylphenylpiperazinium and nicotine, inhibited the responses to all the indirectly acting drugs. Furthermore, mecamylamine, a drug with a less well-defined mode of action, partially inhibited contractions due to 5-hydroxytryptamine in a concentration that blocked those due to nicotine, dimethylphenylpiperazinium and choline phenyl ether. Pempidine, known to act like mecamylamine, did not antagonize 5-hydroxytryptamine. It is concluded that 5-hydroxytryptamine activates specific receptors sited at the intramural parasympathetic ganglion cells.

Several sites of action for 5-hydroxytryptamine have been postulated for the guinea-pig ileum. Rocha e Silva, Valle & Picarelli (1953) offered evidence that 5-hydroxytryptamine specifically stimulated the postganglionic cholinergic fibres in this organ. Gaddum & Hameed (1954) discussed a hypothesis for two kinds of receptor in the intramural ganglion cells, one stimulated by acetylcholine and by

nicotine and blocked by hexamethonium and by large doses of nicotine, the other stimulated by 5-hydroxytryptamine and blocked by excess doses of the same compound. They also considered the possibility that 5-hydroxytryptamine acted at a different kind of cell from the one at which nicotine acted. Later Gaddum & Picarelli (1957) advanced evidence for two separate 5-hydroxytryptamine receptors on the terminal ileum of the guinea-pig. Recently Day & Vane (1963) found that 5-hydroxytryptamine contracted the guinea-pig ileum by acting mainly on receptors in nervous tissue and found that smooth muscle receptors were of negligible importance in eliciting the usual response.

Parasympathetic ganglia are difficult experimental preparations, but a site of action of 5-hydroxytryptamine at the more accessible sympathetic ganglia has been shown. Thus, Trendelenburg (1956) found evidence for the presence of tryptamine receptors in the cat superior cervical ganglion and later (Trendelenburg, 1957) showed that 5-hydroxytryptamine, as well as histamine and pilocarpine, stimulated the ganglion by combining with receptors that seemed to differ from the acetylcholine receptors on the ganglion cells. More recently Bindler & Gyermek (1961) recorded postganglionic action potentials induced by close intra-arterial injections of 5-hydroxytryptamine and dimethylphenylpiperazinium into the cat inferior mesenteric ganglion. Although both drugs stimulated the ganglion, hexamethonium abolished the actions only of dimethylphenylpiperazinium.

This paper describes experiments which localize the site of action of 5-hydroxytryptamine at the intramural parasympathetic ganglion cell of the guinea-pig isolated ileum.

Part of this work was communicated to the British Pharmacological Society in July 1962.

METHODS

Adult female guinea-pigs were killed by a blow on the head and bled. The ileum was excised and 3 cm segments were removed from the middle and terminal regions and suspended, oral-end down, in a 10 ml. organ-bath containing Krebs solution at 37° C bubbled with 95% oxygen and 5% carbon dioxide. Longitudinal contractions were recorded isotonically. The lever was weighted with 0.5 g and had a magnification of ten-times.

Drugs

Agonists. These were acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate, choline phenyl ether bromide, 1,1-dimethyl-4-phenylpiperazinium iodide, nicotine hydrogen tartrate and histamine acid phosphate.

Antagonists. These were hyoscine hydrobromide, procaine hydrochloride, 5-hydroxytryptamine creatinine sulphate, hexamethonium bromide, pentolinium hydrogen tartrate, mecamylamine hydrochloride, pempidine tartrate, nicotine hydrogen tartrate, dimethylphenylpiperazinium iodide, phenoxybenzamine chloride, lysergic acid diethylamide tartrate and 2-bromolysergic acid diethylamide tartrate.

The anticholinesterase used was *N,N'*-diisopropylphosphorodiamidic fluoride (mipafox).

Experiments

In each experiment a dose/response curve was made for each of six agonists, usually employing five or six doses; next, the dose/response curves were repeated in the presence of the antagonists. Finally, the six dose/response curves were established again 1 hr after washing out the antagonist. Two ileal segments from the same region were matched and

the effects of the antagonists on the responses of one were compared with the responses of the control segment. No differences in the results were obtained whichever method was used. The contact time of the agonists was 30 sec and the interval between doses was 2 min. The concentrations of the agonists and antagonists are expressed in μg/ml. of base as final bath concentrations.

Tachyphylaxis with 5-hydroxytryptamine. Tachyphylaxis is a frequent complication of dose/response measurements with 5-hydroxytryptamine on the guinea-pig ileum. It was found that tachyphylaxis could be avoided by the use of long dose cycles or by frequent changes of the bath-fluid. Thus no tachyphylaxis was seen when the bath-fluid was changed six times between doses of 5-hydroxytryptamine given at 2 min intervals.

The composition of the Krebs solution (in g/l. of distilled water) was NaCl 6.92 ; KCl 0.35 ; $CaCl_2$ 0.28 ; $NaHCO_3$ 2.1 ; KH_2PO_4 0.16 ; $MgSO_47H_2O$ 0.29 ; and glucose 2.0.

RESULTS

Hyoscine

The mean results of seven experiments in which the ileum was treated with hyoscine (0.1 μg/ml.) for 45 min before the repetition of the dose/response curves are shown in Fig. 1. The responses to higher concentrations of acetylcholine gave a curve that is parallel to the original and provided evidence of a familiar kind for competitive blockade, but the responses to histamine were unaffected. The contractions due to 5-hydroxytryptamine were blocked and the ganglion-stimulant effects of dimethylphenylpiperazinium, nicotine and choline phenyl ether were reduced to less than 10% of the maximal contraction.

Mipafox

Fig. 2 shows the mean results of ten experiments in which the ileum was treated with mipafox (10 μg/ml.). The responses to acetylcholine, 5-hydroxytryptamine and the ganglion-stimulants were potentiated ; these effects are seen in Fig. 2 as a displacement of the curves to the left. The responses to histamine were unchanged by the action of the anticholinesterase. The effects of hyoscine (0.1 μg/ml.) on the preparations treated with mipafox are also shown.

Procaine

When the ileum was treated with procaine (10 μg/ml.) for 1 hr, only a small displacement to the right of the acetylcholine and histamine dose/response curves was produced. Responses to 5-hydroxytryptamine were inhibited to the same extent as were those to nicotine, choline phenyl ether and dimethylphenylpiperazinium, showing that 5-hydroxytryptamine acted at some point on the intramural nervous system (Fig. 3).

5-Hydroxytryptamine in excess

A concentration of 5-hydroxytryptamine of 5 μg/ml. in contact with the preparation for 45 min specifically blocked the contractions to 5-hydroxytryptamine. The responses to acetylcholine, histamine, choline phenyl ether, nicotine and dimethylphenylpiperazinium were not modified (Fig. 4).

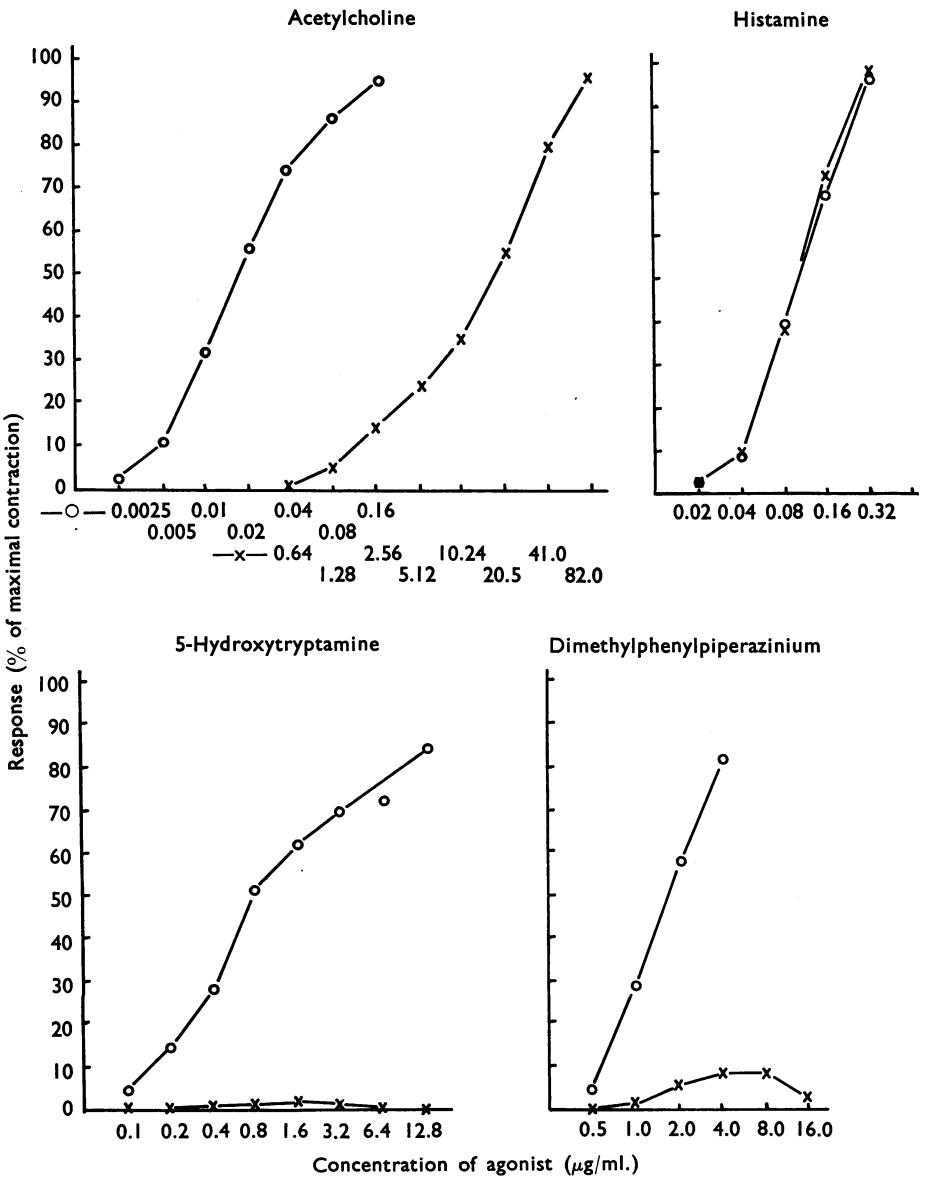

Fig. 1. The effect of hyoscine (0.1 μg/ml.) on the responses of the guinea-pig isolated ileum to acetylcholine, histamine, 5-hydroxytryptamine and dimethylphenylpiperazinium. The results for each agonist are plotted as % of maximal contraction against the dose (μg/ml.) on a log scale. The open circles represent the responses to the agonists and the crosses represent these responses after treatment with hyoscine (0.1 μg/ml.) for 45 min. Higher doses of acetylcholine gave a dose/response curve that is parallel to the original but the responses to histamine were not modified. The responses to 5-hydroxytryptamine and dimethylphenylpiperazinium were greatly inhibited by this concentration of hyoscine. Contractions due to nicotine and choline phenyl ether were affected like those due to dimethylphenylpiperazinium. Each curve represents the mean of seven experiments.

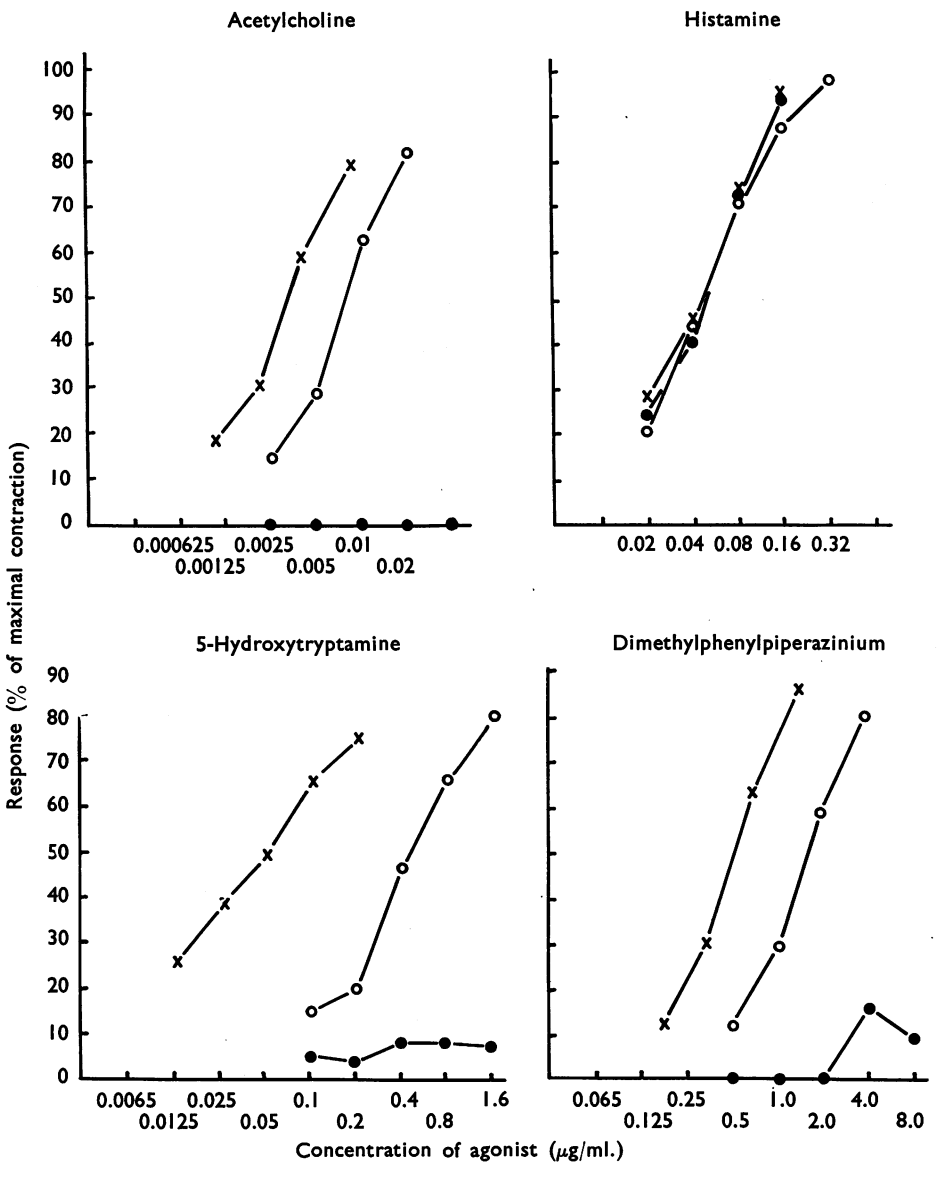

Fig. 2. The effect of mipafox (10 μg/ml.) on the responses of the guinea-pig isolated ileum to acetylcholine, histamine, 5-hydroxytryptamine and dimethylphenylpiperazinium. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses after treatment with mipafox for 1 hr. The curves for acetylcholine, 5-hydroxytryptamine and dimethylphenylpiperazinium were displaced to the left so that they were parallel to the originals. Note that mipafox potentiated 5-hydroxytryptamine more than it did acetylcholine or the ganglion-stimulants. Nicotine and choline phenyl ether were potentiated similarly to dimethylphenylpiperazinium. The histamine responses were unchanged. The closed circles show the effects of exposure to hyoscine (0.1 μg/ml.) for 45 min on the responses to the agonists. Each curve represents the mean of ten experiments.

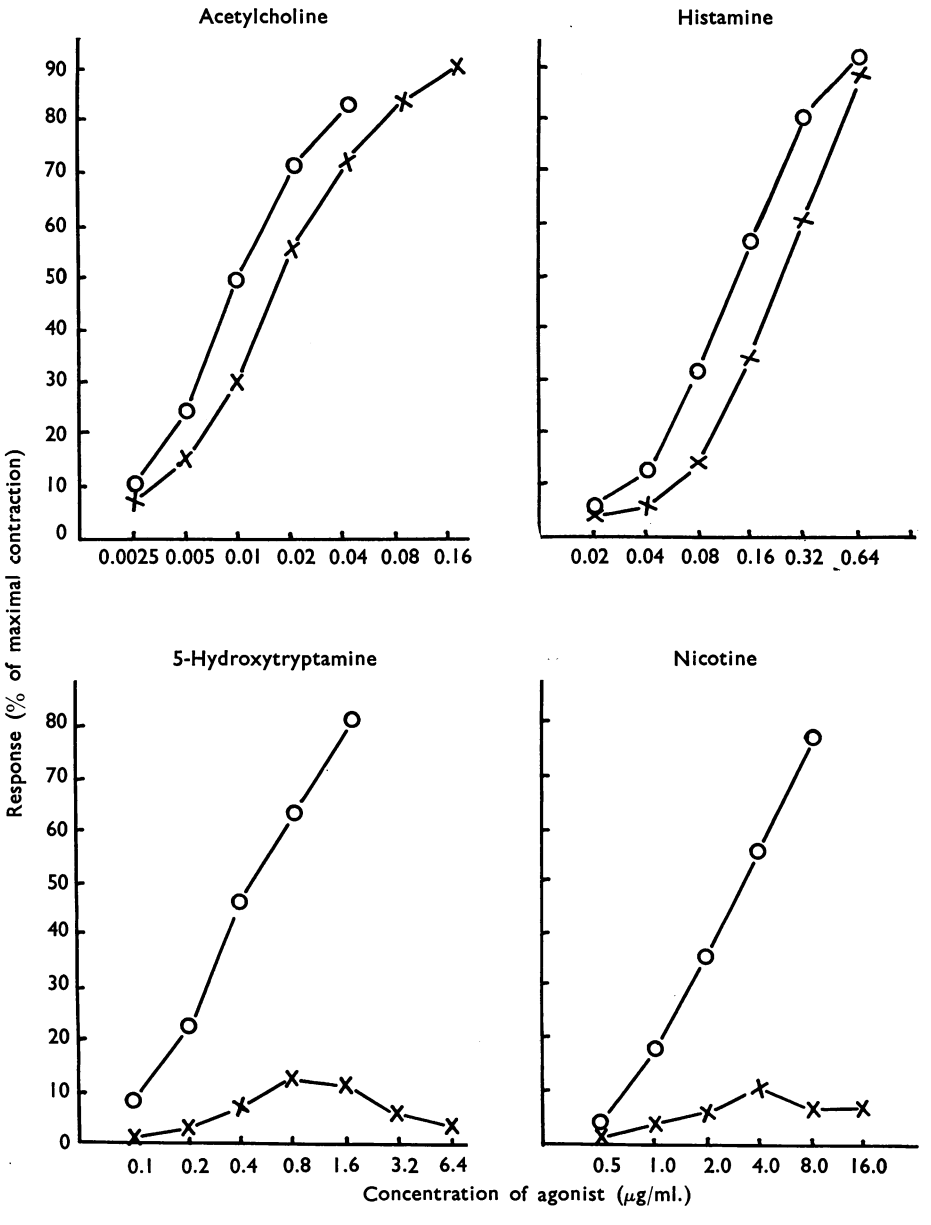

Fig. 3. The effect of treating the guinea-pig isolated ileum for 1 hr with procaine (10 μg/ml.). The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses after treatment with procaine. There was a small parallel displacement to the right of the log dose/response curves for acetylcholine and histamine. The effect of procaine on the responses to 5-hydroxytryptamine was identical with that on the responses to the ganglion-stimulants; all were blocked. The curve for nicotine is similar to those for dimethylphenylpiperazinium and choline phenyl ether. Each curve represents the mean of six experiments.

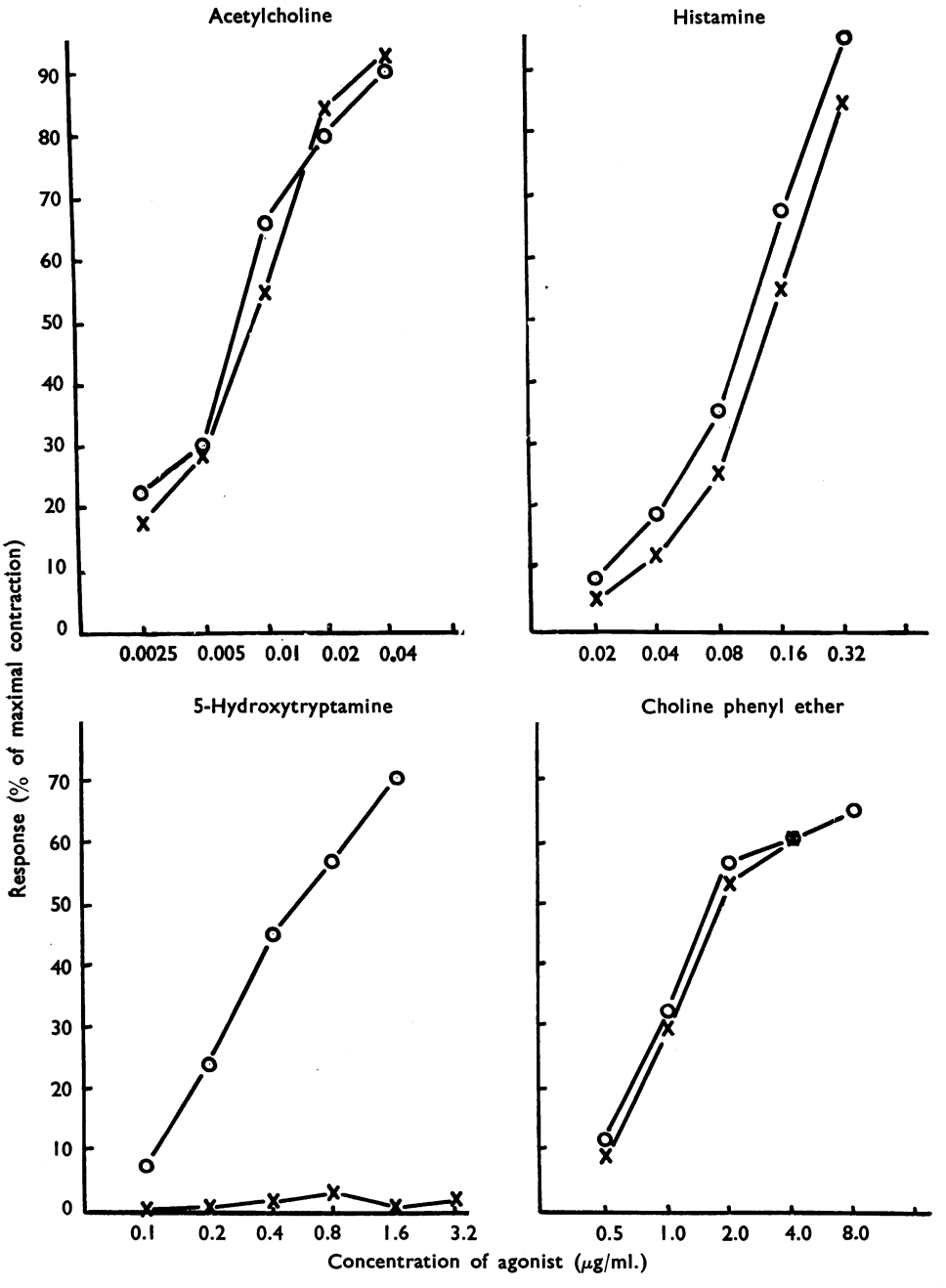

Fig. 4. The effect of treating the guinea-pig isolated ileum with 5-hydroxytryptamine (5 μg/ml.). The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses after treatment with 5-hydroxytryptamine. The responses to 5-hydroxytryptamine were blocked whereas those to acetylcholine, histamine and the ganglion-stimulants were not modified. Dimethylphenylpiperazinium and nicotine gave similar results to choline phenyl ether. Each curve represents the mean of seven experiments.

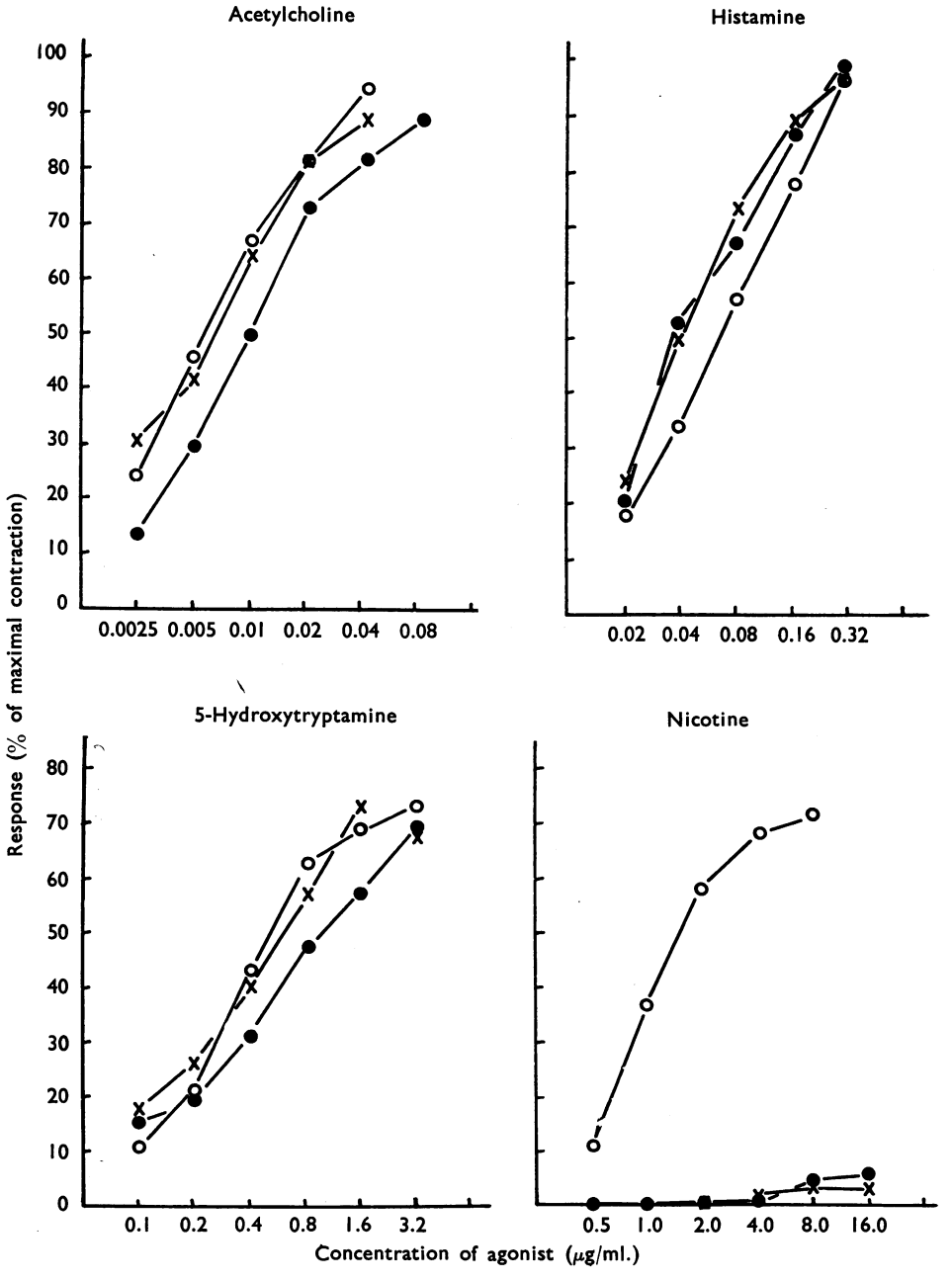

Fig. 5. The effect on the responses to the agonists (open circles) of treating the ileum for 1 hr with pentolinium (5 μg/ml., crosses; 10 μg/ml., filled circles). The ordinates and abscissae as in Fig. 1. Nicotine, choline phenyl ether and dimethylphenylpiperazinium were antagonized by both concentrations of pentolinium whereas there was no significant effect on the responses to acetylcholine, histamine and 5-hydroxytryptamine. The curve for nicotine is similar to those for choline phenyl ether and dimethylphenylpiperazinium.

The ganglion-blocking agents hexamethonium, pentolinium and nicotine

Hexamethonium. When the preparation was treated for 1 hr with hexamethonium (20 μg/ml.), contractions due to nicotine, dimethylphenylpiperazinium and choline phenyl ether were blocked but those due to 5-hydroxytryptamine, acetylcholine and histamine were not affected. Increasing the concentrations of hexamethonium to 40 μg/ml. gave the same results.

Pentolinium. Fig. 5 shows the effects of two concentrations of pentolinium (5 and 10 μg/ml.) on all the dose/response curves. Neither concentration had any significant effect on the responses to acetylcholine, histamine or 5-hydroxytryptamine. The responses to nicotine, dimethylphenylpiperazinium and choline phenyl ether were blocked by both concentrations.

Nicotine, competitive block. Nicotine in large doses blocks ganglia in two ways. Immediately after administration the ganglion cells are discharged and the acetylcholine receptors are blocked by depolarization (Paton & Perry, 1953). The blocking action soon changes to a competitive nature.

Fig. 6 shows the effects of treating the preparations for 1 hr with nicotine (25 and 50 μg/ml.). The results closely resembled those with hexamethonium and pentolinium.

Nicotine, depolarizing block. The action of nicotine changes from a depolarizing to a competitive phase within 3 min and this made it difficult to investigate the depolarizing actions when the drug was in contact with the tissue. However, since the contraction due to a blocking dose of nicotine (40 μg/ml.) faded within 45 sec, it was possible to add the agonist at this time and leave it in contact for a further 30 sec. By this technique it was found that the contractions due to 5-hydroxytryptamine could be blocked, which suggested that the tissue was within the depolarization period.

Difficulty in washing out these large doses of nicotine made unsuccessful attempts to obtain strictly comparative responses with the subsequent depolarizing doses of nicotine (see Fig. 7, lower traces). Nevertheless, this difficulty did not interfere with the interpretation of these results. In these circumstances nicotine blocked all the responses to 5-hydroxytryptamine up to concentrations of 0.8 μg/ml., and the responses to 1.6 μg/ml. of 5-hydroxytryptamine were greatly reduced (Fig. 7).

Dimethylphenylpiperazinium

Dimethylphenylpiperazinium in high concentrations is believed to block ganglia by depolarizing the ganglion cell (Ling, 1959). Dimethylphenylpiperazinium, in a concentration of 5 μg/ml., displaced the acetylcholine but also the histamine curve to the right to the same extent. The responses to 5-hydroxytryptamine, dimethylphenylpiperazinium, choline phenyl ether and nicotine were inhibited equally. Increasing the concentration of dimethylphenylpiperazinium to 10 μg/ml. intensified the block of the actions of all the agonists.

In some experiments with dimethylphenylpiperazinium (2.5 μg/ml.) a specific block of the responses to 5-hydroxytryptamine and the nicotine-like drugs could be obtained. Treating the ileum with dimethylphenylpiperazinium for 2 min was

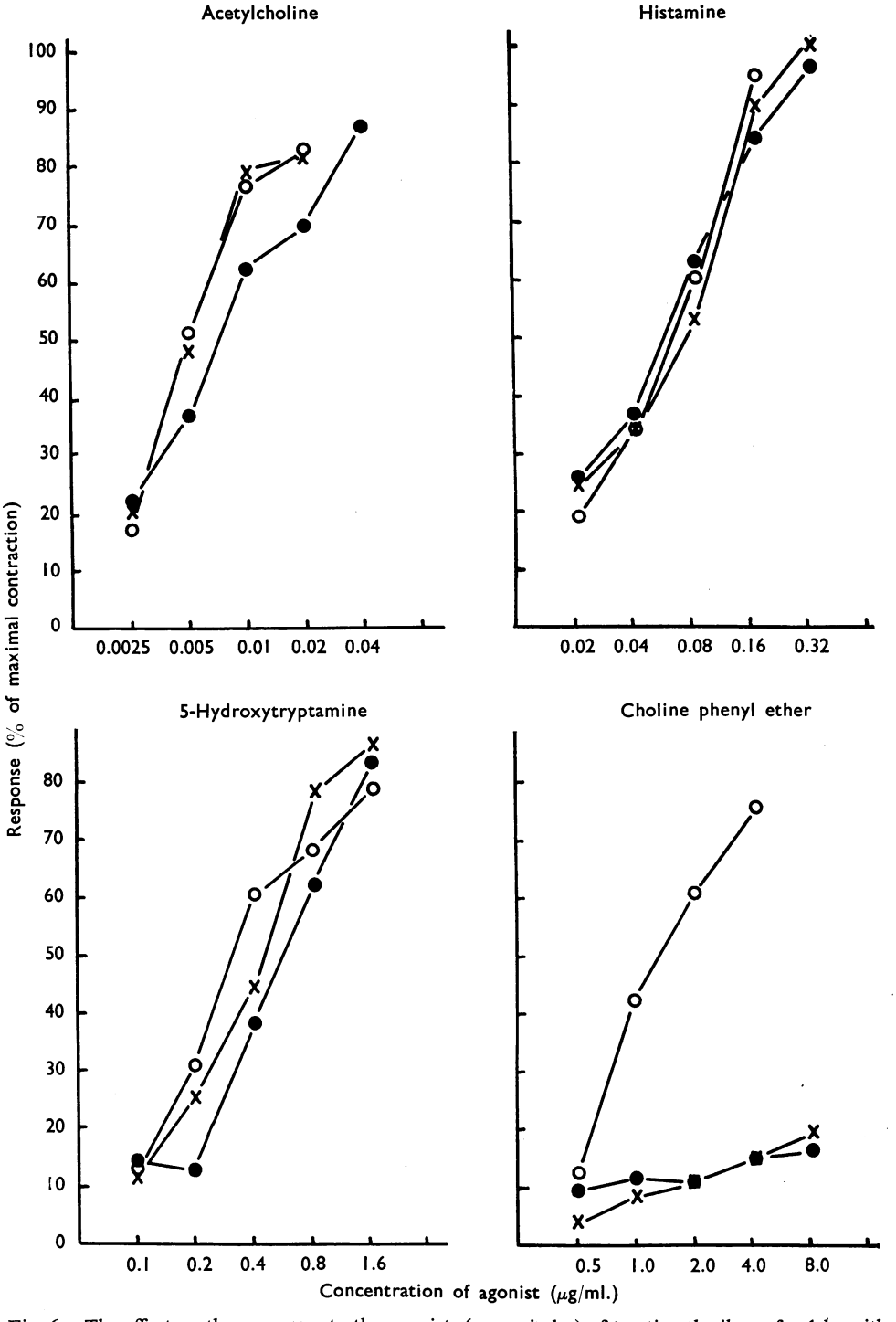

Fig. 6. The effect on the responses to the agonists (open circles) of treating the ileum for 1 hr with ganglion-blocking concentrations of nicotine (25 μg/ml., crosses; 50 μg/ml., filled circles). Ordinates and abscissae as in Fig. 1. Nicotine, choline phenyl ether and dimethylphenylpiperazinium were antagonized by both concentrations of nicotine whereas there was no significant change in the responses to acetylcholine, histamine and 5-hydroxytryptamine. The curve for choline phenyl ether is similar to those for nicotine and dimethylphenylpiperazinium.

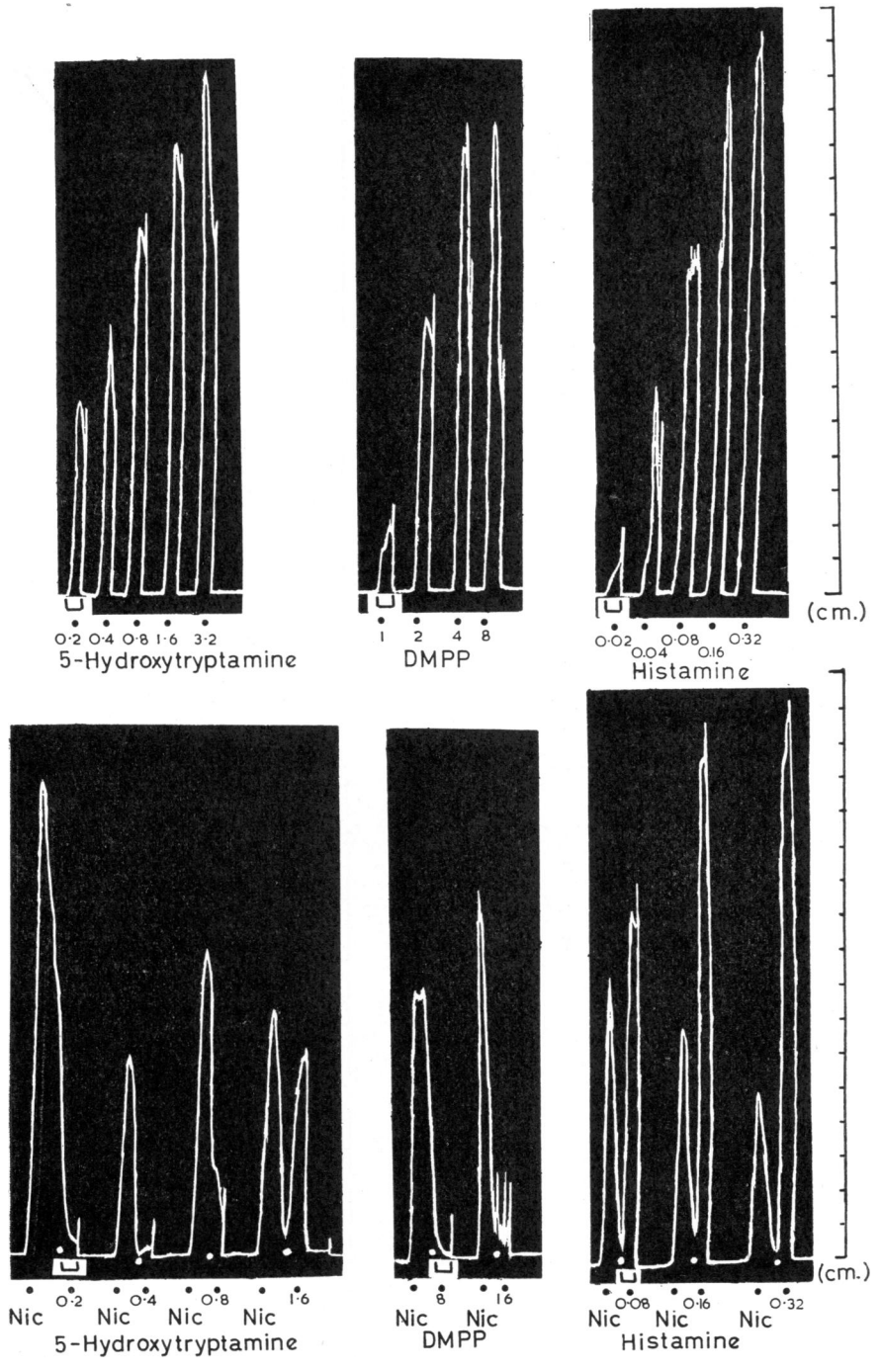

Fig. 7. The upper records show the dose/response relationship of the guinea-pig isolated ileum to 5-hydroxytryptamine, dimethylphenylpiperazinium (DMPP) and histamine. The lower records were made after the guinea-pig ileum had been exposed to 400 μg (40 μg/ml.) of nicotine (Nic) for 45 sec. Doses of agonists were given in the presence of this concentration of nicotine. This treatment leaves the effect of histamine unmodified but those of dimethylphenylpiperazinium and 5-hydroxytryptamine were blocked. The agonist dose-interval was 4 min. All concentrations are in μg/ml. Time mark, 30 sec.

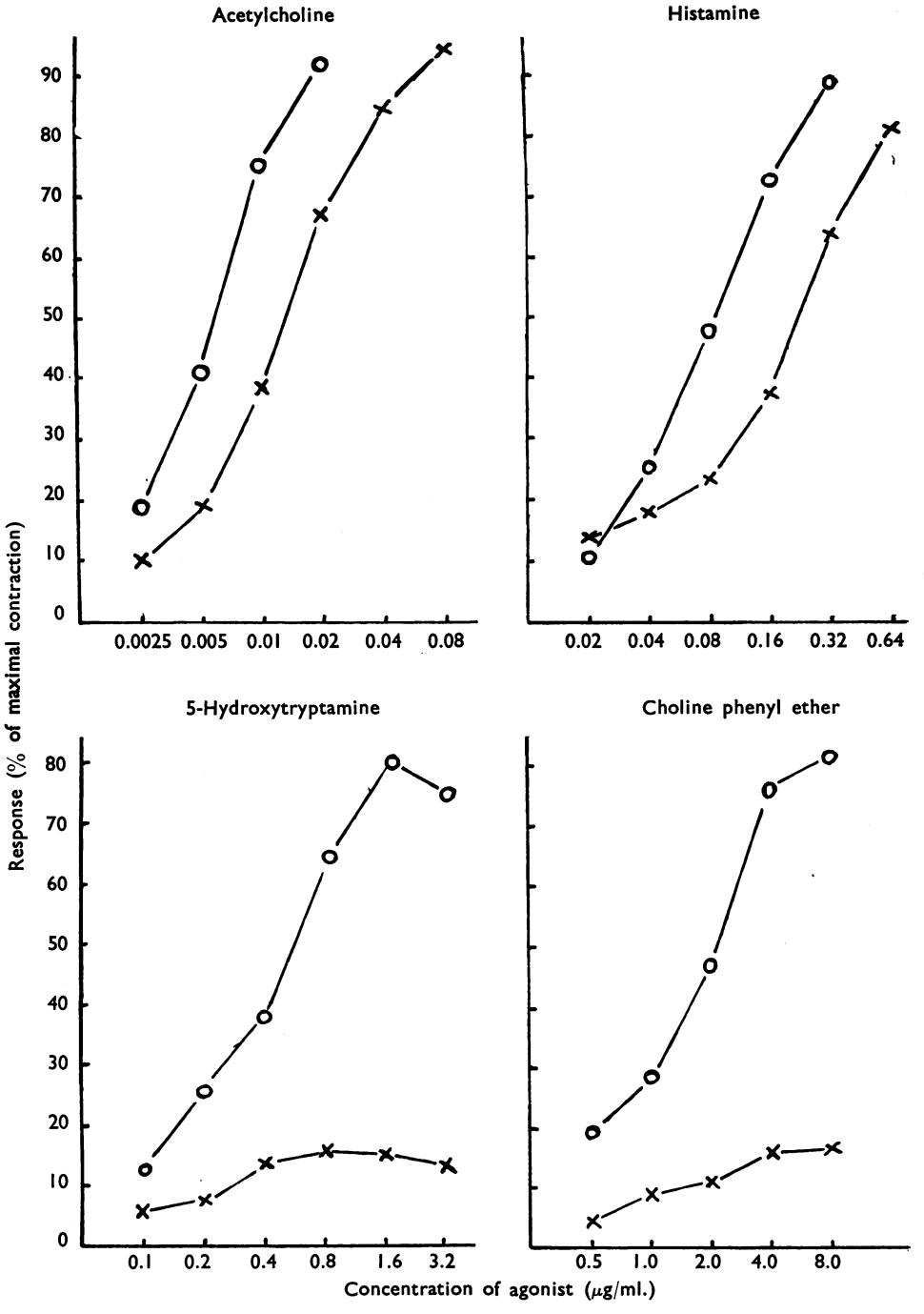

Fig. 8. The effect of dimethylphenylpiperazinium (5 μg/ml.) on the responses of the guinea-pig isolated ileum to acetylcholine, histamine, 5-hydroxytryptamine and choline phenyl ether. Ordinates and abscissae as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses after incubation with dimethylphenylpiperazinium (5 μg/ml.) for 1 hr. The dose/response curves to acetylcholine and histamine were displaced to the right so that they paralleled the original curves. 5-Hydroxytryptamine was antagonized in a similar manner to choline phenyl ether, nicotine and dimethylphenylpiperazinium. The curve for choline phenyl ether is similar to those for nicotine and dimethylphenylpiperazinium. Each curve represents the mean of nine experiments.

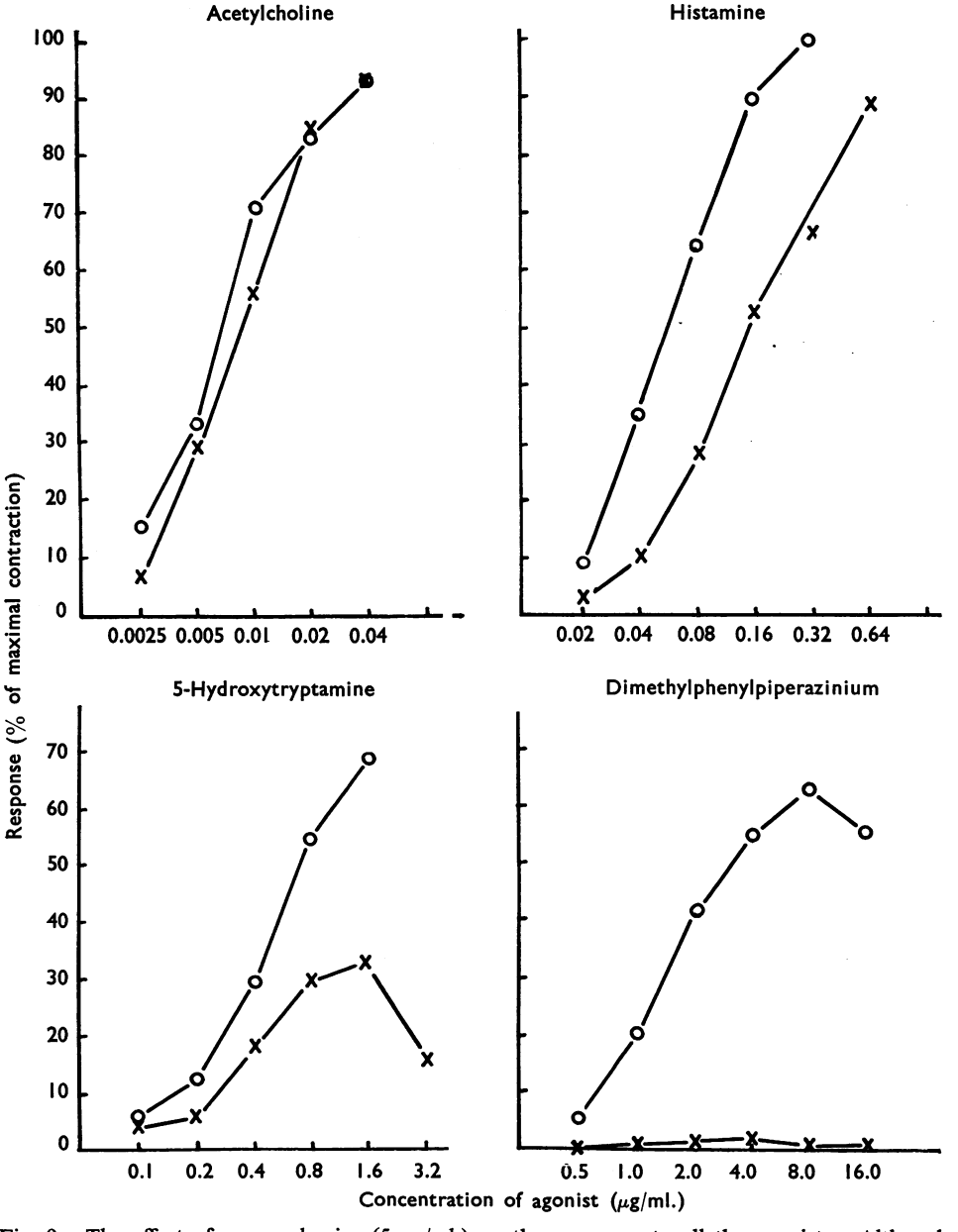

Fig. 9. The effect of mecamylamine (5 μg/ml.) on the responses to all the agonists. Although the responses to acetylcholine were unaffected, those to histamine were slightly inhibited so that its dose/response curve was displaced to the right. The responses to 5-hydroxytryptamine were greatly inhibited in six experiments and unchanged in one; the mean effect from the seven experiments is plotted in this figure. Mecamylamine completely blocked the responses to the ganglion-stimulants; the curve illustrated is for dimethylphenylpiperazinium, and nicotine and choline phenyl ether gave similar curves. The open circles represent the responses of the agonists and the crosses represent these responses after treatment with mecamylamine. The ordinates and abscissae are as in Fig. 1.

sufficient to block the ganglia, but the results shown in Fig. 8 are the means of nine experiments in which the period of treatment was 1 hr, a period used in all our experiments with ganglion-blocking drugs. Dimethylphenylpiperazinium acts by prolonging depolarization and there is no competitive block such as is produced by nicotine (Ling, 1959). The block was reversed rapidly on washing.

Mecamylamine and pempidine

Mecamylamine, in a concentration too low to produce local anaesthesia (5 μg/ml.), was incubated with the preparation for 1 hr. It blocked the contractions due to dimethylphenylpiperazinium, nicotine and choline phenyl ether; responses to 5-hydroxytryptamine were almost completely inhibited in six experiments, and unaltered in one. The mean of the seven experiments is shown in Fig. 9. The responses to acetylcholine were unaffected and those to histamine were inhibited slightly.

Pempidine (5 and 10 μg/ml.) did not inhibit the responses to 5-hydroxytryptamine but behaved in a similar manner to hexamethonium and pentolinium.

Lysergic acid diethylamide, bromolysergic acid diethylamide and phenoxybenzamine

Lysergic acid diethylamide (0.1 μg/ml.), incubated with the preparation for periods of 1, 2 and 2.5 hr, did not block the contractions produced by 5-hydroxytryptamine or any of the agonists used. It was often observed that this concentration of lysergic acid diethylamide potentiated the responses to acetylcholine, 5-hydroxytryptamine, nicotine, choline phenyl ether and dimethylphenylpiperazinium, but left those to histamine unaffected. A higher concentration of lysergic acid diethylamide (1.0 μg/ml.) induced an intense spasm and prolonged increase in the tone and spontaneous activity of the ileum. Experiments with bromolysergic acid diethylamide gave similar results to those with lysergic acid diethylamide, except that the spasm and changes in tone were sometimes seen with a concentration of 0.2 μg/ml. The effects with bromolysergic acid diethylamide (1.0 μg/ml.) were the same as with lysergic acid diethylamide. The preparation so treated was unworkable.

Phenoxybenzamine, incubated with the preparation for 1 hr in a concentration of 0.05 μg/ml., slightly inhibited the effects of all the agonists and, if anything, the response to 5-hydroxytryptamine appeared to be inhibited less than that to acetylcholine. A higher concentration of phenoxybenzamine (1.0 μg/ml.) blocked the responses to all the agonists but did not affect the spontaneous movements of the intestine.

DISCUSSION

These experiments on the guinea-pig ileum show that the main action of 5-hydroxytryptamine is located at autonomic ganglion cells.

Hyoscine, in a concentration of 0.1 μg/ml. incubated with the ileum for 45 min, blocked the action of 5-hydroxytryptamine, and also the actions of acetylcholine, dimethylphenylpiperazinium, nicotine and choline phenyl ether, but that of histamine was not modified. In some experiments on the terminal portion of the ileum a part of the contraction produced by 5-hydroxytryptamine, corresponding to about one-

tenth of the maximal contraction, was not modified by hyoscine. When this effect was observed there was always seen in addition similar residual responses to dimethylphenylpiperazinium, nicotine and choline phenyl ether. From these experiments it may be inferred that 5-hydroxytryptamine acts either on the muscarinic acetylcholine receptor or indirectly, like nicotine, dimethylphenylpiperazinium and choline phenyl ether, through an action on some part of the intramural nerve pathway involving the release of acetylcholine. That the action of histamine was not affected by this concentration of hyoscine agrees with the conclusions of others (Feldberg, 1951 ; Day & Vane, 1963) that histamine acts directly on the smooth muscle.

There is a similarity between these results with hyoscine and those of Rocha e Silva *et al.* (1953) who showed that atropine blocked stimulation of the gut induced by 5-hydroxytryptamine. On the other hand Gaddum & Picarelli (1957) obtained a 50% residual response after atropine. These inconsistencies may be accounted for by the different experimental conditions. The long incubation periods with hyoscine in our experiments were used to ensure maximal muscarinic blockade before the repetition of the six dose/response experiments.

After treatment with the organophosphorus anticholinesterase mipafox, chosen because of its apparent inability to release acetylcholine from nerve terminals (Carlyle, 1963), 5-hydroxytryptamine was potentiated to a greater extent than was acetylcholine or the ganglion-stimulants, an observation also made on the rabbit ileum by Robertson (1954) who inhibited the cholinesterase with the true cholinesterase inhibitor 1:5-di(*p*-allyl-*N*-methylaminophenyl)-pentan-3-one dimethobromide. No explanation is offered for this phenomenon which is now under investigation.

Procaine, in a concentration sufficient to block the effects of nicotine, choline phenyl ether and dimethylphenylpiperazinium, also blocked that of 5-hydroxytryptamine, a demonstration that sites the action of 5-hydroxytryptamine mainly on the nerve pathways, a conclusion also reached by Day & Vane (1963) from their experiments with morphine and with anoxia. Feldberg & Lin (1949a, b) abolished nicotine-induced contractions with a concentration of cocaine similar to that of procaine used in the present experiments. Rocha e Silva *et al.* (1953) found that cocaine did not affect the responses to histamine or acetylcholine, in concentrations that completely blocked the actions of 5-hydroxytryptamine. Their evidence indicated that 5-hydroxytryptamine stimulated the postganglionic cholinergic fibres in the guinea-pig ileum. Gaddum & Picarelli (1957) concluded that 5-hydroxytryptamine acted at two different sites: one, directly on the intestinal muscle at receptors which could be blocked by phenoxybenzamine and by lysergic acid diethylamide (" D " receptors) ; the other, at some point on the intramural nervous system, called " M " receptors since they were blocked by morphine. They suggested that 5-hydroxytryptamine and morphine both acted on the autonomic intestinal ganglia but admitted that there was no direct evidence for this and certainly no proof that they acted on the same receptors. Gaddum & Picarelli's suggestion seemed unlikely to gain acceptance after Lewis (1960) had shown that morphine possessed more general depressant properties than were supposed. Gaddum & Hameed (1954) showed that ergot

alkaloid derivatives, in particular lysergic acid diethylamide, were specific 5-hydroxytryptamine antagonists on the rat uterus and rabbit ear but had little effect on the guinea-pig ileum. In experiments made by us it was found that the lysergic acid derivatives, lysergic acid diethylamide and its bromo-derivative, did not produce any antagonism of 5-hydroxytryptamine even when incubated for 2.5 hr, indeed in most experiments there was a potentiation of all the agonists except histamine by concentrations of less than 1.0 μg/ml.

Costa (1956) and Delay & Thullier (1956) obtained a potentiation of 5-hydroxytryptamine with lysergic acid diethylamide on the rat uterus. Other investigators (Cerletti & Doepfner, 1958) discovered a blocking action only. The present results with lysergic acid and its bromo-derivative do not agree with those of Gaddum & Picarelli (1957) and Barlow & Khan (1959).

A concentration of 1.0 μg/ml. of lysergic acid diethylamide and its bromo-derivative induced an intense spasm and increase of tone. Phenoxybenzamine proved to be a non-specific antagonist of all the agonist drugs ; yet it did not inhibit spontaneous activity.

The specific antagonism of 5-hydroxytryptamine by a large concentration of 5-hydroxytryptamine on the one hand (Gaddum, 1953), and the specific block of response to the nicotine-like drugs by hexamethonium, pentolinium and nicotine (competitive action) on the other, appear to leave little doubt that 5-hydroxytryptamine was acting on specific receptors. The hexamethonium experiments made unlikely the possibility of a preganglionic action of 5-hydroxytryptamine.

The ganglion-blocking agents that act by depolarizing the nerve cell may be expected to block the actions of any drug upon that cell regardless of the position of its receptor. Thus if the 5-hydroxytryptamine receptor is situated on the ganglion cells at some point removed from the nicotine receptor, a depolarizing ganglion-blocking agent should prevent the 5-hydroxytryptamine from acting. Thus the observation that the contractions due to 5-hydroxytryptamine were inhibited by depolarizing actions of nicotine or dimethylphenylpiperazinium seems acceptable evidence for siting the 5-hydroxytryptamine receptor on the ganglion cell.

If the antagonism of acetylcholine by dimethylphenylpiperazinium is looked on as an atropine-like action one must equally note the similar antagonism of histamine.

Mecamylamine and pempidine differ from other ganglion-blocking agents in that they may exert an action intracellularly (Bennet, Tyler & Zaimis, 1957 ; Spinks, Young, Farrington & Dunlop, 1958). In the present experiments mecamylamine differed from pempidine in partially inhibiting the action of 5-hydroxytryptamine, an observation which indicated a difference in their modes of action ; thus, a pathway common to both 5-hydroxytryptamine and the nicotine-like drugs is blocked by mecamylamine. The partial inhibition of responses to histamine was thought to be a histamine-antagonist action of mecamylamine because the hyoscine and procaine experiments eliminated the possibility of a nervous action of histamine on the guinea-pig ileum. The fact that the responses to acetylcholine were unaffected was accepted as further evidence for the specificity of the block of the responses to histamine. Since it has been shown that 5-hydroxytryptamine acts by stimulating receptors on the intramural nervous system the inhibition of the responses to

5-hydroxytryptamine by mecamylamine seemed acceptable evidence for siting the action of 5-hydroxytryptamine at autonomic ganglia. The receptor sites differ from those at which the nicotine-like drugs act and the possibility arises that 5-hydroxytryptamine acts at one of two pharmacologically distinct types of ganglion cell, evidence for the existence of which was given by Shaw, MacCullum, Dewhurst & Mainland (1951) and Hertzler (1961) for sympathetic ganglia.

We are grateful to Dr P. Hey of the Smith, Kline & French Research Institute for the gift of choline phenyl ether bromide, to Parke Davis & Co. for the dimethylphenylpiperazinium and to May & Baker for a gift of pempidine tartrate. One of us (E. S. J.) was supported by a Scholarship from the Medical Research Council.

REFERENCES

BARLOW, R. B. & KHAN, I. (1959). The use of the guinea-pig ileum preparation for testing the activity of substances which imitate or antagonize the actions of 5-HT and tryptamine. *Brit. J. Pharmacol.*, **14**, 553–558.

BENNET, G., TYLER, C. & ZAIMIS, E. (1957). Mecamylamine and its mode of action. *Lancet*, **ii**, 218–222.

BINDLER, E. H. & GYERMEK, L. (1961). Influence of 5-hydroxytryptamine antagonists on the ganglionic action of 5-hydroxytryptamine and DMPP. *Fed. Proc.*, **20**, 319.

CARLYLE, R. F. (1963). The mode of action of neostigmine and physostigmine on the guinea-pig trachealis muscle. *Brit. J. Pharmacol.*, **21**, 137–149.

CERLETTI, A. & DOEPFNER, W. (1958). Comparative study on the serotonin antagonism of amide derivatives of lysergic acid and of ergot alkaloids. *J. Pharmacol. exp. Ther.*, **122**, 124–136.

COSTA, E. (1956). Effects of hallucinogenic and tranquillizing drugs on serotonin evoked uterine contractions. *Proc. Soc. exp. Biol. (N.Y.)*, **91**, 39–41.

DAY, M. & VANE, J. R. (1963). An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum. *Brit. J. Pharmacol.*, **20**, 150–170.

DELAY, J. & THULLIER, J. (1956). Dualité d'action du diéthylamide de l'acide lysergique sur la contraction uterine provoquée par la 5-hydroxytryptamine (sérotonin). *C.R. Soc. Biol. (Paris)*, **150**, 1335.

FELDBERG, W. (1951). Effects of ganglion blocking substances on the small intestine. *J. Physiol. (Lond.)*, **113**, 483–505.

FELDBERG, W. & LIN, R. C. Y. (1949a). The action of local anaesthetics and d-tubocurarine on the isolated intestine of the rabbit and guinea-pig. *Brit. J. Pharmacol.*, **4**, 33–44.

FELDBERG, W. & LIN, R. C. Y. (1949b). Effect of cocaine on the acetylcholine output of the intestinal wall. *J. Physiol. (Lond.)*, **109**, 475–487.

GADDUM, J. H. (1953). Tryptamine receptors. *J. Physiol. (Lond.)*, **119**, 363–368.

GADDUM, J. H. & HAMEED, K. A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **9**, 240–248.

GADDUM, J. H. & PICARELLI, Z. P. (1957). Two kinds of tryptamine receptors. *Brit. J. Pharmacol.*, **12**, 323–328.

HERTZLER, E. C. (1961). 5-Hydroxytryptamine and transmission in sympathetic ganglia. *Brit. J. Pharmacol.*, **17**, 406–413.

LEWIS, G. P. (1960). The inhibition by morphine of the action of smooth muscle stimulants on the guinea-pig intestine. *Brit. J. Pharmacol.*, **15**, 425–431.

LING, H. W. (1959). Action of dimethylpiperazinium. *Brit. J. Pharmacol.*, **14**, 505–511.

PATON, W. D. M. & PERRY, W. L. M. (1953). The relationship between depolarization and block in the cat's superior cervical ganglion. *J. Physiol. (Lond.)*, **119**, 43–57.

ROBERTSON, P. A. (1954). Potentiation of 5-hydroxytryptamine by the true cholinesterase inhibitor 284C51. *J. Physiol. (Lond.)*, **125**, 37*P*.

ROCHA E SILVA, M., VALLE, J. R. & PICARELLI, Z. P. (1953). A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum. *Brit. J. Pharmacol.*, **8**, 378–388.

SHAW, F. H., MACCULLUM, M., DEWHURST, D. J. & MAINLAND, J. F. (1951). The possibility of the dual nature of sympathetic ganglion cells, III. *Aust. J. exp. Biol. med. Sci.*, **27**, 153–160.

SPINKS, A., YOUNG, E. H. P., FARRINGTON, J. A. & DUNLOP, D. (1958). The pharmacological actions of pempidine and its ethyl homologue. *Brit. J. Pharmacol.*, **13**, 501–520.

TRENDELENBURG, U. (1956). The actions of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. *Brit. J. Pharmacol.*, **11**, 74–80.

TRENDELENBURG, U. (1957). Action of morphine on the superior cervical ganglion and on the nictitating membrane of the cat. *Brit. J. Pharmacol.*, **12**, 79–85.