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5-HYDROXYTRYPTAMINE PARTICIPATION IN THE VAGAL INHIBITORY INNERVATION OF THE STOMACH

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SUMMARY

1. Intraluminal pressure was recorded from the isolated guinea-pig and mouse stomach with the vagus and sympathetic nerves attached.

2. The response to vagal stimulation, which consists of an excitatory and an inhibitory component, resembled the response to 5-hydroxytryptamine (5-HT), which has no direct action on the muscle but acts on intrinsic excitatory and inhibitory ganglia.

3. In the presence of hyoscine, the effect of vagal stimulation, of nicotinic compounds and of 5-HT were all purely relaxant. Competitive block of ganglionic receptors for acetylcholine reduced the vagal relaxation without antagonizing 5-HT. Specific desensitization of ganglionic receptors for 5-HT reduced the vagal relaxation without antagonizing nicotinic compounds.

4. During the early phase of the blocking action of nicotine, responses to vagal stimulation and to 5-HT were both abolished. As the non-specific antagonism changed to the later phase of specific antagonism to acetylcholine, the inhibitory (but not the excitatory) component of the vagal response recovered partially, in parallel with the recovery of the relaxant effect of 5-HT.

5. The vagal inhibitory effect was completely abolished only when competitive block of acetylcholine receptors was combined with desensitization of 5-HT receptors.

6. Stimulation of the mouse stomach (after asphyxiation of the mucosa and exclusion of the luminal content) in the presence of hyoscine caused the release of 5-HT; this release was blocked by tetrodotoxin.

7. The results, together with previous observations that 5-HT is contained within preganglionic nerve fibres in the myenteric plexus, are consistent with the hypothesis that 5-HT, with acetylcholine, may be a neurotransmitter in the vagal inhibitory innervation of the stomach.

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INTRODUCTION

It has been known for a long time that the vagus nerves contain fibres which cause relaxation of the stomach (McSwiney & Robson, 1929). This relaxation is partially susceptible to ganglionic blockade by drugs which are competitive antagonists to acetylcholine at nicotinic receptors (Paton & Vane, 1963; Greef, Kasperat & Osswald, 1962). The studies of Martinson (1965*a*, *b*) and Campbell (1966) have shown that these vagal fibres represent a distinct group. They have a high threshold of excitation and they are non-sympathetic since their effects are resistant to adrenergic neurone blocking agents as well as blockers of α- and β-receptors for catecholamines. The final inhibitory transmitter of these fibres is unknown but the partial susceptibility of the pathway to drugs like hexamethonium indicates that a cholinergic synapse is also involved.

Preganglionic nerve endings which are able to synthesize and store 5-HT have recently been demonstrated in the myenteric plexus of the gastro-intestinal tract by means of radioautography (Gershon, Drakontides & Ross, 1965; Gershon & Ross, 1966*a*, *b*). The origin of these endings is not known but they may belong to that part of the vagal inhibitory pathway which is not blocked by antagonists of the action of acetylcholine on ganglia, suggesting a possible participation of 5-HT. The ability of 5-HT to stimulate ganglia is well known (Trendelenburg, 1956*a*, *b*; Hertzler, 1961; Bindler & Gyermek, 1961; Brownlee & Johnson, 1963, 1965; Johnson, 1964) and this effect of 5-HT is not blocked by non-depolarizing antagonists of the nicotinic action of acetylcholine (Trendelenburg, 1956*b*; Bindler & Gyermek, 1961; Brownlee & Johnson, 1965). Moreover, in the guinea-pig stomach, 5-HT has been found to stimulate both excitatory and inhibitory neurones and its action is exclusively mediated through the neural tissue (Gershon, 1967*a*). The role of 5-HT in ganglionic transmission in the vagal inhibitory pathway was therefore investigated.

Some of the results have been communicated to the Physiological Society and to the Symposium on Indole-Derivatives (Bülbring & Gershon, 1966, 1967).

METHODS

Guinea-pigs and mice were used in this study. The animals were stunned and bled. The methods of dissection of the stomachs, of recording intraluminal pressure and of stimulation of vagus and sympathetic nerves have previously been described (Campbell, 1966; Gershon, 1967*a*, *b*). All preparations were set up in Krebs solution at 37° C, equilibrated with 95% O_2 and 5% CO_2.

Output of 5-HT. The combined output of 5-HT was measured from the stomachs of five mice. The stomachs were removed from the animals, the vagus nerves were dissected free of the oesophagus, and the pylorus and oesophagus were ligated. A few mm of oesophagus

and duodenum were left attached to the stomachs which were suspended in oxygenated Krebs solution, supported by ties around the duodenal and oesophageal remnants. After 3 hr suspension with the lumen closed off, the stomachs were removed from the Krebs solution and, after removal of their contents, they were again closed off. They were then resuspended in 10 ml. of Krebs solution and 1 ml. of the solution was withdrawn at intervals for the assay of 5-HT.

The fundus of the rat stomach was used for the bio-assay of 5-HT as described by Vane (1957) with minor modifications. Contractions were recorded with an auxotonic lever, the preparations were not stretched after washing and samples were applied for 2 min at 5 min intervals. Only those preparations sensitive enough to respond to 10^{-11} g/ml. of 5-HT were used. About half the preparations were sufficiently sensitive.

Histological examination. In three experiments the mouse stomachs, after incubation for 3 hr with the lumen closed off, were fixed in 10% formalin buffered to pH 7·4. They were embedded in paraffin, sectioned at 5 μ, stained with haematoxylin and eosin and also for argentaffin cells by the method of Fontana (Pearse, 1953).

Other preparations. The longitudinal muscle strip of guinea-pig ileum (Rang, 1964; Paton & Rang, 1965) was set up in 10 ml. Krebs solution and contractions were recorded auxotonically with a frontal writing lever on a smoked drum. The preparation could be stimulated electrically between two platinum electrodes at the top and the bottom of the organ-bath. square wave pulses of 0·1 msec duration were given at a frequency of 2–6 pulses/min. The stimulus strength was supramaximal.

The hypogastric nerve-vas deferens preparation (Huković, 1961) was mounted in a 50 ml. organ-bath. The nerve was drawn through a pair of platinum ring electrodes and another pair of platinum ring electrodes encircled the vas deferens itself just distal to the point of entry of the hypogastric nerves. The preparations were stimulated alternately through each set of electrodes, with trains of 0·1 msec pulses for 5 sec at 10 c/s. Ganglia are present in the hypogastric nerve in the vicinity of the vas deferens and stimulation of the hypogastric nerves is largely preganglionic and produces a response which is partially susceptible to ganglionic blockade. However, transmural stimulation with pulses of brief duration results in activation of post-ganglionic neural elements and the response is not susceptible to the action of ganglion-blocking drugs (Kuriyama, 1963; Sjöstrand, 1965). This preparation thus makes it possible to stimulate either pre- or post-ganglionic sympathetic nerves.

Drugs used were, acetylcholine bromide, bromlysergic acid diethylamide (BOL 148, Sandoz), bufotenin bioxalate, carbachol, choline phenyl ether bromide, cyproheptadine hydrochloride, dimethylphenylpiperazinium iodide (DMPP), dopamine hydrochloride, eserine sulphate, hexamethonium hydrobromide, 5-hydroxytryptamine creatinine sulphate (5-HT), DL-5-hydroxytryptophan (5-HTP), 5-hydroxy-2-methylindole, 5-hydroxy-N,N,N-trimethyl-tryptamine iodide (bufoteninium iodide), 4-hydroxyindole-N,N-dimethyltryptamine (psilocin) hydrochloride, hyoscine hydrobromide, imipramine hydrochloride, methysergide bimaleate, α-methyltryptamine hydrochloride, morphine sulphate, nialamide (Niamid Pfizer), nicotine hydrogen tartrate, (−)-noradrenaline bitartrate, pentolinium tartrate, phenoxybenzamine hydrochloride, procaine hydrochloride, propranolol hydrochloride, crystalline tetradotoxin, cis-tranylcypromine, tryptamine hydrochloride and tetramethylammonium iodide (TMA). The following biguanides were tested: *o*-anisidine, ethyl aniline, phenyl, *m*-tolyl, *p*-tolyl, *m*-xylidine and *p*-xylidene biguanide. All drug concentrations refer to the salts and are given in g/ml.

We are grateful to Dr E. W. Gill for making bufoteninium iodide and 5-hydroxy-2-methylindole, and to Professor W. D. M. Paton for supplying the biguanides.

RESULTS

Drugs which relax the stomach in the presence of hyoscine

Responses of the stomach to drugs were studied in the presence of hyoscine (10^{-7} g/ml.) which blocks muscarinic responses of the stomach (Paton & Vane, 1963) and thus permits the study of inhibitory effects uncomplicated by coincident excitatory effects. Compounds able to relax the stomach of the guinea-pig under these conditions are listed in Table 1.

TABLE 1. Compounds which relax the guinea-pig stomach in the presence of hyoscine (10^{-7} g/ml.)

Compounds whose action is abolished by tetrodotoxin		
Antagonized by pentolinium and hexamethonium	Not antagonized by pentolinium and hexamethonium	Not affected by tetrodotoxin
Acetylcholine (in concentration > 10^{-6} g/ml.)	5-HT	Noradrenaline
Carbachol	5-hydroxy-2-methylindole	Isoprenaline
Choline phenyl ether	Psilocin	Dopamine
DMPP	Biguanides (all members of class)	—
Nicotine		
Tetramethylammonium iodide		
	Incomplete antagonism	
	Bufotenin	
	Bufoteninium iodide	

They can be divided into two groups by the susceptibility of their action to tetrodotoxin. Tetrodotoxin abolishes nerve-mediated responses but has no effect on those of the smooth muscle itself (Toida & Osa, 1965; Bülbring & Tomita, 1966, 1967; Gershon, 1966, 1967*a*). Table 1 shows that compounds related to acetylcholine and also compounds related to 5-HT activate inhibitory neurones. Only the catecholamines appear to act on the muscle directly. Those compounds whose action is blocked by tetrodotoxin, and is therefore nerve-mediated, can be further subdivided by the action of a cholinergic ganglion blocking drug, e.g. pentolinium, into those which act upon nicotinic receptors and those which do not.

Receptors exist, therefore, on inhibitory neurons which are distinct from those for acetylcholine but which respond to 5-HT and related compounds. This is illustrated in Fig. 1. In the presence of hyoscine the response of the stomach to vagal stimulation, to 5-HT, and to the nicotinic ganglion stimulant, DMPP (Ling, 1959), was a relaxation. Pentolinium reduced the effect of vagal stimulation; it abolished the response to DMPP but had no effect on the response to 5-HT.

Drug effects on vagal responses in the absence of hyoscine

Stimulation of the vagus nerve leads to the simultaneous activation of both excitatory and inhibitory neurones in the wall of the stomach. The

responses in the absence of hyoscine are therefore a complex mixture of excitation and inhibition. In the mouse, and in about 30 % of the guinea-pigs, the response consists of a relaxation during stimulation, a contraction following, and finally an after-relaxation lasting 2–3 min. In about

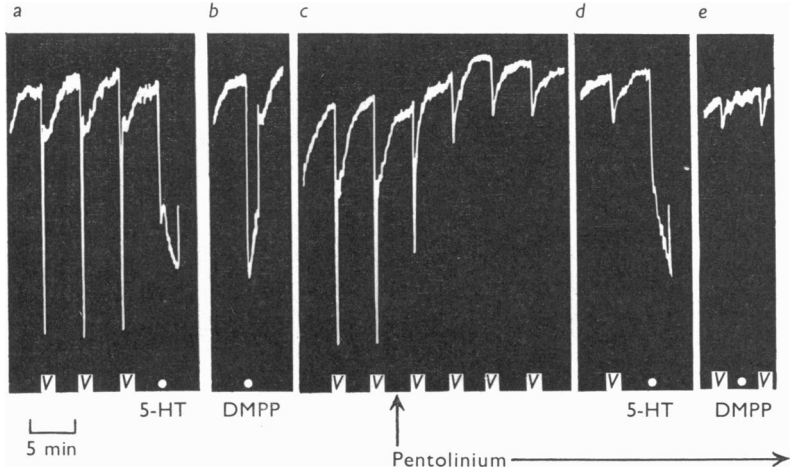

Fig. 1. The effect of pentolinium on the responses of the guinea-pig stomach to vagal stimulation, 5-HT and DMPP. Hyoscine (10^{-7} g/ml.) is present throughout. (*a*) Vagal stimulation (*V*) (10 sec at 5 c/s), 5-HT (10^{-5} g/ml.) and (*b*) DMPP (5×10^{-6} g/ml.) produce relaxation; (*c*) pentolinium (10^{-5} g/ml.) partially blocks vagal responses but, (*d*), does not affect the response to 5-HT (10^{-5} g/ml.) although, (*e*) the response to DMPP (5×10^{-6} g/ml.) is abolished.

70 % of the guinea-pigs the initial relaxation during stimulation was not seen, but even in these preparations the stomach contracted suddenly when stimulation ceased, and the after-relaxation was a constant feature.

In order to determine the nature of the different components in the vagal pathway, the modification by drugs of the response of the stomach to vagal stimulation, to acetylcholine, to the ganglion stimulant DMPP, to nicotine and to sympathetic nerve stimulation was investigated. All the compounds listed in Table 1 reduced the excitatory component of the response to vagal stimulation. The catecholamines, which act by relaxing the smooth muscle directly, reduced the response to acetylcholine. The nicotinic ganglion stimulants either potentiated or did not affect the contractile responses to acetylcholine. However, they blocked the responses to other nicotinic drugs and to 5-HT: thus they appeared to act by blocking ganglia. DMPP (10^{-6} g/ml.) reduced the responses to sympathetic nerve stimulation if it was left in the bath for more than 10–15 min. This adrenergic neurone blocking action has been reported in other preparations (Wilson, 1962; Birmingham & Wilson, 1965; Burnstock,

Campbell & Rand, 1966). For this reason DMPP was not applied for longer than 90 sec.

5-HT and related compounds, while reducing the excitatory component of the vagal response, did not antagonize the action of acetylcholine or DMPP, nor blocked responses to sympathetic nerve stimulation. This is illustrated in Fig. 2. With increasing concentrations of 5-HT, the excitatory

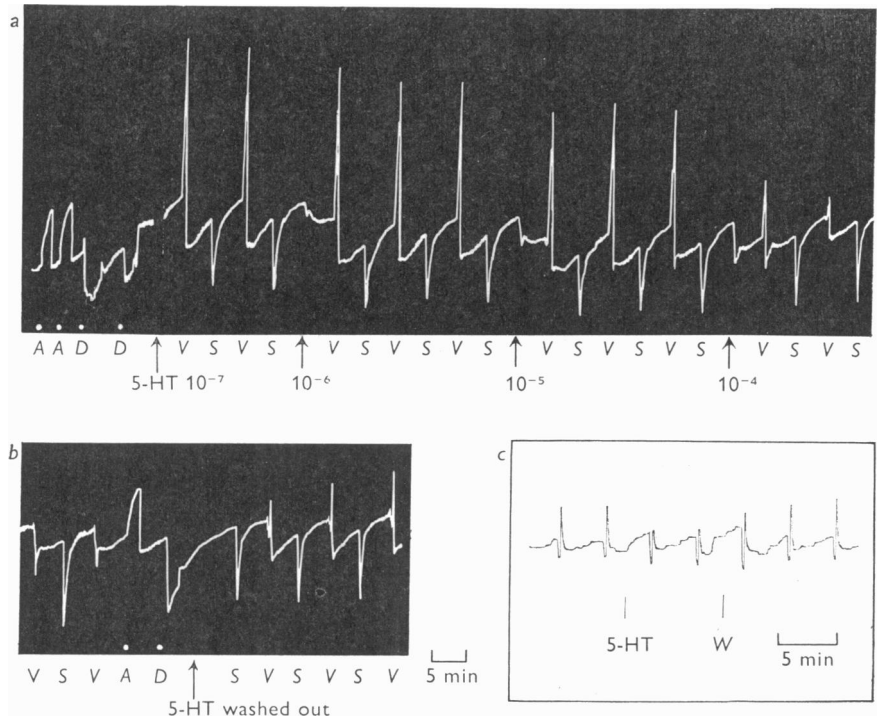

Fig. 2. The effect of 5-HT on the response of the guinea-pig stomach to vagal stimulation. In (*a*) and, directly continued, in (*b*): responses to acetylcholine (10^{-7} g/ml.) (*A*) and DMPP (5×10^{-6} g/ml.) (*D*), to stimulation of vagus nerves (*V*) (10 sec at 10 c/s) and to stimulation of perivascular (sympathetic) nerves (*S*) (10 sec at 50 c/s). As the concentration of 5-HT in the bath is increased progressively from 10^{-7} to 10^{-4} g/ml. the excitatory component of the vagal effect is suppressed, while effects of sympathetic stimulation, acetylcholine and DMPP are unchanged. When 5-HT is washed out vagal excitatory responses begin to recover. (*c*) Mouse stomach. Addition of 5-HT (10^{-5} g/ml.) causes a slight rise in tone, and the vagal responses become relaxant. When 5-HT is washed out (*W*) the vagal excitatory component recovers.

component of the vagal response was reduced and, at 10^{-4} g 5-HT/ml., stimulation of the vagus nerves eventually caused only relaxation. On the other hand, there was no change in the response to stimulation of the sympathetic nerves even at the highest concentration of 5-HT. The effect

of DMPP was not antagonized and the contraction in response to acetylcholine was also unaltered. The reduction of the vagal excitatory response by drugs related to 5-HT is probably, therefore, due to a facilitation of the vagal inhibitory pathway.

Compounds related to 5-HT, which were found to act similarly, include bufotenin, the 4-hydroxyindole (psilocin) and 5-hydroxy-2-methylindole. Tryptamine and α-methyltryptamine were, however, ineffective, indicating that hydroxylation of the indole moiety is essential for this action. Since the tertiary amines, psilocin and bufotenin were active, as was 5-hydroxy-2-methylindole, neither the primary amino group nor the aliphatic side chain of 5-HT appear to be necessary for the modification of the vagal response. The action of the biguanides (Fastier, McDowall & Waal, 1959) was also similar to that of 5-HT though they differed in some aspects (see Table 2).

In the mouse stomach the fundus is phylogenetically different from the remainder of the stomach and the muscle is highly sensitive to the direct action of 5-HT, as in the rat (Vane, 1957; Paton & Vane, 1963; Gershon, 1966, 1967*a*). Consequently, when 5-HT is added to the whole stomach, low concentrations cause contraction of the fundus and a rise in intraluminal pressure which becomes smaller when the 5-HT concentration is higher than 10^{-7} g/ml. Figure 2*c* shows diphasic responses of the mouse stomach to vagal stimulation. 5-HT (10^{-5} g/ml.) causes a small rise in intraluminal pressure and suppresses the contraction, but increases the relaxation in response to vagal stimulation.

Drug effects on vagal responses in the presence of hyoscine

In the presence of hyoscine the relaxation of the stomach in response to vagal stimulation was increased by 5-HT and related compounds, whereas it was decreased by the nicotinic ganglion stimulants. This is illustrated in Fig. 3. In the presence of hyoscine, both 5-HT and DMPP produced relaxation. 5-HT did not prevent the relaxation produced by DMPP (*a*) but DMPP did block the response to 5-HT (*b*). During a 5 min period of sustained vagal stimulation (*c*) DMPP briefly increased then abruptly terminated the vagal relaxation and markedly reduced subsequent responses. When 5-HT was given in the same way, its facilitating effect during vagal stimulation persisted and subsequent vagal responses were enhanced (*d*) and (*e*). The facilitation continued after the wash. 5-HT, therefore appears to produce a sustained stimulation of the inhibitory neurones in the stomach, thereby facilitating vagal relaxation. The nicotinic compounds, on the other hand, produce a transient stimulation of inhibitory neurones which is rapidly followed by a block of vagal ganglionic transmission. All the nicotinic compounds were found to act similarly, except nicotine itself. With nicotine a second phase of block was observed.

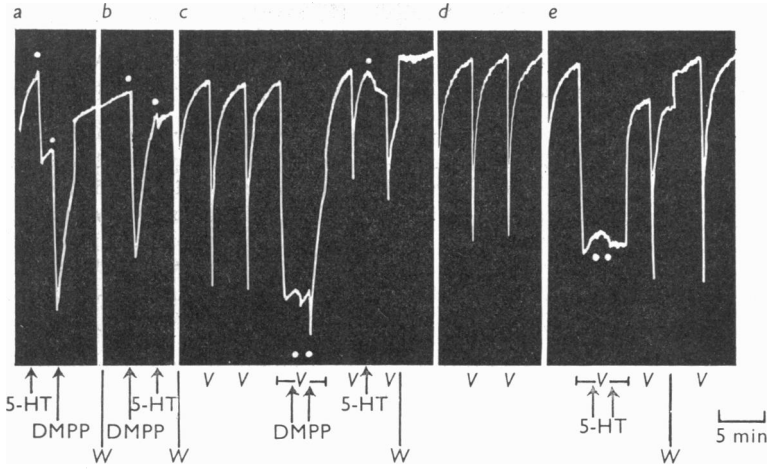

Fig. 3. Interaction of 5-HT, DMPP, and vagal stimulation in the guinea-pig stomach. Hyoscine (10^{-7} g/ml.) is present throughout. (*a*) Relaxation is produced by 5-HT (10^{-5} g/ml.) and, while it is still present, DMPP (10^{-5} g/ml.) causes further relaxation, i.e. 5-HT does not antagonize DMPP. (*b*) A relaxation is produced by DMPP (10^{-5} g/ml.) which quickly fades, after which the response to 5-HT is blocked. (*c*) The vagus nerves are stimulated (*V*) (10 sec at 30 c/s) and, at the bar, for a period (5 min at 30 c/s), during which DMPP (10^{-6} g/ml. first arrow) transiently facilitates and then at concentration 10^{-5} g/ml. (second arrow) causes the vagal relaxation to fade. Subsequent vagal responses and that to 5-HT (10^{-5} g/ml.) are antagonized. (*d*) Recovery of vagal effects. (*e*) Addition of 5-HT during vagal stimulation (10^{-6} g/ml. at first arrow, 10^{-5} g/ml. at second arrow) does not antagonize vagal effects. *W* = wash.

The action of nicotine

Nicotine, like DMPP, always relaxed the guinea-pig stomach. Hyoscine greatly potentiated the relaxation and eserine converted the response to a contraction (Gershon, 1966, 1967*a*). The relaxant response of the stomach to nicotine was transient and, while nicotine was still present, the intraluminal pressure returned rapidly (within 1–2 min) to the original level. At this time vagal responses, effects of other nicotinic compounds and of 5-HT were all inhibited. Figure 4*a* shows that 5-HT, applied 2 min after and in the presence of nicotine, was ineffective. When nicotine was allowed to remain in the bath, the vagal response changed from a much reduced excitatory response to one which was increasingly relaxant (Fig. 4*b*). At this time it was found that the relaxant effect of 5-HT had recovered. When the experiment was repeated in the presence of hyoscine (Fig. 4*d*), the vagal response was relaxant throughout. After addition of nicotine, the vagal relaxation was very much reduced but recovered partially while nicotine remained in the bath. At this time the effect of 5-HT was a prolonged relaxation. Thus, the pronounced antagonism to vagal inhibition

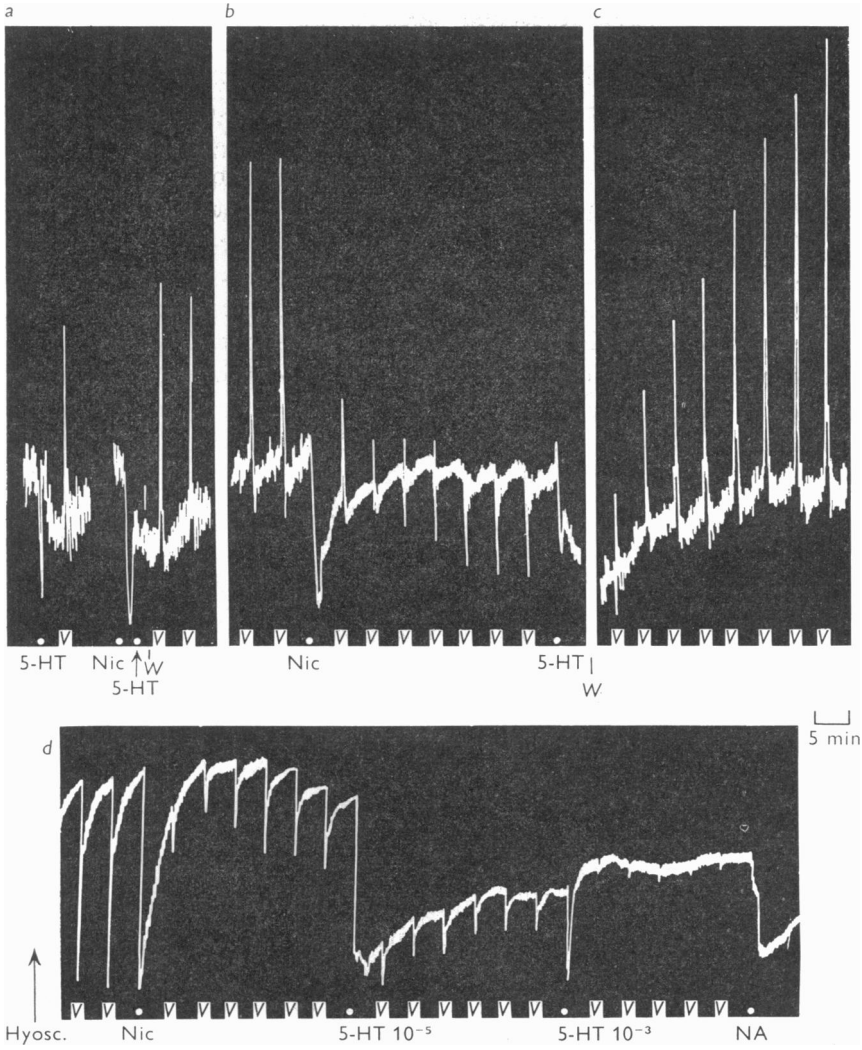

Fig. 4. The effect of nicotine on responses of the guinea-pig stomach to 5-HT and vagal stimulation. (*a*) 5-HT (10^{-5} g/ml.) (marked by dot) causes relaxation, vagal stimulation (10 sec at 5 c/s) mainly contraction (*V*). Nicotine (5×10^{-6} g/ml.) causes a transient relaxation immediately after which the response to 5-HT (10^{-5} g/ml.) (dot) is blocked. After washing out (*W*), vagal responses are normal. (*b*) Nicotine (5×10^{-6} g/ml.) produces a transient relaxation. As it remains in the bath, the response to repeated vagal stimulation becomes relaxant and at this time, 24 min after nicotine was added, 5-HT causes relaxation similar to the control in (*a*). (*c*) After washing out (*W*) the vagal excitatory responses recover and are potentiated. (*d*) In the presence of hyoscine (10^{-7} g/ml.) vagal stimulation (*V*) causes relaxation. Nicotine (5×10^{-6} g/ml.) produces relaxation and, in its presence, the vagal response is reduced, then partially recovers. At this stage 5-HT (10^{-5} g/ml.) causes a relaxation larger than the control in (*a*). Addition of a desensitizing concentration of 5-HT (10^{-3} g/ml.), antagonizes vagal responses without affecting the relaxation caused by (−)-noradrenaline (10^{-8} g/ml.) (NA).

and to 5-HT during the early phase of the nicotine action gradually passed off during the later phase. In contrast, relaxations caused by other nicotinic compounds, e.g. DMPP or high concentrations of acetylcholine in the presence of hyoscine, remained blocked as long as the nicotine remained in the bath.

Paton & Perry (1953) first described an early depolarizing block and a later competitive antagonism by nicotine at acetylcholine receptors. Trendelenburg (1957) has shown that during the second phase, but not during the first, the ganglion cells of the superior cervical ganglion may be stimulated by drugs which, like 5-HT, act on receptors different from those for acetylcholine. Similar observations have been made on ganglia in the intestine (Brownlee & Johnson, 1963). It is therefore probable that the action of nicotine on the guinea-pig stomach is similar to its action on ganglionic transmission elsewhere. The early non-specific block would correspond to the depolarizing phase of its action and the later specific block to its non-depolarizing phase. It is therefore of interest that, as the block induced by nicotine changes to a specific antagonism to acetylcholine, the vagal inhibitory response and the response to 5-HT recover simultaneously.

The action of drugs which antagonize 5-HT

Antagonists of 5-HT which block the direct smooth muscle stimulating action of 5-HT such as methysergide, BOL-148, cyproheptadine and the phenothiazines (for references see Gyermek, 1966) were ineffective in blocking the action of 5-HT on the neural receptors of the guinea-pig and mouse stomach. As shown in Table 2, these compounds only blocked the action of 5-HT when used in very high concentrations, and, although they also blocked vagal responses, they were non-specific and their action cannot be attributed to 5-HT antagonism. Since neither morphine nor phenoxybenzamine (Table 2) had any effect on the inhibitory vagal response or on the inhibitory response to 5-HT, there seems to be no evidence for the existence of *M* and *D* receptors described for guinea-pig ileum (Gaddum & Picarelli, 1957) in the inhibitory mechanism of the stomach.

Gyermek (1961, 1964*a*, *b*, 1966) has drawn attention to the fact that neural receptors for 5-HT are not blocked by drugs which antagonize its direct action on muscle. Neural receptors further differ from muscle receptors in that they are stimulated and subsequently blocked specifically by the biguanides (Fastier *et al.* 1959; Gyermek, 1964*a*), and also by quaternary derivatives of 5-HT (Gyermek, 1964*b*), the hydroxylation of the indole moiety of tryptamines being essential for the stimulation of the neural but not of the muscle receptors. The inhibitory neurones in the stomach showed all the characteristics of 5-HT sensitive neural receptors.

The biguanides stimulated inhibitory ganglia (Table 1) and in higher doses gradually desensitized inhibitory neurones both to their own action and also to that of 5-HT. They did not affect responses to DMPP, although some members of the group, particularly *m*-xylidene biguanide, antagonized the action of (−)-noradrenaline. The quaternary derivative of 5-HT, bufoteninium iodide (2×10^{-6} g/ml.), transiently stimulated inhibitory neurones and also antagonized 5-HT. In contrast to other derivatives of 5-HT the stimulant action of this compound was antagonized by pentolinium and so its action must involve nicotinic receptors. Once the stimulation had passed off, however, responses to DMPP were only slightly reduced while responses to a supramaximal concentration of 5-HT remained completely blocked. Bufoteninium iodide did not affect responses to (−)-noradrenaline or sympathetic nerve stimulation. In concentrations large enough to antagonize 5-HT, both the biguanides and the quaternary derivative of 5-HT also reduced the vagal inhibitory response in the presence of hyoscine.

TABLE 2. Action of compounds tested for antagonism to the relaxant response of the guinea-pig stomach to 5-HT in the presence of hyoscine

Compound	Concentration required to block relaxation in response to 5-HT (10^{-5})	Effect on the relaxation in response to: Vagal stimulation	DMPP (5×10^{-6})	(−) noradrenaline (10^{-7})	Sympathetic nerve stimulation
BOL-148	10^{-5}	Reduced	Block	Block	Block
Methysergide	$2{\cdot}5 \times 10^{-5}$	Reduced	Reduced	Reduced	Reduced
Cyproheptadine	10^{-4} ineffective				
Imipramine	10^{-5}	Block	Block	Block	Block
Phenoxybenzamine (10^{-7})	No effect	No effect	No effect	No effect	No effect
Propranolol	10^{-5}	Reduced	Block	Reduced	Block
Phenoxybenzamine (10^{-7}) + propranolol (10^{-7})	No effect	No effect	No effect	Block	Block
Morphine (10^{-9}–10^{-5})	No effect	No effect	No effect	No effect	No effect
Bufotenin	10^{-4} (incomplete block)	Reduced	No effect	No effect	No effect
Bufoteninium iodide	2×10^{-6}	Reduced	Slightly reduced	No effect	No effect
Tryptamine after MAOI[a]	3×10^{-5}	Reduced	No effect or slightly reduced	No effect	No effect
α-methyltryptamine after MAOI[a]	2×10^{-5}	Reduced	No effect	No effect	No effect
m-xylidene biguanide	10^{-5}	Reduced	No effect	Block	Block
Phenyl biguanide	2×10^{-4} (incomplete block)	Reduced	No effect or slightly reduced	Block	Block
m-tolyl biguanide	5×10^{-5}	Reduced	No effect or slightly reduced	Reduced	Reduced
5-HTP after MAOI[a]	2×10^{-4}	Reduced	No effect	No effect	No effect
5-HT	$7{\cdot}5 \times 10^{-4}$	Reduced	No effect or slightly reduced	No effect	No effect
5-hydroxy-2-methylindole	5×10^{-5} (incomplete block)	Reduced	No effect	No effect	No effect
DMPP	2×10^{-5}	Reduced	Block	Reduced	Block
TMA	2×10^{-5}	Reduced	Block	—	—
Acetylcholine + eserine (10^{-7})	10^{-5}	Reduced	Block	—	—
Nicotine	5×10^{-6} (see Text)	Reduced	Block	—	—
Tetrodotoxin	10^{-7}–5×10^{-7}	Block	Block	No effect	Block
Procaine	3×10^{-5} (lowers tone)	Block	Block	No effect	Block

(*a*) MAOI = monoamine oxidase inhibition. Preparations previously treated with nialamid (10^{-5}) or tranylcypromine (10^{-5}) for 1 hr. Concentrations are in g/ml.

The effect of desensitization to 5-HT and related compounds

(*a*) *Tryptamine.* It has long been known (Gaddum, 1953; Feldberg & Toh, 1953) that tryptamine could desensitize the guinea-pig ileum to the action of 5-HT. Since this organ contains both neural and muscle receptors for 5-HT (Gaddum & Picarelli, 1957; Brownlee & Johnson, 1963), some part of the block of 5-HT by tryptamine must stem from an antagonism on neural receptors. In the mouse stomach in the presence of hyoscine, tryptamine (3×10^{-5} g/ml.) reduced vagal inhibitory responses by about 40–60 % and usually raised the tone slightly. In the guinea-pig stomach the same concentration of tryptamine had no effect on responses to vagal stimulation or to 5-HT. However, Vane (1959) found that the action of tryptamine, in contrast to that of 5-HT, is much potentiated by inhibition of monoamine oxidase. In the present experiments, if the guinea-pig stomach was treated with a monoamine oxidase inhibitor before the application of tryptamine, responses to vagal stimulation were reduced and those to

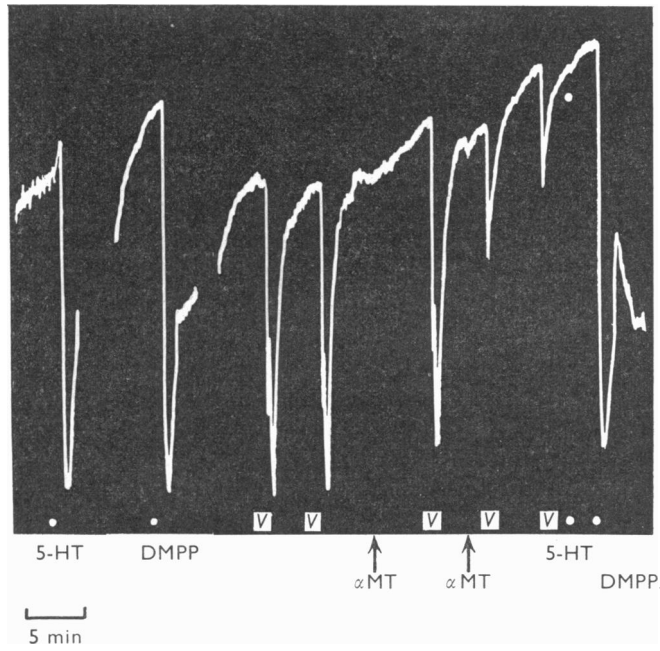

Fig. 5. The effect of α-methyltryptamine (αMT) on the responses of the guinea-pig stomach to 5-HT (10^{-5} g/ml.), DMPP (5×10^{-6} g/ml.) and vagal stimulation (*V*), (10 sec at 5 c/s). The preparation had been treated with a monoamine oxidase inhibitor (tranylcypramine, 10^{-5} g/ml.) 20 min before record started. Hyoscine (10^{-7} g/ml.) is present throughout: α-methyltryptamine (10^{-5} g/ml.) has little effect on vagal stimulation but the response is reduced by α-methyltryptamine (3×10^{-5} g/ml.), and that to 5-HT is abolished. DMPP is not antagonized.

5-HT were blocked. Nialamide (10^{-5} g/ml.), a hydrazine, and tranylcypromine (10^{-5} g/ml.), a non-hydrazine, were used. They were applied for 30–60 min and then washed out before the addition of tryptamine. The tryptamine potentiation and the monoamine oxidase inhibition are both irreversible, suggesting that monoamine oxidase is involved. On the other hand, both inhibitors also potentiated the blocking action of α-methyltryptamine (which is itself a weak inhibitor of monoamine oxidase, but is not a substrate for the enzyme), an action also seen in the central nervous system (Lessin, Long & Parkes, 1965).

The combination of monoamine oxidase inhibitors and tryptamine or α-methyltryptamine which, in the presence of hyoscine, blocked the relaxation of the guinea-pig stomach by 5-HT and reduced the responses to vagal stimulation, did not affect the responses to DMPP, noradrenaline, or sympathetic nerve stimulation. Some of these results are shown in Fig. 5. Hyoscine and tranylcypromine were given before the record started. 5-HT, DMPP and vagal stimulation produced relaxation: α-methyltryptamine reduced the vagal responses, abolished the 5-HT response but had no effect on the relaxation produced by DMPP. Vagal inhibitory responses can therefore be antagonized by desensitizing 5-HT receptors without blocking nicotinic receptors.

(*b*) *Desensitization by 5-HT.* It is well known that in the guinea-pig ileum the sustained administration of a high concentration of 5-HT blocks its own action without affecting responses to other agonists (Gaddum, 1953; Brownlee & Johnson, 1963). An example of this is shown in Fig. 6. Post-ganglionic cholinergic nerves of the innervated longitudinal muscle strip of the guinea-pig ileum were stimulated electrically with pulses of brief duration (0·1 msec) (Paton, 1955) causing contractions. DMPP also caused contraction. 5-HT (10^{-4} g/ml.) also contracted the ileum but during the continued presence of 5-HT, the muscle soon relaxed. Neither the response to electrical stimulation nor that to DMPP were antagonized by the presence of 5-HT. Thus, 5-HT desensitization does not abolish the responsiveness of the muscle; it does not block nerve conduction, acetylcholine release, nor nicotinic receptors. The rapid desensitization of 5-HT receptors on excitatory ganglion cells contrasts with the resistance of inhibitory ganglion cells (cf. Fig. 2).

Specific desensitization of the inhibitory ganglia in the stomach could only be achieved by applying massive concentrations of 5-HT, i.e. $7{\cdot}5 \times 10^{-4}$ or 10^{-3} g/ml. For example, desensitization is shown in the experiment Fig. 4*d*, in which the cholinergic receptors of muscle and ganglia had been blocked by hyoscine and nicotine. In this condition 5-HT (10^{-5} g/ml.) caused a long lasting relaxation during which vagal inhibitory responses were still obtainable, though reduced because the stomach was already

partly relaxed. However, when the 5-HT concentration was raised to 10^{-3} g/ml., it caused a momentary relaxation which faded rapidly beyond the original level and vagal inhibitory responses were then abolished.

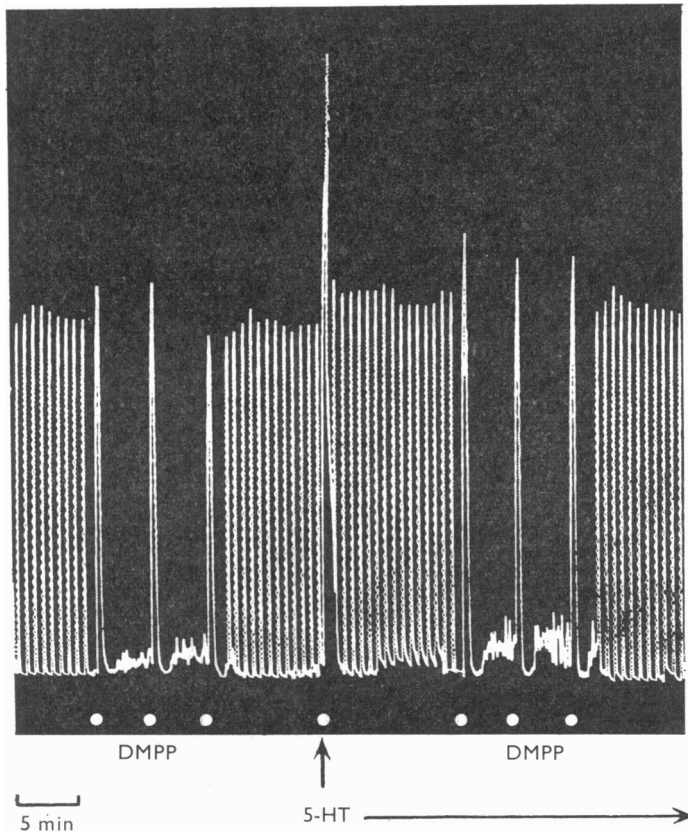

Fig. 6. Contractions of the innervated longitudinal muscle strip of guinea-pig ileum. Electrical stimulation (single 0·1 msec supramaximal shocks) and DMPP (5×10^{-6} g/ml.) (given 3 times (at dots) and washed out after 1 min exposure) cause contraction of the muscle. 5-HT (2×10^{-5} g/ml.) also causes contraction which fades while 5-HT remains in the bath. Desensitization to 5-HT has no antagonistic effect on the response to electrical stimulation or to DMPP.

In preparations in which nicotinic receptors were not blocked, a concentration of 10^{-3} g 5-HT/ml. blocked its own action, reduced the vagal inhibitory responses, but left the relaxant effect of DMPP unchanged, in the same way as is shown in Fig. 5 for α-methyltryptamine after pretreatment with an amine oxidase inhibitor.

The stomach could also be desensitized to 5-HT by the administration of its precursor, 5-HTP, but only after pretreatment with a monoamine

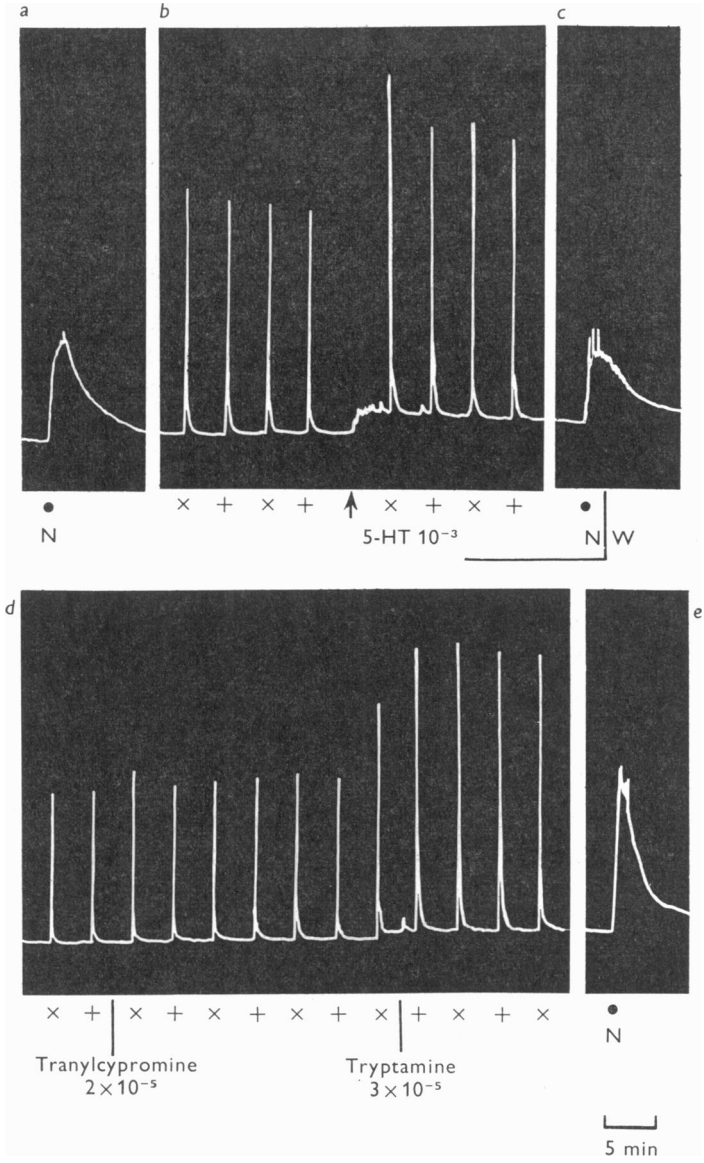

Fig. 7. Guinea-pig hypogastric nerve–vas deferens preparation. (*a*) Contraction in response to (−)-noradrenaline 2×10^{-5} g/ml. (*b*) Contraction in responses to stimulation of hypogastric nerve (×) (5 sec, 0·1 msec at 10 c/s) and to electrical field stimulation (+) (5 sec, 0·1 msec at 10 c/s). 5-HT (10^{-3} g/ml.) facilitates both preganglionic and post-ganglionic stimulation. (*c*) 5-HT has no effect on the response to (−)-noradrenaline. (*d*) The monoamine oxidase inhibitor, tranylcypromine (2×10^{-5} g/ml.) has no effect by itself. The addition of tryptamine (3×10^{-5} g/ml.) after the inhibition of monoamine oxidase increases responses of the vas deferens to preganglionic and post-ganglionic nerve stimulation and to (−)-noradrenaline.

oxidase inhibitor, as for tryptamine. 5-HTP, in a concentration of 2×10^{-4} g/ml. gradually antagonized both vagal inhibitory responses, and responses to 5-HT (10^{-5} g/ml.). Maximal antagonism of vagal and 5-HT responses by 5-HTP was reached 30–60 min after the application of the precursor. Responses to DMPP were not antagonized. Neither the monoamine oxidase inhibitors, nialamide and tranylcypromine, nor 5-HTP had any demonstrable effect on the stomach of the normal guinea-pig or mouse when applied alone.

The specificity of a very high concentration of 5-HT and of tryptamine after monoamine oxidase inhibition was tested on a third preparation, which contained a sympathetic ganglionic synapse. Figure 7 shows the contraction of the isolated hypogastric nerve–vas deferens preparation of the guinea-pig caused by (−)-noradrenaline and by alternating pre- and post-ganglionic nerve stimulation. Both 5-HT and tryptamine after monoamine oxidase inhibition potentiated the contractions produced by pre- and post-ganglionic stimulation equally, indicating that the potentiation was due to an action distal to the ganglia. There was no inhibition of ganglionic transmission and no antagonism to the sympathetic transmitter or to applied noradrenaline.

Reserpine

Since procedures which antagonize 5-HT specifically also reduced vagal inhibition of the stomach, the question arose whether depletion of 5-HT stores would have the same effect. An attempt was made with reserpine, although it is known that the stores of 5-HT in this organ are particularly resistant to reserpine depletion (Bennett, Bucknell & Dean, 1966). Reserpine was dissolved in a 20 % solution of ascorbic acid and was administered to guinea-pigs in 5 mg/kg doses intraperitoneally on 2–4 successive days. Animals were used on the day following their last injection. The vagus and sympathetic nerves to the stomach were both stimulated. If the sympathetic response was abolished the reserpine treatment was considered to have been effective. This criterion was achieved in only two out of sixteen guinea-pigs. In these two animals in which reserpine had abolished sympathetic responses, the vagal responses appeared to be weaker than usual, and the relaxant response to 5-HT (10^{-5} g/ml.) larger than in normal preparations. While in normal animals the relaxation caused by this concentration of 5-HT was about half the amplitude of the maximal vagal relaxation, it was more than twice as large in the reserpinized animals. Moreover, in both preparations, the vagal responses were transiently increased after 90 sec exposure to 5-HT (Fig. 8). DMPP also relaxed the stomach but it had no facilitating action.

In reserpinized animals the vagal inhibitory effects were much more susceptible to fatigue than in normal animals. This observation was made in all sixteen animals treated with reserpine (compare Figs. 8 and 9 with Fig. 3). The fatigue of the vagal response in the animals treated with reserpine could be prevented by the addition of 5-HT during vagal stimulation, but not by the addition of DMPP. Instead, DMPP antagonized vagal responses as in normal animals (Fig. 9).

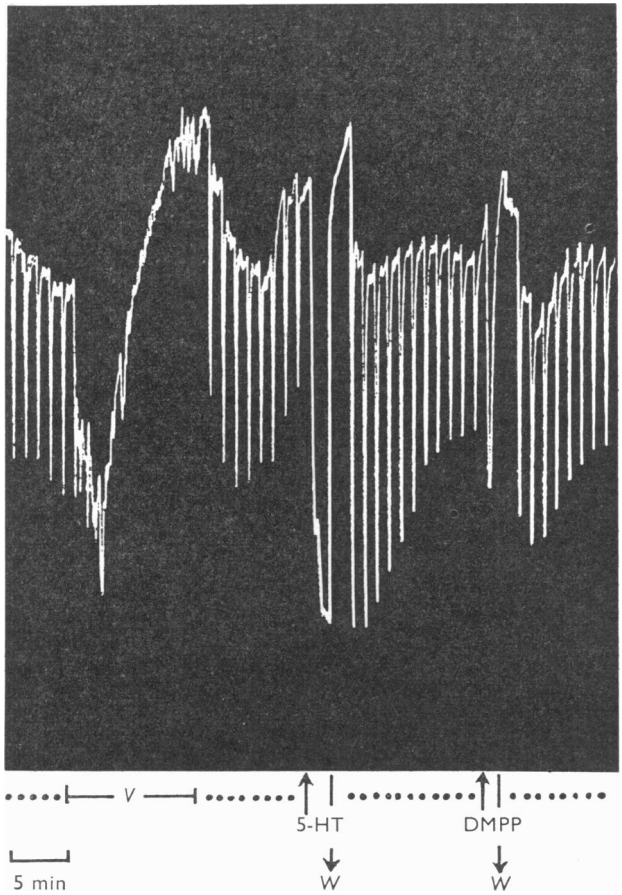

Fig. 8. Responses of the stomach of a guinea-pig treated with reserpine. Hyoscine (10^{-7} g/ml.) is present throughout. The dots indicate vagal stimulation (V) for 10 sec at 20 c/s. The bar indicates sustained vagal stimulation for 8 min at 30 c/s. The vagal response fatigues. The relaxation in response to 5-HT (10^{-5} g/ml.) is approximately twice that in response to 10 sec vagal stimulation. After 5-HT had been applied for 90 sec the vagal response is transiently facilitated. DMPP (10^{-5} g/ml.) also causes relaxation but it does not facilitate vagal stimulation. W = wash.

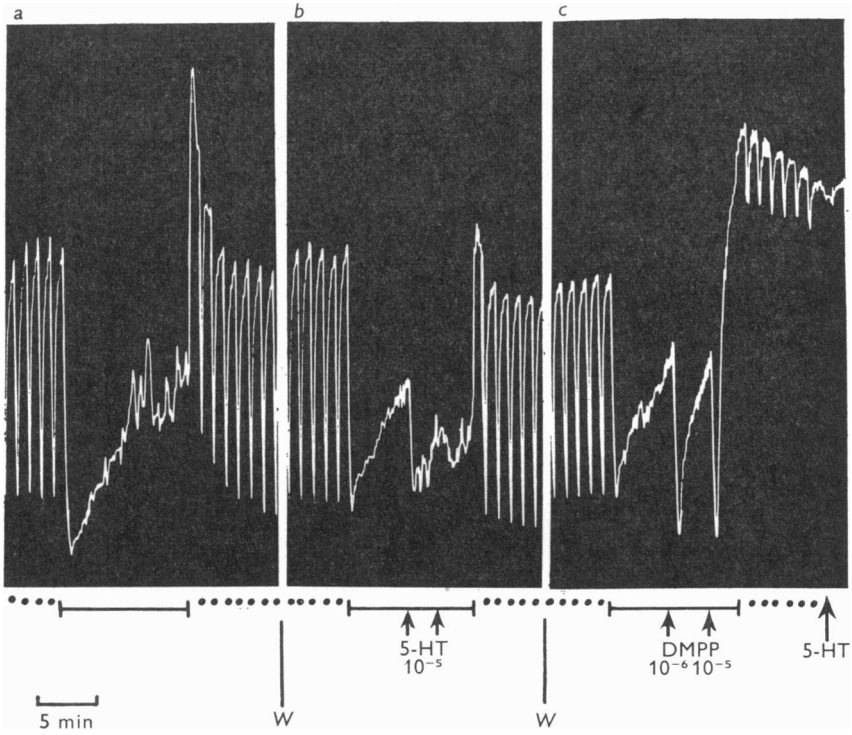

Fig. 9. Reserpinized animal. Hyoscine (10^{-7} g/ml.) present throughout. (*a*) The dots indicate 10 sec, the bar 10 min vagal stimulation (at 30 c/s). The response of the stomach fatigues during sustained stimulation, and the stomach contracts on cessation of the sustained stimulus. (*b*) 5-HT (first arrow 10^{-5} g/ml.; second arrow 2×10^{-5} g/ml.) during sustained vagal stimulation prevents the development of fatigue. (*c*) DMPP (first arrow 10^{-6} g/ml.) during sustained vagal stimulation causes transient relaxation. At second arrow, DMPP (10^{-5} g/ml.) antagonizes vagal stimulation and abolishes the effect of 5-HT (10^{-5} g/ml.)

The immediate action of methysergide in reserpinized animals was equally unspecific as in normal animals. In a concentration of 10^{-5} g/ml. it abolished the response to the same concentration of 5-HT and reduced the vagal inhibitory response in the presence of hyoscine. The responses to DMPP (10^{-5} g/ml.) and (−)-noradrenaline were reduced as well, although not abolished as 5-HT. However, 30 min after methysergide was washed out, the responses to DMPP and (−)-noradrenaline had returned to normal but both responses to 5-HT and to vagal stimulation remained antagonized.

Release of 5-HT from the stomach

When Krebs solution containing 5-HT is perfused through the blood vessels of the guinea-pig stomach the 5-HT is either destroyed or taken up by the tissue since only a small percentage is recovered in the perfusate (G. Campbell, unpublished observations). For this reason the 5-HT release into the bath fluid was measured. Mouse stomach was used and,

since it is very small, the combined output from five stomachs was determined. Current pulses of 0·1 msec duration were passed, at different frequencies, between two platinum electrodes at the top and bottom of the 10 ml. organ-bath in which the stomachs were suspended. Records of the response produced by field stimulations were indistinguishable from those

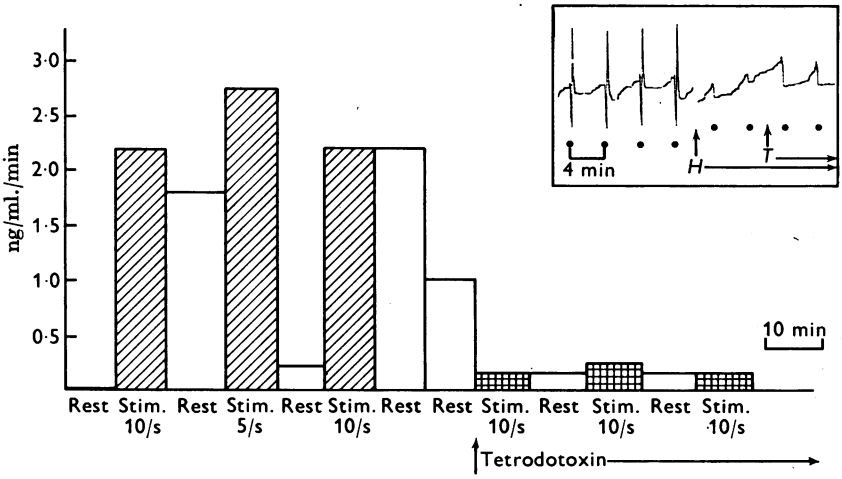

Fig. 10. The combined output of 5-HT from five mouse stomachs assayed on the fundus of the rat stomach. Hyoscine (10^{-7} g/ml.) is present throughout in both donor and recipient baths. Each column represents a successive collection period. Open columns indicate periods of rest, diagonal lines periods of stimulation with 0·1 msec pulses at indicated frequencies consisting of stimulation for 1 min alternating with 1 min rest. The cross-hatched columns indicate periods of stimulation in the presence of tetrodotoxin (10^{-7} g/ml.). Inset: responses of mouse stomach to field stimulation (cf. Fig. 2*b*) before and after adding hyoscine (10^{-7} g/ml.) (*H*). Tryptamine (10^{-5}g/ml.) (*T*) reduces responses.

produced by stimulation of the vagus nerves (see inset Fig. 10). In the presence of hyoscine the responses were purely inhibitory. They were reduced, like the vagal responses, by pentolinium or tryptamine, indicating that they were due to activation of preganglionic vagal fibres.

In order to avoid the possible contamination by 5-HT from non-neural sources (e.g. argentaffin cells), the stomachs were first incubated with the lumen closed off for 3 hr to asphyxiate the mucosa. (Histological examination of fifteen stomachs from three experiments taken after pre-incubation for 3 hr, revealed remnants of mucosa but no demonstrable argentaffin cells. It was therefore unlikely that mucosal 5-HT contributed significantly to the total 5-HT output.) For the experiment, the intraluminal contents were washed out after the 3 hr pre-incubation period and the stomachs were resuspended, again with the lumen closed off. The output of 5-HT was assayed on the fundus of the rat stomach.

Hyoscine was added to the donor bath in order to prevent the contraction of the stomachs during stimulation and the possible mechanical release of 5-HT (Bülbring & Crema, 1959; Paton & Vane, 1963; Bennett *et al.* 1966). Hyoscine was also added to the recipient rat fundus preparation in order to prevent contraction by acetylcholine if present in the samples. The 5-HT receptors of the rat fundus muscle are easily blocked by low concentrations of methysergide which are specific (Bennett *et al.* 1966) and they are not affected by hyoscine (Vane, 1957) or tetrodotoxin (Gershon, 1966, 1967*a*). A contraction of the rat fundus was considered to have been due to 5-HT if it was blocked by methysergide (10^{-7} g/ml.).

In all preparations stimulation led to the release of 5-HT. In three out of five experiments no resting release could be detected, but in two experiments there was a resting release which was increased, in one threefold and in the other tenfold, by stimulation at 5 c/s. One experiment, in which there was no detectable resting release before stimulation, is illustrated in Fig. 10. Stimulation at 10 c/s caused the release of 5-HT and a similar amount was released during the subsequent rest period. The increased release of 5-HT by stimulation was abolished by the addition of tetrodotoxin, indicating a neural origin. The increased release during the period following stimulation was not affected by tetrodotoxin, but remained elevated. However, when the output of 5-HT had fallen to the original level, it was not increased again by stimulation in the presence of tetrodotoxin. The persistent output of 5-HT after a period of stimulation cannot, therefore, be attributed to continuing nervous activity but may be due to slow diffusion out of the stomach wall. Since the release of 5-HT was initiated by stimulation of the stomach after asphyxiation and exclusion of the mucosa and after the addition of hyoscine to prevent contraction, and since the effect was abolished by tetrodotoxin, it seems most likely that the 5-HT was released from nerves in the myenteric plexus.

DISCUSSION

The evidence which has been described has led to the hypothesis that 5-HT, with acetylcholine, is a neurotransmitter in the vagal pathway to the stomach. While acetylcholine appears to be the sole pre- and postganglionic transmitter in the excitatory portion, both 5-HT and acetylcholine appear to participate as preganglionic transmitters in the inhibitory portion of the vagal pathway.

The hypothesis is based, in the first place, on the observation that the action of 5-HT resembles that of vagal stimulation and, secondly, that the vagal inhibitory effects and those of 5-HT are similarly affected by the same antagonistic drugs. The results also indicate that the vagal inhibitory neurones possess separate receptors for acetylcholine and for 5-HT.

5-HT produces a long lasting stimulation of inhibitory neurones and facilitation of vagal inhibitory responses, so strong as to suppress the excitatory component of the vagal effect. Nicotinic drugs, on the other hand (e.g. DMPP) stimulate inhibitory neurones only transiently, then antagonize transmission in the vagal pathway and also block the response to 5-HT. Non-depolarizing ganglion blockers, however (e.g. pentolinium) do not affect the response to 5-HT. This situation is most clearly seen in the presence of nicotine: during the early depolarizing phase of its action, nicotine non-specifically antagonizes both vagal ganglionic transmission and the response to 5-HT. Later, when the blocking action of nicotine is competitive and specific for acetylcholine receptors, the inhibitory response to 5-HT recovers in parallel with the partial recovery of the vagal inhibitory response. This suggests that vagal inhibitory ganglia receive both cholinergic and '5-hydroxytryptaminergic' preganglionic fibres which act synergistically on the same cells.

If this were true, it should be possible to reduce inhibitory vagal responses by 5-HT antagonists without reducing responses to nicotinic compounds, just as the non-depolarizing antagonists of acetylcholine reduce vagal responses without affecting the responses to 5-HT. It was found that the 5-HT receptors of the inhibitory neurones in the stomach behaved in the same way as neural 5-HT receptors in other tissues. They were stimulated and subsequently blocked by the biguanides and the quaternary derivatives of 5-HT (Fastier *et al.* 1959; Gyermek, 1966). They were also desensitized by massive concentrations of 5-HT itself, or, after inhibition of monoamine oxidase, by 5-HTP, or tryptamine, or α-methyltryptamine. The reason for the requirement of monoamine oxidase inhibition (which, by itself, was entirely ineffective) for the block by tryptamine, α-methyltryptamine and 5-HTP is not clear and needs further investigation. While the responses to 5-HT were blocked, those to DMPP, (−)-noradrenaline and sympathetic nerve stimulation were unaffected. The antagonism (except with some biguanides) was specific for 5-HT and was always accompanied by a reduction of the vagal inhibition of the stomach. The relaxant effect of vagal stimulation in the presence of hyoscine was completely abolished only when a competitive block of acetylcholine receptors was combined with desensitization of 5-HT receptors.

An attempt was made to deplete 5-HT stores by reserpine treatment in order to see whether this interfered with the vagal inhibitory pathway. Amine depletion as evident from the failure of sympathetic nerve stimulation, was successful in only two out of sixteen animals. In these, vagal inhibition of the stomach was weak while relaxation by 5-HT was larger than in normal preparations. Exposure to 5-HT, for as little as 90 sec, markedly increased subsequent vagal inhibitory responses, indicating

replenishment of depleted 5-HT stores. In all sixteen animals treated with reserpine, the vagal response fatigued rapidly and this fatigue was prevented by addition of 5-HT to the bath.

The effect of reserpine cannot be ascribed to depletion of catecholamines (Carlsson, 1966). The combination of propranolol and phenoxybenzamine blocked the inhibitory effect of sympathetic nerve stimulation and (−)-noradrenaline without affecting the vagal inhibition (Table 2). This observation confirmed the view of Martinson (1965*a*, *b*) and Campbell (1966) that the vagal inhibitory nerves are not adrenergic.

If 5-HT is involved as neurotransmitter it should be released during vagal stimulation. The detection of this neural release is, however, complicated by the fact that large amounts of 5-HT are known to be present in the gastric mucosa and may be released by pressure, stretch, peristalsis and other contractions of the muscles in the gastrointestinal wall (for references see Bülbring, 1961).

A release of 5-HT during vagal or transmural stimulation of the stomach, with intact mucosa, has already been reported. While Paton & Vane (1963) examined the fluid collected from the lumen separately from that collected from the bath, Bennett *et al.* (1966) tested both together, so that much of the 5-HT must have come from argentaffin cells. Their results are therefore not strictly relevant to the question of a neural origin.

In the present experiments, the neural origin of the 5-HT released on stimulation of the mouse stomach with pulses of brief duration is indicated by the abolition of the release with tetrodotoxin. Release from argentaffin cells is unlikely since the experiments were performed after asphyxiation of the mucosa, with the lumen closed off, and in the presence of hyoscine to prevent contractions.

It remains to be proved that the preganglionic nerve terminals in the myenteric plexus in which 5-HT has been localized by radioautography are really from preganglionic vagal fibres. The release of 5-HT on vagal stimulation together with the observations that vagal effects and 5-HT effects are both inhibitory in the presence of hyoscine and that they are altered in parallel fashion by nicotine, 5-HT antagonists, and 5-HT desensitization, support the view that 5-HT participates as transmitter in the vagal inhibitory pathway to the stomach.

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