

# Analysis of the motor effects of gastrin and pentagastrin on the human alimentary tract *in vitro*

A. BENNETT, J. J. MISIEWICZ, AND SHEILA L. WALLER

*From the Department of Surgery, King's College Hospital Medical School, London, and the Medical Research Council Gastroenterology Research Unit, Central Middlesex Hospital, London*

**EDITORIAL COMMENT** This careful *in vitro* analysis shows that gastrin and its synthetic analogue pentagastrin stimulate motor activity in gastric antral muscle and perhaps in the colon but seem to have virtually no effect upon small intestinal muscle.

Exogenous gastrin and its synthetic analogue pentagastrin (I.C.I. 50,123) stimulate the secretion of gastric juice in man (Makhlouf, McManus, and Card, 1964; Wormsley, Mahoney, and Ng, 1966), but little is known about their effect on human gastrointestinal muscle. It is not yet clear whether all parts of the alimentary tract are contracted by gastrin, or how closely the effects of the pentagastrin resemble those of the hormone itself. Nor is it known whether the motor effects of gastrin and pentagastrin in man are due to stimulation of intrinsic nerves or to a direct action on the smooth muscle cell. We have examined these problems by studying isolated strips of human gastrointestinal muscle.

## METHODS AND MATERIALS

Human gastrointestinal tissue was taken from macroscopically normal parts of gut removed at operations for various gastrointestinal diseases. The mucosa and submucosa were removed and strips of muscle measuring approximately  $20 \times 3$  mm. were cut parallel to the longitudinal or circular layers. The strips were suspended at a tension of 0.5 to 1.5 g. in an organ bath filled with Krebs' solution aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at 37°C. The responses of the tissue were measured with an isotonic lever and recorded either on a smoked drum or with a transducer and a pen writer. Drugs were added to the bath fluid and washed out at fixed time intervals. When reproducible submaximal responses were obtained, the drugs were administered while appropriate potentiating or blocking agents were present. The drugs used were: gastrin I and II, pentagastrin (N-t-butyloxycarbonyl- $\beta$ -Ala. Try. Met. Asp. Phe-L-phenylalanine amide), acetylcholine hydrochloride or perchlorate, atropine sulphate, cocaine hydrochloride, hexamethonium bromide, (-) hyoscine hydrobromide, lignocaine hydrochloride, neostigmine methylsulphate,

physostigmine sulphate and procaine hydrochloride. Drug concentrations are expressed in micrograms of base per millilitre of fluid in the bath.

## RESULTS

Experiments were performed on 173 strips of longitudinal and circular muscle from 111 patients.

The number of strips studied from each site and the number which were contracted by gastrin I or II, or by pentagastrin, is shown in Table I. There was no difference between the effects of gastrin I or II. All the strips were sensitive to acetylcholine within the range of doses previously reported by Bennett and Whitney (1966a) for human tissue.

**OEESOPHAGUS AND STOMACH** Nine strips from seven patients were cut from the distal 5 cm. of the oesophagus. Gastrin (0.05 to 0.5  $\mu$ g./ml.) caused contractions of four out of six strips from both muscle layers, and one of the remaining three strips contracted to pentagastrin.

The 88 strips of gastric muscle came from 57 patients. Gastrin (0.05 to 0.6  $\mu$ g./ml.) or pentagastrin (1 to 5  $\mu$ g./ml.) caused contractions of 42 out of the 88 strips from the body and antrum (Table I). The contractions of gastric strips were frequently vigorous and after gastrin was washed out the tissue relaxed slowly (Fig. 1); the response to a subsequent dose of acetylcholine was often potentiated. A similar potentiation also occurred in four other experiments in which gastrin did not cause a contraction. The responses of eight gastric preparations to repeated doses of gastrin were reproducible but in the remaining strips the responses became smaller or absent. Tachyphylaxis was even more marked with pentagastrin and repeatable responses were never obtained.

TABLE I

Site	Drug	Total No. of Strips	Circular Muscle		Longitudinal Muscle	
			No. of Strips	No. Contracting	No. of Strips	No. Contracting
Lower oesophagus	Gastrin I or II	6	2	2	4	2
	Pentagastrin	3	2	1	1	None
Stomach body	Gastrin I or II	23	16	6	7	3
	Pentagastrin	22	8	3	14	7
Stomach antrum	Gastrin I or II	22	13	7	9	8
	Pentagastrin	21	8	3	13	5
Duodenum	Gastrin I	2	1	None	1	None
	Pentagastrin	4	2	None	2	None
Jejunum	Gastrin I or II	7	3	None	4	None
	Pentagastrin	4	3	None	1	None
Terminal ileum	Gastrin I	6	3	1	3	1
	Pentagastrin	9	7	None	2	None
Ascending colon	Gastrin I	5	2	1	3	2
	Pentagastrin	5	1	1	4	2
Transverse and descending colon	Gastrin I or II	4	4	None	—	—
	Pentagastrin	4	1	None	3	None
Sigmoid	Gastrin I or II	13	7	4	6	3
	Pentagastrin	13	4	None	9	4

**SMALL INTESTINE** In contrast to oesophageal and gastric muscle, none of the 17 duodenal or jejunal strips from 13 patients were contracted by gastrin (0.05 to 10  $\mu\text{g./ml.}$ ) or by pentagastrin (1 to 25  $\mu\text{g./ml.}$ ). Pentagastrin did, however, potentiate the effect of acetylcholine on one longitudinal duodenal preparation. The 15 strips from the terminal ileum (nine patients) were also unaffected, except that the circular and longitudinal muscle from one patient

responded to gastrin with a very small contraction (Table I).

**COLON** The 44 colonic strips were taken from 25 patients. Muscle from the ascending colon was contracted by gastrin (0.5 to 1.0  $\mu\text{g./ml.}$ ) or pentagastrin (5.0 to 10.0  $\mu\text{g./ml.}$ ) in six of 10 experiments; 11 of 26 preparations from the sigmoid colon (usually the taenia) responded to gastrin (0.4 to 1.0  $\mu\text{g./ml.}$ ) or

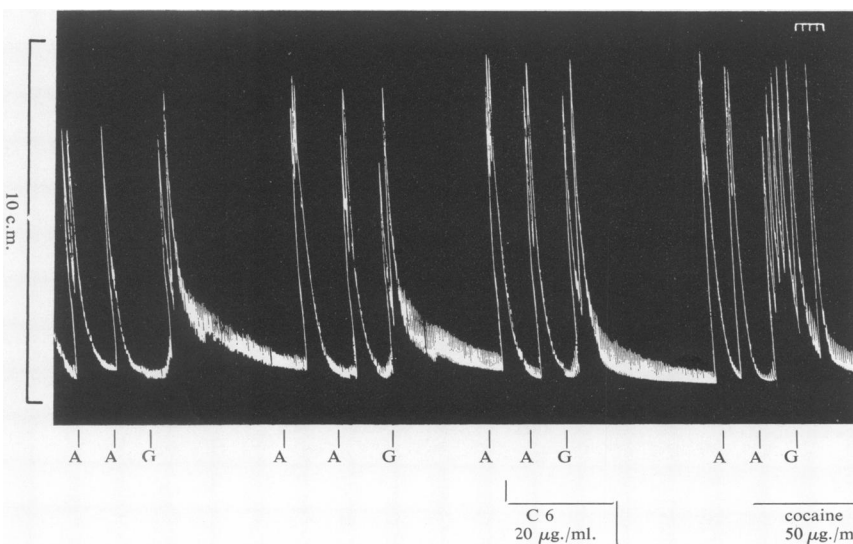


FIG. 1. The responses of an isolated strip of longitudinal muscle from the gastric antrum to acetylcholine 1.2  $\mu\text{g./ml.}$  (A) and to gastrin 4.0  $\mu\text{g./ml.}$  (G). The responses are unaltered by hexamethonium (C6) or cocaine. Time trace 1 min.

pentagastrin (6.0 to 10.0  $\mu\text{g./ml.}$ ). These contractions, however, were very small in comparison with the responses of the gastric strips. With the exception of one preparation from the ascending colon, it was not possible to obtain more than one contraction to either gastrin or pentagastrin. The transverse and descending colon was unaffected by either substance.

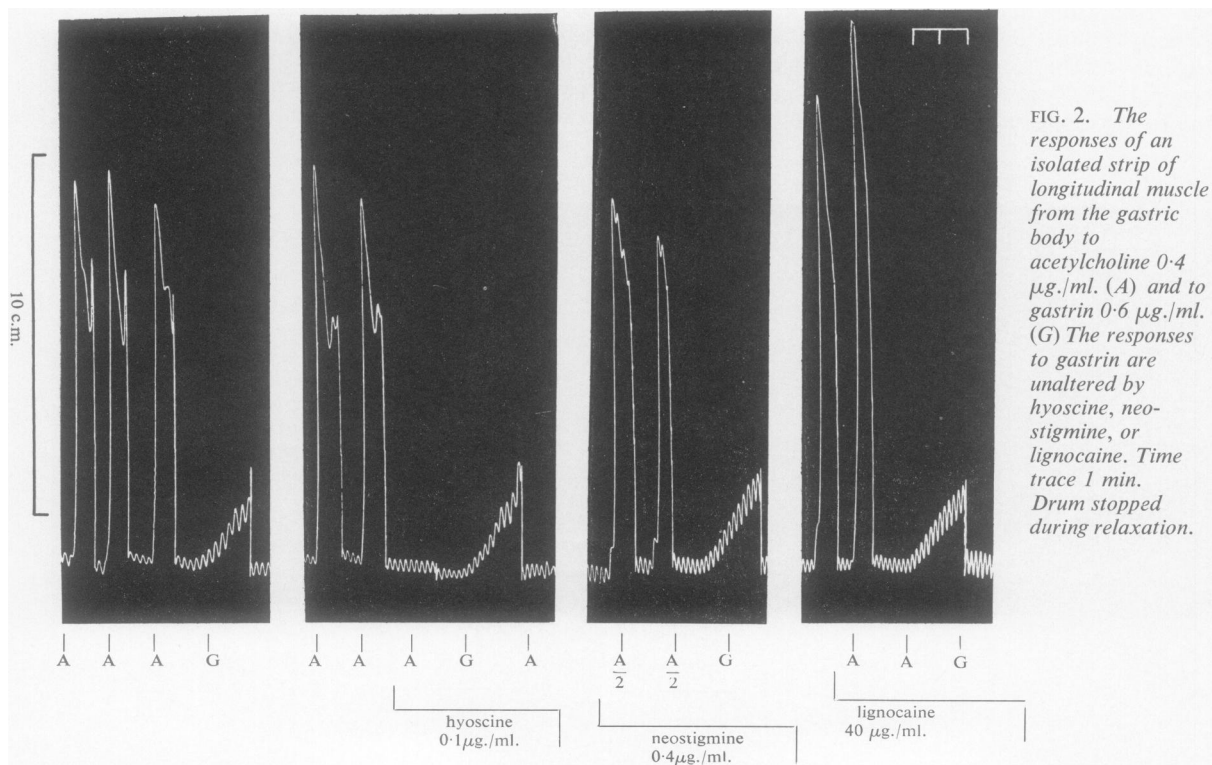
**PHARMACOLOGICAL ANALYSIS OF THE ACTION OF GASTRIN AND PENTAGASTRIN** Drugs which modify the response to acetylcholine or block nerve conduction were used to determine whether gastrin and pentagastrin caused contractions by stimulating the intrinsic nerves or by acting directly on the muscle. Observations of this nature could only be made with strips showing little or no tachyphylaxis. Eight gastric strips proved suitable for pharmacological analysis since reproducible contractions were obtained when gastrin was added at intervals of at least 15 minutes. Hexamethonium (20  $\mu\text{g./ml.}$ ), in doses previously shown to block the excitatory effects of nicotine on intrinsic autonomic ganglia (Bennett and Whitney, 1966b), did not affect the response to gastrin in two experiments (Fig. 1) and anaesthesia of the intrinsic nerves with cocaine (50  $\mu\text{g./ml.}$ , Fig. 1) or with lignocaine (40  $\mu\text{g./ml.}$ , Fig. 2) also had no effect. The anticholinesterases neostigmine and physostigmine (0.1 to 0.6  $\mu\text{g./ml.}$ ) potentiated the response to

acetylcholine, but had no effect on the response to gastrin in seven experiments (Fig. 2). In one preparation, however, neostigmine did cause a slight potentiation which was abolished by hyoscine. In five further experiments, hyoscine (0.1 to 0.5  $\mu\text{g./ml.}$ ) prevented the response to acetylcholine, but had no effect on the response to gastrin (Fig. 2).

Because of tachyphylaxis, it was possible to study the mode of action of gastrin on other regions of the gut in only two experiments. Contractions of an oesophageal strip to gastrin were not affected by neostigmine (0.2  $\mu\text{g./ml.}$ ) or hyoscine (0.2 to 0.4  $\mu\text{g./ml.}$ ) and the latter drug did not alter the contractor effect of gastrin on a strip from the ascending colon.

Tachyphylaxis also interfered with the analysis of the action of pentagastrin on gastric muscle. Nevertheless it was possible to show that contractions to pentagastrin occurred in the presence of hexamethonium (20  $\mu\text{g./ml.}$ , one experiment), procaine (50  $\mu\text{g./ml.}$ , one experiment), atropine (0.2 to 10  $\mu\text{g./ml.}$ , two experiments), or hyoscine (0.4  $\mu\text{g./ml.}$ , one experiment).

In order to determine whether gastrin and pentagastrin acted on the same receptors, two strips from the same specimen were studied simultaneously in separate organ baths on four occasions. In this way it was possible to show that cross tachyphylaxis



could occur between gastrin and pentagastrin, but pentagastrin had no effect on two strips which were subsequently contracted by gastrin.

#### DISCUSSION

The most striking result of this study is that whilst gastrin and pentagastrin contracted gastric muscle, their effect on the colon was weak and the small intestine was virtually unaffected. The effects of both substances were qualitatively similar, but gastrin was more active and less prone to tachyphylaxis than pentagastrin. The lower activity of pentagastrin when compared with gastrin thus includes both its motor and its gastric secretory effects (Barrett, 1966).

The pharmacological analysis of the mode of action of gastrin suggests that the hormone causes contractions by stimulating receptors on or in the muscle cell. An action on intrinsic nerves is excluded since ganglion blockade, local anaesthetics, anticholinesterases, or hyoscine did not alter the response of the muscle strips to gastrin. The mechanism of action of gastrin on isolated human gastrointestinal muscle is therefore similar to that on the hamster stomach (Mikos and Vane, 1967) but different from that on the guinea-pig ileum and the rat colon (Bennett, 1965; Mikos and Vane, 1967).

Our *in vitro* results are not entirely compatible with some of the *in vivo* observations made by others in man. In a preliminary study Smith and Hogg (1966) suggested that a generalized increase in gut motility followed injections of gastrin, and that the effect of the hormone was reduced by atropine. Logan and Connell (1966) found that sigmoid and rectal motility was sometimes increased after intravenous injections of pentagastrin, but this observation has not been confirmed by the recent more extensive studies of Misiewicz, Holdstock, and Waller (1967). On the other hand the present data on gastric muscle *in vitro* agree well with the observations *in vivo* (Misiewicz *et al.*, 1967) that the motor activity of the antrum was stimulated by intravenous infusion of pentagastrin in doses causing submaximal secretion of gastric acid.

There are several possible reasons for the differences between the *in vitro* and *in vivo* observations. *In vitro* techniques measure the effects of drugs which diffuse into the tissue from the surrounding bath fluid; the responses may not be the same when the substances reach the muscle through its vascular supply. Further, vagal tone *in vivo* potentiates gastric secretion induced by gastrin (De la Rosa, Linares, Woodward, and Dragstedt, 1966); it might also enhance the motor effects of the hormone. The reduced effect of gastrin on gastric motility, which Smith and Hogg (1966) observed after atropine,

might have been due in part to an effect on vagal tone. Reduced motor activity would also result from antagonism of acetylcholine released continuously in the gut wall. Thus their results are compatible with our conclusion that gastrin does not cause contraction by stimulating cholinergic nerves.

*In vitro* methods measure only the local effects of a drug. It is possible that stimulation of intestinal motility might be due to an indirect mechanism such as the release of other substances following acidification of the duodenum. Evidence for such a hypothesis has been presented by Misiewicz *et al.* (1967).

Gastrin stimulated some strips of stomach muscle in doses as low as 0.05  $\mu\text{g./ml.}$ , and on a molar basis was approximately 10 times more potent than acetylcholine. As with other substances, such as 5-hydroxytryptamine and histamine (Fishlock, Parks, and Dewell, 1965; Bennett and Whitney, 1966b), not all gastric strips responded to gastrin or pentagastrin, but *in vivo* the antrum was nearly always stimulated by pentagastrin (Misiewicz *et al.*, 1967). It seems possible therefore that gastrin plays a direct role in controlling gastric motor activity. It is of particular interest that the same chemical transmitter may affect both the secretory and motor functions of the stomach.

#### SUMMARY

The effects of gastrin and pentagastrin (I.C.I. 50,123) on isolated strips of human gastrointestinal muscle have been studied. Both substances caused contractions of gastric muscle, but upper and lower small intestine were unresponsive. Strips from the ascending and sigmoid colon responded with small contractions. A pharmacological analysis indicates that both gastrin and pentagastrin stimulate receptors on or in the smooth muscle cell. It is suggested that gastrin may play a physiological role in the control of gastric motor activity.

We thank Dr. D. A. Long and Dr. B. A. L. Hurn of Wellcome Research Laboratories and Dr. J. D. Fitzgerald of I.C.I. for the generous gift of gastrin, and Dr. J. D. Fitzgerald for supplies of pentagastrin (Peptavlon). We are grateful to the many surgeons of King's College and Central Middlesex Hospitals who have provided us with specimens and to Mrs. Vivien Binks for much technical assistance.

#### REFERENCES

- Barrett, A. M. (1966). Specific stimulation of gastric acid secretion by a pentapeptide derivative of gastrin. *J. Pharm. Pharmacol.*, **18**, 633-639.
- Bennett, A. (1965). Effect of gastrin on isolated smooth muscle preparations. *Nature (Lond.)*, **208**, 170-173.
- , and Whitney, B. (1966a). A pharmacological study of the motility of the human gastrointestinal tract. *Gut*, **7**, 307-316.
- , — (1966b). A pharmacological investigation of human isolated stomach. *Brit. J. Pharmacol.*, **27**, 286-298.

- De La Rosa, C., Linares, C. A., Woodward, E. R., and Dragstedt, L. R. (1966). Effect of vagotomy on the gastric secretory response to endogenous gastrin. *Arch. Surg.*, 93, 583-585.
- Fishlock, D. J., Parks, A. G., and Dewell, J. V. (1965). Action of 5-hydroxytryptamine on the human stomach, duodenum, and jejunum *in vitro*. *Gut*, 6, 338-342.
- Logan, C. J. H., and Connell, A. N. (1966). The effect of a synthetic gastrin-like pentapeptide (I.C.I. 50,123) on intestinal motility in man. *Lancet*, 1, 996-999.
- Makhlouf, G. M., McManus, J. P. A., and Card, W. I. (1964). Dose-response curves for the effect of gastrin II on acid gastric secretion in man. *Gut*, 5, 379-384.
- Mikos, E., and Vane, J. R. (1967). Effects of gastrin and its analogues on isolated smooth muscles. *Nature (Lond.)*, 214, 105-107.
- Misiewicz, J. J., Holdstock, D. J., and Waller, S. L. (1967). Motor responses of the human alimentary tract to near-maximal infusions of pentagastrin. *Gut*, 8, 463-469.
- Smith, A. N., and Hogg, D. (1966). Effect of gastrin II on the motility of the gastrointestinal tract. *Lancet*, 1, 403-404.
- Wormsley, K. G., Mahoney, M. P., and Ng, M. (1966). Effect of a gastrin-like pentapeptide (I.C.I. 50,123) on stomach and pancreas. *Ibid.*, 1, 993-996.