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A PHARMACOLOGICAL INVESTIGATION OF HUMAN ISOLATED STOMACH

BY

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Although gastrointestinal muscle is frequently removed from patients at operation, there have been relatively few pharmacological investigations of smooth muscle preparations from man. Graham (1949) studied isolated longitudinal muscle strips from the human stomach, and Walder (1953) studied gastric muscularis mucosae. Since then, there have been pharmacological investigations of oesophagus (Trounce, Deuchar, Kauntze & Thomas, 1957; Ellis, Kauntze, Nightingale & Trounce, 1960), colon (Trounce & Nightingale, 1960; Fishlock & Parks, 1963a; Bucknell & Whitney, 1964), jejunum (Whitney, 1965), ileum (Bennett, 1965) and duodenum (Bennett & Whitney, 1966).) The present paper reports results of a pharmacological investigation of human stomach.

METHODS

Specimens obtained from stomach resected for gastric carcinoma or for gastric or duodenal ulceration, were taken to the laboratory in Krebs solution at room temperature. Macroscopically normal strips of stomach wall, approximately 3 mm. wide and 3 cm. long, were cut parallel to the circular or longitudinal muscle fibres at least 6 cm. from the diseased area. The mucosa and submucosa were removed and the strips suspended in Krebs solution at 37° C. in a 5 ml. bath aerated with 95% oxygen and 5% carbon dioxide. Acetylcholine and potassium were generally left in the bath for 45 sec. The other agonists were usually left in for 1 min. When a drug produced no response, an inhibitory effect was looked for by adding acetylcholine or potassium to the bath with the drug still present. The bath was then washed out by overflow. Whenever possible, doses were given at 3 min intervals. The tissue responses were magnified $\times 8$ by a frontal writing isotonic lever and were recorded on a smoked drum. The load on the tissue was 0.8–1.4 g. Most specimens were used the same day, but some were stored overnight in Krebs solution at 4° C. Neither storage nor removal of the mucosa and submucosa appeared to affect the responses of the tissues.

Drugs. The drugs used were: acetylcholine perchlorate, (—)-adrenaline bitartrate, cocaine hydrochloride, dimethylphenylpiperazinium iodide (DMPP), guanethidine sulphate, hexamethonium bromide, histamine acid phosphate, "Hydergine" (a mixture of equal parts of dihydroergocornine, dihydroergokryptine and dihydroergocrystine as methanesulphonates), 5-hydroxytryptamine creatinine sulphate (5-HT), (—)-hyoscine hydrobromide, (±)-isoprenaline sulphate, lignocaine hydrochloride, mepyramine maleate, methysergide hydrogen maleate, neostigmine methylsulphate, nicotine acid tartrate, (—)-noradrenaline bitartrate, pentolinium tartrate (±)-phenylephrine hydrochloride, physostigmine sulphate, potassium chloride, and pronethalol hydrochloride. Drug concentrations are expressed as μg/ml. of the final bath concentration of the base, except for "Hydergine" which is expressed as the salt. All the drugs were dissolved in Krebs solution. Ascorbic acid (10 μg/ml.) was added to solutions of adrenaline, isoprenaline and noradrenaline.

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RESULTS

The effect of the agonist drugs are listed in detail in Table I. The responses of circular and longitudinal muscle strips from any one region were similar.

Spontaneous activity almost always occurred in strips of gastric body (corpus) and antrum, but seldom occurred in strips of pyloric ring (pyloric sphincter). Strips of corpus tended to shorten at the start of the experiment. Inhibitory drugs relaxed most strips of corpus and a few strips of antrum and pyloric ring. In those preparations which were not relaxed by inhibitory drugs the responses to acetylcholine or potassium were reduced.

TABLE 1

THE RESPONSES OF STOMACH MUSCLE STRIPS TO DRUGS

Drug	Part of stomach	No. of strips studied		Approximate lowest effective drug concentration (μg/ml.)	Response					
					Contraction		Relaxation or inhibition		No effect	
		Circ.	Long.							
Acetyl-choline	Corpus	22	15	0·1 – 0·8	37		0		0	
	Antrum	43	36	0·05– 0·8	79		0		0	
	Pyloric ring	8	4	1 –10	12		0		0	
Sympatho-mimetic amines	Corpus	11	6	Phenylephrine 4 –20	0		17		0	
	Antrum	25	14	Noradrenaline 0·02–1	0		39		0	
	Pyloric ring	8	4	Adrenaline 0·02–1 Isoprenaline 0·04–1	0		12		0	
					Circ.	Long.	Circ.	Long.	Circ.	Long.
5-HT	Corpus	16	11	0·1–10	14	7	0	0	2	4
	Antrum	11	9	0·2–16	0	0	10	5	1	4
	Pyloric ring	3	2	10 –20	0	0	2	1	1	1
Histamine	Corpus	16	7	0·5–10	9	6	2	0	5	1
	Antrum	15	10	0·2–10	5	5	10	5	0	0
	Pyloric ring	3	2	5 –20	0	0	1	1	2	1
DMPP or nicotine	Corpus	16	9	3–25	8	3	3	5	5	1
	Antrum	6	18	3–25	1	5	4	6	1	7
	Pyloric ring	5	1	6–25	0	0	4	0	1	1

Acetylcholine. All muscle strips, including circular and longitudinal muscle strips cut from the pyloric ring, were contracted by acetylcholine. In general, the antrum was more responsive than the corpus; it was much more responsive than the pyloric ring which was capable of only small contractions. The effects of acetylcholine on preparations from each region were potentiated by physostigmine or neostigmine (0.1 μg/ml.) and abolished by hyoscine (0.1 μg/ml.; Fig. 1). The contractions were either unaffected by hexamethonium (20–40 μg/ml.; eight experiments, Figs. 7, 8), or were reduced by 10 to 60% (seven experiments).

Sympathomimetic amines—Phenylephrine, noradrenaline, adrenaline and isoprenaline always inhibited spontaneous contractions and sometimes produced an overt relaxation (Fig. 2) of corpus, antrum and pyloric ring. They reduced the responses to acetylcholine and potassium (Figs. 3, 4, 10) in the other preparations. A measure of the inhibitory effect of adrenaline on antral preparations was obtained using dose ratios (Gaddum, Hameed, Hathaway & Stephens, 1955). These are the ratios of equi-effective doses of agonist in the presence and absence of antagonist. Adrenaline (0.4 μg/ml.) reduced the response to a standard dose of acetylcholine by 63 to 100% (six experiments) and the dose

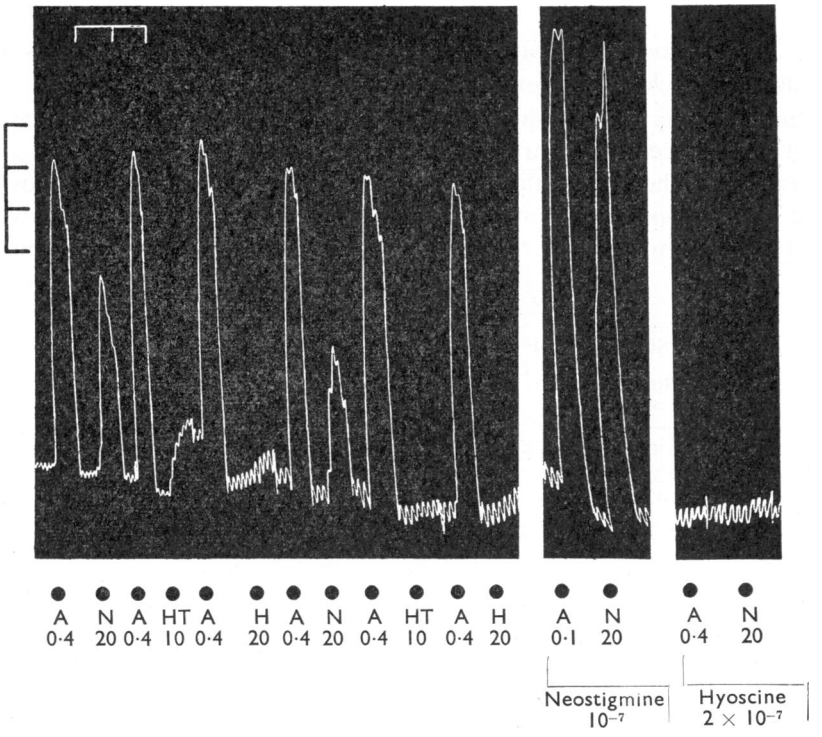

Fig. 1. The effect of drugs on a longitudinal muscle strip of corpus. Acetylcholine (A), nicotine (N), 5-HT (HT) and histamine (H) cause contractions, but tachyphylaxis to the three latter drugs occurs. After 5-HT has been washed out, the tissue does not relax fully. Neostigmine potentiates, and hyoscine blocks, the responses to acetylcholine and nicotine. The numbers represent drug concentrations in μg/ml. of bath fluid. Time trace, 1 min. Vertical scale in cm.

of agonist had to be increased 2 to 20 times to restore the response to its original size. The same concentration of adrenaline reduced the response to potassium by 10 to 88% (five experiments ; Fig. 3), but the dose ratio with this agonist was only 1.2 to 2.

The adrenaline α-receptor antagonist "Hydergine" (1μg/ml.) abolished both the relaxations and the inhibitory effects due to phenylephrine and noradrenaline. It reduced the effect of adrenaline (Fig. 2) but not of isoprenaline. The adrenaline β-receptor antagonist pronethalol (8 μg/ml.) blocked the effect of isoprenaline, reduced that of adrenaline and sometimes reduced the effects of noradrenaline and phenylephrine. Both antagonists together abolished the actions of any of the sympathomimetic amines (Figs. 2, 6, 10). They also sometimes reduced the response to acetylcholine (Figs. 6, 10).

5-Hydroxytryptamine. 5-HT produced small contractions of strips from the corpus. The tissue often tended to stay contracted after the drug had been removed from the bath (Fig. 1). The effects of 5-HT on the antrum and pyloric ring, however, were inhibitory ; it reduced the responses to both acetylcholine and potassium (Figs. 3, 4). Some preparations from each region were unresponsive to 5-HT.

A prolonged tachyphylaxis to both the contractor and inhibitory actions of 5-HT often occurred. This made the analysis of drug action difficult. In order to determine the increase in the dose of acetylcholine or potassium required to overcome the inhibitory effect of 5-HT on antral strips it was necessary to use only one dose to prevent the influence of tachyphylaxis. Accordingly, 5-HT was added to the bath for 1 min and doses of agonist were added at intervals of 45 sec until the response was restored to its original height. 5-HT (2 μg/ml.) reduced the response to acetylcholine by 25 to 58%. The dose ratio required to restore the response was 2 to 3 (five experiments). The same concentration of 5-HT reduced the responses of antral strips to potassium by 13 to 45% ; the dose ratio was 1.2 to 1.5 (two experiments ; Fig. 3).

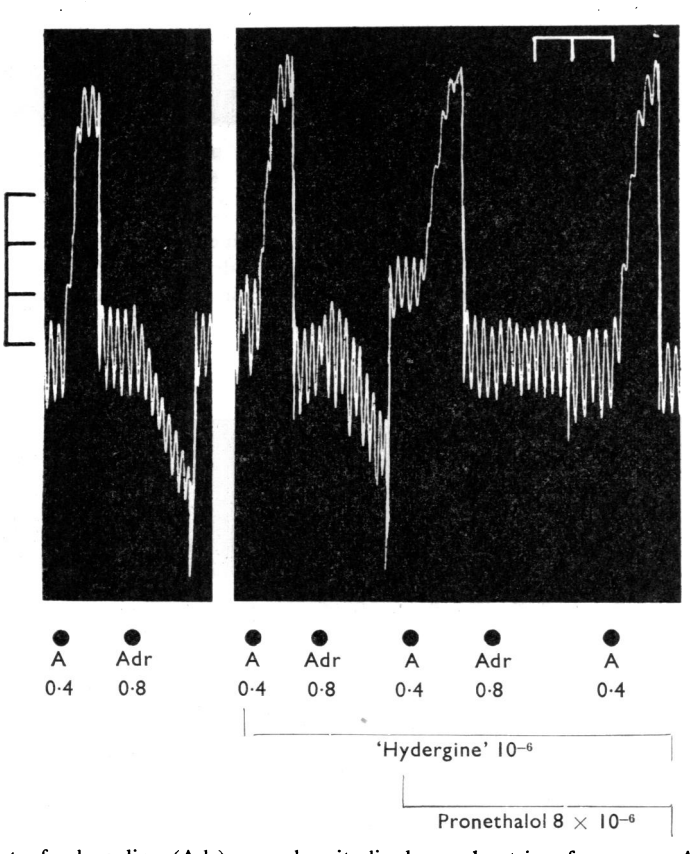

Fig. 2. The effect of adrenaline (Adr) on a longitudinal muscle strip of corpus. Adrenaline relaxes the tissue ; its effects, but not those of acetylcholine (A), are reduced by " Hydergine " and prevented by " Hydergine " and pronethalol. The numbers represent drug concentrations in μg/ml. of bath fluid. Time trace, 1 min. Vertical scale in cm.

The contractile and inhibitory effects of 5-HT were abolished by methysergide (0.05–0.3 μg/ml.). Further analysis was difficult because of tachyphylaxis but it was found that guanethidine (8 μg/ml.) in one experiment, and pronethalol (8 μg/ml.) in another did not alter the inhibitory effect of 5-HT on responses of antral strips to acetylcholine.

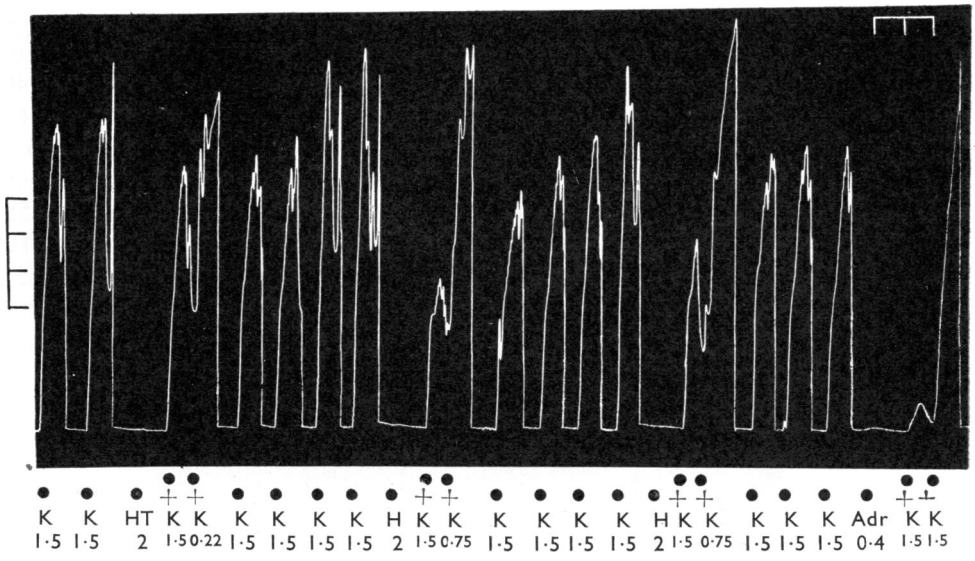

Fig. 3. The effects of 5-HT (HT), histamine (H) and adrenaline (Adr) on the responses of a longitudinal muscle strip of antrum to potassium (K). The drugs reduced the effect of potassium, but addition of more agonist restored the responses to their original level. The numbers represent drug concentrations in μg/ml. of bath fluid, except for potassium which is in mg/ml. Time trace, 1 min. Vertical scale in cm.

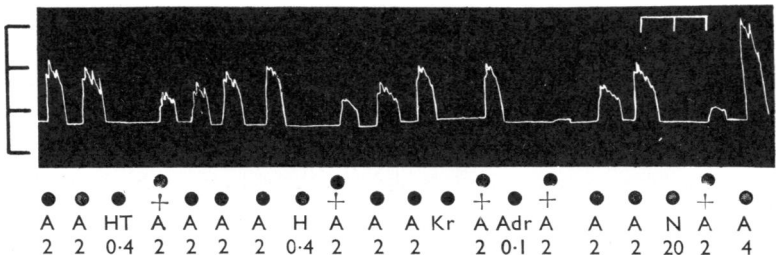

Fig. 4. The response of a circular muscle strip from the pyloric ring to 5-hydroxytryptamine (HT), histamine (H), adrenaline (Adr) and nicotine (N). Kr represents 0.2 ml. of Krebs solution. All the drugs reduce the contraction produced by acetylcholine (A), but Krebs solution has no effect. The numbers represent drug concentrations in μg/ml. of bath fluid. Time trace 1 min. Vertical scale in cm.

Histamine. The effects of histamine were variable. Strips from the corpus generally responded with small contractions (Figs. 1, 5). However, small relaxations occurred in two experiments, and some preparations were unaffected. Histamine either produced small contractions of antral preparations or inhibited the responses to acetylcholine or potassium (Figs. 3, 5, 6); in two experiments a small relaxation occurred. Histamine either inhibited the response of strips of pyloric ring to acetylcholine (Fig. 4), or had no effect.

A prolonged tachyphylaxis to histamine often occurred, but was generally not as marked

as with 5-HT. Thus the response sometimes increased with the dose (Fig. 5). With those experiments on antral strips in which histamine (2 μg/ml) was inhibitory, the responses to acetylcholine were reduced by 30 to 90% and the dose ratio ranged from 2.5 to 5.5 (five experiments). With potassium as the agonist, the responses were reduced by 18 to 61% and the dose ratio was 1.2 to 1.5 (two experiments).

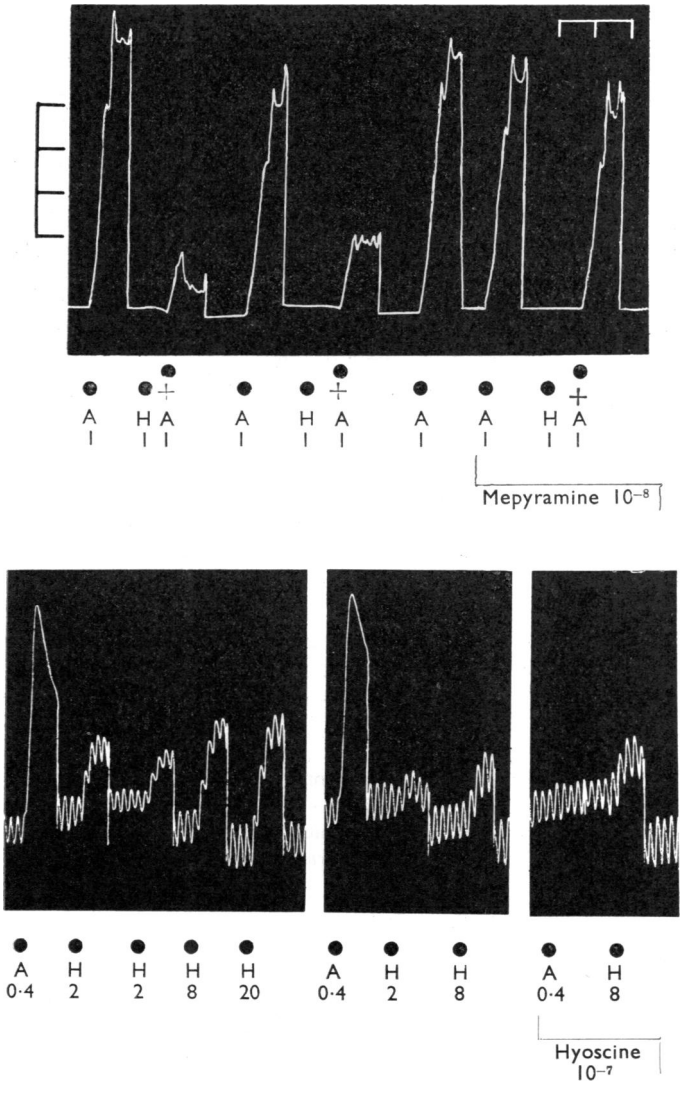

Fig. 5. *Top trace.* The effect of histamine (H) on the response of a circular muscle strip of antrum to acetylcholine (A). Histamine reduces the effect of acetylcholine ; the reduction is antagonized by mepyramine. *Bottom trace.* The effect of histamine (H) on a longitudinal muscle strip of corpus. Histamine causes contractions which increase with higher drug concentrations. Hyoscine blocks the effect of acetylcholine (A) but not of histamine. The numbers represent drug concentrations in μg/ml. of bath fluid. Time trace, 1 min. Vertical scale in cm.

Mepyramine (10–100 ng/ml.) prevented both the contractile and the inhibitory effects of histamine. The contractions were unaffected by hyoscine (0.1 μg/ml.; corpus, three experiments, Fig. 5; antrum, one experiment) or hexamethonium (20 μg/ml.; antrum, one experiment). The relaxations or the inhibitions of responses to acetylcholine were unaffected by "Hydergine" (1 μg/ml.) and pronethalol (8 μg/ml.; antrum, two experiments, Fig. 6), guanethidine (8μg/ml.; antrum, one experiment) or hexamethonium (20 μg/ml.; corpus, one experiment).

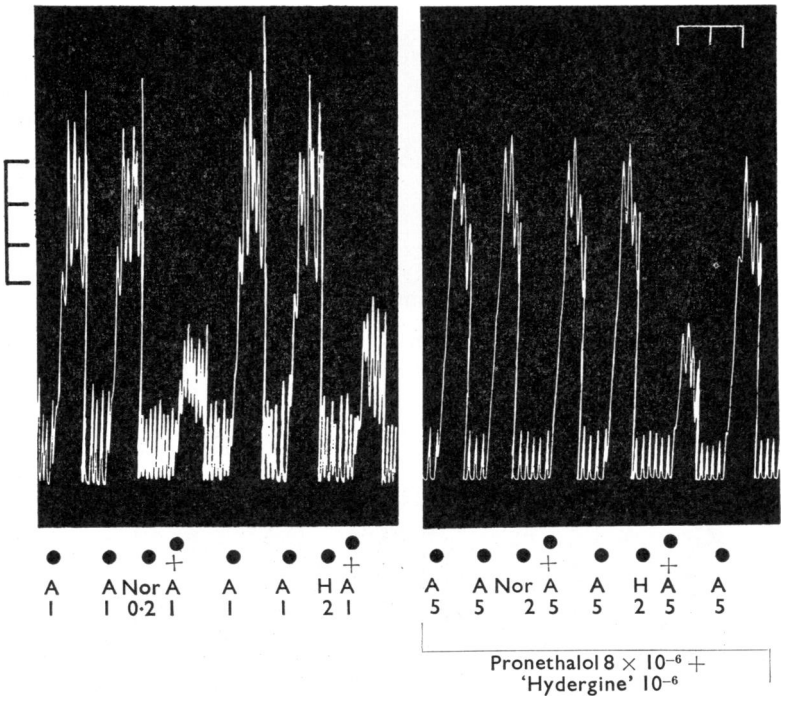

Fig. 6. The effect of blocking the adrenaline receptors in a circular muscle strip of antrum on the relaxant effect of histamine (H). Pronethalol + "Hydergine" prevent noradrenaline (Nor) but not histamine from reducing the response of acetylcholine (A). The numbers represent drug concentrations in μg/ml. of bath fluid. Time trace 1 min. Vertical scale in cm.

DMPP and Nicotine. Strips of corpus responded to DMPP and nicotine with small contractions (Fig. 1) or relaxations. These drugs either contracted antral strips or inhibited the responses to acetylcholine (Figs. 7, 8, 9) or potassium; small relaxations, however, occurred in four preparations. DMPP and nicotine either relaxed strips of pyloric ring or inhibited the responses to acetylcholine (Fig. 4). Some of the preparations from each region were unaffected by the drugs. As with 5-HT and histamine, tachphylaxis often developed. With those experiments on antral strips in which nicotine (20 μg/ml.) was inhibitory the responses to acetylcholine were reduced by 35 to 90% and the dose ratio varied from 2 to 6 (four experiments); the responses to potassium were reduced by 10 to 50% and the dose ratio was 1.2 to 2 (four experiments). Using DMPP (20 μg/ml.), the reduction of response was 28 to 85% with acetylcholine and 10 to 26% with potas-

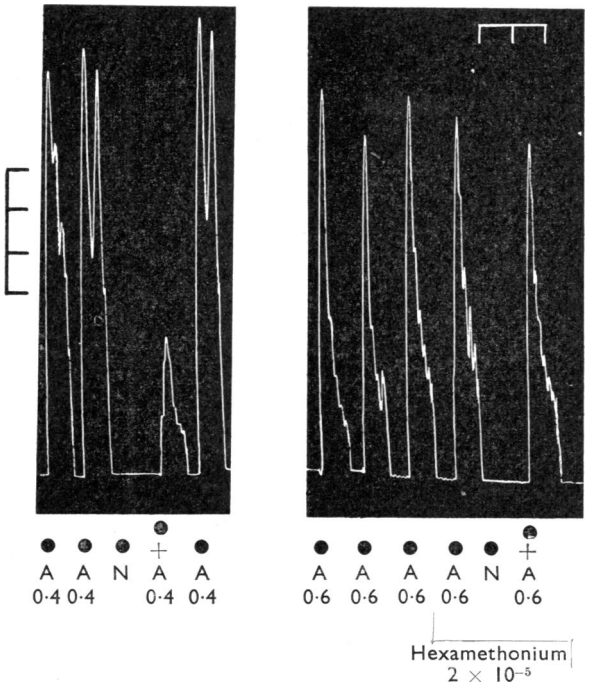

Fig. 7. The effect of hexamethonium on the inhibitory effect of nicotine (N) on longitudinal antral muscle. Because of tachyphylaxis, two strips from the same specimen were set up simultaneously. Nicotine reduced the response to acetylcholine (A) in the left hand but in the presence of hexamethonium (right hand tracing) nicotine has no effect. The numbers represent drug concentrations in μg/ml. of bath fluid. Time trace, 1 min. Vertical scale in cm.

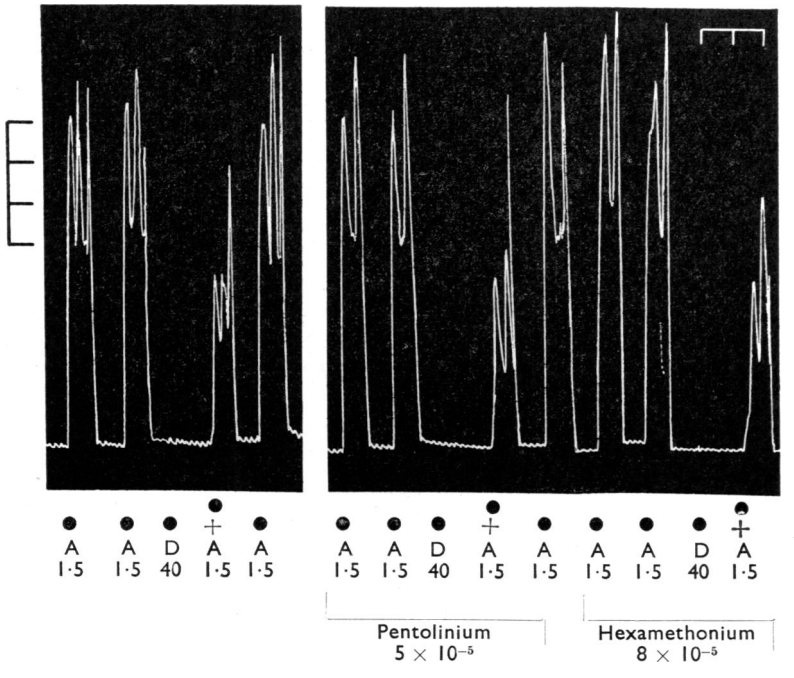

Fig. 8. The effect of ganglion blocking drugs on the inhibitory action of DMPP (D) on a longitudinal antral muscle strip. The ability of DMPP to reduce the response to acetylcholine is unaffected by pentolinium or hexamethonium. The numbers represent drug concentrations in μg/ml. of bath fluid. Time trace, 1 min. Vertical scale in cm.

sium. The dose ratios were respectively 2, 4 and 4 (three experiments) and 1.2, 1.4 and 1.5 (three experiments). To overcome the problem of tachyphylaxis, it was sometimes necessary to set up simultaneously two preparations from the same specimen, and add a drug antagonist to one preparation before its first dose of DMPP or nicotine (Fig. 7).

The actions of DMPP and nicotine were blocked by hexamethonium 20 to 40 μg/ml.; Fig. 7). The relaxations and inhibitions were blocked by " Hydergine " (1 μg/ml.) and pronethalol (8 μg/ml.). The contractions were potentiated by physostigmine or neostigmine (0.1 μg/ml., nine experiments) and blocked by hyoscine (0.1 to 0.2 μg/ml.; five experiments, Fig. 1). In two experiments with strips from the corpus, hyoscine converted the contractions into relaxations, and in another experiment neostigmine converted the relaxation to a contraction.

As antral strips seldom possessed " tone," the inhibitory effect of DMPP was demonstrated in the initial experiments as a reduced response to acetylcholine (Fig. 8). This inhibition differed from that of nicotine since it could be obtained repeatedly on the same tissue, whereas tachyphylaxis to nicotine developed quickly. The various procedures described below indicated that this repeatable inhibitory effect of DMPP was due to antagonism of acetylcholine. As the inhibitory effect of DMPP in the initial experiments on antral strips may have been due to antagonism of acetylcholine and not to stimulation of a relaxant mechanism, these results have been excluded from Table I.

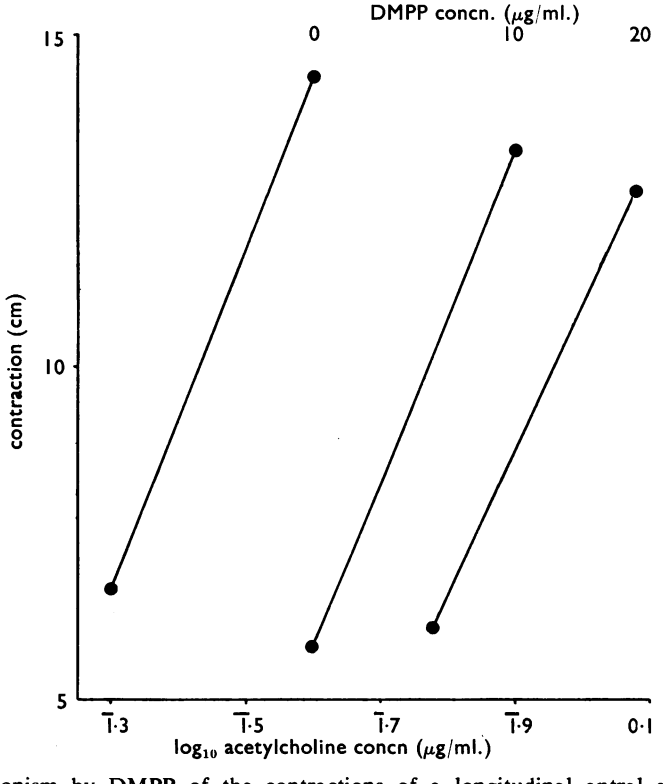

Fig. 9. The antagonism by DMPP of the contractions of a longitudinal antral strip produced by acetylcholine.

The reduction of the response of antral strips to acetylcholine increased with the dose of DMPP (Fig. 9). The reduction occurred in the presence of hexamethonium (20–80 μg/ml.; six experiments), pentolinium (20–50 μg/ml.; one experiment, Fig. 8), " Hydergine " (1 μg/ml.) and pronethalol (8 μg/ml.; three experiments, Fig. 10), guanethidine (8 μg/ml.; one experiment), cocaine (10–20 μg/ml.; two experiments) and lignocaine (20 μg/ml.; one experiment). The effects of DMPP and nicotine were then examined on the response of antral strips to potassium (0.5–1.5 mg/ml.), since this substance produced a contraction which was not affected by hyoscine (0.1–1 μg/ml. or hexamethonium (20–40 μg/ml.). Both ganglion stimulants caused an initial reduction of the contraction but subsequent doses were ineffective. This reduction was prevented by hexamethonium (20–40 μg/ml.) or by " Hydergine " (1 μg/ml. and pronethalol (8 μg/ml.).

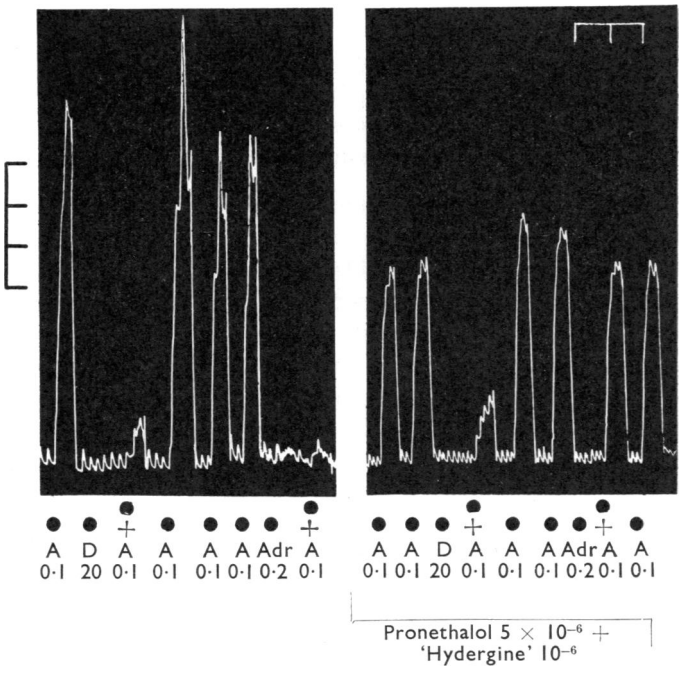

Fig. 10. The effect of blocking the adrenaline receptors in a circular muscle strip of antrum on the inhibitory effect of DMPP (D). Pronethalol + " Hydergine " prevent adrenaline (Adr) from reducing the response to acetylcholine (A) but have no effect on the response to DMPP. The numbers represent drug concentrations in μg/ml. of bath fluid. Time trace, 1 min. Vertical scale in cm.

DISCUSSION

Bucknell & Whitney (1964) found that the responses of tissues to drugs were not influenced by the disease for which the operation was performed. Fishlock & Parks (1963b, 1966) found that variations in premedication and anaesthesia made no difference to the responses of colonic muscle to acetylcholine or nicotine. It therefore seems likely that results from specimens removed at operation represent the pharmacology of untreated isolated tissue.

The responses of strips of stomach and pyloric ring to acetylcholine were due to stimulation of " muscarinic " receptors, since the contractions were potentiated by anticholinesterases and prevented by hyoscine. Part of the contraction, however, may sometimes have been due to stimulation of parasympathetic ganglia since hexamethonium sometimes reduced the response.

Since phenylephrine, noradrenaline, adrenaline and isoprenaline were only inhibitory, and were antagonized by " Hydergine " and pronethalol, it appears that the corpus, antrum and pyloric ring contain α- and β- (Ahlquist, 1948) adrenaline inhibitory receptors. The classical view (e.g. Goodman & Gilman, 1955) that adrenaline contracts the gastrointestinal sphincters does not appear to be true for the human isolated pyloric sphincter.

Fishlock, Parks & Dewell (1965) reported that 5-HT contracted longitudinal muscle strips from the corpus. A contraction was seldom evoked from circular muscle strips of corpus and never from antral strips. We, however, have been unable to find any difference between longitudinal and circular muscle strips from the corpus in their response to 5-HT. Our results confirm that 5-HT does not contract antral muscle, and show in addition that 5-HT inhibits the responses of this tissue to acetylcholine and potassium.

The action of 5-HT was difficult to examine because of tachyphylaxis. The results indicate that 5-HT acts directly on the muscle ; its effects were prevented by methysergide which blocks the direct action of 5-HT (Day & Vane, 1963), and its inhibitory action occurred in the presence of guanethidine or pronethalol. The possibility that the inhibibition is mediated through non-adrenergic inhibitory nerves (Burnstock, Campbell & Rand, 1966) has not been excluded. Such an explanation seems unlikely, however, since the action of 5-HT is unaffected by pronethalol which is a potent local anaesthetic (Gill & Vaughan Williams, 1964). Fishlock *et al.* (1965) did not report tachyphylaxis to 5-HT in their experiments. They found that the contractions of strips of corpus were antagonized by lysergic acid diethylamide but not by atropine or procaine. They, too, concluded that 5-HT acts directly on the muscle.

As with 5-HT, the results indicate that histamine acts directly on the muscle ; its effects were abolished by mepyramine, the contractions were unaffected by hyoscine, and the relaxant and inhibitory actions were unaffected by adrenergic neurone blockade or by " Hydergine " and pronethalol. The site of action of histamine is therefore the same as in the rest of the human gastrointestinal tract (Bennett & Whitney, 1966). This is unlike the guinea-pig and kitten in which histamine contracts the stomach by acting on nervous tissue (Paton & Vane, 1963).

The experiments with DMPP and nicotine demonstrated, as did most of the other drugs studied, how unreactive isolated preparations of the human stomach are compared with the rest of the gastrointestinal tract. As with histamine, the effects of DMPP and nicotine were variable and tachyphylaxis developed quickly. The contractions were apparently due to stimulation of parasympathetic ganglia since they were blocked by hexamethonium or hyoscine and potentiated by anticholinesterases. The relaxations or the inhibitory effects on acetylcholine and potassium were apparently due to stimulation of adrenergic nervous tissue (see Bennett & Whitney, 1966) since they were prevented by hexamethonium or by " Hyergine " and pronethalol.

The inhibitory effects of sympathomimetic amines, 5-HT, histamine, DMPP and nicotine on preparations which were not relaxed by these drugs were demonstrated by their ability to reduce the responses to acetylcholine and potassium. It seems probable that these inhibitions were due to physiological antagonism since these drugs sometimes relaxed strips from other specimens, they affected acetylcholine and potassium similarly, they were blocked by specific antagonists, and the dose ratios were small.

During the course of this study, another pharmacological action of DMPP was encountered. An inhibitory effect of DMPP, but not of nicotine, could be repeatedly produced with the same antral strip when acetylcholine was the agonist used. With potassium, only the first dose of either DMPP or nicotine was inhibitory, apparently by an adrenergic mechanism; subsequent doses had no effect because of tachyphylaxis. The repeatable effect of DMPP on responses to acetylcholine was not due to stimulation of nervous tissue or to the release of sympathomimetic amines, since the phenomenon was unaffected by local anaesthetic drugs or by adrenaline antagonists. The inhibition therefore appears to be due to a direct antagonism of acetylcholine. It is known that DMPP can block ganglia (Chen & Portman, 1954) and the skeletal neuromuscular junction (Kaller, 1956; Ling, 1959). Day & Vane (1963) have shown that it is a weak agonist at "muscarinic" sites, and we conclude that DMPP reduces the response to acetylcholine by occupying "muscarinic" receptors. The antagonism is weak, however, since the dose ratio is small with quite large doses.

SUMMARY

(1) A study has been made of the effects of acetylcholine, sympathomimetic amines, 5-HT, histamine, DMPP and nicotine on human isolated stomach muscle. The latter four drugs produced only small effects or none, and tachyphylaxis to them often occurred. Strips of circular and longitudinal muscle from any one region responded similarly to each other.

(2) Acetylcholine contracted all muscle strips by stimulating "muscarinic" receptors. The degree of response of different regions varied (antrum>corpus>pyloric ring).

(3) The sympathomimetic amines relaxed all muscle strips or inhibited the responses to acetylcholine or potassium.

4. 5-HT appeared to act directly on the muscle. It contracted strips of corpus but inhibited antral muscle.

5. Histamine either contracted or inhibited strips from the corpus and antrum by acting directly on the muscle.

6. DMPP and nicotine either contracted or inhibited preparations from the corpus and antrum. The contractions were due to stimulation of parasympathetic ganglia, and the relaxations were apparently due to an action on adrenergic tissue.

7. DMPP has a weak blocking action at "muscarinic" receptors.

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