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An analysis of the action of drugs on the circular muscle strips from the caecum of the guinea-pig

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The taenia-free circular muscle strips of the caecum of the guinea-pig contracted to acetylcholine or histamine. 5-Hydroxytryptamine produced small contractile responses but high concentrations (40 μg/ml or more) induced relaxation. The contractions to 5-hydroxytryptamine were enhanced by the organophosphorus anticholinesterase drug mipafox. The responses to nicotine were always relaxations but contractions were obtained after incubating the preparation with mipafox. Hence circular muscle strips treated with mipafox were used to investigate the mechanism of contractions to all the drugs. The site of action of acetylcholine or histamine was located on the smooth muscle cells because the responses were not blocked by pentolinium, dimethylphenylpiperazinium, procaine or cocaine. Hyoscine blocked the responses to acetylcholine but left those to histamine unchanged. The contractions to nicotine were blocked by hyoscine, local anaesthetics or ganglion blocking drugs. Nicotine is thought to activate cholinergic ganglion cells. 5-Hydroxytryptamine produced contractions which were potentiated by mipafox, blocked by hyoscine and almost abolished by local anaesthetics. The responses were not modified by pentolinium but were reduced by dimethylphenylpiperazinium at a concentration which did not block the responses to acetylcholine or histamine. It is concluded that the action of 5-hydroxytryptamine was wholly indirect, on nervous tissues, part of which was located on cholinergic ganglion cells.

THE action of drugs on the circular muscle strips of the mammalian intestine has been investigated by many workers. Thus nicotine contracted the circular muscle preparation of the ileum of the cat; after treatment of this preparation with botulinum toxin, the nicotine induced contraction was replaced by an inhibition (Ambache & Lessin, 1955). Harry (1963) and Brownlee & Harry (1963) showed that the circular muscle strips of the guinea-pig ileum were insensitive to acetylcholine and contracted to histamine, nicotine or 5-hydroxytryptamine (5-HT) only after treatment with an anticholinesterase drug. Similarly, circular muscle strips from the rabbit ileum were insensitive to muscarinic drugs and did not respond at all to nicotine, histamine, 5-HT or dimethylphenylpiperazinium (Tweeddale, 1963). The circular muscle preparations of the human ileum and colon reacted with inhibitory responses only to nicotine, while 5-HT caused contraction of the preparations from the ileum but produced relaxation of those from the colon (Fishlock & Parks, 1963; 1966; Fishlock, 1964).

The caecum of the guinea-pig, like the human colon, has three bands of longitudinal muscle (the taeniae). The circular muscle is easily separated from the taeniae and thus each muscle layer can be studied in isolation. The present investigation is concerned with the mechanism of action of drugs on the caecal circular muscle strips of the guinea-pig.

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Methods

Guinea-pigs weighing less than 500 g were killed by stunning and bleeding. The abdomen was opened and the two taeniae furthest from the mesenteric border were dissected. The caecum was cut open along its mesenteric border, washed and pinned out on a cork pad under Krebs solution. A taenia-free circular muscle strip (3 cm × 4 mm) was cut and suspended in an organ bath containing 20 ml of Krebs solution at 37° gassed with a mixture of oxygen 95% and carbon dioxide 5%. The responses, magnified 8 times, were recorded on smoked paper with an isotonic frontal-writing lever. The load on the circular muscle was about 350 mg.

The preparations were incubated with 50 μg/ml of mipafox (*NN*-diisopropylphosphorodiamidic fluoride, an organophosphorus anticholinesterase) for 1 hr, and after removing mipafox from the bath fluid a dose-response curve was made for acetylcholine, histamine, nicotine and 5-HT. This treatment with mipafox was necessary to obtain contractile responses to nicotine. Thirty min after incubating the circular muscle with an antagonist drug, and in its presence, a second dose-response curve was made.

The contact time for the agonists was 45 sec and the interval between doses was 4 min during which period the preparation was washed 6 times.

DRUGS

These were acetylcholine chloride, histamine acid phosphate, nicotine acid tartrate, 5-hydroxytryptamine creatinine phosphate, *NN*-di-isopropylphosphorodiamidic fluoride (mipafox), hyoscine hydrobromide, neostigmine methylsulphate, dimethylphenylpiperazinium iodide, cocaine hydrochloride, procaine hydrochloride and pentolinium tartrate. The concentrations of the drugs, except mipafox, are expressed as the final bath concentration in μg/ml of the base.

The composition of the Krebs solution (in g/litre of 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

The caecal circular muscle strip when suspended in Krebs solution slowly went into a well-maintained contracture which reached a maximum in 1 to 2 hr. This contracture will be referred to as "tone". The tone was maintained for over 8 hr and was not abolished by hyoscine (0·1 to 1·0 μg/ml) (Fig. 1). The extent of tone exhibited by circular muscle strips varied greatly; preparations from small guinea-pigs (less than 500 g) showed less tone than those from large animals (600 g or more). The circular muscle strips were often spontaneously active.

RESPONSES TO DRUGS

Acetylcholine. The caecal circular muscle responded to acetylcholine with a contraction, the threshold dose being about 0·1 μg/ml (Fig. 1). The responses were potentiated about 25 times by mipafox and were abolished

by hyoscine (0·1 μg/ml). In the presence of this concentration of hyoscine, high doses of acetylcholine (more than 50 μg/ml) produced a biphasic effect consisting of a short-lived relaxation, followed by contraction.

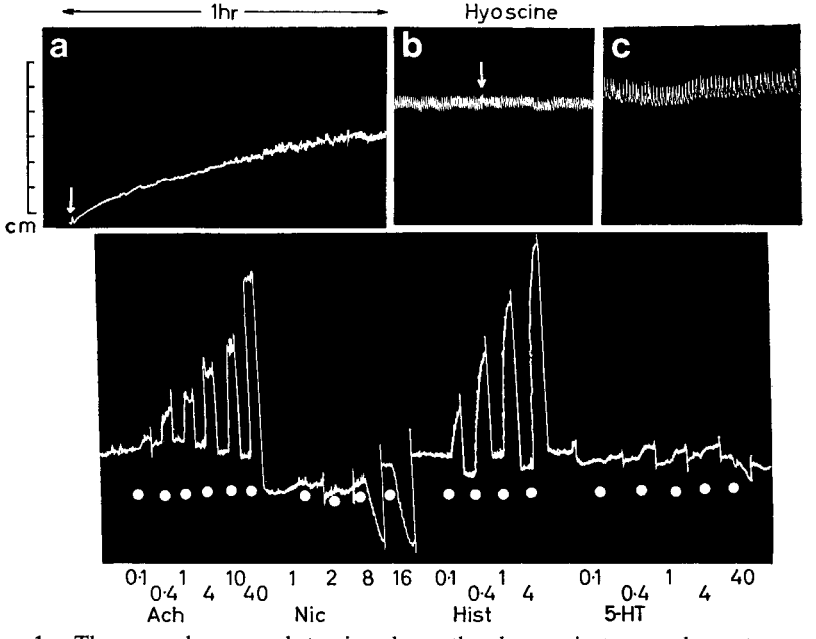

FIG. 1. The upper kymograph tracing shows the changes in tone and spontaneous activity exhibited by a caecal circular muscle strip from the guinea-pig. The preparation was set up in Krebs solution at 37°. The increase in tone (starting from the arrow) within the first hour is shown by (a). The second record (b) and the third record (c) were obtained 3 and 5 hr later respectively. Note that hyoscine (1 μg/ml) added to the bath at the arrow in b and left in bath throughout the duration of the experiment, did not reduce the tone. The lower tracing shows the typical effect of acetylcholine, nicotine, histamine and 5-HT on the circular strip. Nicotine produced only relaxation of the preparation. 5-HT caused small contractions but a high concentration (40 μg/ml) produced relaxation. The numbers represent the concentrations of the drugs in μg/ml of the bath fluid.

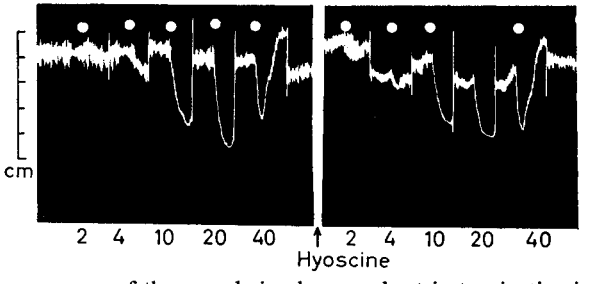

FIG. 2. The responses of the caecal circular muscle strip to nicotine in the absence and presence of hyoscine (1·0 μg/ml). Note that the high concentration of nicotine (40 μg/ml) produced a relaxation followed by a contraction. The contraction was not modified by hyoscine. The numbers refer to the concentrations of nicotine in μg/ml of bath fluid.

Histamine. The responses of the circular muscle strips to histamine were contractions. The contractions were neither potentiated by mipafox (50 μg/ml) nor depressed by hyoscine (0·1 μg/ml).

5-*Hydroxytryptamine.* It was found that 5-HT caused contraction of the caecal circular muscle and that the threshold dose was about 0·1 μg/ml.

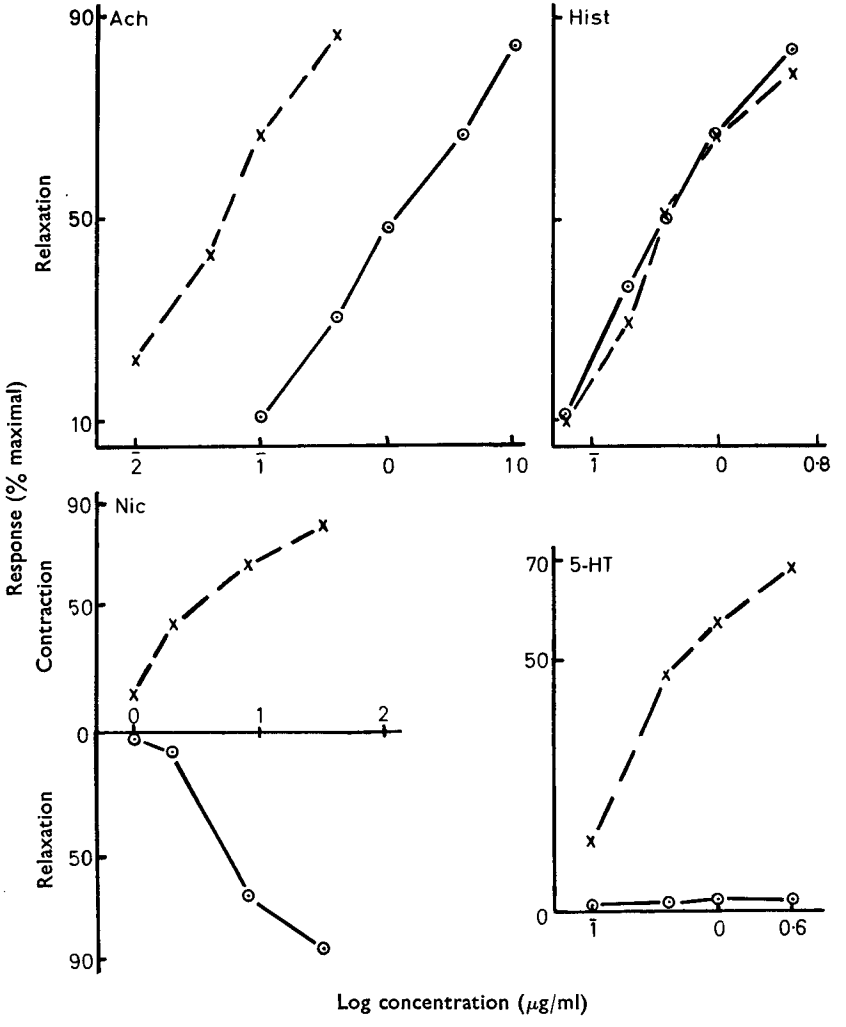

FIG. 3. The effect of treating caecal circular muscle strips of the guinea-pig with mipafox (50 μg/ml) for 1 hr on the responses to acetylcholine, histamine, nicotine and 5-HT. The results are plotted as % of maximal response (contraction or relaxation) against the log concentration in μg/ml. The circles represent the responses to the agonists and the crosses represent these responses after treating the preparations with mipafox. The responses to acetylcholine or 5-HT were enhanced but those to histamine were not. The inhibitory responses of nicotine were blocked and replaced by contractions after treatment with mipafox. Each curve represents the mean of six experiments.

These contractions were usually small and were reversed to inhibition with high doses (Fig. 1). Mipafox (50 μg/ml) enhanced the contractions and hyoscine (0·1 μg/ml) abolished or reversed them to inhibition.

Nicotine. The typical effect of nicotine is shown in Figs 1 and 2. Nicotine produced relaxation of the preparations and the response increased with dose. When a high concentration (40 μg/ml or more) of

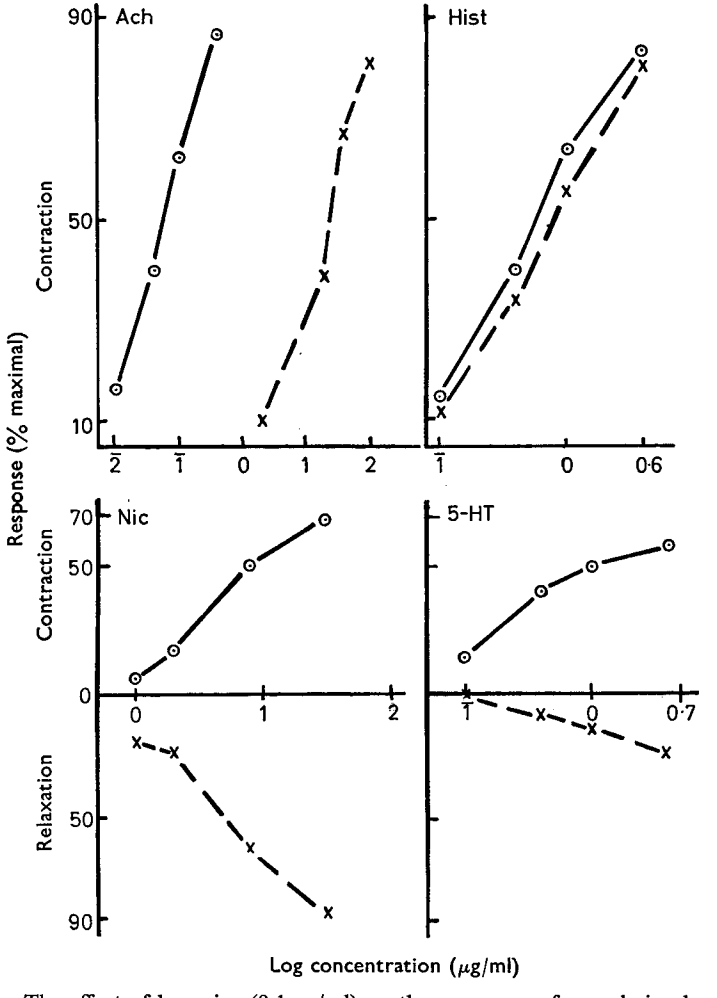

FIG. 4. The effect of hyoscine (0·1 μg/ml) on the responses of caecal circular strips of the guinea-pig to acetylcholine, histamine, nicotine and 5-HT. The control responses (circles) were obtained after the preparations had been treated with mipafox (50 μg/ml) for 1 hr. The crosses represent these responses in the presence of hyoscine (0·1 μg/ml). The results are plotted as % maximal response (contraction or relaxation) against log concentration in μg/ml. Hyoscine produced a parallel displacement to the right of the dose-response curve to acetylcholine. The responses to histamine were not modified. The contractile responses to nicotine or 5-HT were replaced by inhibitory responses. Each curve represents the mean of six experiments.

nicotine was used, a small relaxation followed by a contraction was seen. This contraction was not modified by hyoscine (1 μg/ml). After the preparations were treated with mipafox (50 μg/ml), the inhibitory responses were reversed and the contractions were then seen. For this reason the mechanism of the contractile responses to all the agonists was investigated after treating the preparations with mipafox (50 μg/ml).

The effects of anticholinesterase drugs. Fig. 3 shows the effect of an organophosphorus anticholinesterase drug, mipafox (50 μg/ml) on the dose-response curves to acetylcholine, histamine, nicotine or 5-HT. The responses to acetylcholine or 5-HT were potentiated but those of histamine were not modified. The inhibitory responses of the caecal circular muscle to nicotine were reversed and were replaced by contractions.

It was observed that the extent of potentiation to acetylcholine produced by 20 μg/ml of mipafox was the same as that produced by 50 μg/ml. Nicotine produced biphasic responses on preparations treated with mipafox (20 μg/ml) but only contractions after treatment with mipafox (50 μg/ml) and these contractions were bigger than those observed with the lower concentration of mipafox. The enhancement of the contractions to 5-HT was greater after treatment with 50 μg/ml than after 20 μg/ml of mipafox. The reason for these differences in the extent of potentiation by various concentrations of mipafox is being investigated.

Potentiation of the responses to acetylcholine or 5-HT and the reversal of the inhibitory responses to nicotine, were also produced by neostigmine (1–5 μg/ml). These concentrations of neostigmine induced a high degree of tone and the extent of the potentiation could not be determined.

Dimethylphenylpiperazinium resembled nicotine in producing relaxation of the caecal circular muscle. This inhibitory effect was replaced by contractile responses after treatment with mipafox (50 μg/ml).

The influence of hyoscine on the responses. After incubating the circular muscle strips with mipafox, the contractile responses to acetylcholine were abolished by hyoscine (0·1 μg/ml). High doses of acetylcholine produced a dose-response curve parallel to the original. The contractions to nicotine or 5-HT were reversed and replaced by inhibition but the responses to histamine were not modified (Fig. 4).

The action of local anaesthetics. Cocaine (10 μg/ml) eliminated the responses to nicotine, reduced those to 5-HT but left the responses to acetylcholine or histamine unchanged. A higher concentration of cocaine (20 μg/ml) almost abolished the responses to 5-HT but did not modify those to histamine or nicotine. A concentration of procaine (10 μg/ml) which did not antagonise the responses to histamine, greatly reduced the responses to 5-HT and almost abolished the contractions to nicotine. The dose-response curve to acetylcholine was slightly displaced to the left. The effect of cocaine (10 μg/ml) is shown in Fig. 5.

The effect of cooling on the responses. The contractile responses to all agonists were first established at 37° and then repeated after a 30 min equilibration period at any other temperature.

At temperatures lower than 37° the delay in onset of contraction was increased for all agonists but was greatest for nicotine or 5-HT. The time

taken for a response to reach maximum was also prolonged. It was found necessary to leave the drugs in the bath for 2–3 min.

At 18° the responses to 5-HT remained unchanged but those to acetylcholine, histamine or nicotine were enhanced. When the circular muscle preparation was equilibrated at 15° the contractions to histamine were potentiated or not affected and those to acetylcholine or nicotine were not modified but the responses to 5-HT were reduced. All the four agonists produced smaller contractions at 12° than at 37°. In all experiments,

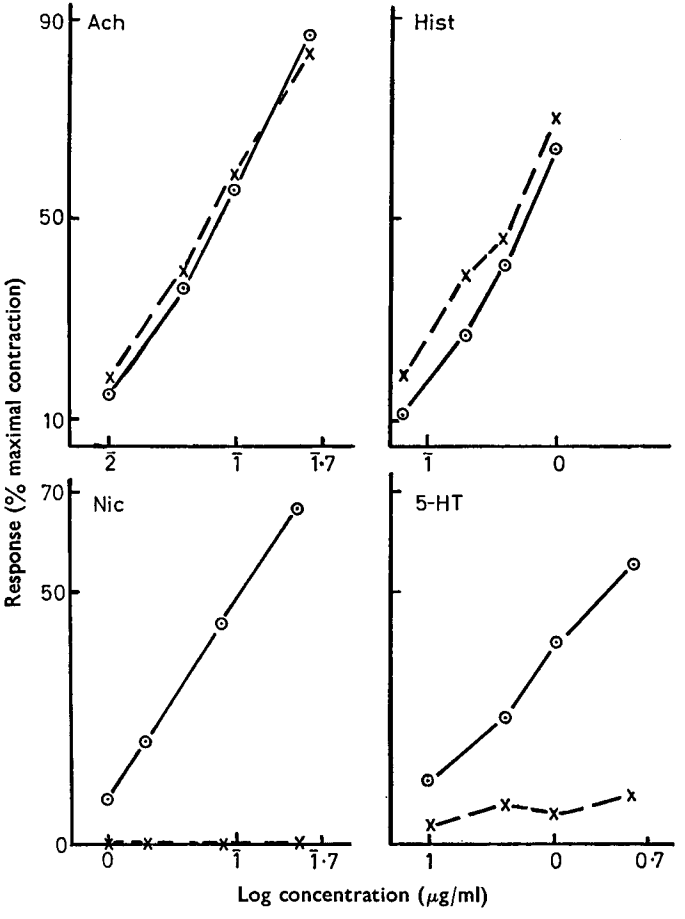

FIG. 5. The effect of cocaine (10 μg/ml) on the responses of caecal circular muscle strips to acetylcholine, histamine, nicotine and 5-HT. The preparations were treated with mipafox (50 μg/ml) for 1 hr before the control responses (circles) were established. The crosses are these responses in the presence of cocaine. The results are plotted as % of maximal contraction against log concentration in μg/ml. The contractions to nicotine were blocked, those to 5-HT were greatly reduced, but the responses to acetylcholine or histamine were not reduced. The contractions to histamine were slightly enhanced. Each curve represents the mean of eight experiments.

the contractions returned completely when the temperature was raised to 37°.

The effect of ganglion blocking drugs. The contractions to nicotine were blocked by pentolinium (5 μg/ml) but those to acetylcholine, histamine or 5-HT were not affected. Dimethylphenylpiperazinium (4 μg/ml) almost completely inhibited the responses to nicotine, greatly reduced those to 5-HT but did not modify those to acetylcholine or histamine (Fig. 6). A higher concentration of dimethylphenylpiperazinium (10 μg/ml) reduced the responses to all agonists.

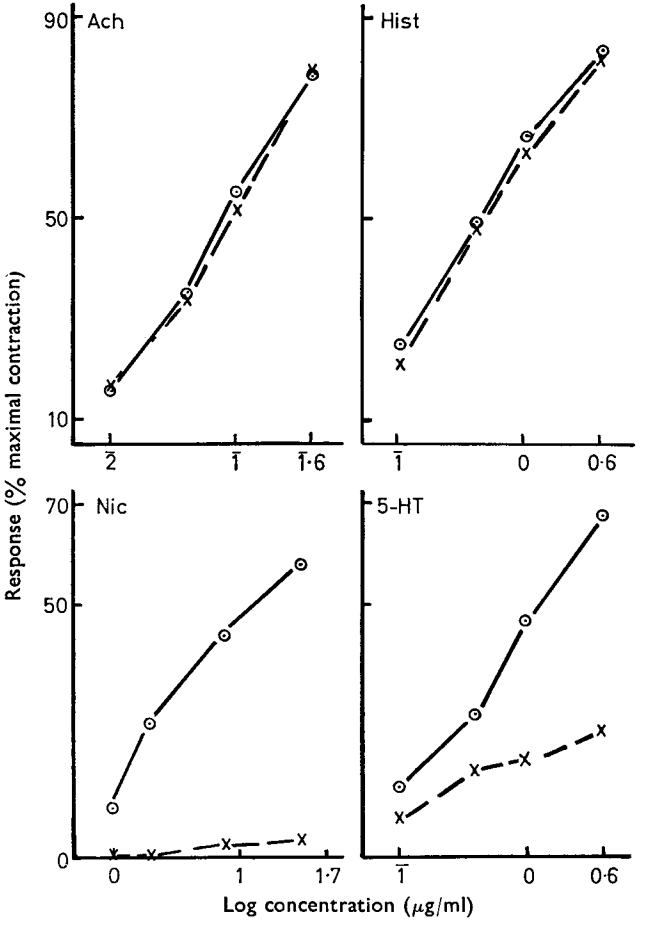

FIG. 6. The action of dimethylphenylpiperazinium (4 μg/ml) on the contractions of caecal circular muscle strips. The circles represent the control responses of the preparations after treatment with mipafox (50 μg/ml) for 1 hr. The crosses represent these responses in the presence of dimethylphenylpiperazinium. The ordinates and the abscissae are as in Fig. 5. The dose-response curves to acetylcholine and to histamine were unchanged but the responses to 5-HT were reduced and those to nicotine were almost eliminated. Each curve represents the mean of four experiments.

The effect of 5-hydroxytryptamine in excess. After treating the caecal circular muscle with mipafox, the dose-response curves to acetylcholine, histamine, 5-HT or nicotine were made in the absence of and also in the presence of 10 μg/ml of 5-HT. The responses to 5-HT were blocked but those to acetylcholine, histamine or nicotine were not reduced (Fig. 7).

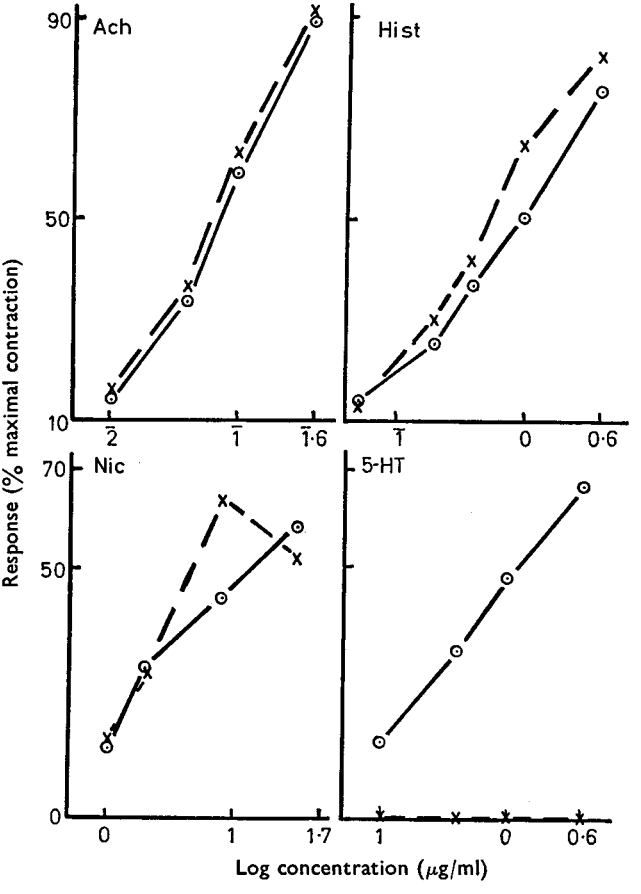

FIG. 7. The effect of 5-HT (10 μg/ml) on the contractions of the caecal circular muscle after treatment with mipafox (50 μg/ml) for 1 hr. The circles represent the control responses and the crosses represent these responses in the presence of 5-HT. The ordinates and the abscissae are the same as in Fig. 5. The responses to 5-HT were blocked but those to acetylcholine, histamine or nicotine were not reduced. Each curve represents the mean of four experiments.

Discussion

The contractions of the caecal circular muscle induced by acetylcholine were potentiated by anticholinesterase drugs, and were blocked by hyoscine but were not modified by ganglion blocking drugs or by cocaine. It seems therefore that acetylcholine stimulated muscarinic receptors sited

on the smooth muscle fibres. Procaine displaced the dose-response curve to acetylcholine slightly to the right, an action which is best explained by its known anti-acetylcholine property (Sinha, 1953; Wiedling & Tegner, 1963).

Experiments made at temperatures lower than 37° were unhelpful in producing results which could be used to explain the mechanism of action of any of these drugs. For example, at 18°, the responses to all the agonist drugs except 5-HT were enhanced. Similar potentiation of the responses to acetylcholine or histamine at temperatures lower than 37° has been reported for the guinea-pig ileum (Day & Vane, 1963) and for the guinea-pig trachealis muscle (Carlyle, 1963).

Histamine produced contractions which were neither potentiated by mipafox nor depressed by hyoscine, local anaesthetics nor by ganglion blocking drugs. Thus the action of histamine did not appear to involve the stimulation of nervous tissue. This is unlike the action of histamine on cholinergic nerves within the circular muscle strips of the guinea-pig ileum demonstrated by Harry (1963). It seems probable that such cholinergic nerves with histamine receptors are not present in the guinea-pig caecum.

Nicotine produced only a relaxation of caecal circular strips untreated with other agents. But high doses of nicotine produced a relaxation followed by a contraction. The contraction was of great interest in that it was not antagonised by hyoscine. This hyoscine-resistant action of nicotine may represent a direct effect on the smooth muscle cells. Day & Vane (1963) have suggested from their experiments on the isolated ileum of the guinea-pig, that nicotine might have a direct effect on smooth muscle fibres. An alternative consideration is that the hyoscine-resistant contraction observed in the present investigation was a 'rebound' effect resulting from the prior relaxation.

The absence of hyoscine-sensitive contractile responses to nicotine in normal doses on preparations not treated with an anticholinesterase drug, seemed to suggest an absence of cholinergic ganglion cells within the circular muscle strips. However, after treatment with mipafox, nicotine induced contractions in doses which previously produced only relaxation. These contractions were abolished by hyoscine at a concentration which also blocked the responses to acetylcholine. The contractile responses to nicotine were eliminated by ganglion blocking drugs or by cocaine at concentrations which did not modify the responses to acetylcholine or histamine. Hence the responses resulted very probably from the stimulation of cholinergic ganglion cells. Thus treatment with mipafox revealed the presence of these cells within the preparation.

The reversal of the inhibitory action of nicotine to contraction was not only produced by mipafox but also by neostigmine. Also dimethylphenylpiperazinium caused relaxation of the caecal circular muscle and this response was reversed and replaced by contraction after treatment with mipafox. This is good evidence that the observations made with nicotine or mipafox did not arise from an unusual property of either compound.

It is not known why nicotine did not cause contraction of the caecal circular muscle strips before treatment with mipafox. It seems probable that a high concentration of cholinesterases may be located around cholinergic nerve endings and thus their inhibition is needed to reveal cholinergic activation by nicotine. Another possibility is that there was a large store of easily releasable inhibitory substances within the circular muscle, which masked the effect of cholinergic nerve stimulation in untreated preparations.

The contractions of the circular muscle strips induced by 5-HT like those produced by acetylcholine, were potentiated by mipafox and blocked by hyoscine. The responses were antagonised by cocaine at a concentration which blocked the effect of nicotine but not that of histamine or acetylcholine. Hence the action of 5-HT like that of nicotine, appears to be on cholinergic nerves.

The concentration of dimethylphenylpiperazinium which did not modify the responses of the caecal preparation to acetylcholine or histamine, antagonised the relaxation caused by 5-HT and by nicotine. It is therefore very likely that the action of 5-HT involved the stimulation of ganglion cells. A similar ganglionic action of 5-HT has been demonstrated on the guinea-pig ileum (Brownlee & Johnson, 1963) and on the taenia caeci (Akubue, 1966).

The contractions caused by 5-HT were attributed to activation of specific receptors because they were abolished by high concentration of 5-HT which did not modify the responses to acetylcholine, histamine or nicotine. Thus 5-HT activated specific receptors on the cholinergic nerves. One part of this action is located on ganglion cells.

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