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# ACTIONS OF 5-HYDROXYTRYPTAMINE AND HISTAMINE ON THE NEURAL STRUCTURES AND MUSCULARIS MUCOSAE OF THE GUINEA-PIG OESOPHAGUS

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The striped external muscle of the isolated guinea-pig oesophagus is insensitive to drugs and the lumen does not seem to be patent; thus the preparation does not respond to drugs until they have penetrated the external muscle and stimulated the intramural nerves or the plain muscle of the muscularis mucosae. The innervation of the external muscle of the guinea-pig oesophagus is not blocked by hexamethonium and is apparently without ganglia, so a stimulant of ganglion-cells produces a contraction of the muscularis mucosae only, which is distinguishable from a contraction of the external muscle. Furthermore, the external muscle and the muscularis mucosae can be separated, so that the actions of drugs on the muscularis mucosae *in situ* can be compared with those on the separated muscularis mucosae (Bartlet, 1968a, b). Hence the structure of the guinea-pig oesophagus makes it a suitable preparation for analysing the actions of drugs on the intramural nervous plexuses and muscularis mucosae of the alimentary tract, and a description of the actions of 5-hydroxytryptamine (5-HT) and histamine on this preparation follows.

## METHODS

Preparations of muscularis mucosae, external muscle, oesophagus and oesophagus with vagus nerve attached were made as before (Bartlet, 1968a). The term " oesophagus " in the text, tables and figures refers to the whole organ, and the terms " external muscle " and " muscularis mucosae " refer to the separated oesophageal layers. The preparations were exposed to agonists for 1–3 min in every 15 min. Antagonists were added to the Krebs solution bathing the preparation when reproducible responses to an agonist had been obtained, only one agonist being tested in any such experiment with an antagonist. The effect of antagonists was expressed in terms of the dose ratio (Gaddum, Hameed, Hathway & Stephens, 1955), which is the ratio of equi-active doses of agonist in the presence and absence of antagonist. The inverse ratio was used to express the effect of drugs which potentiated agonists.

The following drugs were used: acetylcholine chloride, acetyl-$\beta$-methylcholine chloride, atropine sulphate, carbachol chloride, cocaine hydrochloride, eserine salicylate, hexamethonium bromide, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, hyoscine hydrobromide, mepyramine maleate, methysergide bimaleate, muscarine iodide and tubocurarine chloride. The muscarine iodide was kindly supplied by J. R. Geigy & Co., Basel, Switzerland. Quantities of the drugs in the text, tables and figures refer to the above salts.

## RESULTS

### *5-Hydroxytryptamine*

#### *Muscularis mucosae*

On most preparations of muscularis mucosae 5-HT, at a concentration of 0.1–0.5 μg/ml., produced a reproducible contraction for several hours. Cocaine (10 μg/ml.) potentiated 5-HT and acetylcholine to about the same extent (three experiments) (Fig. 1), the contractions returning to their original height when cocaine was washed out of the organ bath.

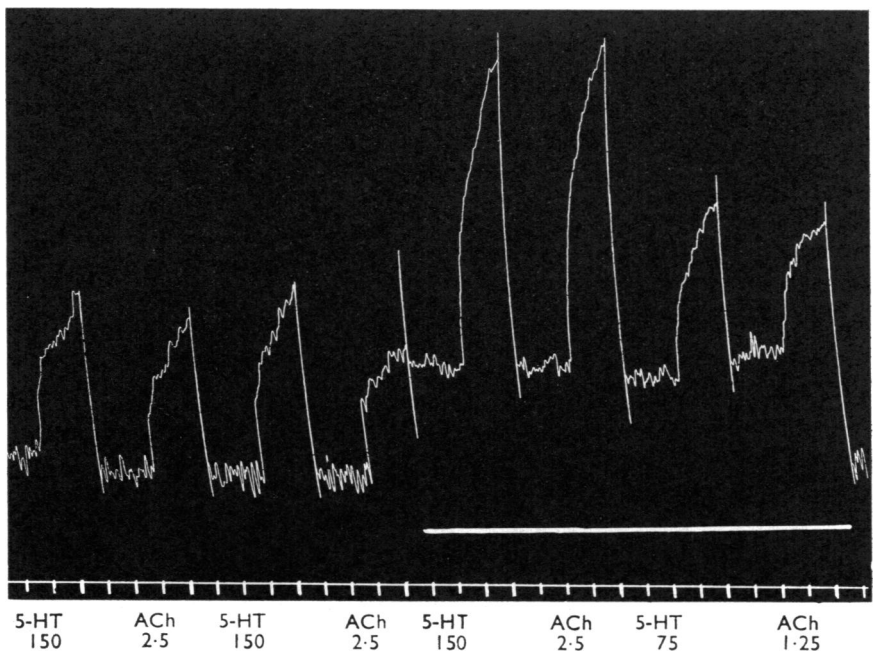


Fig. 1. Muscularis mucosae of guinea-pig oesophagus. Numerals refer to organ bath concentrations (ng/ml.) of acetylcholine (ACh) and 5-HT. The white line marks the presence of cocaine (10 μg/ml.). Time, 1 min.

The action of 5-HT on the muscularis mucosae was partially antagonized by methysergide and hyoscine. Thus in one experiment methysergide (10 ng/ml.) partially antagonized 5-HT, but the residual contraction was not further antagonized even when the concentration of methysergide was increased to 5 μg/ml. Hyoscine (10 ng/ml.) blocked the methysergide-resistant action of 5-HT. In a second experiment methysergide (10 ng/ml.) antagonized 5-HT to give a dose ratio of 6, but no further antagonism occurred when the concentration of methysergide was increased to 1 μg/ml. Hyoscine (10 ng/ml.) blocked the action of 5-HT on six preparations of muscularis mucosae but a dose ratio was not obtained because the antagonism was insurmountable.

Eserine (250 ng/ml.) potentiated the action of 5-HT on the muscularis mucosae by a factor of 3–6, but it did not potentiate contractions to carbachol (three experiments).

*External muscle*

In concentrations up to 250 μg/ml., 5-HT was without action on the external muscle.

*Oesophagus*

5-Hydroxytryptamine (0.025–0.25 μg/ml.) produced reproducible contractions of the oesophagus for several hours. The contractions were usually monophasic, but large doses of 5-HT sometimes produced biphasic contractions. Cocaine blocked 5-HT acting on the oesophagus. The antagonism of 5-HT by cocaine (1 μg/ml.) was slow in onset and insurmountable and cocaine (10 μg/ml.) promptly abolished the contraction to 5-HT (five experiments).

Hyoscine antagonized 5-HT acting on the oesophagus, but tubocurarine and hexamethonium did not (Table 1). In three experiments a dose ratio was obtained for 5-HT in the presence of hyoscine (1 ng/ml.) but the antagonism did not become steady (Table 1). In three further experiments the action of 5-HT was promptly abolished by hyoscine (10 ng/ml.) and the antagonism was insurmountable.

TABLE 1

ANTAGONISM OF 5-HT, HISTAMINE AND ACETYL-β-METHYLCHOLINE ON THE GUINEA-PIG OESOPHAGUS

Most of the dose ratios were measured when the antagonism became constant, but an asterisk denotes that it had not fully developed at the time of measurement, the time of exposure of the tissue to the antagonist being 75 min (*), 90 min (**) or 135 min (***). Where more than two observations have been made, results are quoted as means and standard errors with the number of observations in parenthesis.

| | | Dose ratio | | |
|---|---|---|---|---|
| Antagonist | Concentration (μg/ml.) | 5-HT | Histamine | Acetyl-β-methylcholine |
| Hexamethonium | 20 | 1·0 | 1·0 | 1·0 |
| *d*-Tubocurarine | 5 | 1·0 | 1·0 | 1·0 |
| Hyoscine | 0·001 | 12·5*, 10**, 20** | 1·5±0·4 (3) | 11·0± 3·3 (4) |
| | 0·01 | Abolished (3) | 1·4 | 115·0±49·8 (4) |
| | 0·1 | | 1·5±0·1 (4) | |
| | 1 | | 1·2, 1·3 | |
| | 10 | | 1·7±0·2 (4) | |
| Atropine | 0·1 | | 2·0 | |
| | 1 | | 1·8 | |
| Mepyramine | 0·001 | | 7·4±1·4 (7) | |
| | 0·01 | 1·0 | | 1·0 |
| Methysergide | 0·0001 | 5·0**, 13*** | | |
| | 0·01 | Insurmountable (6) | 1·0 | 1·0 |
| | 1 | | | 1·0 |

Eserine (25 ng/ml.) was tested on two preparations which were contracting to 5-HT and histamine. In both experiments eserine potentiated 5-HT by a factor of 3, but it did not potentiate histamine.

Methysergide specifically antagonized 5-HT (Table 1). This antagonism was insurmountable, but a residual action of 5-HT was not blocked by methysergide. In the presence of methysergide, 5-HT produced small biphasic contractions which were blocked by hyoscine. Methysergide (100 ng/ml.) did not antagonize the contractions produced by submaximal stimulation of a vagus nerve (two experiments).

*Histamine*

*Muscularis mucosae*

The muscularis mucosae contracted to histamine (0.2–1.0 μg/ml.) but steady responses were obtained only on preparations made from guinea-pigs with a body weight less than 350 g. In five experiments mepyramine (1 ng/ml.) antagonized histamine, the dose ratio being $3.2 \pm 0.9$. Hyoscine (1 μg/ml.) did not antagonize histamine acting on the muscularis mucosae (four experiments).

The effects of mepyramine and hyoscine on the actions of histamine and acetyl-β-methylcholine on the muscularis mucosae have been compared with those on the oesophagus (Table 2). The antagonism of histamine and acetyl-β-methylcholine by mepyramine and hyoscine respectively, tended to be weaker on the muscularis mucosae than on the oesophagus, but the differences between the preparations were not significant at the 5% level.

TABLE 2

EFFECT OF SEPARATING THE EXTERNA FROM THE MUCOSA ON THE ACTIONS OF MEPYRAMINE AND HYOSCINE

| | | Dose ratio | | |
|---|---|---|---|---|
| Antagonist | Agonist | Oesophagus | Muscularis mucosae | *P* |
| Mepyramine, 1 ng/ml. | Histamine | $7{\cdot}4 \pm 1{\cdot}4$ (7) | $3{\cdot}2 \pm 0{\cdot}9$ (5) | >0·05 |
| Mepyramine, 10 ng/ml. | Acetyl-β-methyl-choline | 1·0 | 1·0 | |
| Hyoscine, 1 ng/ml. | Acetyl-β-methyl-choline | $11{\cdot}0 \pm 3{\cdot}3$ (4) | $8{\cdot}3 \pm 1{\cdot}9$ (6) | >0·3 |
| Hyoscine, 100 ng/ml. | Histamine | $1{\cdot}5 \pm 0{\cdot}1$ (4) | 1·0 | <0·02 |

*External muscle*

Histamine (50–250 μg/ml.) produced a very small contraction on some preparations of external muscle but not on others. The action of acetylcholine was always potentiated after exposure to histamine. Although the contractions produced by these drugs were small, the addition of 175 mg of potassium chloride to the 40 ml. organ bath always produced a large contraction of the external muscle.

*Oesophagus*

Histamine (0.5–10 μg/ml.) produced a slow contraction of the oesophagus after a delay of 30 sec, relaxation of the preparation after removing histamine from the organ bath being very slow. In Fig. 2, drugs were added to the organ bath for 2 min; the kymograph was then stopped for 5 min while the Krebs solution in the organ bath was changed three times. The oesophagus was not relaxed 1 hr after washing out histamine although it relaxed within 5 min of washing out 5-HT or acetyl-β-methylcholine.

The relaxation of the oesophagus after histamine was particularly slow when histamine was alternated with doses of acetylcholine, but when histamine was the only agonist tested the oesophagus relaxed fast enough to allow tests at 15 min intervals. A few preparations of oesophagus were very insensitive to acetylcholine, but in these experiments the action of acetylcholine was greatly enhanced after testing histamine.

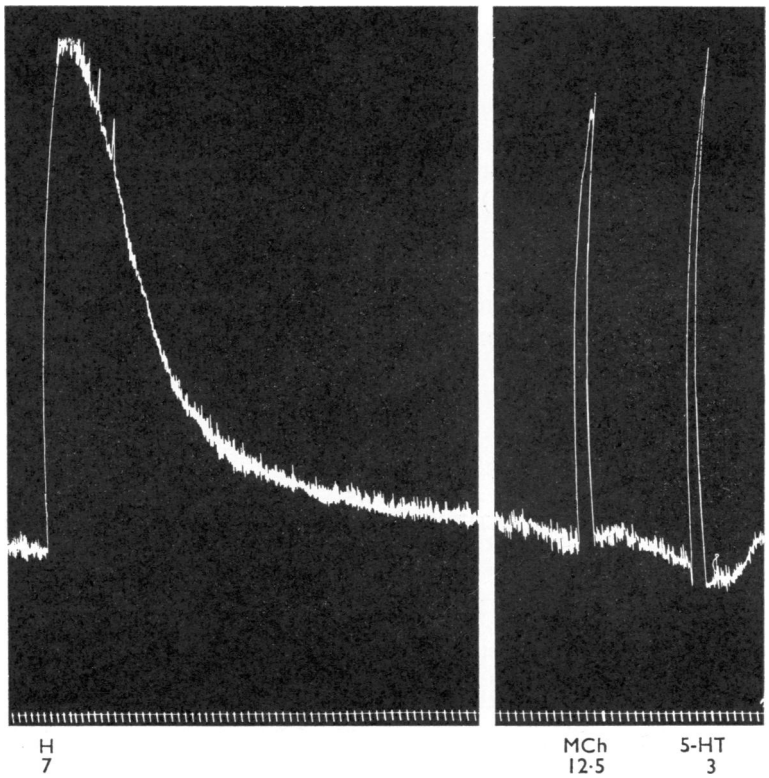


Fig. 2. Longitudinal contractions of guinea-pig oesophagus. Numerals refer to organ bath concentrations ($\mu$g/ml.) of histamine (H), acetyl-$\beta$-methylcholine (MCh) and 5-HT. Time, 1 min.

Contractions to histamine sometimes became progressively smaller. The tachyphylaxis was related to the age of the guinea-pig, because it only occurred when preparations were made from large animals. Preparations from guinea-pigs with a body weight less than 350 g gave reproducible contractions to histamine for several hours, and were always selected for experiments with this drug.

Hyoscine and atropine antagonized histamine acting on the oesophagus, but tubocurarine and hexamethonium did not (Table 1). The dose ratio for histamine did not increase as the concentration of hyoscine was raised from 1 ng/ml. to 10 $\mu$g/ml. The dose ratios for histamine in the presence of hyoscine ranged from 1.2–2.0, which means that 17–50% of the action of histamine was antagonized. The dose ratio for acetyl-$\beta$-methylcholine, however, increased with the concentration of hyoscine (Table 1).

Cocaine potentiated histamine. In each of three experiments cocaine (10 $\mu$g/ml.) potentiated histamine by a factor of 1.25; and hyoscine (1 $\mu$g/ml.) failed to antagonize histamine in the presence of cocaine. Cocaine potentiated acetyl-$\beta$-methylcholine more than histamine; in four experiments cocaine (10 $\mu$g/ml.) potentiated acetyl-$\beta$-methylcholine by a factor of $1.9 \pm 0.2$. Hyoscine antagonized acetyl-$\beta$-methylcholine in the presence of cocaine.

Eserine (250 ng/ml.) was tested on four preparations which were contracting to histamine and muscarine. In one experiment eserine potentiated histamine 2–4 times (Fig. 3), and in the other experiments histamine was potentiated by a factor of 2 although the contractions to muscarine were not potentiated.

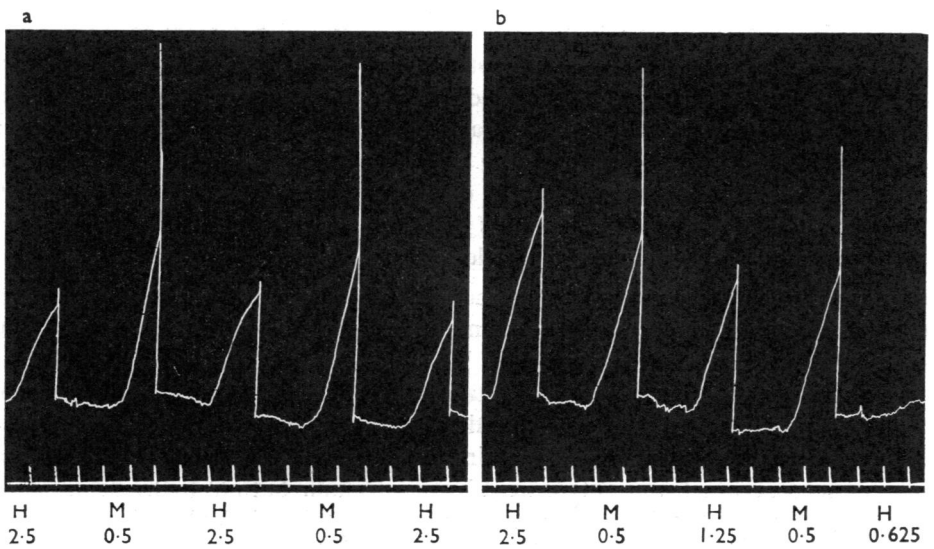


Fig. 3. Longitudinal contractions of guinea-pig oesophagus. Numerals refer to organ bath concentrations (μg/ml.) of histamine (H) and muscarine (M). In (b) eserine (25 ng/ml.) was present. Time, 1 min.

Mepyramine (10 ng/ml.) was a specific antagonist of histamine on the guinea-pig oesophagus (Table 1). Both the hyoscine-sensitive and the hyoscine-resistant actions of histamine were antagonized by mepyramine, for in seven experiments the mean dose ratio for histamine in the presence of mepyramine (1 ng/ml.) was 7.4 with a standard error of 1.4. Mepyramine (100 ng/ml.) did not antagonize the contractions produced by submaximal stimulation of a vagus nerve (two experiments).

## DISCUSSION

The separated externa of the guinea-pig oesophagus did not contract to 5-HT, which shows that the striped external muscle is insensitive to this drug. However, 5-HT produced a contraction of the separated muscularis mucosae which was antagonized by hyoscine and potentiated by eserine, suggesting that 5-HT stimulated the plain muscle indirectly through the action of an acetylcholine-like substance, but because cocaine potentiated 5-HT acting on the muscularis mucosae, it is unlikely that 5-HT was stimulating a neural structure. Methysergide partially antagonized 5-HT acting on the muscularis mucosae of the guinea-pig oesophagus, so that 5-HT seemed to be combining with both methysergide-sensitive and methysergide-resistant receptors. Atropine did not antagonize 5-HT acting on the muscularis mucosae of the chicken oesophagus (Bartlet & Hassan, 1968), so the action of 5-HT on the muscularis mucosae is not the same in all species.

Tubocurarine did not antagonize 5-HT acting on the guinea-pig oesophagus; thus there was no evidence that 5-HT indirectly stimulated the striped external muscle. The contraction of the oesophagus to 5-HT was potentiated by eserine and abolished by cocaine or hyoscine, suggesting that 5-HT stimulated innervation linked with the muscularis mucosae. Hexamethonium did not antagonize the contraction of the oesophagus to 5-HT, however, so that the 5-HT stimulation did not seem to involve a pre-ganglionic structure. Methysergide did not antagonize the response of the oesophagus to vagal stimulation although it antagonized the neural action of 5-HT; thus the neural receptors which were stimulated by 5-HT and blocked by methysergide were associated with a nervous pathway not excited during vagal stimulation. The absence of a contribution of striped muscle to the contraction of the oesophagus to 5-HT is in accord with the view that 5-HT stimulated ganglia, as the innervation of the longitudinal external muscle is apparently without ganglia (Bartlet, 1968a).

Histamine produced a very small contraction of the separated externa in some experiments only, showing that the striped external muscle is insensitive to this drug. After exposure of the preparation to histamine, however, the action of acetylcholine on the externa was always potentiated. It is uncertain whether acetylcholine stimulates receptors on the striped muscle or its innervation (Bartlet, 1968b), so the mode of action of histamine on the externa remains obscure. Histamine produced a contraction of the separated muscularis mucosae which was antagonized by mepyramine but not by hyoscine, showing that histamine directly stimulates the plain muscle. The tachyphylaxis to histamine which occurred on preparations of muscularis mucosae made from large guinea-pigs was also observed in experiments with the chicken oesophagus and has been discussed before (Bartlet & Hassan, 1968).

The action of histamine on the guinea-pig oesophagus was partially antagonized by hyoscine and potentiated by eserine, which implies that it stimulated receptors associated with the release of acetylcholine. This may have been an action on a neural structure because hyoscine failed to antagonize histamine in the presence of cocaine. This suggests that cocaine blocked the hyoscine-sensitive action of histamine, the overall potentiation of histamine by cocaine being caused by a potentiation of its hyoscine-resistant action. The neural action of histamine is more prevalent on the chicken oesophagus than on the guinea-pig oesophagus (Bartlet & Hassan, 1968). Histamine seems to stimulate neural structures throughout the alimentary tract of the guinea-pig (Busse, Wolf & Lendle, 1953; Harry, 1963; Paton & Vane, 1963), however, although the location of the receptors is uncertain. In the present experiments hexamethonium and tubocurarine did not antagonize histamine acting on the oesophagus; thus histamine did not seem to have a pre-ganglionic action or to produce a contraction by acting indirectly on the striped external muscle. Mepyramine did not antagonize the response of the oesophagus to vagal stimulation, although it blocked the neural action of histamine; thus the neural histamine receptors were associated with an afferent nervous pathway or an efferent pathway which was not activated during vagal stimulation. None of the evidence is incompatible with an action of histamine on the intramural ganglia similar to that on the superior cervical ganglion of the cat (Trendelenburg, 1957).

Although 5-HT and histamine acting on the oesophagus produced contractions of the muscularis mucosae *in situ*, these contractions were pharmacologically dissimilar

to those produced by the same drugs on the separated muscularis mucosae. Two phenomena seem to be involved in the differences between the actions of drugs on the separated muscularis mucosae and the muscularis mucosae *in situ*. First, the absence of neural actions on the separated muscularis mucosae—for example, the cocaine-sensitive action of 5-HT, the hyoscine-sensitive action of histamine and the cocaine-sensitive action of acetylcholine (Bartlet, 1968b) were shown on the muscularis mucosae *in situ* but not on the separated muscularis mucosae. The most likely explanation for this phenomenon is that the neural structures responding to the drugs were severed or damaged in separating the externa from the mucosa. Second, certain actions of drugs were shown on the separated muscularis mucosae but not on the muscularis mucosae *in situ*—for example, cocaine-resistant contractions were produced by 5-HT and acetylcholine (Bartlet, 1968b) on the separated muscularis mucosae only. 5-HT and acetylcholine did not seem to penetrate to their receptors in the muscularis mucosae *in situ*, presumably because of inactivation during diffusion through the tissues of the oesophagus. The rapid relaxation of the oesophagus after washing 5-HT or acetylcholine from the organ bath supports this assumption, as does the presence of cholinesterase in the guinea-pig oesophagus (Gerebtzoff & Bertrand, 1957). In contrast, the oesophagus was slow to relax after exposure to histamine, which had a direct action on the muscularis mucosae *in situ*, which indicates that its clearance from the guinea-pig oesophagus was slow. The potentiation of acetylcholine after exposure of the oesophagus or external muscle to histamine may also be due to the slow clearance of histamine from the preparations.

SUMMARY

1. 5-Hydroxytryptamine produced a contraction of the separated muscularis mucosae of the guinea-pig oesophagus which was antagonized by methysergide or hyoscine and potentiated by eserine or cocaine.

2. 5-Hydroxytryptamine produced a contraction of the whole oesophagus which was dissimilar to the contraction of the muscularis mucosae in being blocked by cocaine.

3. Histamine produced a contraction of the separated muscularis mucosae which was antagonized by mepyramine but not by hyoscine.

4. Histamine had a dual action on the oesophagus, one action being blocked by mepyramine only, and the other by mepyramine or hyoscine or cocaine.

5. Histamine potentiated the action of acetylcholine on the external muscle and oesophagus.

6. Reproducible contractions to histamine were obtained with preparations of oesophagus and muscularis mucosae from small guinea-pigs, but preparations from large animals showed tachyphylaxis.

7. The response of the oesophagus to vagal stimulation was not antagonized by mepyramine or methysergide.

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