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Effect of motilin on the lower oesophageal sphincter[1, 2]

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SUMMARY The effect of motilin on lower oesophageal sphincter (LES) pressure has been studied in unanaesthetised specially trained dogs using an infusion manometric technique. Motilin produced significant rises in resting pressure and contractions of the LES after doses ranging from 0·009 μg/kg to 0·05 μg/kg. Doses greater than 0·05 μg/kg resulted in repetitive high amplitude contractions. Atropine 30 μg/kg completely abolished the effect of the lower doses of motilin. Higher doses of motilin in atropinised dogs still caused a small rise in baseline pressure and contractile activity still appeared. Hexamethonium 2 mg/kg resulted in both a diminished rise in LES pressure and the disappearance of contractions after motilin. Hexamethonium and atropine together completely abolished the LES response to motilin. We conclude that motilin increases LES pressure by acting on preganglionic cholinergic neurones to release acetylcholine which excites other cholinergic neurones supplying the circular muscle of the LES.

Alkalinisation of the duodenum stimulates motor activity of a denervated canine gastric fundal pouch (Brown *et al.*, 1966). Crude duodenal extracts have the same action and recently Brown *et al.* (1971, 1973) have succeeded in isolating and identifying the complete amino acid sequence of the peptide responsible for this action. This substance, motilin, in a dose of 2 μg/kg intravenously results in a marked increase in both the frequency and strength of antral and fundal contractions.

The lower oesophageal sphincter (LES)[5] responds to all known gastrointestinal hormones (Cohen and Lipshutz, 1971; Thomas and Earlam, 1973). Purified natural porcine motilin induces phasic changes in LES pressure in anaesthetised dogs (Jennewein *et al.*, 1975). This study was performed to evaluate the type and mechanism of action of motilin on the lower oesophageal sphincter in the unanaesthetised animal.

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[5]Abbreviations used in this paper: LES—lower oesophageal sphincter, BW—body weight, ID—internal diameter, OD—external diameter.

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Methods

The experiments were done on three healthy adult dogs specially trained to swallow the pressure tube assembly and to lie quietly with the tube *in situ* throughout the period of study. All studies were done after a 12 hour fast.

The pressure tube assembly consisted of six fused polyvinyl catheters (ID 1·11 mm, OD 1·65 mm, total OD 5·0 mm). Each tube had one lateral opening equal in diameter to its circumference. The openings of the four middle tubes faced the same direction and were spread over a total distance of 0·8 cm (2-3 mm apart). The most distal opening was placed 0·5 cm from the distal end of the tube assembly and 5 cm below the four closely spaced holes. The last tube had its recording orifice 5 cm above the closely spaced holes. Each tube was connected to a Statham P23 BB transducer[6], and thence to a Beckman Type R411 Dynograph recorder[7]. Water was constantly infused by a Harvard infusion pump[8] at 0·38 ml/min through each tube during the study.

The tube assembly was swallowed and all recording orifices introduced into the stomach. It was then withdrawn until the central four recording catheters were located in the lower oesophageal sphincter. Throughout the study the position of the tube was maintained so that the respiratory inversion point

[6]Statham Laboratories Inc., Hato Rey, Puerto Rico.
[7]Beckman Instruments Inc., Schiller Park, Illinois, 60176.
[8]Harvard Apparatus Co. Inc., Millis, Mass.

lay below the opening of the second and above the opening of the fifth tube. The proximal tube then recorded intraoesophageal pressure 5 cm above the LES and the distal tube recorded gastric pressure 5 cm below the LES. The LES pressure was expressed in centimetres of water with gastric pressure as the zero reference. The LES pressure values reported in this paper are the mean of end-inspiratory and end-expiratory pressures recorded in the tube closest to the respiratory reversal point. In construction of the dose response curves the maximal resting LES pressures recorded in the 10 minute period immediately preceding and after motilin were used. Swallows and contractions of the LES were excluded in measurements of resting sphincter pressure. Quantitation of the LES response to high doses of motilin was difficult because of the appearance of repetitive contractions with these doses. For this reason, when the effects of various antagonists were compared after high doses of motilin, the maximal LES pressures recorded including the peak of contractions were measured for the 10 minutes immediately before and after the injection of motilin. Respiration was simultaneously recorded by a strain gauge transducer attached to the dog's chest.

Highly purified natural porcine motilin (Brown *et al.*, 1972) was stored at a temperature of +4°C as a solution (100 μg/10 ml saline) and injected doses were diluted fresh for each experiment.

Four series of tests were performed in the present study:

1. The effect of doses of motilin from 0·001 to 0·5 μg/kg body weight was determined on different days. LES pressure was recorded for 10 minutes before and 30 minutes after the intravenous bolus injection of motilin.

2. An intravenous bolus of atropine 30 μg/kg was given after a 10 minute basal period. Ten minutes later an intravenous bolus of motilin was given. Doses of motilin from 0·009 to 0·05 μg/kg body weight were tested after atropine. In an additional eight experiments, instead of motilin, methacholine bromide 12 μg/kg was given as an intravenous bolus 10 minutes after atropine 30 μg/kg.

3. An intravenous bolus of hexamethonium 2 mg/kg was given after a 10 minute basal period. Ten minutes later motilin 0·2 μg/kg was given as an intravenous bolus.

4. A combined intravenous bolus of hexamethonium 2 mg/kg and atropine 30 μg/kg was given after a 10 minute basal recording. Motilin 0·2 μg/kg was given as an intravenous bolus 10 minutes later.

Each of the above tests was done three times in each dog. All were performed on different days and in random order. A minimal interval of three days was allowed in each dog between doses of atropine and hexamethonium.

Results

An intravenous bolus injection of motilin resulted in three characteristic changes in the pressures we recorded from the LES and stomach: a rise of resting LES pressure, the appearance of spontaneous contractions in the LES and contractions of the body of the stomach. There were no effects on the body of the oesophagus. The above changes were all dose dependent.

Motilin in doses of 0·001, 0·003, 0·006, and 0·008 μg/kg had no effect on LES pressure or on stomach contractions. The doses of 0·009, 0·012, 0·025, and 0·05 μg/kg produced statistically significant rises in resting LES pressure ($P < 0.02$, Fig. 1) and sphincter contractions. The latent period (time interval between motilin injection and a rise of 5 cm H_2O in LES pressure) was inversely proportional to dose. The shortest latent period was 23 seconds after the largest dose given (0·5 μg/kg) and 104 seconds after the smallest dose that produced a significant effect (0·09 μg/kg).

Repetitive contractions of the LES appeared at doses as low as 0·009 μg/kg. These contractions occurred in the absence of swallows (Fig. 2a). They increased in frequency, duration, and amplitude as the dose of motilin increased. A resting LES pressure could not be accurately measured at doses greater than 0·05 μg/kg because of the contractions. The frequency of LES contractions increased with the dose of motilin administered and in this study the highest number (three/minute) was recorded at the highest dose, 0·5 μg/kg (Table). The duration of contractions ranged from 77 to 277 seconds and was also directly related to dose. Contractions of the stomach, like those of the LES, were recorded after doses of motilin as low as 0·009 μg/kg. With higher doses these contractions were often repetitive. There was no consistent relationship between stomach and LES contractions. They frequently

Table *Number of contractions in LES and body of stomach after administration of different doses of motilin*

Dosage (μg/kg BW)	*Contractions**	
	LES	*Stomach*
0·012	12·3 ± 3·12	5·1 ± 1·72
0·025	12·7 ± 4·10	9·0 ± 3·10
0·05	12·1 ± 3·22	5·7 ± 1·68
0·01	20·6 ± 4·35	10·8 ± 1·32
0·2	24·3 ± 3·08	11·3 ± 2·87
0·5	29·6 ± 2·10	17·5 ± 2·01

*The values represent the mean ± SEM of contractions observed in 10 minute periods after motilin injection.

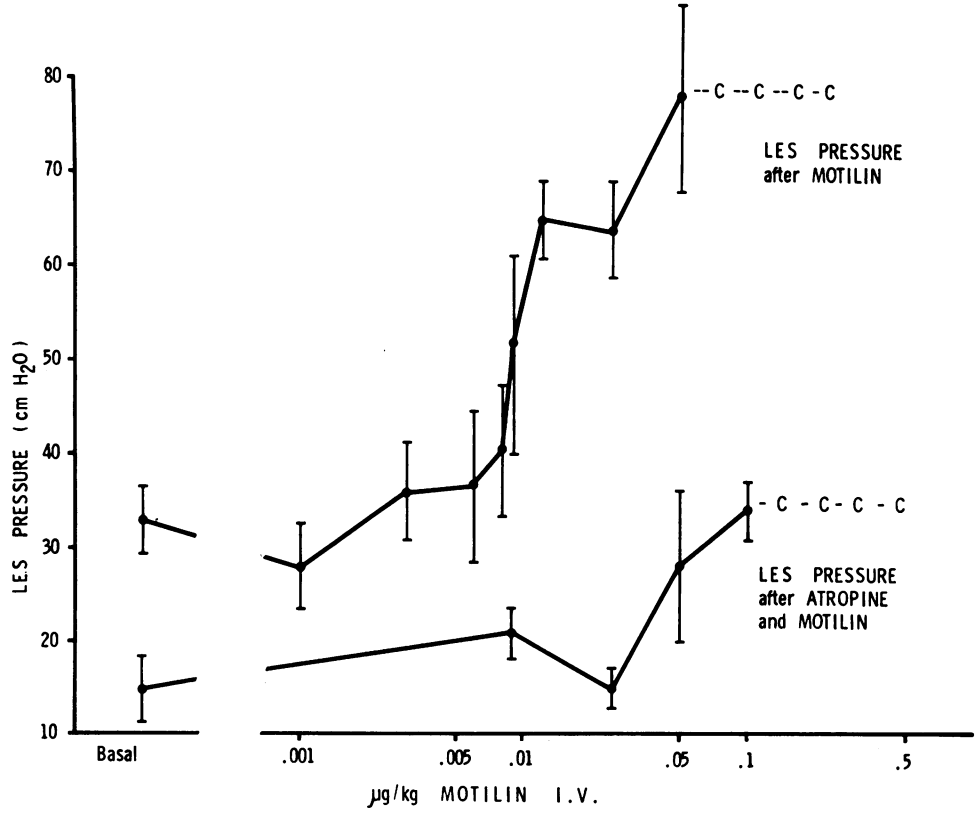

Fig 1 *Dose response curve for the effect of motilin on canine resting LES pressure without pretreatment (upper curve) and after pretreatment (lower curve) with atropine 30 μg/kg. Each point represents the mean ± SEM of the maximal resting LES pressure observed in the 10 minute period immediately before or after motilin. C denotes repetitive LES contractions.*

occurred simultaneously but often one or the other would appear alone. The incidence of contractions was less in the stomach than in the LES at all doses studied (Table).

Intravenously administered atropine caused a statistically significant decrease in the resting LES pressure ($P < 0.001$, Fig. 3). Methacholine bromide (12 μg/kg) had no effect when given after atropine but produced marked increases in LES pressure in the absence of atropine. Atropine also prevented the effect of motilin in all doses lower than 0·1 μg/kg. However, a significant increase in LES pressure was still observed after 0·1 μg/kg or larger doses of motilin ($P < 0.05$, Figs. 1 and 3). These doses of motilin still caused repetitive contractions of both the stomach and LES but they were at a much lower amplitude than those observed in the control experiments (Fig. 2b).

After hexamethonium (2 mg/kg intravenously) the effects of motilin (0·2 μg/kg) on resting LES pressure were reduced as they were after atropine. A slight but significant rise in LES pressure was observed (Fig. 4). However, the repetitive contractile response that motilin produced in atropinised dogs was not observed after hexamethonium but small contractile responses of the stomach still occurred (Fig. 2c).

Motilin 0·2 μg/kg had no effect on the LES pressure or on stomach when it was given to dogs pretreated with both hexamethonium and atropine. No contractions were observed (Fig. 2d) and there was no significant increase in pressure (Fig. 4).

Discussion

This study confirms a stimulatory action of motilin on the LES. The effect of small doses of motilin is abolished and the effect of large doses markedly

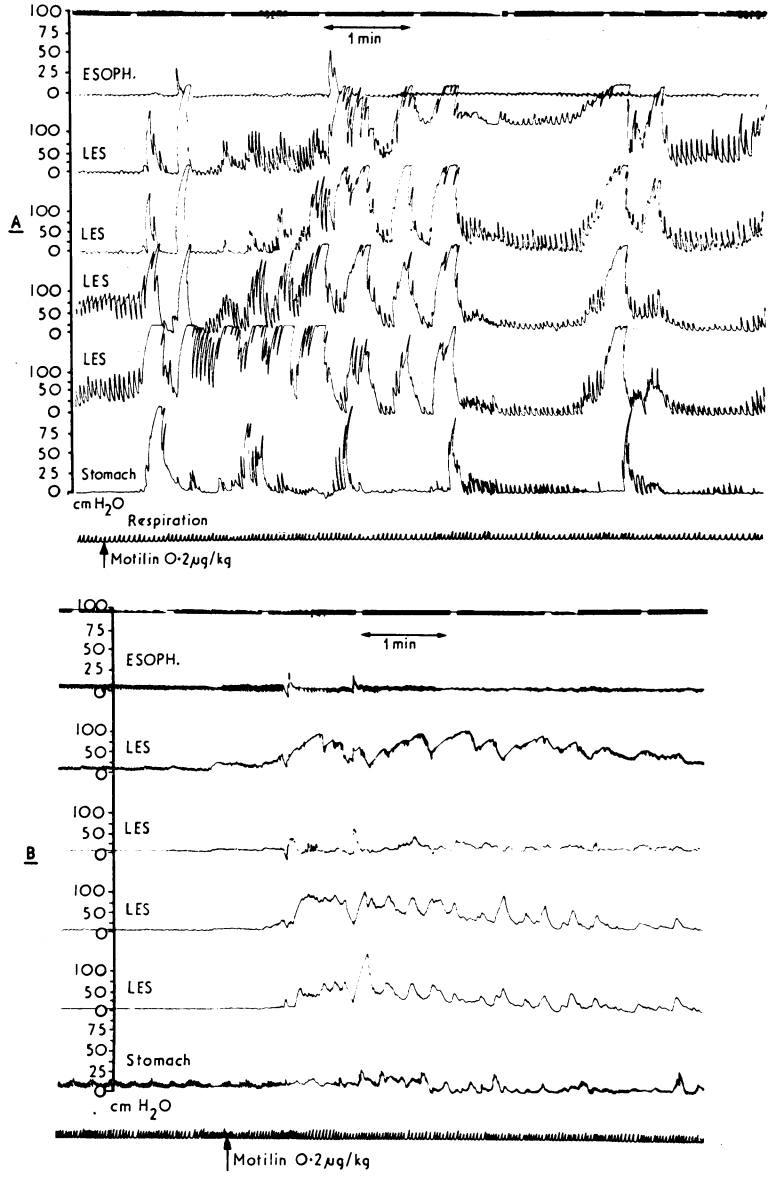

Fig 2 *Oesophageal, LES, and gastric pressure before and after an intravenous bolus of motilin 0·2 µg/kg in (a) untreated dog, (b) dog pretreated with atropine, (c) dog pretreated with hexamethonium, and (d) dog pretreated with both atropine and hexamethonium.*

diminished by atropine or hexamethonium. We have previously found, in the same dogs, that these antagonists were not capable of blocking the effect of pentagastrin on the LES (Zwick *et al.*, 1976). Their action in markedly attenuating the effect of motilin, then, is not due to a peripheral, non-specific action directly on the LES. The dose of atropine used was effective in blocking a large supramaximal dose of methacholine (Zwick *et al.*, 1976) and was previously used to inhibit vagal stimulation of antral motility (Sarna and Daniel, 1974). The hexamethonium dose was chosen as one effective in blocking vagal effects on heart rate (Daniel, unpublished). Thus, both drugs were given in effective, but selective doses.

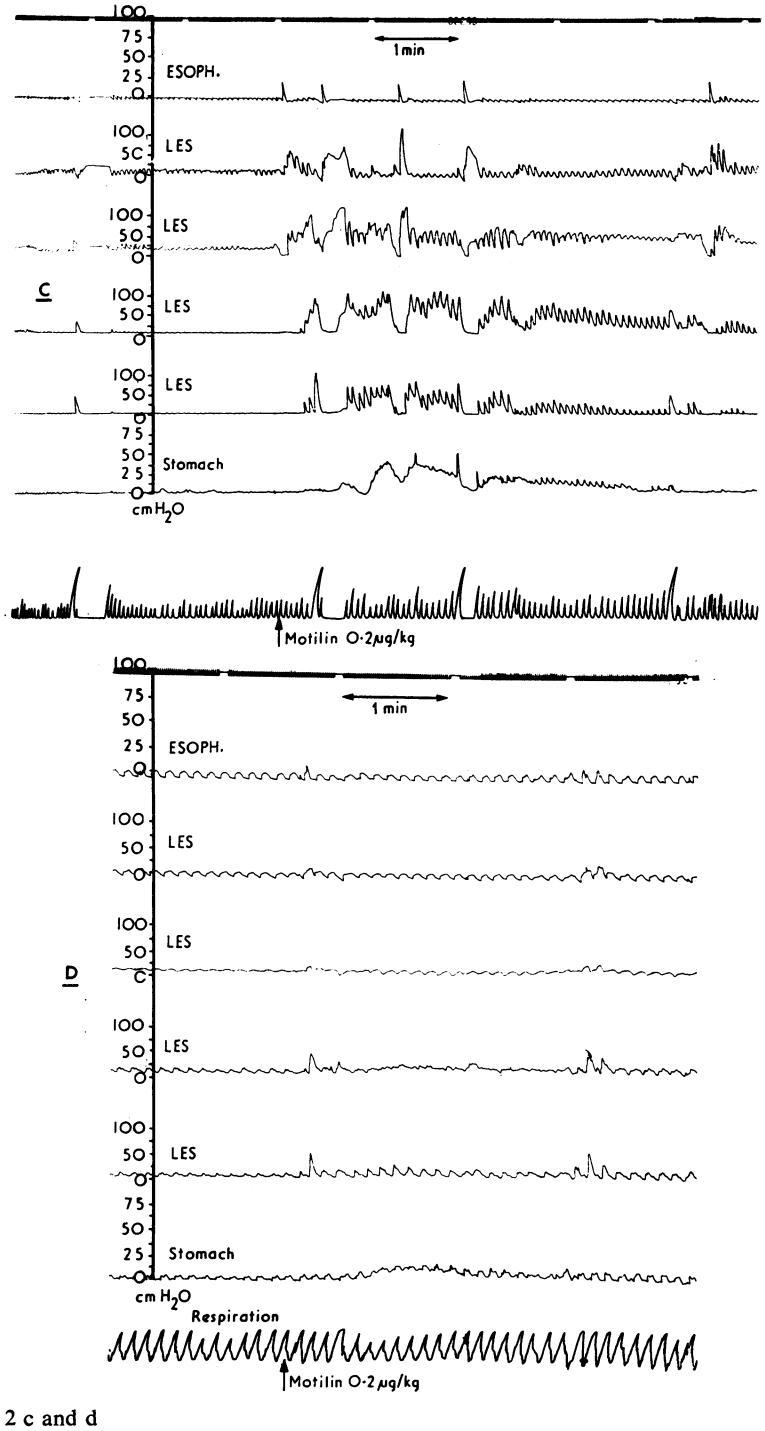

Fig 2 c and d

Presumably atropine blocks the effects of acetylcholine on muscarinic receptors of the LES, while hexamethonium blocks the effects of acetylcholine on nicotinic receptors presumably located in

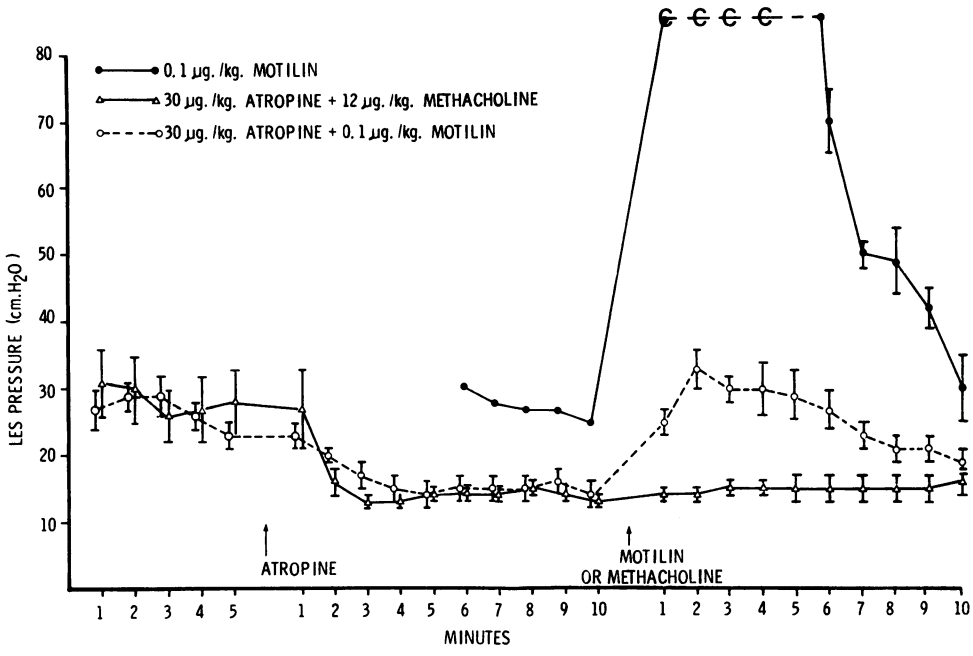

Fig 3 *Resting LES pressures in response to 0·1 µg/kg motilin before and after atropine 30 µg/kg. The absence of a response to 12 µg/kg methacholine after this dose of atropine is also depicted. Each point represents the mean ± SEM of nine observations. C denotes repetitive LES contractions that made measurement of a resting LES pressure impossible.*

the ganglia of the vagal efferent pathway. This action could, therefore, be mediated mainly by release of acetylcholine from preganglionic cholinergic nerves causing in turn firing of cholinergic neurons supplying LES circular muscle.

An unusual feature of the LES response to motilin is the appearance of repetitive contractions in larger doses. Methacholine and pentagastrin, in our experience, result in a smooth rise in resting LES pressure even with very large doses (Zwick *et al.*, 1976). LES contractions after these agents appear only in association with oesophageal contractions and the latter were not noted after motilin.

The LES contractions were frequently, but not always, associated with stomach contractions recorded in the fundus and were abolished by hexamethonium and reduced by atropine. The LES contracts when the stomach contracts and it is possible that the LES changes were due to a reflex from the stomach musculature (Diamant and Akin, 1972). In this study the frequency of LES contractions was always greater than that observed in the stomach but never exceeded the maximum expected for gastric contractions (five/minute). Intraluminal pressure, as recorded, is not however an absolute index of gastric contractions. Pressure will rise with a contraction only if the recording orifice lies in a closed space. If the pylorus were open, a rise in intraluminal pressure might not occur with each contraction. It is thus possible that each contraction observed in the LES was reflexly induced by one of the contractions produced in the stomach by motilin. If so, the effect of hexamethonium suggests that a nicotinic cholinergic synapse is in the pathway from gastric stimulus to LES response.

The blocking effect of atropine may result from inhibition of muscarinic receptors in the LES muscle. Less likely is an action of atropine on muscarinic receptors in ganglia; no evidence exists that muscarinic receptors that exist in ganglia control contraction of LES.

An alternative explanation for the repetitive contractions is that they represent the intermittent appearance of a repetitive relaxation due to stimulation by high doses of motilin of non-adrenergic inhibitory nerves activated through cholinergic ganglionic receptors. Goyal and Rattan (1975) have postulated such a pathway for LES relaxation induced by vagal stimulation. A third possibility is that the contractions or relaxations are due to preganglionic stimulation of another nerve whose effect is mediated by the intermittent release of a

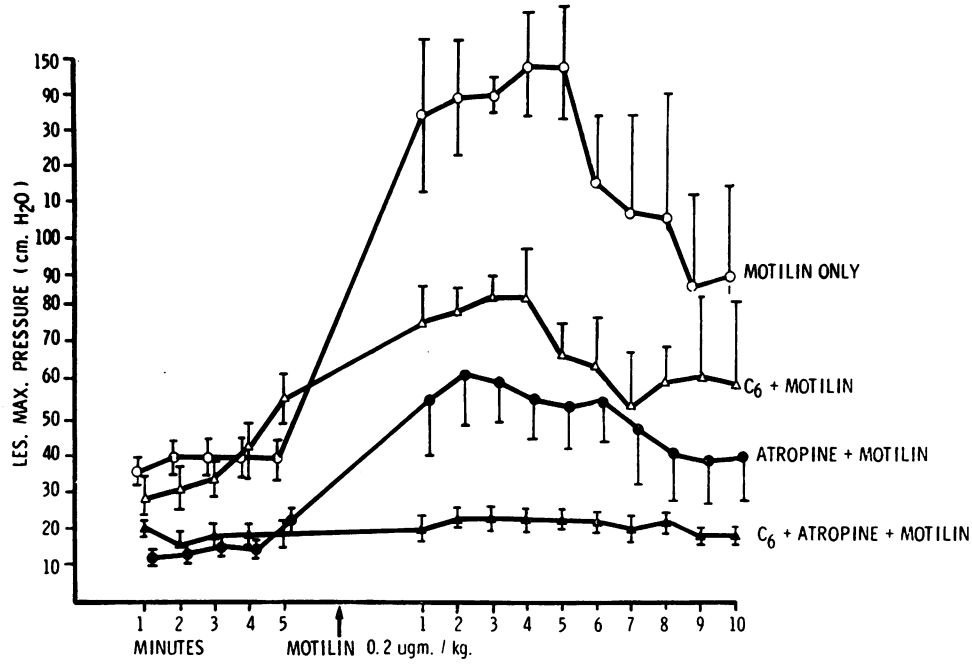

Fig 4 *Maximum LES pressures (including contractions) in response to 0·2 μg/kg motilin before and after 30 μg/kg atropine alone, after 2 mg/kg hexamethonium and after both atropine and hexamethonium.*

mediator other than acetylcholine. At this time, the exact mechanism producing the repetitive contractions remains conjectural.

Recently Struntz *et al.* (1975) found that a synthetic motilin, 13-Norleucine motilin, stimulated human and rabbit gastric muscle strips *in vitro* in the presence of atropine. The action was abolished by the Ca^{++} antagonistic compound verapamil and they postulated that motilin acts directly on smooth muscle through an action on calcium transport. They found motilin to have no effect on rat and guinea-pig stomach and small intestine muscle strips and in the rabbit it acted on circular muscle of the colon but not on the taenia, whereas in human colon the reverse effect occurred. Our study was performed *in vivo*, on a different species, and on a different smooth muscle. The extrapolation of results from species to species is always dangerous and appears to be especially so in studies of motilin.

LES pressure increases after feeding (Nebel and Castell, 1972). A complex mixture of neural and hormonal events occur after eating and it would be premature to attribute these changes to any particular agent or agents. This study demonstrates that exogenous motilin has the ability to raise LES pressure and cause repetitive LES contractions. We do not know if sufficient motilin is released under physiological conditions to have this effect. Jennewein *et al.* (1975) found the threshold dose for motilin to be similar in the duodenum antrum and LES. In our experiments the LES seemed to be more sensitive to motilin than the stomach, but our technique was not optimal for recording gastric contractions. If motilin is released in significant concentrations after a physiological stimulus, the LES will probably be an important site of action.

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