J. Pharm. Pharmacol., 1965, **17**, 710–720 Received July 12, 1965

A direct and an indirect action of 5-hydroxytryptamine on the distal part of the isolated colon of the rat

KAREN ULRICH*

The motor response of 5-hydroxytryptamine (5-HT) on the distal part of the isolated rat colon was investigated by constructing dose: response curves to 5-HT, acetylcholine and nicotine; these were repeated in the presence of different antagonists and an anticholinesterase. Hyoscine abolished the responses to acetylcholine, almost completely blocked the effect of nicotine, but reduced the contractions to 5-HT only to about half of the original level. The anticholinesterase *NN'*-diisopropylphosphorodiamidic fluoride (mipafox) potentiated the responses to acetylcholine, 5-HT or nicotine. Procaine and cocaine inhibited to the same extent the large doses of 5-HT, but had no effect on the small doses. Both drugs reduced the contractions with nicotine. Three ganglion-blocking agents were used. Hexamethonium had no effect on acetylcholine or 5-HT, but antagonised nicotine. Mecamylamine had no effect on acetylcholine; it blocked the responses to nicotine and reduced the large doses of 5-HT. The action of dimethylphenylpiperazinium on the three agonists was similar to that of mecamylamine. 2-Bromolysergic acid diethylamide had no effect on the responses to acetylcholine, but reduced equally the contractions due to 5-HT and nicotine. It was concluded that 5-HT acted indirectly by stimulating the intramural parasympathetic ganglia and directly by an action on the muscle fibres. The direct action was pronounced with small doses, the indirect action with higher doses of 5-HT.

SMOOTH muscle is contracted by 5-hydroxytryptamine (5-HT) in two known ways. One is by a direct action on the muscle fibres, the second is via the nerves and involves a cholinergic pathway.

An indirect action was demonstrated by Rocha e Silva, Valle & Picarelli (1953), Robertson (1953) and Gaddum & Hameed (1954). Gaddum & Picarelli (1957) found both a direct and an indirect action of 5-HT on the terminal part of the guinea-pig ileum, while the evidence of an indirect mechanism was submitted by Day & Vane (1963), who concluded that the amine acted mainly on receptors in the nervous tissue. Brownlee & Johnson (1963), in a formal analysis of the mechanism of action of 5-HT in the guinea-pig ileum, located the precise site on the intramural parasympathetic ganglia.

A direct action of 5-HT was reported by Vane (1957) and by Paton & Vane (1963) on fundal strips of the guinea-pig, kitten and rat stomach.

This paper describes experiments on the distal part of the rat isolated colon and provides evidence of both a direct and an indirect action of 5-HT in this tissue.

Experimental

METHODS

Adult albino male rats, 175 to 325 g, were killed by a blow on the head and bled. The colon was excised, and a 2 cm segment from the descending part was removed after discarding 2 cm nearest the rectal junction.

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

*Present address: The Laboratory of Danmarks Apotekerforening, Lergravsvej, 59, Copenhagen 5.

The oral end of the tissue was tied to a supporting hook and the other end to the recording lever. Organ baths containing 20 or 60 ml Krebs solution at 37° and aerated with a mixture of 95% oxygen and 5% carbon dioxide were used. Longitudinal contractions were recorded with isotonic frontal writing balsa levers loaded with 0·5 g. The magnification was ten times.

The preparation was set up and allowed to stabilise for 30 min. Dose: response curves were then constructed using five to seven doses of the three agonists. A contact time of 30 sec was used within a dose cycle of 4 min; between the doses the bath fluid was changed four times. The antagonist was then added to the bath and after 45 min the dose: response curves repeated; antagonist concentrations were re-established after each washing cycle. Subsequently the tissue was washed for an hour and the dose: response curves established again. Control experiments were made using preparations in which dose: response curves were repeated in the absence of the antagonist.

In the experiments with mipafox, this anticholinesterase was kept in contact with the tissue for 90 min; the tissue was then washed with Krebs solution for 30 min and the dose: response curves to the agonists repeated. Each agonist: antagonist experiment was made on preparations from at least four rats.

Most tissues were spontaneously active; those with marked and irregular spontaneous activity were not used. Frequently there was a change in tone during the experiments; if this change became too great the experiment was discontinued.

The composition of the Krebs solution (in g/litre of distilled water) was: 6·92 NaCl, 0·35 KCl, 0·28 $CaCl_2$, 2·1 $NaHCO_3$, 0·16 KH_2PO_4, 0·29 $MgSO_4.7\,H_2O$, and 2·0 glucose.

DRUGS

Agonists. These were acetylcholine chloride (Hopkins & Williams), histamine acid phosphate (L. Lights), 5-hydroxytryptamine creatinine sulphate (May and Baker), nicotine hydrogen tartrate (British Drug Houses).

Antagonists. These were hyoscine hydrobromide (Burroughs Wellcome and Co.), cocaine hydrochloride (British Drug Houses), procaine hydrochloride (British Drug Houses), hexamethonium bromide (L. Lights), mecamylamine hydrochloride (Merck, Sharp and Dohme), 1,1-dimethyl-4-phenylpiperazinium iodide (Fluka), 2-bromolysergic acid diethylamide tartrate (Sandoz).

The anticholinesterase used was *NN'*-diisopropylphosphorodiamidic fluoride (mipafox) (L. Lights).

All concentrations are quoted as final bath concentrations in μg/ml in terms of base except those of 2-bromolysergic acid diethylamide, which are expressed in terms of the salt.

Results

Acetylcholine. Acetylcholine (0·001 to 0·256 μg/ml) contracted the tissue and gave reproducible contractions with repeated doses.

5-Hydroxytryptamine. 5-HT (0·003 to 12·8 μg/ml) contracted the tissue. Frequently, tachyphylaxis occurred when it was administered repeatedly despite washing the preparation several times for 15 min between the doses. The sensitivity of the tissue to 5-HT could be maintained by interpolating 2 to 3 doses of acetylcholine between each dose of 5-HT.

Nicotine. The dose: response curves to nicotine were constructed in a manner similar to those to 5-HT, because tachyphylaxis was often observed with repeated doses. Nicotine gave a motor response within the dose range used (1·0 to 32·0 μg/ml). In a few experiments nicotine gave either no response or a relaxation.

Histamine was used in concentrations of 1 μg to 5 mg/ml, all of which were inactive.

Hyoscine. After a treatment for 45 min with hyoscine (0·1 μg/ml) the dose: response curves to acetylcholine, 5-HT and nicotine were repeated (Fig. 1). Hyoscine blocked the responses to previously effective

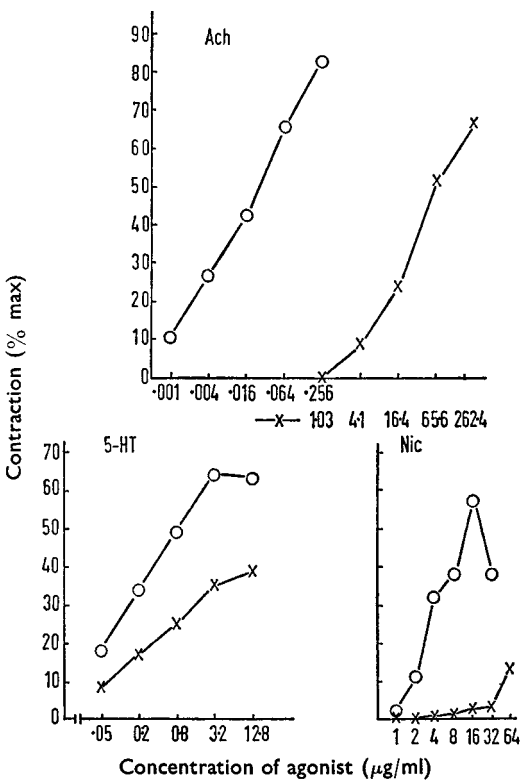

FIG. 1. The effect of hyoscine (0·1 μg/ml) on the dose: response curves to acetylcholine, 5-hydroxytryptamine and nicotine, on the distal part of the isolated colon of the rat. The responses to the agonists are plotted as per cent maximal contraction to acetylcholine against the log dose in μg/ml. Each curve represents the mean results from four experiments. The open circles show the responses to the agonists, the crosses show the responses in the presence of hyoscine. The previously effective doses of acetylcholine were blocked. The responses to 5-hydroxytryptamine were inhibited and those to nicotine nearly abolished.

doses of acetylcholine; higher concentrations of acetylcholine gave a dose: response curve parallel to the original curve (competitive blockade).

In the presence of the same concentration of hyoscine the responses to 5-HT and nicotine were opposed. The dose: response curve to 5-HT was shifted to the right, but the contractions due to nicotine were abolished except at the highest dose used.

Mipafox. Concentrations lower than 20 μg/ml of mipafox had no effect on the responses to acetylcholine. A concentration of 20 μg/ml of mipafox which slightly potentiated the contractions due to acetylcholine, also potentiated 5-HT and nicotine (Fig. 2). A higher concentration of mipafox (100 μg/ml) induced a sustained spasm of the tissue, so it became unworkable.

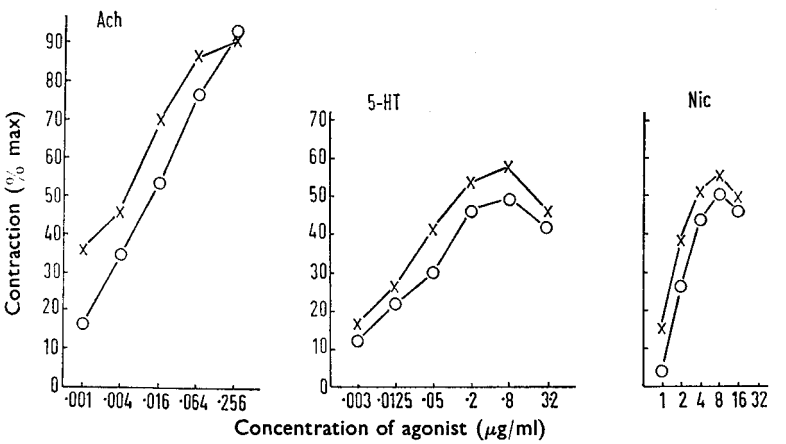

FIG. 2. The effect of mipafox (20 μg/ml) on the responses to acetylcholine, 5-hydroxytryptamine and nicotine, on the distal part of the isolated colon of the rat The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses after treatment with mipafox for 90 min. The dose: response curves to acetylcholine, 5-hydroxytryptamine and nicotine were shifted to the left and to the same extent. Each curve represents the mean of four experiments.

Cocaine. Fig. 3 shows the effect of cocaine, 5 and 20 μg/ml, on the dose: response curves to acetylcholine, 5-HT, and nicotine. Cocaine, 5 μg/ml, had no effect on the responses to acetylcholine and no significant effect on the dose: response curve to 5-HT, but it reduced the contractions due to nicotine to about one third of the maximal contraction to nicotine.

Cocaine, 20 μg/ml, shifted the dose: response curve to acetylcholine to the left (potentiation) and depressed the higher doses to 5-HT to about one half of its maximal response. This concentration of cocaine caused no further reduction of the responses to nicotine.

Procaine. Procaine, 10 μg/ml, displaced the dose: response curve to acetylcholine slightly to the right; it caused a pronounced depression of the responses to the higher doses of 5-HT and almost completely abolished the contractions due to nicotine (Fig. 4).

Hexamethonium. In concentrations of 20 and 60 μg/ml, hexamethonium caused no shift in the dose: response curves due to acetylcholine and 5-HT. With both concentrations the dose: response

curves to nicotine were shifted to the right; 60 μg/ml of hexamethonium was more effective than 20 μg/ml (Fig. 5).

Mecamylamine. Mecamylamine, 5 and 10 μg/ml, did not affect the dose: response curves to acetylcholine, while the low concentration

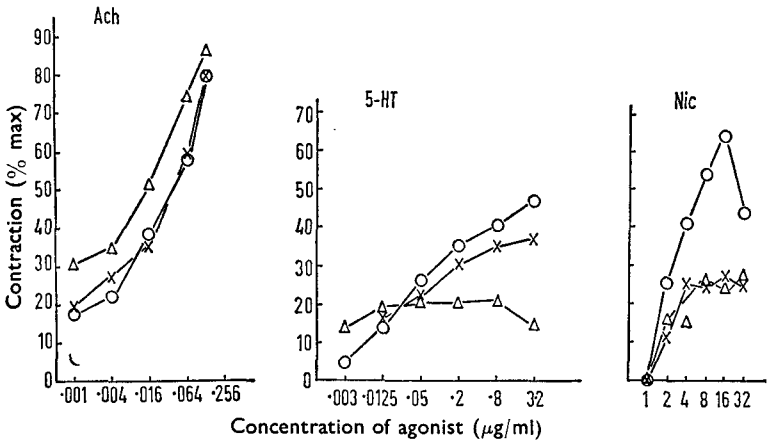

FIG. 3. The effect of cocaine (5 and 20 μg/ml) on the responses to the three agonists. The ordinates and abscissae are as in Fig. 1. The open circles show the responses to the agonists, the crosses represent these responses in the presence of cocaine 5 μg/ml, and the triangles the responses in the presence of cocaine 20 μg/ml. The low concentration of cocaine had no significant effect on the dose: response curves to acetylcholine and 5-hydroxytryptamine, but it inhibited the responses to nicotine. 20 μg/ml of cocaine had no further effect on the nicotine contractions, but it inhibited the upper part of the dose: response curve to 5-hydroxytryptamine and displaced the dose: response curve to acetylcholine to the left. Each curve represents the mean of six experiments.

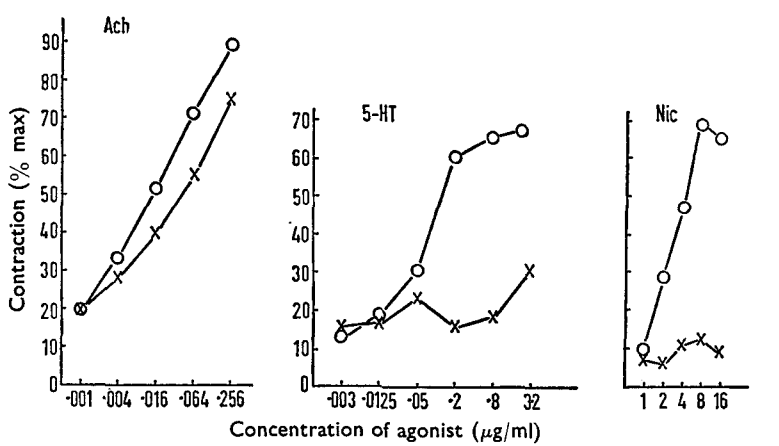

FIG. 4. The effect of treating the distal part of the isolated colon of the rat with procaine (10 μg/ml). The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses show these responses in the presence of procaine. The dose: response curve to acetylcholine was displaced slightly to the right, while the responses to nicotine and the higher doses of 5-hydroxytryptamine were inhibited. Each curve represents the mean of five experiments.

abolished the responses to nicotine. Both concentrations depressed the dose: response curve to 5-HT due to concentrations higher than 0·05 μg/ml to about one half of its maximal response (Fig. 6). In one experiment mecamylamine, 5 μg/ml, failed to reduce the contractions due to 5-HT.

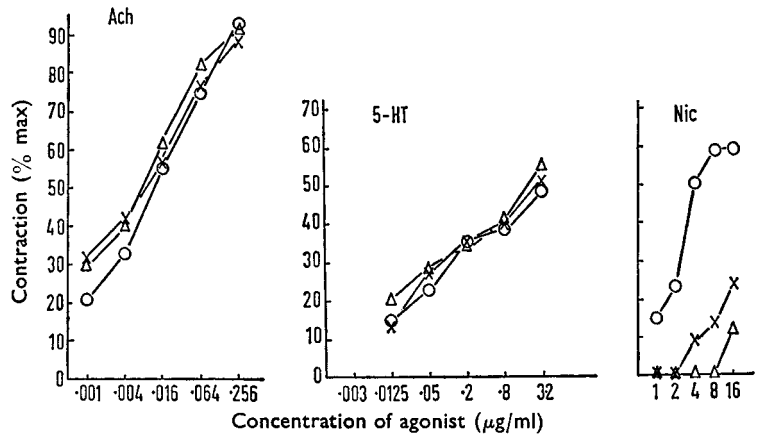

FIG. 5. The effect of hexamethonium (20 and 60 μg/ml) on the responses to the agonists. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses of the agonists, the crosses show these responses in the presence of hexamethonium 20 μg/ml and the triangles the responses in the presence of hexamethonium 60 μg/ml. Neither concentration of hexamethonium affected the dose: response curves to acetylcholine and 5-hydroxytryptamine, but inhibited the responses to nicotine; 60 μg/ml of hexamethonium was more effective than 20 μg/ml on the dose: response curve to nicotine. Each curve represents the mean of eight experiments.

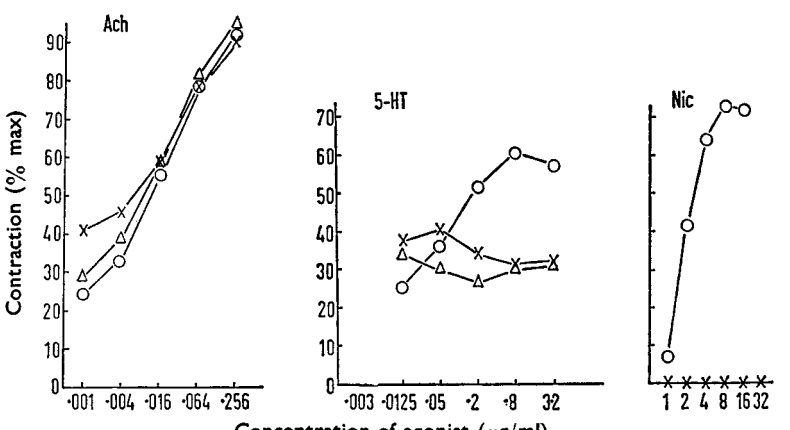

FIG. 6. The effect of mecamylamine (5 and 10 μg/ml) on the responses to the three agonists. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists, the crosses represent these responses in the presence of mecamylamine 5 μg/ml, and the triangles the same responses in the presence of mecamylamine 10 μg/ml. The dose: response curves to acetylcholine were not affected. Both concentrations depressed the upper part of the dose: response curve to 5-hydroxytryptamine, while 5 μg/ml of mecamylamine abolished the responses to nicotine. Each curve represents the mean of four experiments.

Dimethylphenylpiperazinium. After a concentration of 5 μg/ml of dimethylphenylpiperazinium, the dose: response curve to acetylcholine was little changed or slightly moved to the right, and all responses due to nicotine were reduced. This concentration of dimethylphenylpiperazinium depressed only the contractions caused by the higher doses of 5-HT to about one half of the maximal response to the amine (Fig. 7).

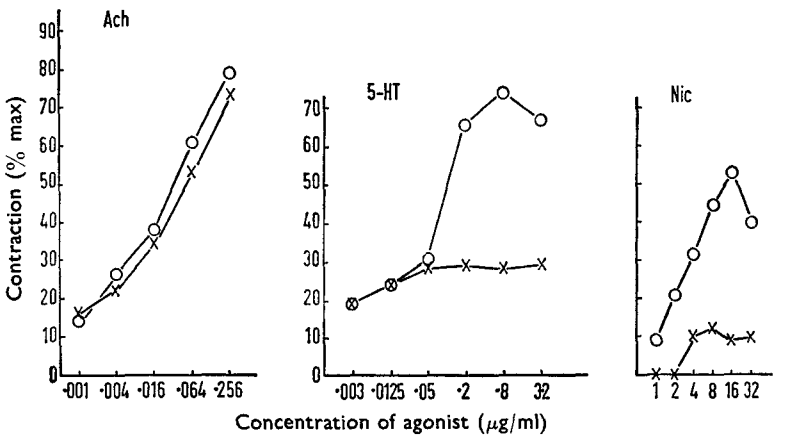

FIG. 7. The effect of dimethylphenylpiperazinium (5 μg/ml) on the responses to acetylcholine, 5-hydroxytryptamine and nicotine. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists, the crosses represent these responses in the presence of dimethylphenylpiperazinium. The dose: response curve to acetylcholine was slightly shifted to the right, all the responses due to nicotine and the contractions caused by higher doses of 5-hydroxytryptamine were depressed. Each curve represents the mean of four experiments.

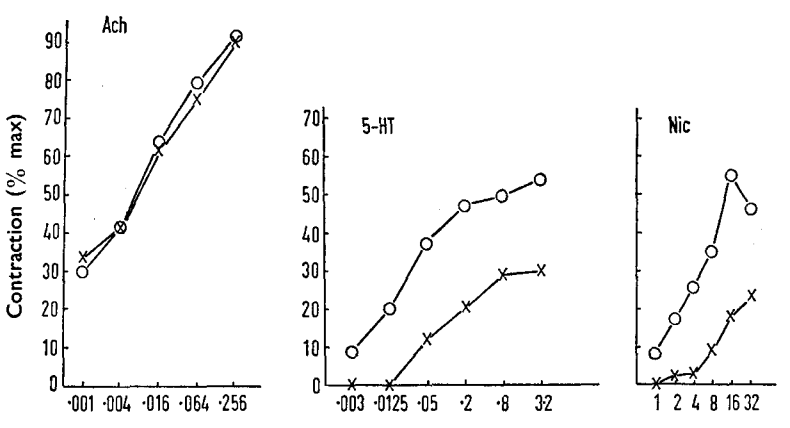

FIG. 8. The effect of 2-bromolysergic acid diethylamide (0·2 μg/ml) on the responses to acetylcholine, 5-hydroxytryptamine and nicotine. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the three agonists, the crosses show these responses in the presence of 2-bromolysergic acid diethylamide. The dose: response curve to acetylcholine was not affected, but the contractions due to 5-hydroxytryptamine and nicotine were inhibited to the same extent. Each curve represents the mean of five experiments.

2-Bromolysergic acid diethylamide. A concentration of 0·2 μg/ml of 2-bromolysergic acid diethylamide did not abolish the contractions due to acetylcholine, but shifted the dose: response curves to 5-HT and nicotine to the right. A higher concentration (0·5 μg/ml) of bromolysergic acid diethylamide did not block the responses to 5-HT completely, but the interpretation of these results was complicated by an increase in tone caused by the antagonist.

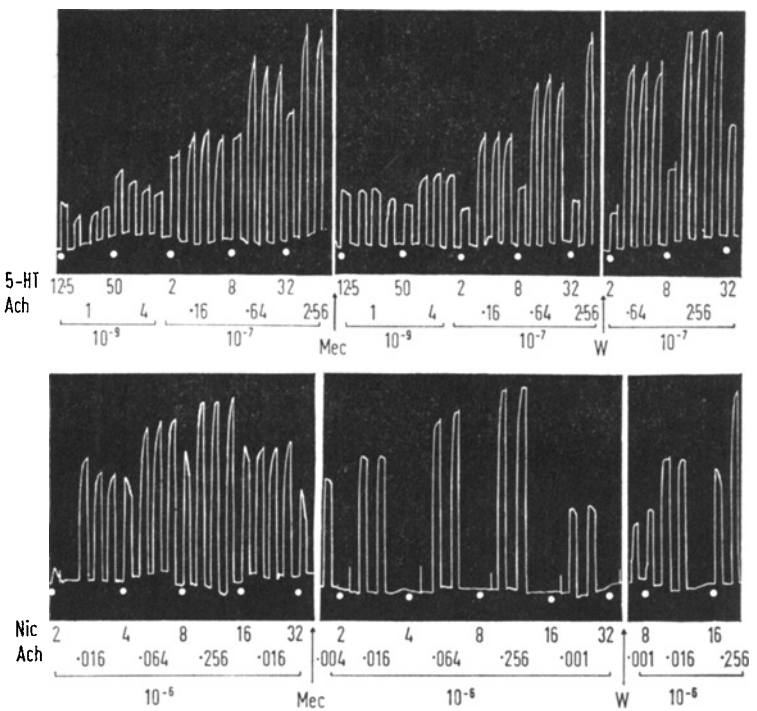

FIG. 9. The upper records show dose: response curves to 5-hydroxytryptamine and acetylcholine. Each dose of 5-hydroxytryptamine was spaced with three doses of acetylcholine. The responses due to 5-hydroxytryptamine are represented by the white dots. At Mec, mecamylamine (10 μg/ml) was added to the bath, and after a contact time of 45 min the dose: response curves were repeated in the presence of mecamylamine. At W the preparation was washed for an hour, and the dose: response curves were established again. The lower records show a similar experiment with nicotine in which mecamylamine (5 μg/ml) was added at Mec. The responses to nicotine are shown by the white dots.

Discussion

These experiments are concerned with a quantitative agonist: antagonist analysis of the site of action of 5-HT on the isolated colon of the rat. The agonists acetylcholine, 5-HT and nicotine all produced contraction of the preparation, while histamine even at concentrations as high as 5 mg/ml had no effect, suggesting the absence of histamine receptors in the tissue.

In the presence of a concentration of hyoscine which abolished the responses to acetylcholine and nearly blocked the effect of nicotine the contractions due to 5-HT were reduced to about half of the original level.

From these results it may be suggested that 5-HT contracts the tissue partly by an action involving a cholinergic mechanism—either an action on the muscarinic acetylcholine receptors or, like nicotine and dimethylphenylpiperazinium, by release of acetylcholine through the nerve pathway, and also partly by an action directly on the smooth muscle.

In the guinea-pig ileum the action of 5-HT is abolished by muscarinic blocking agents, as shown by Rocha e Silva & others (1953) and Brownlee & Johnson (1963). On the terminal part of the guinea-pig ileum Cambridge & Holgate (1955) and Gaddum & Picarelli (1957) found that atropine blocked only 50% of the responses to 5-HT. In other tissues the muscarinic blocking agents had no effect on the action of 5-HT; this was reported also by Vane (1957) on the fundal strip of the rat stomach and by Sleisenger, Law, Smith, Pert & Lewis (1955) on the distal colon of the dog *in vivo*.

The fact that the anticholinesterase mipafox potentiated the contractions due to acetylcholine, 5-HT or nicotine and to the same extent, provided further evidence of the involvement of a cholinergic link.

The accepted challenge for the presence of a nervous involvement is the effect of cooling to 20°, the use of cocaine or procaine, the use of botulinum toxin, the reduction in calcium ions or the increase of magnesium ions (Harry, 1962). Each of these has the effect of reducing the amount of acetylcholine set free at postganglionic nerve terminals. The one chosen here was the use of cocaine or procaine.

The results obtained with the local anaesthetic drugs, procaine and cocaine, site part of the action of 5-HT on the cholinergic nerve pathway and part of the action as a direct stimulation of muscle fibres. A concentration of procaine which abolished the contractions due to nicotine caused a reduction of the responses to the higher doses of 5-HT, but did not affect the contractions to the smaller doses. A reduction similar to that produced by procaine of the higher doses of 5-HT was obtained with the high concentration of cocaine. The specificity of these results may be questioned, because cocaine in both concentrations failed to block the contractions due to nicotine completely and the high concentration of cocaine potentiated the responses to acetylcholine. Trendelenburg (1962) analysed this potentiation of acetylcholine by cocaine on the nictitating membrane and found that cocaine potentiated only the lower part of the dose: response curve to acetylcholine. Moreover, Trendelenburg (1956) showed that cocaine potentiated the direct action of 5-HT on the nictitating membrane and abolished the stimulating effect on the superior cervical ganglion.

Whether the indirect action of 5-HT involved a ganglionic synapse was challenged by the use of ganglionic blocking agents. Hexamethonium, in producing a competitive blockade of the ganglia, did not antagonise 5-HT or acetylcholine in concentrations which antagonised nicotine. These results excluded the possibility of a preganglionic action of 5-HT.

Similar results were obtained by Feldberg (1951), Kosterlitz & Robinson (1958), and Brownlee & Johnson (1963).

Dimethylphenylpiperazinium, 5 μg/ml, which in a high concentration is considered to block ganglia by depolarisation (Ling, 1959), shifted the dose: response curve to acetylcholine slightly to the right, but produced a pronounced reduction to the response of nicotine. This concentration of dimethylphenylpiperazinium depressed only the responses to the higher doses of 5-HT to about one half of their maximal contraction.

Mecamylamine which produces an action intracellularly (Bennet, Tyler & Zaimis, 1957) reduces the responses to 5-HT to an extent similar to those of procaine, cocaine, and dimethylphenylpiperazinium in concentrations which blocked nicotine.

The persistence of contractions due to 5-HT in the presence of hexamethonium also excluded an action on the nicotinic ganglionic receptors, while the results obtained with dimethylphenylpiperazinium and mecamylamine site the indirect cholinergic part of the action of 5-HT on or in autonomic ganglia. These results are similar to those obtained by Brownlee & Johnson (1963), who sited the action of 5-HT in the guinea-pig ileum on the intramural parasympathetic ganglia. Furthermore, Trendelenburg (1956) gave evidence of specific tryptamine receptors in the cat superior cervical ganglion; these receptors were not blocked by hexamethonium. Similar results were reported by Bindler & Gyermek (1961) in the cat inferior mesenteric ganglion. Moreover, the results, together with the evidence of hyoscine and the local anaesthetic drugs, show that small doses of 5-HT acted directly on 5-HT receptors in the muscle while large doses acted indirectly.

There is a certain similarity between these results and those obtained by Gaddum & Picarelli (1957) on the terminal part of the guinea-pig ileum. They concluded that 5-HT activated two different receptors, one sited in the intramural nervous system (M-receptors) which was blocked by morphine, the other one sited on the intestinal muscle (D-receptors) which could be blocked by phenoxybenzamine and lysergic acid diethylamide.

Gaddum & Hameed (1954) showed that lysergic acid diethylamide was a specific antagonist of 5-HT on the rabbit ear and rat uterus. In the experiments described in this paper its bromo-derivative abolished the effects of small doses of 5-HT and reduced those of large doses; no conclusion can be drawn from this result because nicotine was simila rly affected by bromolysergic acid diethylamide.

Brownlee & Johnson (1963) found that 5-HT stimulated the guinea-pig ileum mainly by an indirect action, while Gaddum & Picarelli (1957) concluded that only 50% of the action of 5-HT was due to an indirect action on the terminal part of the guinea-pig ileum. The experiments described in this paper suggest that about 30% of the action of 5-HT is due to a direct action. Furthermore, Dalgliesh, Toh & Work (1953) and Feldberg & Toh (1953) have used the atropinised proximal part of the isolated rat colon for the assay of 5-HT in the tissue extracts showing that a direct action is involved in this preparation.

Brownlee & Johnson (1963) found that, with the guinea-pig ileum,

the dose: response line for 5-HT was steep. On the isolated colon of the rat the dose: response line was more shallow. This is consistent with the dual nature of the action involving both indirect and direct effects.

Acknowledgement. I would like to express my thanks to Professor G. Brownlee for his helpful criticism and advice throughout this work and in the preparation of this manuscript.

References

Bennet, G., Tyler, C. & Zaimis, E. (1957). *Lancet*, **2**, 218–222.
Bindler, E. H. & Gyermek, L. (1961). *Fed. Proc.*, **20**, 319.
Brownlee, G. & Johnson, E. S. (1963). *Brit. J. Pharmacol.*, **21**, 306–322.
Cambridge, G. W. & Holgate, J. A. (1955). *Ibid.*, **10**, 326–335.
Dalgliesh, C. E., Toh, C. C. & Work, T. S. (1953). *J. Physiol. (Lond.)*, **120**, 298–310.
Day, M. & Vane, J. R. (1963). *Brit. J. Pharmacol.*, **20**, 150–170.
Feldberg, W. (1951). *J. Physiol. (Lond.)*, **113**, 483–505.
Feldberg, W. & Toh, C. C. (1953). *Ibid.*, **119**, 352–362.
Gaddum, J. H. & Hameed, K. A. (1954). *Brit. J. Pharmacol.*, **9**, 240–248.
Gaddum, J. H. & Picarelli, Z. P. (1957). *Ibid.*, **12**, 323–328.
Harry, J. (1962). *Ibid.*, **19**, 42–55.
Kosterlitz, H. W. & Robinson, J. A. (1958). *Ibid.*, **13**, 296–303.
Ling, H. W. (1959). *Ibid.*, **14**, 505–511.
Paton, W. D. M. & Vane, J. R. (1963). *J. Physiol. (Lond.)*, **165**, 10–46.
Robertson, P. A. (1953). *Ibid.*, **121**, 54P.
Rocha e Silva, M., Valle, J. R. & Picarelli, Z. P. (1953). *Brit. J. Pharmacol.*, **8**, 378–388.
Sleisenger, M. H., Law, D. H., Smith, F. W., Pert, J. H. & Lewis, C. M. (1959). *J. clin. Invest.*, **38**, 2119–2130.
Trendelenburg, U. (1956). *Brit. J. Pharmacol.*, **11**, 74–80.
Trendelenburg, U. (1962). *J. Pharmacol.*, **135**, 39–44.
Vane, J. R. (1957). *Brit. J. Pharmacol.*, **12**, 344–349.