Br. J. Pharmac. (1972), **44**, 100–108.

Effects of 5-hydroxytryptamine on isolated strips of the guinea-pig stomach

T. YAMAGUCHI

Department of Physiology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Summary

1. The effects of 5-hydroxytryptamine (5-HT) on isolated strips of the longitudinal and circular muscles of the guinea-pig stomach were investigated.

2. 5-HT (0·1–1 μg/ml) increased the resting tension of the longitudinal muscle while it decreased that of the circular muscle. These effects were blocked by lysergic acid diethylamide (LSD), but were not affected by tetrodotoxin, hyoscine or morphine.

3. Electrical stimulation caused contraction in the longitudinal muscle, and contraction followed by relaxation in the circular muscle. In both the longitudinal and circular muscles, the evoked contractions were potentiated by 5-HT. This effect was blocked by tetrodotoxin, hyoscine and morphine, but was not affected by LSD.

4. It is concluded that, in the stomach as well as the intestine of the guinea-pig, there are two kinds of 5-HT receptors: the morphine-sensitive M receptor is situated on the intramural nerves and the D-receptor on the smooth muscle cells.

Introduction

There are two kinds of tryptamine receptors in the guinea-pig ileum: the M receptors, which can be blocked with morphine, are present in nervous structures and the D receptors, which can be blocked with dibenzyline or its analogues, are in the smooth muscle fibres (Gaddum & Picarelli, 1957 ; Gaddum, 1958).

The rat stomach probably contains almost entirely D receptors (Vane, 1957), because it is rather insensitive to the inhibiting action of morphine. On the other hand, the guinea-pig stomach may contain mainly M receptors, because the effects of 5-hydroxytryptamine (5-HT) (0·1–1 μg/ml) on intraluminal pressure are blocked by tetrodotoxin (Gershon, 1967 ; Bülbring & Gershon, 1967). In the presence of tetrodotoxin, only a high concentration of 5-HT (10–100 μg/ml) produces a slight contraction, followed by a slight relaxation (Paton & Vane, 1963 ; Gershon, 1967).

In order to investigate further the action of 5-HT, strips of the longitudinal and circular muscles were dissected from the guinea-pig stomach, as described for the preparations of the rat stomach by Vane (1957). The effects of 5-HT were studied on the resting tension and on response to electrical field stimulation. With these methods, it was demonstrated that the smooth muscles of the guinea-pig stomach contain the D receptor as well as the M receptor.

Methods

Guinea-pigs of either sex were stunned and bled. The stomachs were taken out and their contents were removed by an incision along the lesser curvature. Three different strips of preparation were made. The first group of preparations was made by cutting along the longitudinal muscle fibres. The second group was dissected along the circular muscle fibres at right angle to the longitudinal muscle fibres. The third group contained mainly the sphincter antri. Muscle fibres of the sphincter run in parallel with those of the circular muscle, but they can be distinguished by their thickness and whitish colour.

The mucosal layer was removed from the preparations under a stereomicroscope. All strips had similar dimensions, that is about 25 mm long and 2 mm wide. The preparation was suspended in an organ-bath which contained 25 ml Krebs solution, bubbled with 97% O_2 and 3% CO_2, and kept at 32° C.

The preparations were stimulated between two large Ag-AgCl electrodes placed at opposite ends of the organ bath. Stimulation with a single pulse (1–10 ms) and repetitive pulses (0·5–2 ms, 10 Hz) with constant stimulus strength, was applied alternately every 2–3 minutes. The tension was measured isometrically with a strain gauge and displayed on a potentiometric pen recorder.

Drugs used were 5-hydroxytryptamine creatine sulphate (5-HT), hyoscine hydrobromide, tetrodotoxin citrate, morphine sulphate, and lysergic acid diethylamide (LSD). All drug concentrations refer to the salts and are given in μg/ml.

Results

Effects of 5-HT

Longitudinal muscle

At the beginning of the experiment, a strip of longitudinal muscle was stretched to give about 0·1 g of resting tension. Usually, during the first 30 min, the resting tension increased gradually to various degrees in different preparations (up to 0·5 g), and spontaneous rhythmic contractions developed. The size of spontaneous contractions was different from preparation to preparation (Fig. 1); it was usually irregular and varied between 0·01 and 0·3 g tension. The frequency of contractions (three–six/min) was rather constant in any particular preparation throughout the experiment.

Stimulations with a single pulse (1–10 ms duration) and with repetitive pulses (0·5–2 ms duration, 10 Hz for 10 s) caused contractions. However, the size of contraction varied greatly from preparation to preparation.

5-HT (0·1–1 μg/ml) caused an increase in the resting tension (0·1–1 g in different preparations), but this was not maintained even in the continuous presence of 5-HT but gradually declined to the original level within 3–10 min (Fig. 1). In some preparations, the spontaneous rhythmic contractions were augmented during the tension increase caused by 5-HT (Fig. 1a). However, in other preparations, the spontaneous activity was not affected either in size or in frequency.

The responses to single and repetitive stimuli were greatly potentiated by 5-HT (Fig. 1). This potentiation lasted much longer than the increase in resting tension, and it took more than 30 min to return to control values after washing out 5-HT.

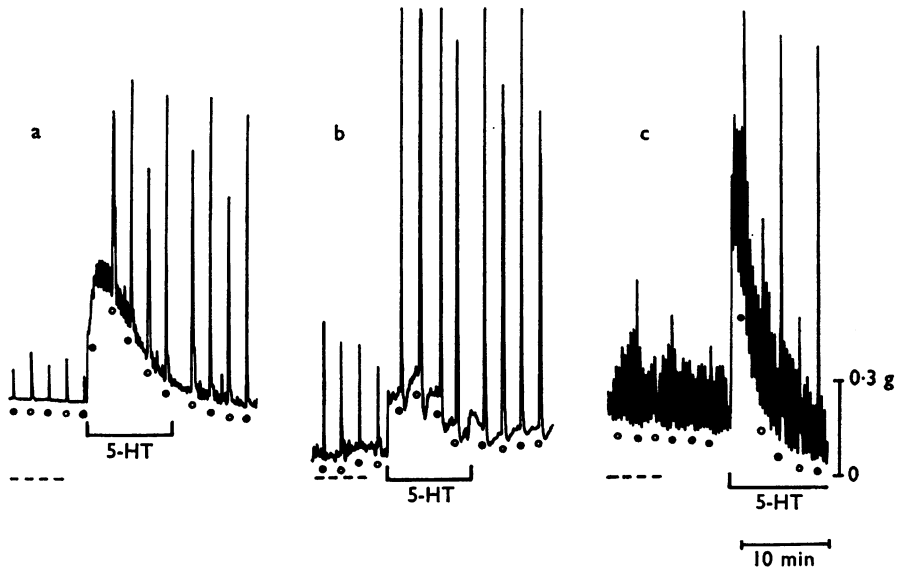

FIG. 1. Effects of 5-HT (1 μg/ml) on the longitudinal muscle of preparations of the guinea-pig stomach which had different patterns of mechanical activity. (– – –), Zero tension level. (●), Single pulse stimulation (pulse duration: 3 ms in (a), 2 ms in (b), 5 ms in (c). (○), Repetitive stimulation 10 Hz for 10 s (pulse duration: 1 ms in (a) and (b), 2 ms in (c). 5-HT was injected into the organ bath and its concentration remained constant until washing was started.

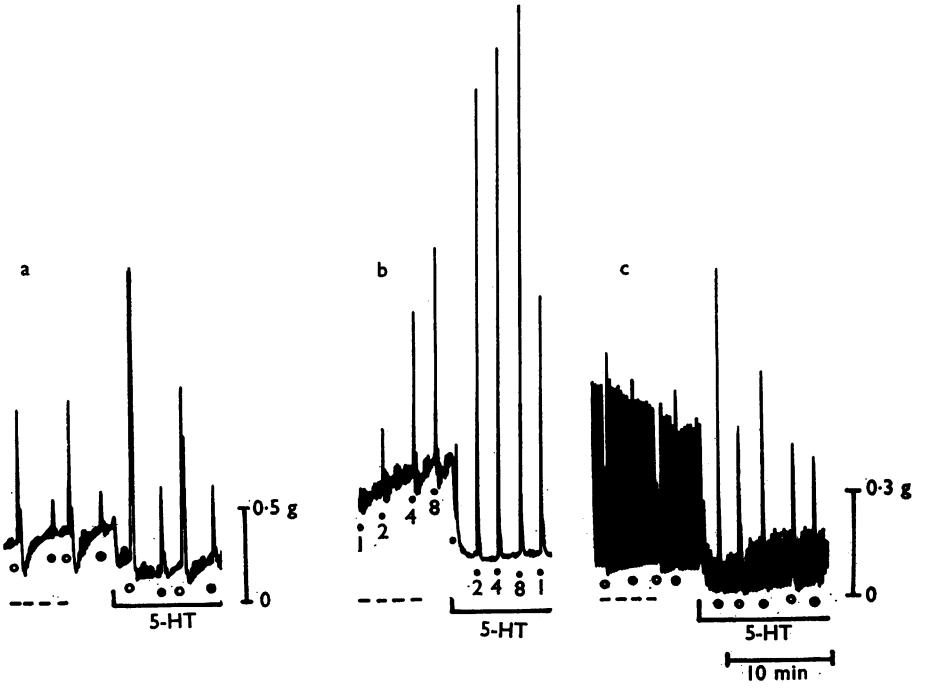

FIG. 2. Effects of 5-HT (1 μg/ml) on circular muscle of preparations with different patterns of activity. (○), repetitive stimulation at 10 Hz for 10 s (pulse duration: 1 ms in (a), (c). (●), Single pulse stimulation (pulse duration: 5 ms in (a) 3 ms in (c), and as indicated in (b). (– – –), Zero tension level.

Circular muscle

Patterns of the spontaneous activity of circular muscle were similar to those of longitudinal muscles (Fig. 2). However, fluctuations of the resting tension were greater in the circular than in the longitudinal muscle. Stimulation with single pulses produced contractions but, with pulse duration of more than 5 ms, the contraction was usually followed by relaxation. Repetitive stimulation evoked one or two contractions followed by relaxation. There was no relationship between the size of the preceding contraction and the subsequent relaxation.

In most circular muscle preparations, 5-HT (1 μg/ml) caused relaxation, and no recovery was observed during exposure for 10 min to 5-HT (Fig. 2). The resting tension was reduced nearly to zero, so that the relaxation was larger in a tissue with higher resting tension. During the relaxation, the spontaneous rhythmical contractions were usually reduced in size, but the frequency remained the same. In some preparations, the spontaneous activity was abolished by 5-HT.

Contractions evoked by electrical stimulations were potentiated by 5-HT (Fig. 2), as in the longitudinal muscle. However, the relaxation following the contraction produced by electrical stimulation was reduced or abolished, although this effect may be secondary, due to the decrease in resting tension caused by 5-HT.

Sphincter antri

The sphincter antri had quite a different pattern of contractions. The resting tension was rather stable in this tissue, but in many preparations the size of spontaneous and evoked contractions fluctuated irregularly (Fig. 3). Therefore, the evoked response was sometimes difficult to differentiate from the spontaneous contractions.

5-HT suppressed the spontaneous contractions to a varying extent. It reduced the resting tension only in some preparations, even when the resting tension was first increased by acetylcholine (0·1 μg/ml). No clear effect of 5-HT on the responses to electrical stimulations was observed.

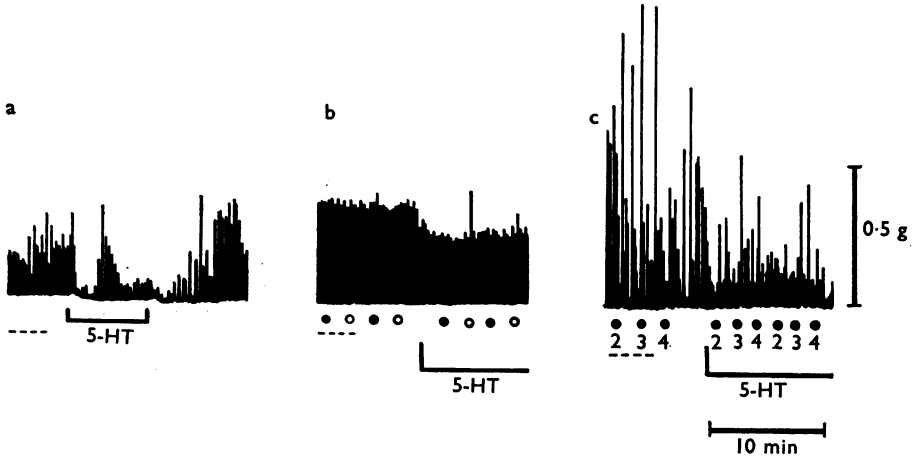

FIG. 3. Effects of 5-HT on sphincter antri. Three different preparations, (a), (b), (c). (– – –), Zero tension level. (●), Single pulse stimulation (5 ms in (b), as indicated in (c)). (○), Repetitive stimulation (10 Hz for 10 s; pulse duration: 1 ms).

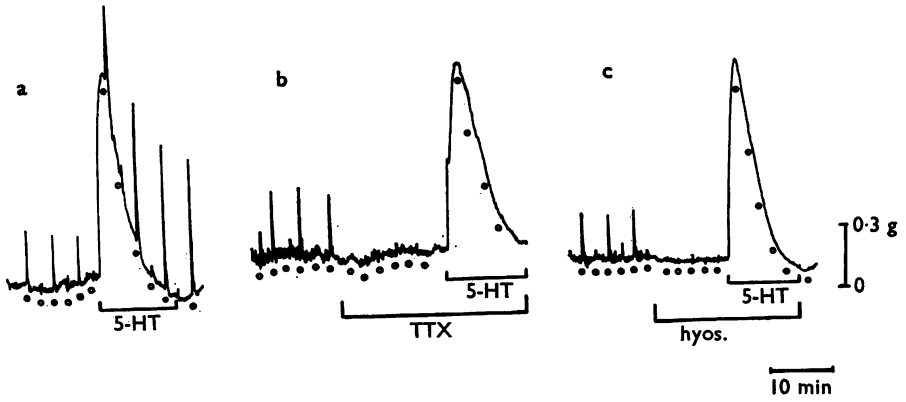

FIG. 4. Effects of tetrodotoxin (TTX) and hyoscine (hyos) on the contractions of the longitudinal muscle induced by 5-HT; one preparation. (●), Single pulse stimulation (5 ms duration). (○), Repetitive stimulation (pulses of 2 ms duration at 10 Hz for 10 s). (a), Effects of 5-HT (1 μg/ml). (b), TTX (0·1 μg/ml) blocked the responses to electrical stimulation, but not the contraction caused by 5-HT. (c), Hyoscine (1 μg/ml) had effects similar to TTX.

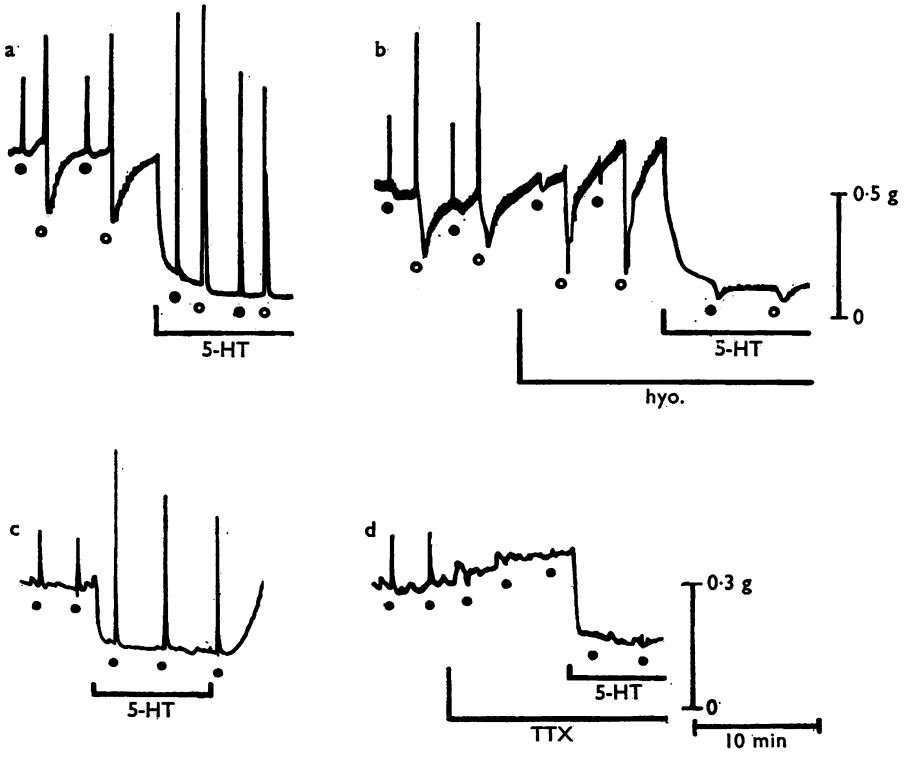

FIG. 5. Effects of hyoscine (hyo) and tetrodotoxin (TTX) on the action of 5-HT on circular muscles. (●), Single pulse stimulation (4 ms duration in (a), (b); 5 ms in (c), (d). (○), Repetitive stimulation (pulses of 0·7 ms duration at 10 Hz for 10 s). (a), 5-HT (1 μg/ml) reduced the resting tension and potentiated the responses to electrical stimulations. (b), Hyoscine (0·1 μg/ml) inhibited the contractions and potentiated the relaxations evoked by electrical stimulation. 5-HT still reduced the resting tension but the evoked relaxations were now small. (c), Effects of 5-HT in another preparation. (d), The evoked responses were blocked by TTX (0·1 μg/ml) but the decrease of resting tension due to 5-HT was not affected.

Effects of tetrodotoxin and hyoscine

In the longitudinal muscle, tetrodotoxin (0·1 μg/ml) and hyoscine (0·1 μg/ml) blocked the responses to electrical stimulation (Fig. 4). In many preparations, they also reduced the size of spontaneous contractions. In the presence of tetrodotoxin or of hyoscine, electrical stimulation did not cause contractions in the absence or presence of 5-HT (Fig. 4b, c), but the increase in resting tension caused by 5-HT was not affected and was the same as the control.

As in the longitudinal muscle, tetrodotoxin and hyoscine blocked the contractions of the circular muscles evoked by electrical stimulation, but they did not affect the relaxing action of 5-HT on the resting tension (Fig. 5b, d). Hyoscine and also tetrodotoxin occasionally changed the resting tension, but no consistent result was obtained. The relaxation caused by electrical stimulations was either unchanged or sometimes potentiated by hyoscine. When 5-HT was applied in the presence of hyoscine, the relaxation in response to electrical stimulation became small or was abolished (Fig. 5b). This effect may be due to the fact that the muscle was already in a relaxed state. All responses to electrical stimulations were blocked by tetrodotoxin.

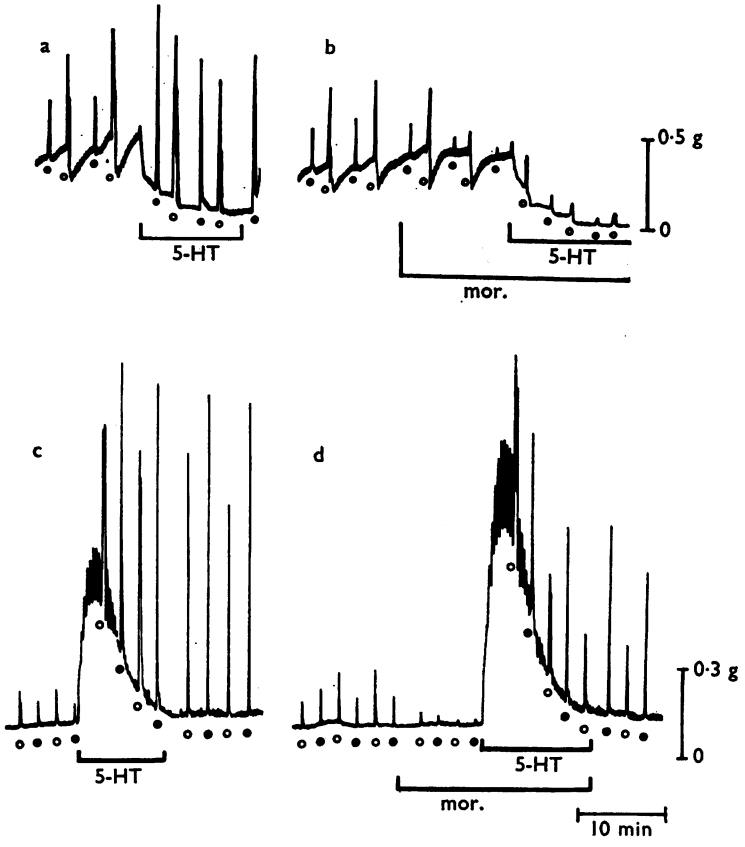

FIG. 6. Effects of morphine (mor.) on the action of 5-HT on circular muscle (a, b) and longitudinal muscle (c, d). (●), Single pulse stimulation (3 ms duration). (○), Repetitive stimulation (pulses of 0·7 ms duration at 10 Hz for 10 s). (a), Control. (b), Morphine (1 μg/ml) reduced the evoked contractions but did not affect the reduction in resting tension due to 5-HT (1 μg/ml). (c), Control. (d), Morphine (1 μg/ml) reduced the responses evoked by electrical stimulation, but potentiated the contraction caused by 5-HT.

Effects of morphine and lysergic acid diethylamide

Morphine

Morphine inhibits markedly the contractions of the guinea-pig isolated ileum caused by 5-HT (Gaddum & Picarelli, 1957; Kosterlitz & Robinson, 1958). This effect may be due to a depressant action on acetylcholine release from the nerve fibres (Paton, 1957; Schaumann, 1957).

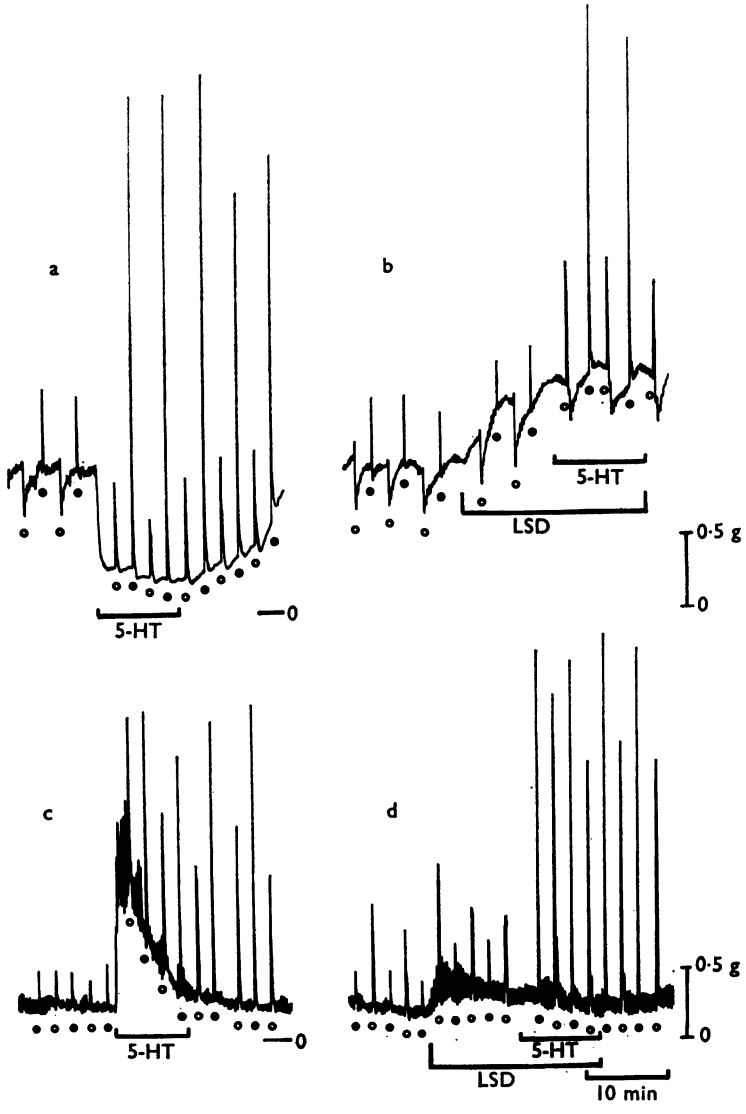

FIG. 7. Effects of LSD (1 μg/ml) on the actions of 5-HT in circular muscle (a, b) and longitudinal muscle (c, d). (○), Repetitive stimulation (pulses of 1 ms duration at 10 Hz for 10 s). (●), Single pulse stimulation (2 ms duration). (a), Control. (b), LSD increased resting tension and potentiated relaxation evoked by electrical stimulation. The decrease in resting tension by 5-HT was blocked but the contractor responses to electrical stimulation were still potentiated by 5-HT. (c), Control. (d), LSD increased spontaneous activity and blocked the increase in tension induced by 5-HT; the evoked contractions were still potentiated with 5-HT.

Morphine (1 μg/ml) had little effect on the resting tension of the circular and the longitudinal muscles (Fig. 6b, d). The contractions evoked by electrical stimulation were reduced by morphine. However, the relaxations of the circular muscle in response to electrical stimulation were not affected. Thus, morphine had actions similar to those of hyoscine, but the effect appeared to be weaker.

When 5-HT was applied in the presence of morphine, the effect on the resting tension was similar to that in the control (Fig. 6). However, the potentiation of the evoked contractions by 5-HT was reduced by morphine. The change of the resting tension by 5-HT was often larger in the presence of morphine, particularly in the longitudinal muscles (Fig. 6d).

Lysergic acid diethylamide (LSD)

LSD (1 μg/ml) caused an increase in the resting tension in the longitudinal and in the circular muscles. This observation is in accord with the report that LSD increases the intraluminal pressure of the guinea-pig stomach (Paton & Vane, 1963). The responses to electrical stimulation appeared to be not much affected by LSD.

The changes in resting tension caused by 5-HT were abolished by LSD, as Gaddum (1958) found for the isolated rat uterus. On the other hand, the potentiation by 5-HT of the responses to electrical stimulations was not significantly affected (Fig. 7b, d). The relaxation in response to repetitive stimulation was slightly increased by LSD, probably due to the increase in resting tension. 5-HT reduced this response to its original magnitude (Fig. 7b).

Discussion

In the guinea-pig intestine, the contraction caused by 5-HT is mediated through two kinds of receptors, M and D (Gaddum & Picarelli, 1957 ; Gaddum, 1958). The M receptors are blocked by morphine and atropine, whereas the D receptors are blocked with dibenyline or its analogues. In both the longitudinal and circular muscles of guinea-pig stomach, the effects of 5-HT are also mediated through two different actions.

The effects are complex. In the longitudinal muscle 5-HT causes contraction, but in the circular muscle it causes relaxation. Nevertheless, in both muscle layers, 5-HT potentiates the contractions in response to electrical stimulation. Since these responses are abolished by hyoscine or by tetrodotoxin, 5-HT seems either to potentiate the release of the transmitter from the nervous elements or to increase the sensitivity of the smooth muscles to the transmiter. The transmitter is probably acetylcholine, which causes contraction of the longitudinal and circular muscles ; moreover, the contractor responses to electrical stimulation and to acetylcholine are abolished by hyoscine or atropine. Finally, the contractions evoked by electrical stimulation and the effects of 5-HT on these contractions are augmented by eserine (unpublished observation), while they are reduced with morphine, which reduces acetylcholine release from nerve fibres in the ileum of the guinea-pig (Paton, 1957 ; Schaumann, 1957).

In the isolated intact stomach of the guinea-pig, 5-HT caused either a reduction in intraluminal pressure or a diphasic response, namely, an increase followed by a decrease (Gershon, 1967 ; Bülbring & Gershon, 1967). Since all responses to 5-HT in concentrations of up to 1 μg/ml were abolished by tetrodotoxin, it was concluded

that, in the guinea-pig stomach, 5-HT acts indirectly through the nervous components. However, in the light of the present experiments, this conclusion must be reconsidered. In isolated strips of longitudinal and circular layers the direct action of 5-HT on the D receptors could be clearly demonstrated because the contraction of the longitudinal muscle and the relaxation of the circular muscle induced by 5-HT were not affected by hyoscine or tetrodotoxin but inhibited by LSD. It is possible that, in the intact isolated stomach, 5-HT can not reach the smooth muscle from the serosal surface; moreover, there may be in the intact organ an interaction between longitudinal and circular muscles as described for the intestine (Kottegoda, 1969). Finally, the measurement of intraluminal pressure may not be sufficiently sensitive to detect the responses.

It may be that 5-HT can increase transmitter release only when the nervous elements are active and that, in the isolated intact stomach, the nervous elements have spontaneous activity, whereas the nerves in the dissected preparation are inactive. Therefore, electrical stimulation is necessary for a demonstration of the existence of M receptors in the isolated strips.

The increase in the intraluminal pressure caused by vagus stimulation is suppressed by 5-HT (Bülbring & Gershon, 1967). It is difficult to reconcile this observation with the present finding that the contraction caused by electrical stimulation is potentiated by 5-HT in both the longitudinal and circular muscles. The difference could be explained on the basis that the responses to stimulation of extramural vagal fibres are reduced while those to stimulation of intramural fibres innervating the muscle cells are augmented by 5-HT.

On the basis of the present investigation, the sphincter antri seems to be very poorly innervated and to have only sparse D receptors in the muscle fibres.

I should like to thank Dr. T. Tomita for his advice in this study. I am also grateful to Professor N. Toida and Professor Y. Ikemi for their hospitality and encouragement, and to Professor E. Bülbring for her help in preparing the manuscript.

REFERENCES

BÜLBRING, E. & GERSHON, M. D. (1967). 5-Hydroxytryptamine participation in the vagal inhibitory innervation of the stomach. *J. Physiol., Lond.*, **192**, 823–846.

GADDUM, J. H. (1958). Drugs which antagonize the actions of 5-hydroxytryptamine on peripheral tissues. In: *5-Hydroxytryptamine*, ed. Lewis, G. P., pp. 195–201. London: Pergamon Press.

GADDUM, J. H. & PICARELLI, Z. P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmac. Chemother.*, **12**, 323–328.

GERSHON, M. D. (1967). Effects of tetrodotoxin on innervated smooth muscle preparations. *Br. J. Pharmac. Chemother.*, **29**, 259–279.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1958). The inhibitory action of morphine on the contraction of the longitudinal muscle coat of the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **13**, 296–303.

KOTTEGODA, S. R. (1969). An analysis of possible nervous mechanisms involved in the peristaltic reflex. *J. Physiol., Lond.*, **200**, 687–712.

PATON, W. D. M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **12**, 119–127.

PATON, W. D. M. & VANE, J. R. (1963). An analysis of the responses of the isolated stomach to electrical stimulation and to drugs. *J. Physiol., Lond.*, **165**, 10–46.

SCHAUMANN, W. (1957). Inhibition by morphine of the release of acetylcholine from the intestine of the guinea-pig. *Br. J. Pharmac. Chemother.*, **12**, 115–118.

VANE, J. R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

(*Received September* 3, 1971)