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THE SITE OF ACTION OF DRUGS ON THE ISOLATED TAENIA CAECI FROM THE GUINEA-PIG

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The taenia from the guinea-pig caecum has been much used for electrophysiological experiments (see Burnstock, Holman & Prosser, 1963) but its pharmacology has been largely neglected. However, Bülbring (1954, 1955) in her electrophysiological studies described the effect of acetylcholine, histamine or adrenaline on the membrane potential and on the tension of the taenia. Later, the effects of 5-hydroxytryptamine were described; it resembled acetylcholine or histamine in depolarizing the cell membrane and increasing the tension developed by the preparation, (Bülbring & Burnstock, 1960).

The present experiments were designed to locate the sites of action of acetylcholine, histamine, 5-hydroxytryptamine and nicotine on the taenia caeci. This name is preferred to "taeniae coli" because the preparation was obtained from the caecum; moreover, taeniae are not found on the guinea-pig colon.

METHODS

Adult guinea-pigs weighing 250 to 450 g of either sex were stunned by a blow on the head and bled. The abdomen was opened and a 5 cm length of the taenia caeci was dissected free from the circular muscle. The preparation was set up in an organ bath containing 10 ml. Krebs solution at 37° C. Both the solution in the organ bath and that in the reservoir were gassed with a mixture of 95% O_2 and 5% CO_2. The taenia caeci was tied to a light side-writing balsa wood lever (Foster, 1963). Isotonic responses, magnified four times, were recorded on smoked paper. The load on the tissue was 300 mg.

The bath fluid was changed by upward displacement with warm Krebs solution from a reservoir. This ensured that the preparation was constantly immersed in bath fluid even when a drug was being washed out.

The state of tone of the taenia caeci

The taenia caeci, when placed in Krebs solution at room temperature (about 20° C) shortened to approximately one half of its original length *in situ* in the animal. After being transferred to Krebs solution at 37° C, it slowly relaxed to its *in situ* length (called the initial length) in about 15 min. This stage was followed by one in which there was a gradual increase in contraction which was complete in one hr but sometimes lasted as long as two hr. This state of tone was maintained for up to six hr. However, treatment with drugs modified the tone.

The extent of tone exhibited by the taenia caeci varied a great deal. Rhythmic spontaneous activity was often observed as was previously reported by Weis (1962). It was also found that preparations from small guinea-pigs (250 to 450 g) usually exhibited less tone than those from large ones (600 g or more). The taeniae caeci from small animals were more likely to relax during the repeated dosing with acetylcholine than were those from larger animals.

Contractile Responses

For the analysis of the action of the drugs, the preparation, after 30 min equilibration period, was now treated repeatedly with acetylcholine (0.01 or 0.02 μg/ml.) until it relaxed to its initial length and reproducible responses to acetylcholine were obtained. Preparations which did not relax to about the initial length within 2 hr, or which changed tone during an experiment, were discarded.

Once the tissue had relaxed to its initial length, a dose-response curve was made for each of the four agonists, 5-hydroxytryptamine, nicotine, acetylcholine and histamine. A dose of a drug was added in a volume of 0.1 or 0.2 ml. and left in contact with the taenia caeci for 45 sec, and then washed by upward displacement. A total of six washes was made between doses to avoid tachyphylaxis. The interval between doses was 3 min.

The preparation was then incubated with an antagonist drug for 30 min and dose-response curves were repeated in the presence of the antagonist. Some preparations were incubated for one hr with the irreversible anticholinesterase, mipafox, after establishing control dose-response curves. This drug was removed from the bath fluid before the dose-response curves to the four agonists were repeated.

Drugs

These were acetylcholine chloride, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, nicotine acid tartrate, hyoscine hydrobromide, *N,N'*-diisopropylphosphorodiamidic fluoride (mipafox), procaine hydrochloride, cocaine hydrochloride, morphine hydrochloride, hexamethonium bromide, mecamylamine hydrobromide, dimethylphenylpiperazinium iodide, D-lysergic acid diethylamide tartrate and mepyramine maleate.

Concentrations of all the drugs except mipafox are expressed as μg base/ml. final bath concentration.

Krebs Solution

The composition of the Krebs solution (in g/l. distilled water) was NaCl 6.92 ; KCl 0.35 ; $CaCl_2$ 0.28 ; $NaHCO_3$ 2.1 ; KH_2PO_4 0.16 ; $MgSO_4 7H_2O$ 0.29 ; and glucose 2.0.

RESULTS

Acetylcholine, histamine or 5-hydroxytryptamine produced contraction of the taenia caeci. The effect of nicotine depended upon the state of tone of the preparation ; if there was little or no tone then only contraction was seen ; if there was much tone then a biphasic effect was observed in which the contraction was preceded or was followed by relaxation. Contractions produced by nicotine produced a progressive dose-response effect in the range 0.5 to 4 μg/ml., above this range (8–20 μg/ml.) the contractions were sometimes reduced below the height produced by the 4 μg dose.

The effects of hyoscine on the responses

Hyoscine (0.005 μg/ml.) antagonized the responses of the taenia caeci to acetylcholine and the dose-response line was displaced in parallel to the right. The responses to nicotine were almost completely abolished and those to 5-hydroxytryptamine were reduced. The effect of histamine was not modified by treatment with hyoscine (Fig. 1).

When a higher concentration of hyoscine (0.1 μg/ml.) was used, the responses to acetylcholine in the usual concentration range were blocked. In the presence of this concentration of hyoscine and when tone was present, large doses of acetylcholine of 20 μg/ml. or more produced a biphasic effect, a short-lived relaxation followed by contraction. Nicotine, under similar conditions, caused only relaxation of the taenia caeci.

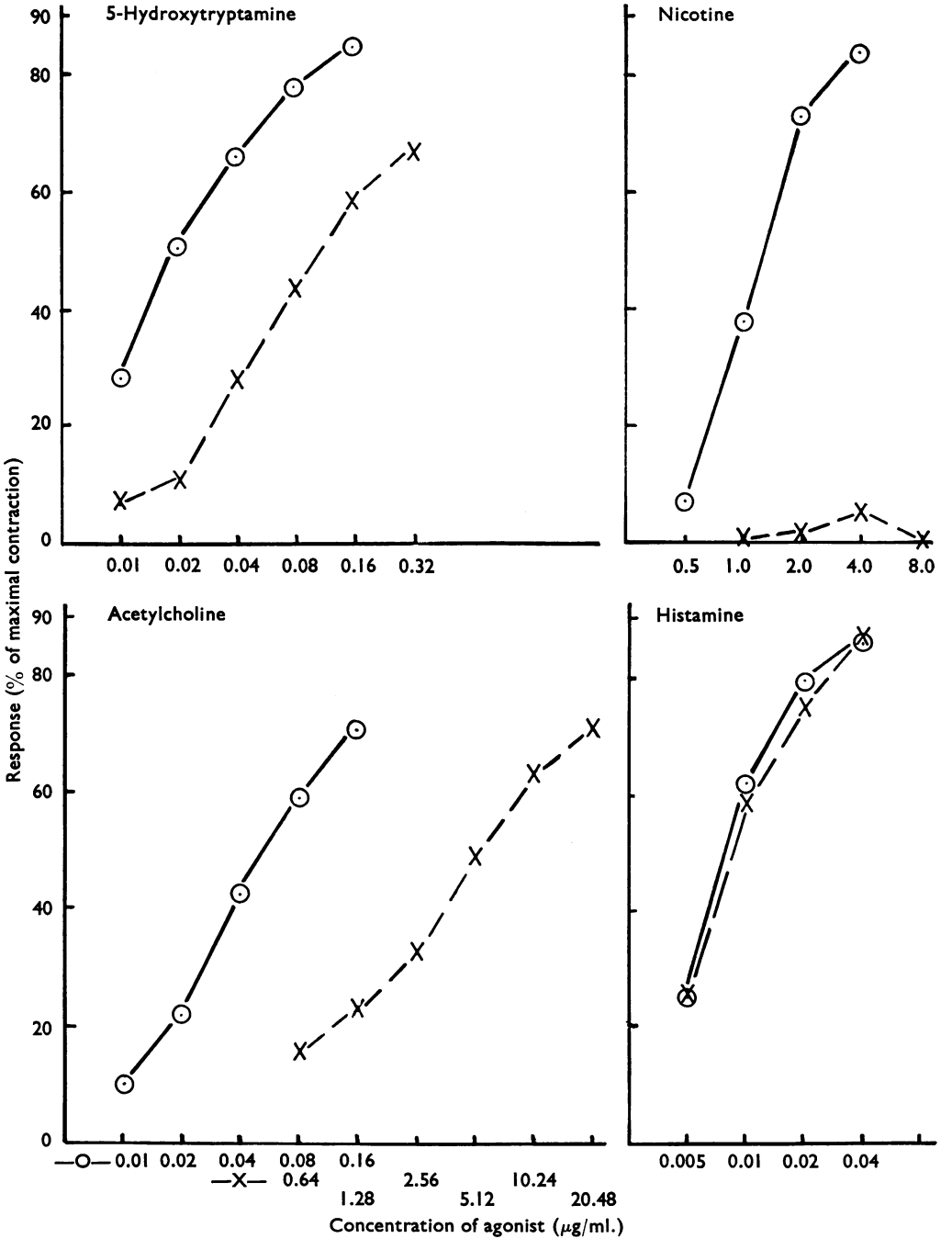

Fig. 1. The effect of hyoscine on the responses of the isolated taenia caeci of the guinea-pig to 5-hydroxytryptamine, nicotine, acetylcholine and histamine. The responses are plotted as % of maximal contraction against the concentration (μg/ml.) on a log scale. The open circles represent the responses to the agonists and the crosses represent these responses in the presence of hyoscine (0.005 μg/ml.). The responses to 5-hydroxytryptamine were reduced and those to nicotine were almost abolished. The responses to acetylcholine were abolished but higher doses produced a dose-response curve parallel to the original. The responses to histamine were not modified. Each curve represents a mean of five experiments; the standard errors are not greater than ± 5% for any mean (Akubue, P. I., Ph.D. Thesis Univ. Lond., 1966).

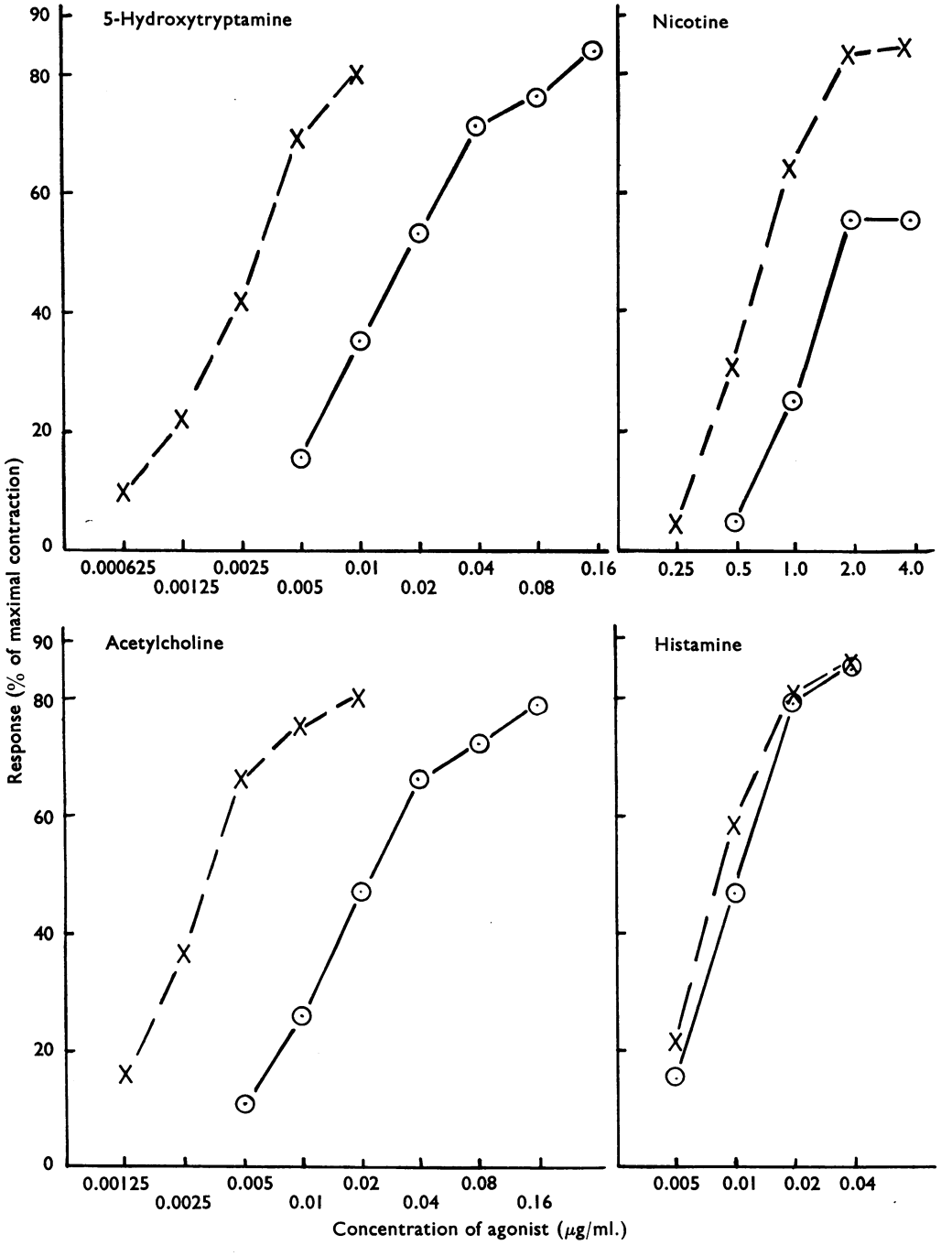

Fig. 2. The effect of treating the taenia caeci with the anticholinesterase drug mipafox (20 μg/ml.) for one hr on the dose-response curves to 5-hydroxytryptamine, nicotine, acetylcholine and histamine. The ordinates and the abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses after treatment with mipafox. The dose-response curves to 5-hydroxytryptamine, nicotine and acetylcholine were displaced to the left. The responses to histamine were not modified. Each curve represents the mean of eight experiments.

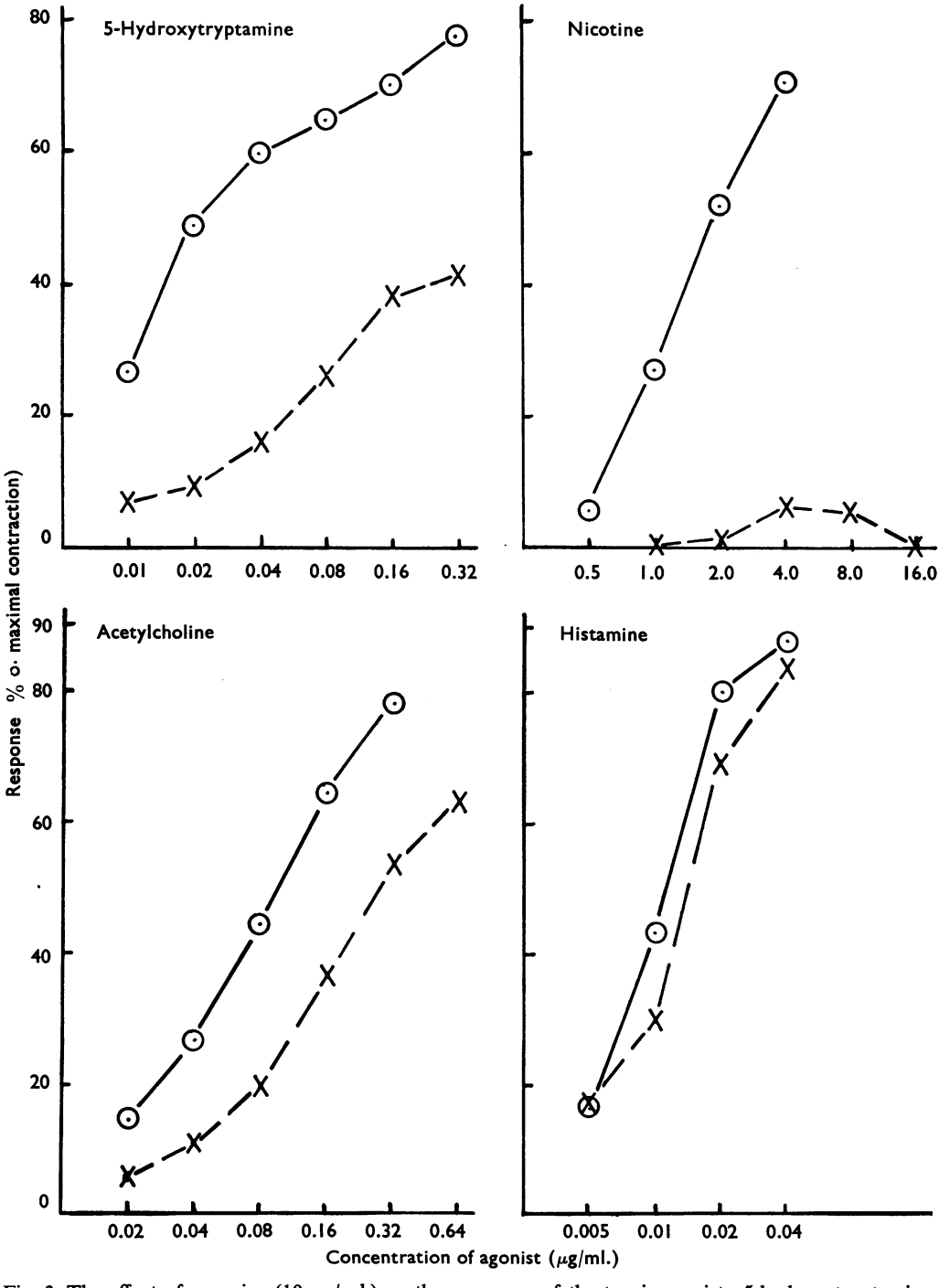

Fig. 3. The effect of procaine (10 μg/ml.) on the responses of the taenia caeci to 5-hydroxytryptamine, nicotine, acetylcholine and histamine. The ordinates and the abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses in the presence of procaine. The responses to 5-hydroxytryptamine were greatly reduced and those to nicotine were almost abolished. The curves to acetylcholine were displaced to the right but those to histamine were not. Each curve represents the mean of six experiments.

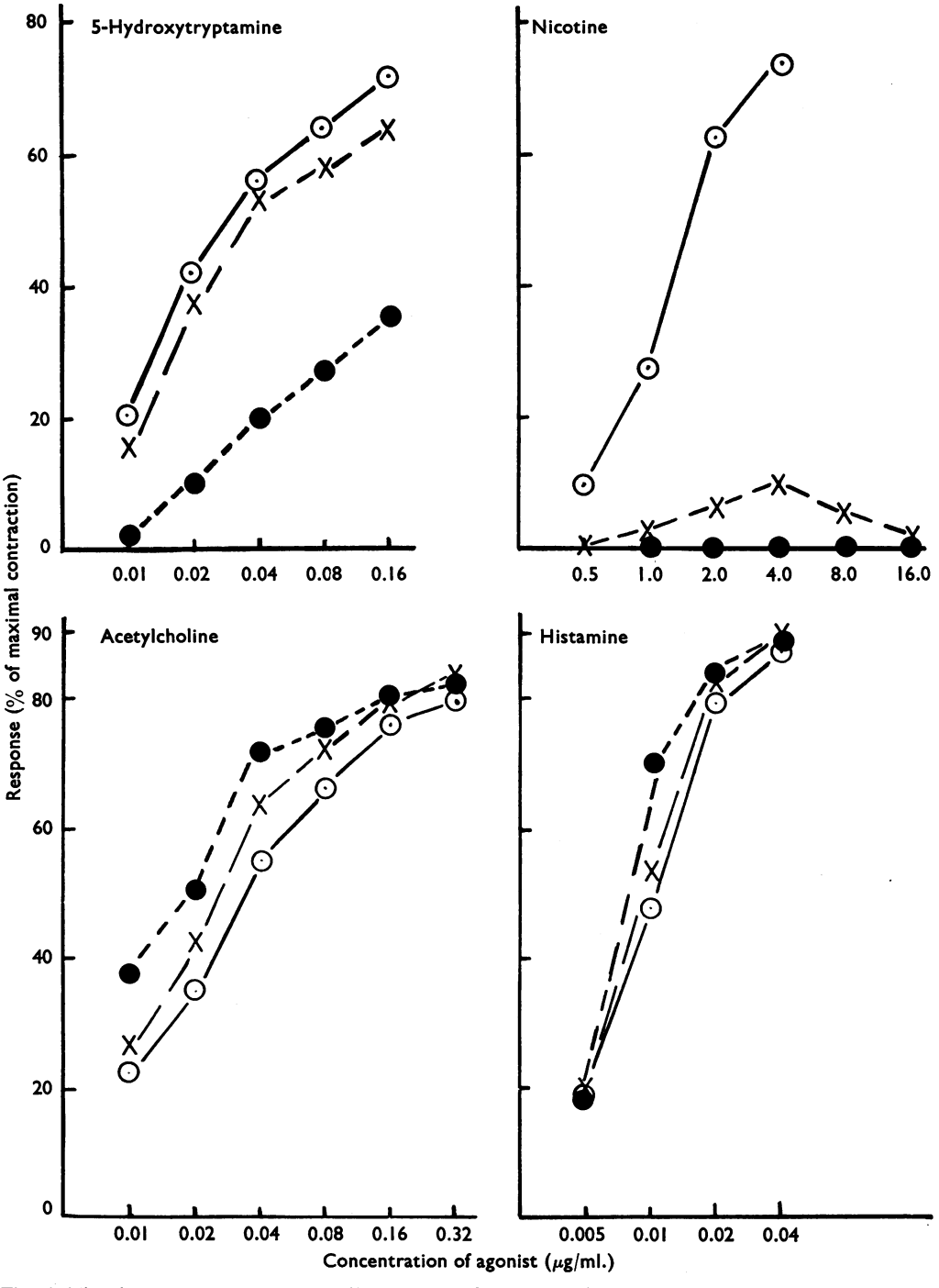

Fig. 4. The dose-response curves to all agonists before (open circles) and in the presence of cocaine (1 μg/ml., crosses; 20 μg/ml., filled circles). Details as in Fig. 1. The responses to 5-hydroxytryptamine were depressed and those to nicotine were eliminated by the higher concentration of cocaine. The responses to histamine were not affected but those to acetylcholine were slightly enhanced. Each curve represents the mean of six experiments.

The responses to the usual concentrations of 5-hydroxytryptamine were greatly reduced although never abolished. Higher concentrations of 5-hydroxytryptamine (1 μg/ml. or more) produced a biphasic effect similar to that observed with acetylcholine. The effect of histamine was not modified even by this concentration of hyoscine.

The effect of mipafox, an anticholinesterase drug

When the preparation was treated with 20 μg/ml. of mipafox, the dose-response curves to acetylcholine and to 5-hydroxytryptamine were displaced to the left, and to a similar extent. The responses to nicotine were also potentiated by mipafox but those to histamine remained unchanged (Fig. 2).

The action of local anaesthetics

Procaine

The effect of procaine (10 μg/ml.) on the dose-response curves to the four agonists is shown in Fig. 3. The responses to acetylcholine were slightly depressed but those to histamine were not. Procaine abolished almost completely the responses of the taenia caeci to nicotine and greatly inhibited those to 5-hydroxytryptamine. In two out of six experiments, the responses to 5-hydroxytryptamine were almost abolished and those to nicotine were blocked.

Cocaine

Fig. 4 shows the effect of two concentrations of cocaine on the dose-response curves of the four agonists. The responses to nicotine were almost completely inhibited by cocaine (1 μg/ml.). This concentration of cocaine only slightly antagonized the responses to 5-hydroxytryptamine. A higher concentration of cocaine (20 μg/ml.) completely eliminated the responses to nicotine and reduced those to 5-hydroxytryptamine. The reduction of the responses to 5-hydroxytryptamine was almost complete in two out of six experiments. Neither of these two concentrations of cocaine antagonized the responses to acetylcholine or histamine. In fact, the effect of acetylcholine was slightly enhanced.

The effect of morphine on the responses

The contractile responses to nicotine and to 5-hydroxytryptamine were antagonized to the same extent by morphine (1 μg/ml.). The effects of acetylcholine and of histamine were not affected (Fig. 5). It was observed that the inhibition of the responses to nicotine or 5-hydroxytryptamine was almost complete when the dose-response curve was made within one hr of incubation of the taenia caeci with morphine. After this time, the extent of inhibition was slightly reduced. A similar type of "tolerance" to morphine was reported by Paton (1957).

The influence of temperature on the responses

Attempts to differentiate between the directly and the indirectly acting drugs by lowering the bath temperature were not successful. After 30 min at 20° C the taenia caeci showed spontaneous rhythmic activity which was variable in size and greater than the activity frequently observed at 37° C. At 15° C the rate at which these spontaneous

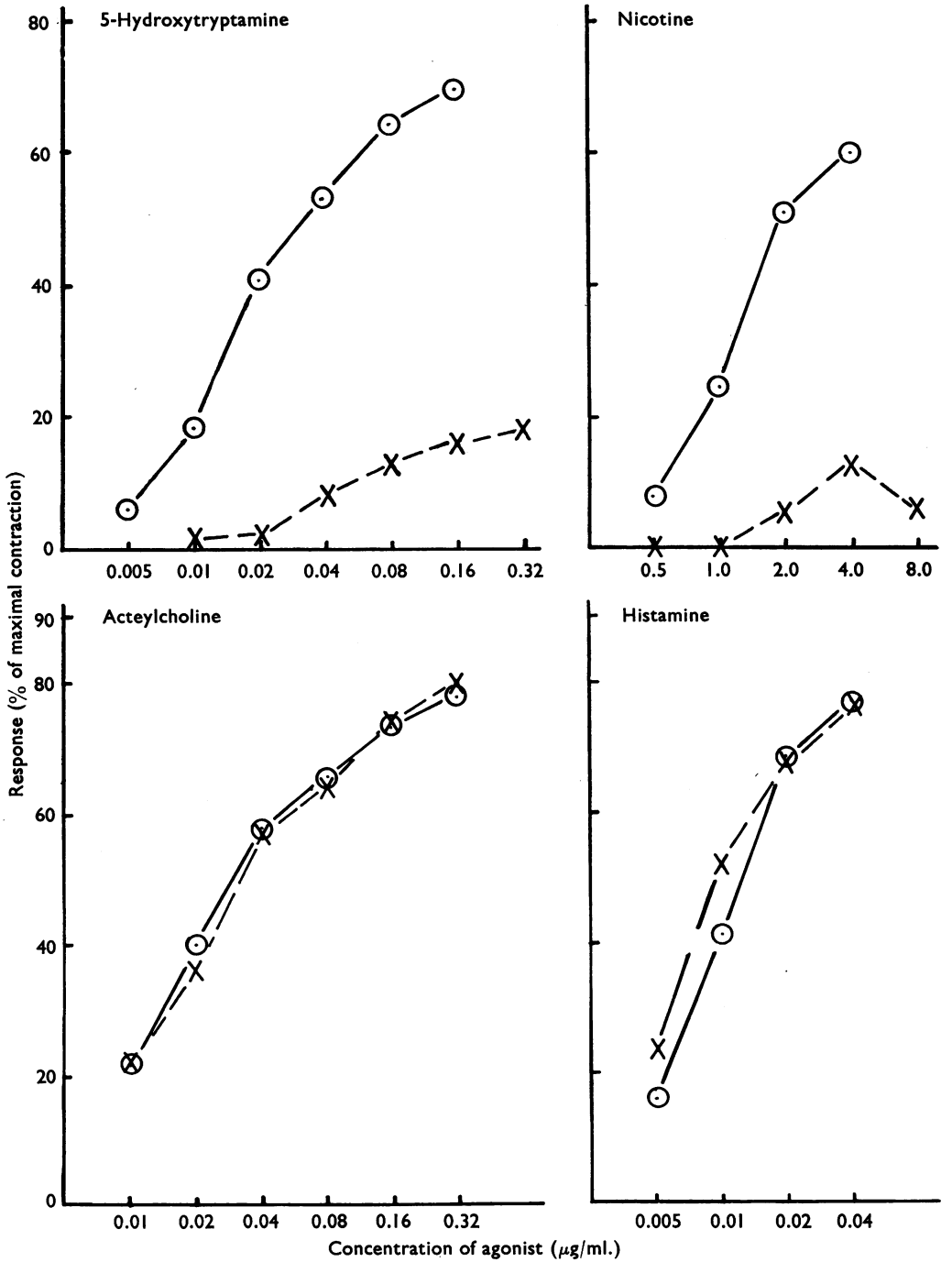

Fig. 5. The effect of treating the taenia caeci with morphine (0.1 μg/ml.) on the responses to agonists. Details as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses in the presence of morphine. The responses to 5-hydroxytryptamine or nicotine were depressed but those to acetylcholine or histamine were not. Each curve represents the mean of five experiments.

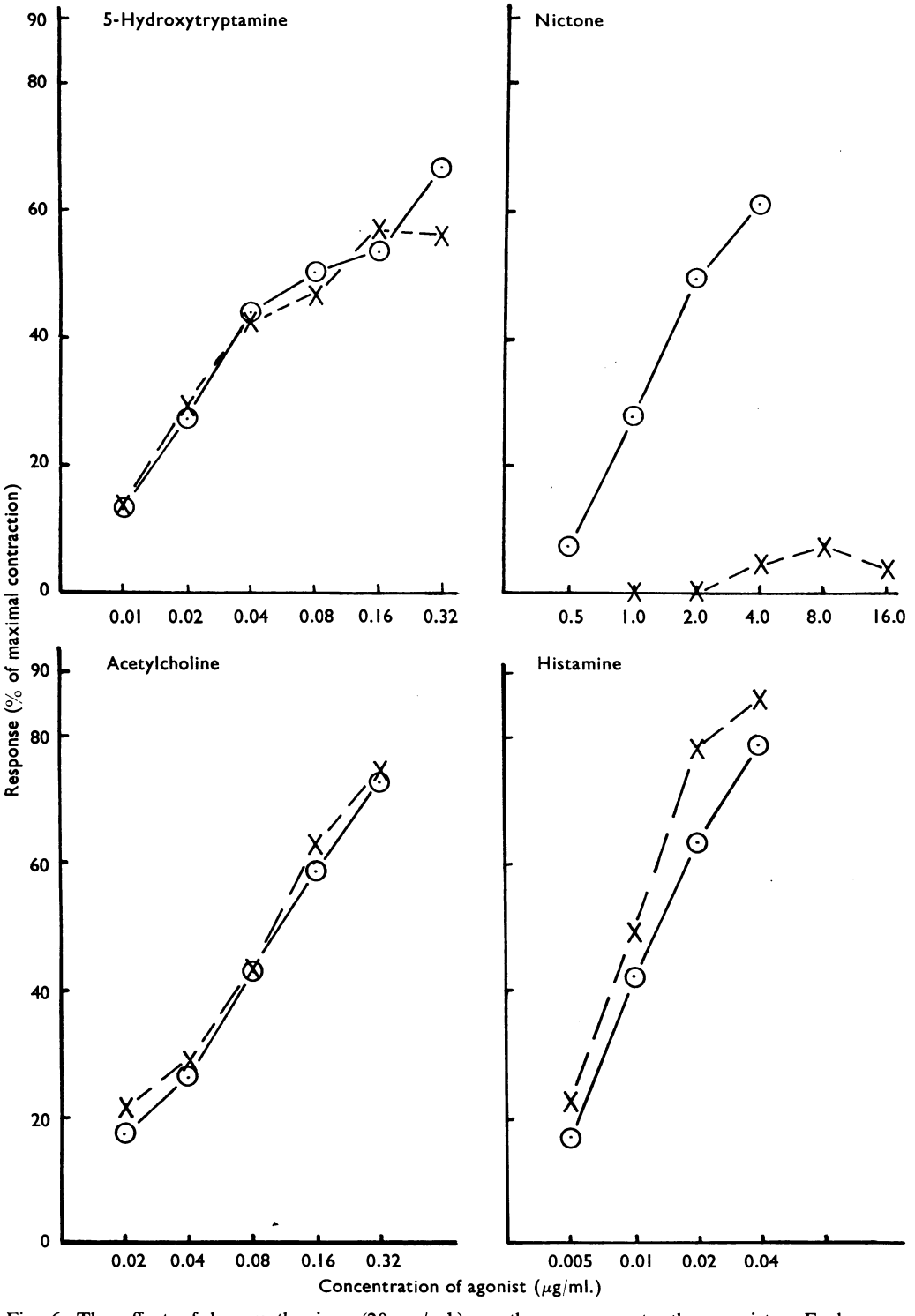

Fig. 6. The effect of hexamethonium (20 μg/ml.) on the responses to the agonists. Each curve represents a mean of six experiments, and the ordinates and the abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses in the presence of hexamethonium. The responses to nicotine were almost abolished but those to 5-hydroxytryptamine, acetylcholine or histamine remained unchanged.

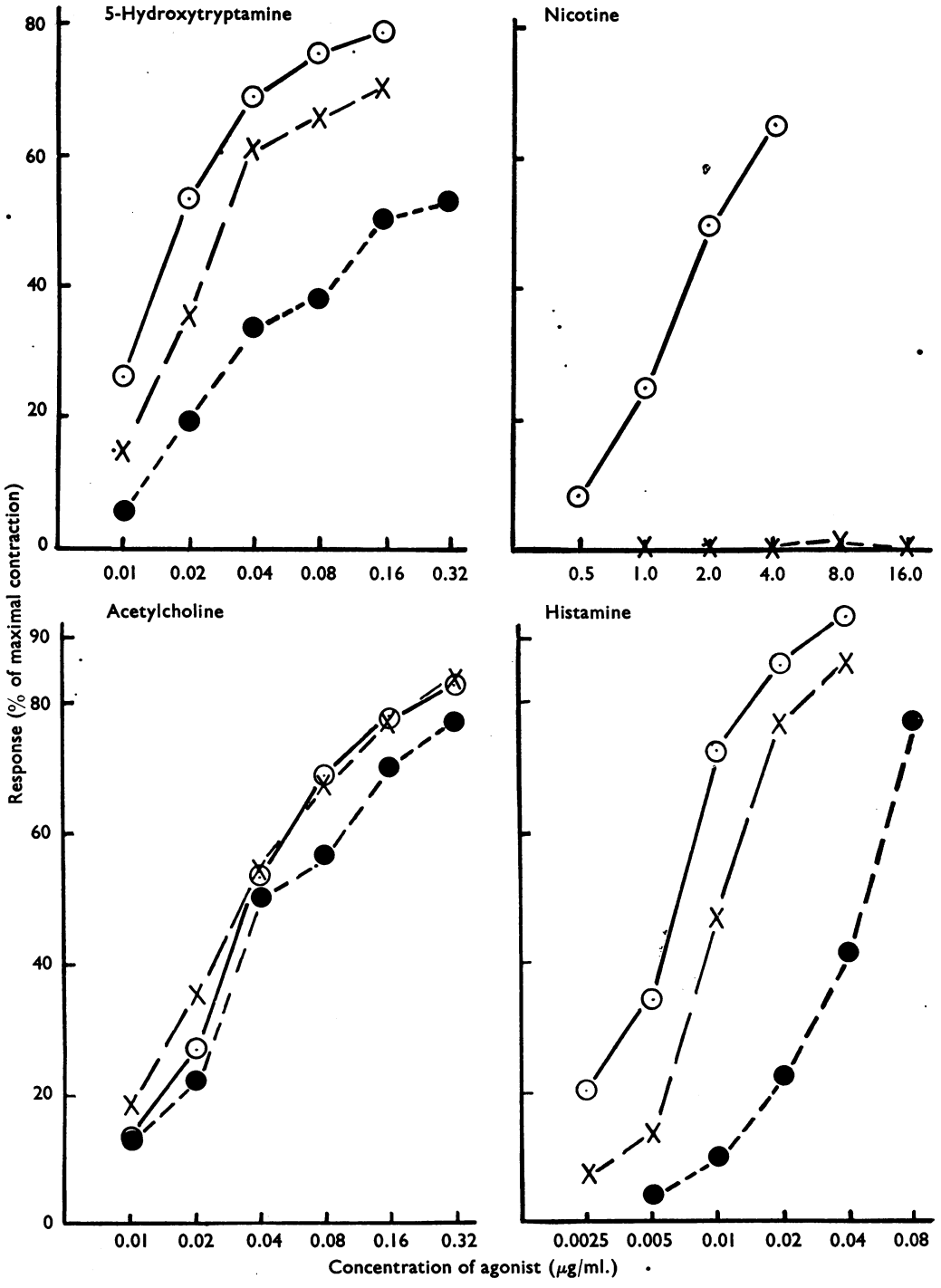

Fig. 7. The effect on the responses to the agonists (open circles) of mecamylamine (1 μg/ml., crosses; 5 μg/ml., filled circles). The ordinates and the abscissae are the same as in Fig. 1. The responses to nicotine were blocked and those to 5-hydroxytryptamine were reduced by both concentrations of mecamylamine. The dose-response curves to histamine were displaced to the right but the responses to acetylcholine were not modified. Each curve represents the mean of six experiments.

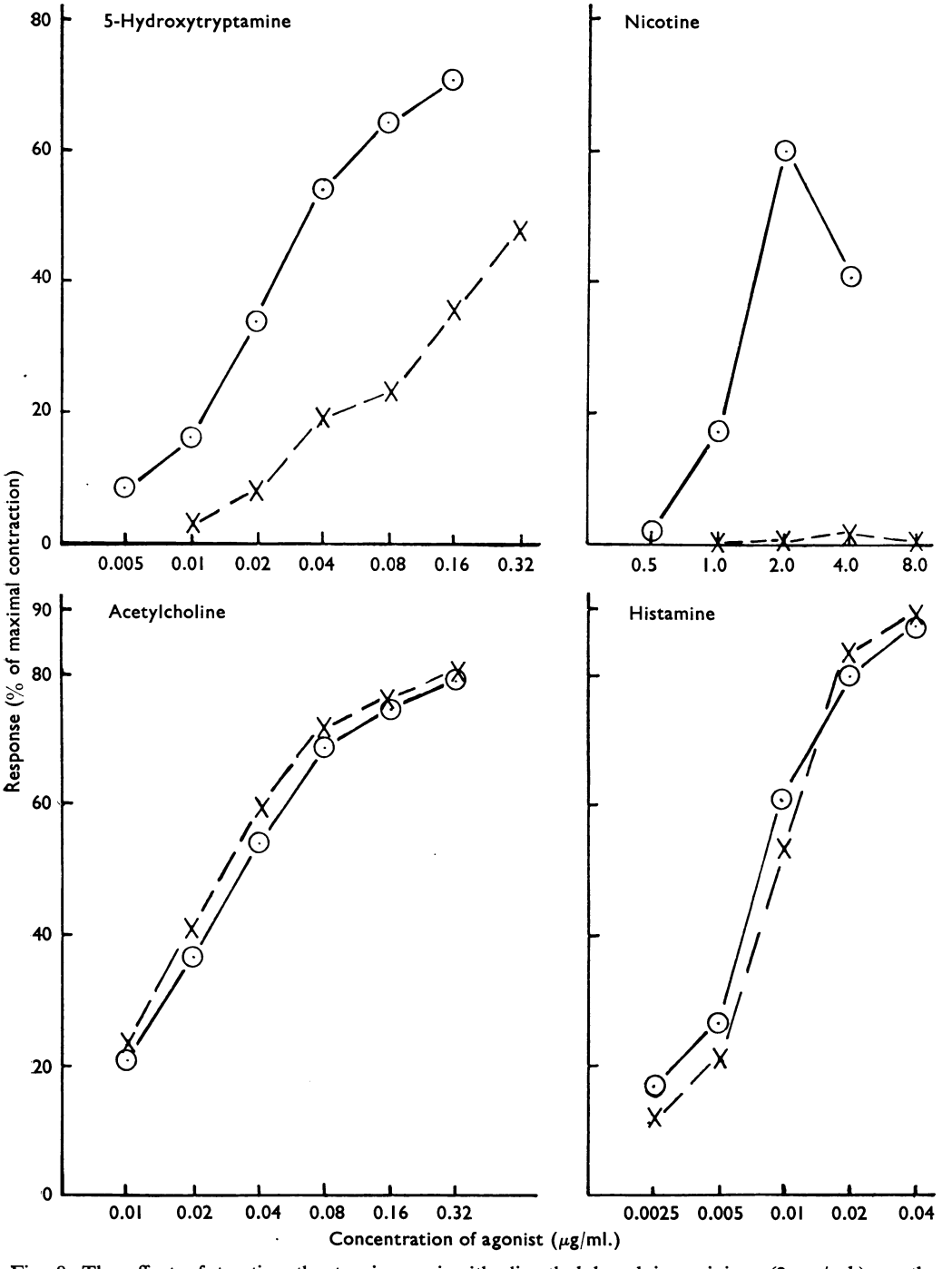

Fig. 8. The effect of treating the taenia caeci with dimethylphenylpiperazinium (2 μg/ml.) on the responses to the agonists. Ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses in the presence of dimethylphenylpiperazinium. The responses to nicotine were abolished and those to 5-hydroxytryptamine were reduced but the responses to acetylcholine or histamine remained unchanged. Each curve represents the mean of seven experiments.

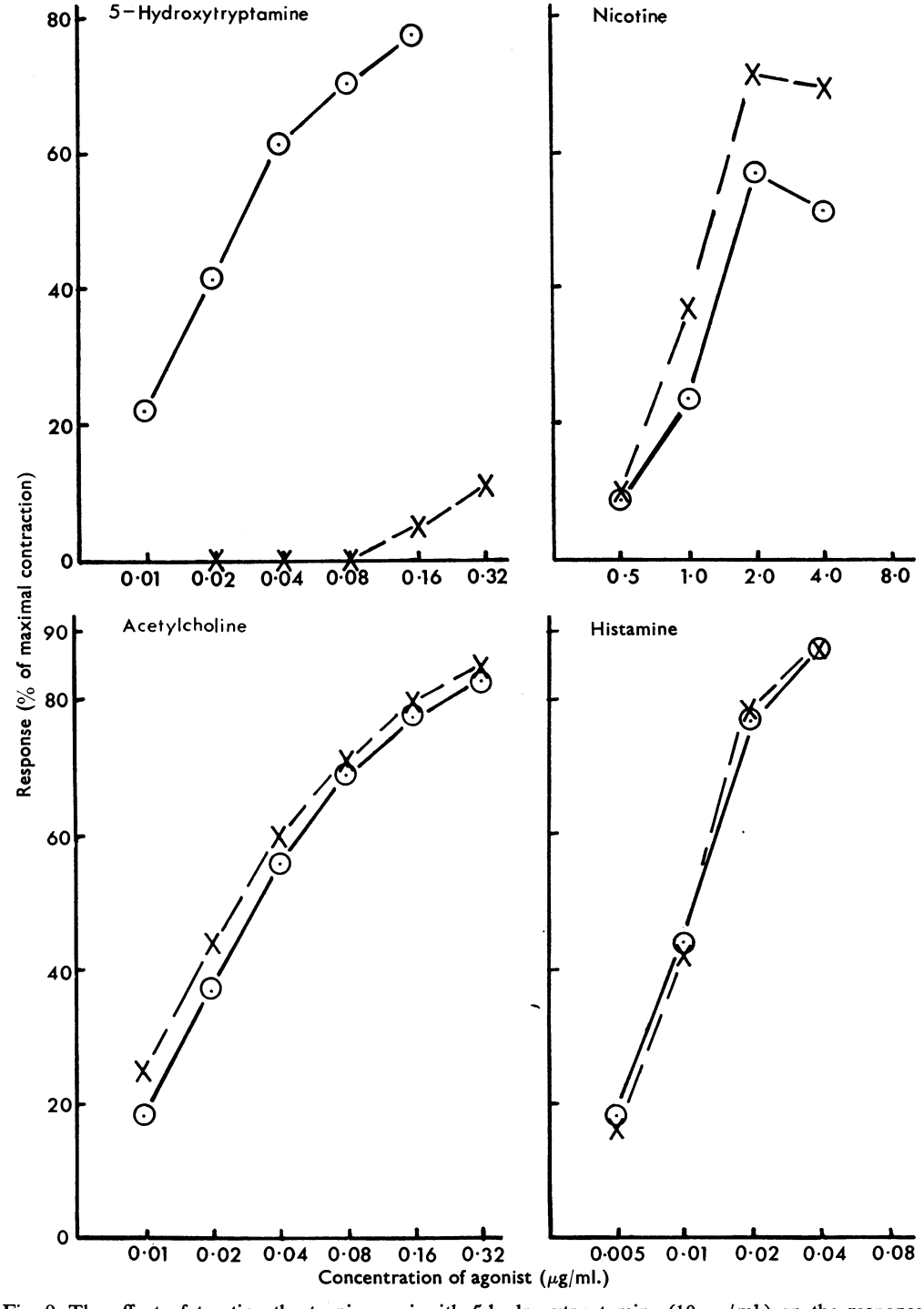

Fig. 9. The effect of treating the taenia caeci with 5-hydroxytryptamine (10 μg/ml.) on the responses to the agonists. The ordinates and the abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses represent these responses after treatment with 5-hydroxytryptamine. The responses to 5-hydroxytryptamine were almost eliminated but those to acetylcholine or histamine were unchanged. The responses to nicotine were slightly enhanced. Each curve represents the mean of five experiments.

contractions appeared, diminished but their size increased. These spontaneous contractions were often about 50% of the maximal contraction of the preparation. When the temperature of the bath fluid was maintained at 13° C for 30 min, the spontaneous activity was eliminated but the responses to the agonists were not graded and often appeared only after a contact time of 2 to 3 min. The taenia caeci at this temperature did not respond to 5-hydroxytryptamine.

The effects of ganglion blocking drugs

Hexamethonium

In the presence of hexamethonium (20 μg/ml.) the responses to nicotine were almost eliminated but those to acetylcholine, histamine or 5-hydroxytryptamine were not depressed. The dose-response curve to histamine was slightly displaced to the left (Fig. 6).

Mecamylamine

Two concentrations of mecamylamine were used (Fig. 7). The lower concentration 1 μg/ml.) blocked the responses to nicotine, reduced those to histamine and to 5-hydroxytryptamine but not those to acetylcholine. The displacement to the right of the dose-response curve to histamine or 5-hydroxytryptamine was increased by a higher concentration of mecamylamine (5 μg/ml.), although the responses to acetylcholine remained unaffected.

Dimethylphenylpiperazinium

A concentration of 2 μg/ml. dimethylphenylpiperazinium displaced the dose-response curve to 5-hydroxytryptamine to the right and blocked the responses to nicotine but did not antagonize the responses to acetylcholine or histamine (Fig. 8). The responses to the four agonists were depressed by a higher concentration of dimethylphenylpiperazinium (5 μg/ml.).

The effect of high concentrations of 5-hydroxytryptamine

The taenia caeci was incubated with 5-hydroxytryptamine (10 μg/ml.) for 30 min and the bath fluid was replaced with Krebs solution containing 1 μg/ml. 5-hydroxytryptamine. This treatment blocked the responses of the preparation to 5-hydroxytryptamine but not those to acetylcholine, histamine or nicotine. This effect is illustrated in Fig. 9.

The action of lysergic acid diethylamide

In one of six experiments, lysergic acid diethylamide (0.1 μg/ml.) depressed the responses to 5-hydroxytryptamine but did not modify those to acetylcholine, histamine or nicotine. The effects of all the four agonists were depressed in five other experiments but the responses to 5-hydroxytryptamine were most affected. This concentration of lysergic acid diethylamide induced tone and often initiated spontaneous activity of the preparations.

Fig. 10 illustrates the typical effect of hyoscine alone, and of a mixture of hyoscine and lysergic acid diethylamide on the responses to acetylcholine or 5-hydroxytryptamine. Hyoscine (0.1 μg/ml.) blocked the responses to acetylcholine but only reduced those to

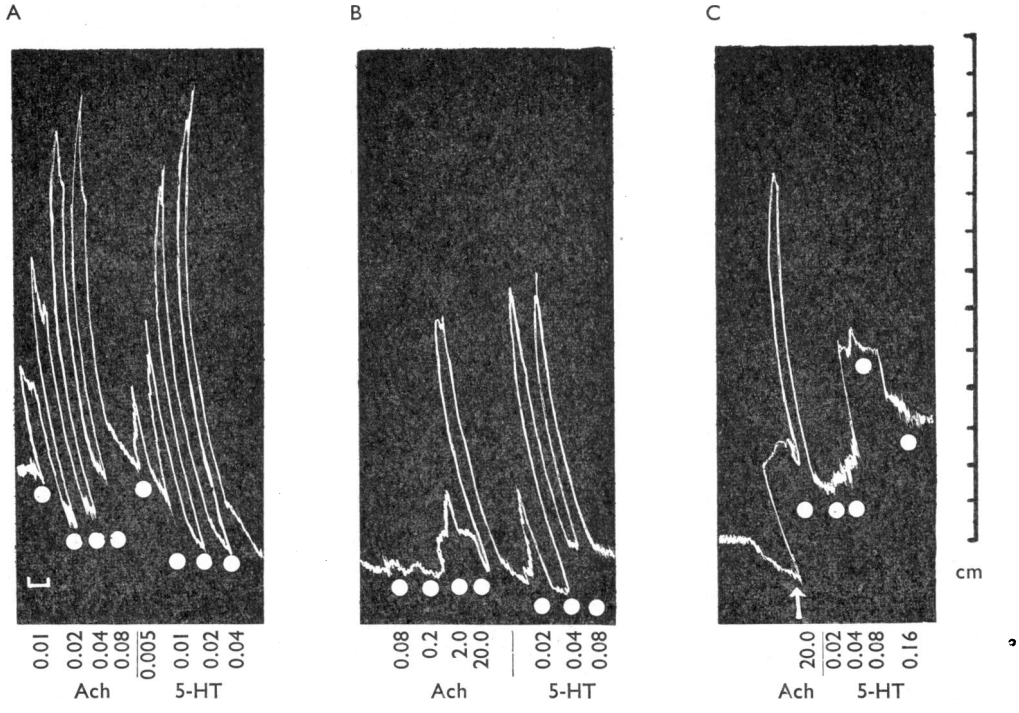

Fig. 10. The effect of hyoscine (0.1 μg/ml.) and lysergic acid diethylamide (0.1 μg/ml.) on the responses of the taenia caeci to acetylcholine (Ach) and 5-hydroxytryptamine (5-HT). Panel (A) shows the control responses and panel (B) shows the responses in the presence of hyoscine. The contractions to acetylcholine were inhibited by the hyoscine treatment, but the inhibition was overcome by increasing the concentration of acetylcholine. The responses to 5-hydroxytryptamine were reduced. Panel (C) shows the responses to acetylcholine and 5-hydroxytryptamine in the presence of lysergic acid diethylamide and hyoscine. Lysergic acid diethylamide was added at the arrow and itself caused a contraction. Note that the responses to acetylcholine were not reduced but the responses to 5-hydrotryptamine were abolished. The doses are expressed in μg/ml. and time mark is 45 sec.

5-hydroxytryptamine. A high concentration of acetylcholine (20 μg/ml. produced a contraction of the preparation. When lysergic acid diethylamide (0.1 μg/ml.) was added in the presence of hyoscine, the responses to 5-hydroxytryptamine were eliminated but the contraction to acetylcholine was not further reduced.

The effect of mepyramine on the responses

Mepyramine (0.1 μg/ml.) abolished the responses to histamine but did not modify those to acetylcholine or 5-hydroxytryptamine. Nicotine was not used in these experiments.

DISCUSSION

The contractions of the taenia caeci produced by acetylcholine or nicotine were abolished by hyoscine; but those caused by 5-hydroxytryptamine were only reduced. The responses to histamine were not modified. These results indicated that nicotine

or acetylcholine activated either directly or indirectly the muscarinic receptors and that part, at least, of the response to 5-hydroxytryptamine was located at this site also. Histamine had no action at the muscarine receptors.

It was observed that when the taenia caeci exhibited tone and in the presence of hyoscine, nicotine produced relaxation; but high doses of acetylcholine produced a biphasic response, a relaxation followed by a contraction. Similar biphasic responses were obtained with 5-hydroxytryptamine. The mechanisms of these inhibitory responses are being investigated.

The responses of the smooth muscle to acetylcholine, nicotine or 5-hydroxytryptamine, but not those to histamine, were potentiated by the anticholinesterase drug mipafox. This is further evidence that the action of nicotine and of 5-hydroxytryptamine involved a cholinergic mechanism.

The local anaesthetic agents, procaine or cocaine, abolished the responses to nicotine and greatly reduced those to 5-hydroxytryptamine but did not modify those to histamine. The effect of acetylcholine was slightly reduced by procaine but not by cocaine. This suggests an atropine-like action of procaine (Sinha, 1953; Wiedling & Tegner, 1963).

Paton (1957) and Schaumann (1957) showed that morphine inhibited the output of acetylcholine from segments of the guinea-pig ileum during coaxial electrical stimulation. They suggested that morphine antagonized the release of acetylcholine from cholinergic nerves. Morphine modified the action of nicotine or 5-hydroxytryptamine but not that of histamine or acetylcholine on the guinea-pig isolated ileum (Kosterlitz & Robinson, 1958: Gaddum & Picarelli, 1957; Day & Vane, 1963). Harry (1963), and Brownlee & Harry (1963) showed that morphine blocked the indirect action of nicotine, histamine or 5-hydroxytryptamine on the circular muscle strip of the guinea-pig ileum, but the direct action of histamine on the longitudinal muscle of the ileum was not affected. In the present investigation the responses of the taenia caeci to nicotine or 5-hydroxytryptamine were antagonized by morphine, but those to histamine or acetylcholine were not affected.

Cooling has been extensively used to analyse the action of drugs on the intestinal smooth muscle (Ambache, Dixon & Wright, 1945; Ambache, 1946; Innes, Kosterlitz & Robinson, 1957; Day & Vane, 1963). In the present investigation cooling increased the spontaneous contractions. At the temperature of 13° C the contractions were eliminated but the response to various doses of the agonist drugs were not graded. Axelsson & Bülbring (1961) observed that the spontaneous spike discharge of the taenia caeci was increased when the temperature in which the preparation was suspended was lowered from 37° C to 23° C. They suggested that lowering the temperature decreased the metabolic rate and thus decreased the energy necessary for the stabilization of the smooth muscle membrane. This may explain the increased spontaneous activity observed at temperatures lower than 37° C in the present experiments.

The experiments with local anaesthetic agents or morphine leave little doubt that acetylcholine or histamine activated the muscle directly but nicotine or 5-hydroxytryptamine had an indirect mechanism of action. It remains to consider the probable sites of action of these drugs on the intramural plexus.

The competitive ganglion blocking agent, hexamethonium, blocked the responses to nicotine but not those to acetylcholine, histamine or 5-hydroxytryptamine. Mecamylamine

has been shown to produce ganglion blockade by a mechanism different from that of hexamethonium (Bennett, Tyler & Zaimis, 1957). The responses of the taenia caeci to nicotine or 5-hydroxytryptamine were modified by mecamylamine but the responses to acetylcholine remained unchanged. The effect of histamine was reduced by mecamylamine but this was not thought to be the result of a ganglionic effect of mecamylamine because procaine, cocaine or morphine did not antagonize the responses to histamine. This effect is probably indicative of an antihistamine activity of mecamylamine as was suggested by Brownlee & Johnson (1963) who observed a similar effect in the guinea-pig ileum.

Additional evidence for the ganglionic origin of the action of nicotine or 5-hydroxytryptamine was provided by the experiments with dimethylphenylpiperazinium. This depolarizing ganglion blocking agent (Leach, 1957; Ling, 1959) blocked the responses to nicotine and reduced those to 5-hydroxytryptamine but did not affect those to acetylcholine or histamine. Leach (1957) reported that dimethylphenylpiperazinium depressed the emptying reaction of peristaltic reflex of the guinea-pig ileum without any effect on the longitudinal contractions to transmural stimulation. The concentration of dimethylphenylpiperazinium used in the present investigation was lower than that found to reduce the responses of the guinea-pig ileum to coaxial stimulation by Birmingham & Wilson (1965). Hence the present observations made it likely that the site of action of nicotine and part of the action of 5-hydroxytryptamine on the taenia caeci was on the ganglion cells within the preparation.

Gaddum & Picarelli (1957) showed that 5-hydroxytryptamine stimulated two types of receptors which they called the "D" and the "M" receptors. The "M" receptors were thought to be on the cholinergic nerves and were blocked by morphine or atropine while the "D" receptors were located on the smooth muscle and were blocked by phenoxybenzamine or lysergic acid diethylamide. On the taenia caeci, specific antagonism of the responses to 5-hydroxytryptamine by lysergic acid diethylamide was observed in one experiment. In five other experiments the effects of all the agonists were suppressed but the responses to 5-hydroxytryptamine were most affected. It is likely that 5-hydroxytryptamine activated receptors sensitive to lysergic acid diethylamide. The specific action of lysergic acid diethylamide against 5-hydroxytryptamine was demonstrated when it was used in the presence of hyoscine. Hyoscine reduced the response to 5-hydroxytryptamine, and the combination of hyoscine and lysergic acid diethylamide abolished them. The effect of acetylcholine in the presence of hyoscine was not further modified by the addition of lysergic acid diethylamide.

The rise in tone of the taenia caeci induced by lysergic acid diethylamide may be attributed at least in part to its anticholinesterase activity (Thompson, Tickner & Webster, 1955). Similar stimulant actions of lysergic acid diethylamide were observed on the guinea-pig isolated ileum (Brownlee & Johnson, 1963).

The evidence presented above can be interpreted to mean that 5-hydroxytryptamine stimulated the smooth muscle in part directly. The receptors involved may be the "D" receptors of Gaddum & Picarelli (1957). The remainder of the action of 5-hydroxytryptamine was concluded to be indirect, probably by activating cholinergic ganglion cells. A similar indirect action of 5-hydroxytryptamine was demonstrated by Brownlee & Johnson (1963) on the guinea-pig ileum. These workers successfully detected and estimated the acetylcholine released by 5-hydroxytryptamine (Brownlee & Johnson, 1965).

5-Hydroxytryptamine activated receptors on the intramural plexuses and these receptors differed from those activated by nicotine. Hexamethonium blocked the responses to nicotine but had no effect on the responses to 5-hydroxytryptamine. Similar hexamethonium-resistant receptors have been demonstrated in the isolated inferior mesenteric ganglion of the cat (Bindler & Gyermek, 1961) and in the superior cervical ganglion of the cat (Trendelenburg, 1956). Many workers have suggested that the sympathetic ganglion cells are heterogeneous (Shaw, MacCullum, Dewhurst & Mainland, 1951 ; Hertzler, 1961 ; Appelgren, Hansson & Schmiterlöw, 1963 ; Hamberger, Norberg & Sjöqvist, 1963). If this is true of cholinergic ganglia also, 5-hydroxytryptamine and nicotine may be acting not only on different receptors but upon different ganglion cells.

The action of histamine on the taenia caeci resulted from the stimulation of specific receptors sited on the smooth muscle fibres.

SUMMARY

1. The mechanisms of the contractions of the taenia from the guinea-pig caecum (the taenia caeci) to acetylcholine, histamine, nicotine and to 5-hydroxytryptamine were investigated.

2. Hyoscine blocked the responses to acetylcholine and to nicotine, reduced those to 5-hydroxytryptamine but did not modify those to histamine. The organophosphorus anticholinesterase drug, mipafox, potentiated the responses to acetylcholine, nicotine and 5-hydroxytryptamine but not those to histamine.

3. The responses to nicotine were almost abolished by procaine and those to 5-hydroxytryptamine were greatly reduced. The effect of histamine was not modified by procaine but that of acetylcholine was slightly reduced. Cocaine or morphine antagonized the responses to nicotine or 5-hydroxytryptamine but not those to acetylcholine or histamine.

4. Hexamethonium blocked the responses to nicotine but left those of other agonists unchanged. Mecamylamine or dimethylphenylpiperazinium blocked the contractions to nicotine, reduced those to 5-hydroxytryptamine but not those to acetylcholine. The contractions to histamine were reduced by mecamylamine but not by dimethylphenylpiperazinium.

5. The contractions to 5-hydroxytryptamine were reduced by hyoscine or lysergic acid diethylamide but were abolished by a combination of the two antagonist drugs.

6. High concentration of 5-hydroxytryptamine inhibited the responses to 5-hydroxytryptamine but did not affect those to acetylcholine, histamine or nicotine.

7. Mepyramine blocked the responses to histamine but not those of acetylcholine or 5-hydroxytryptamine.

8. It was concluded that acetylcholine or histamine activated receptors sited on the smooth muscle cells. Nicotine stimulated cholinergic ganglion cells. The action of 5-hydroxytryptamine was partly direct on the smooth muscle cells and partly indirect on the cholinergic ganglion cells.

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