amphetamine, despite the fact that, like 4-chloroamphetamine (Meek, Fuxe & Carlsson, 1971) it is apparently a substrate for the amine pump on the neuronal membrane. This suggests that the ability to be taken into the neuron by the amine pump is not sufficient to account for the long duration of action of 4-chloroamphetamine.

March 31, 1976

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Effect of synthetic motilin and related polypeptides on contraction of gastrointestinal smooth muscle

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Brown, Johnson & Magee (1966) found that when the duodenum of the dog was made alkaline with tris buffer (pH 9·0), powerful motor activity was observed in transplanted pouches of the fundic gland area of the stomach. The observation caused these authors to suggest that the increased motor activity could be due to the release of a stimulatory substance from duodenal mucosa. This hypothesis was seen supported by their experimental results that intravenous injection of duodenal extract containing pancreozymin-cholecystokinin produced a similar increase in motor activity in fundic pouches (Brown & Parkes, 1967).

This substance, named motilin, is a docosapeptide which is distinct from other gastrointestinal hormones extracted from the same area of the small intestine. The entire amino-acid sequence of porcine motilin was determined by Schubert & Brown (1974), after a minor correction of their previous formula (Brown, Cook & Dryburgh, 1973).

Yajima, Kai & Kawatani (1975) have synthesized the peptide corresponding to the newly revised sequence of motilin by a method different from that employed by Wünsch, Brown & others (1973) who synthesized [13-Nle, 14-Glu]-motilin. 13-Nle-motilin has been shown to have a contraction-promoting effect—mainly on the smooth muscle of the gastric antrum, the duodenum and the colon in the rabbit and man, but little or no effect on other parts of the intestine (Domschke, Strunz & others, 1974). However, no studies have so far investigated the effect of synthetic motilin on contraction of gastrointestinal smooth muscle. Besides motilin, the docosapeptide, Yajima & others (1975) have synthesized three shorter chain peptides related to motilin whose amino-acid sequences are shown in Table 1.

The aim of the present experiment was to investigate the influences of synthetic motilin and related polypeptides on contraction of gastrointestinal smooth muscle and to clarify the amino-acid sequence necessary for the effect.

The smooth muscle strips (Table 2) were suspended in a 20 ml organ bath containing Tyrode solution gassed with air and maintained at 30 ± 2°. For stomach, a strip of longitudinal muscle approximately 0·5 cm wide and 2 cm in length was removed along

Table 1. *Amino-acid sequences of synthetic motilin and related polypeptides.*

Motilin	H-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH
M[6–22]	H-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH
M[9–22]	H-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH
M[14–22]	H-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH

† Correspondence.

Table 2. *Gastrointestinal smooth muscles tested.*

	Guinea-pig	Rat	Rabbit	Pig	Dog
Stomach	+	+	+	+	+
Duodenum	+	+	+	+	+
Jejunum			+		
Ileum	+		+	+	
Colon			+		

the greater curvature. Muscle contraction was recorded isotonically with a writing lever on a smoked drum. The load applied to the tissue was approximately 1·5 g which allowed the preparation to develop sufficient spontaneous contraction and tone. The motilin and related polypeptides were dissolved in saline solution which had previously been boiled for 3 min and bubbled with nitrogen-gas for 10 min, and were kept in a siliconized tube at 3° for up to 2 weeks before being added to the organ bath.

Among the smooth muscle tested rabbit duodenal and colonic muscle were most sensitive to motilin. Duodenal muscle contraction was remarkably increased with $1{\cdot}7 \times 10^{-9}$M of motilin (Fig. 1). M[6–22] also increased the contraction of rabbit duodenal muscle but when the contraction height induced by M[6–22] ($4{\cdot}3 - 8{\cdot}6 \times 10^{-7}$M) was compared with that of motilin ($3{\cdot}4 - 6{\cdot}8 \times 10^{-9}$M) the effect of M[6–22] was found to be 1/100 to 1/150 of that of motilin. Neither M[9–22] nor M[14–22] affected the smooth muscle of rabbit duodenum and colon at a concentration of 5×10^{-6}M. The muscle contraction of rabbit gastric antrum and jejunum was increased with motilin at concentrations of $3{\cdot}4 \times 10^{-7}$M and $1{\cdot}7 \times 10^{-8}$M, respectively. The rabbit ileum and any part of the gastrointestinal smooth muscle from guinea-pig, rat, pig and dog were practically insensitive to motilin as well as related polypeptides since the contraction was not affected with concentrations of the peptides up to 5×10^{-6}M.

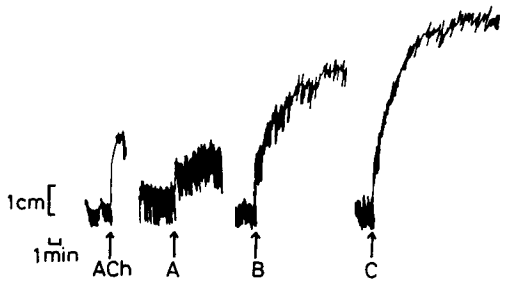

FIG. 1. Effect of synthetic motilin on rabbit duodenal muscle. The bath was washed out with Tyrode solution after maximal contraction. ACh: Acetylcholine, $5{\cdot}5 \times 10^{-9}$M. A: Motilin, $1{\cdot}7 \times 10^{-9}$M, B: Motilin, $3{\cdot}4 \times 10^{-9}$M, C: Motilin, 10^{-8}M.

These results indicate that the synthetic motilin has a similar contraction-promoting effect to that of 13-Nle-motilin on the gastrointestinal smooth muscle of the rabbit. Since the lack of *N*-terminal pentapeptide in motilin resulted in a decrease in contraction-promoting effect on rabbit duodenal muscle, and subsequent removal of Thr–Tyr–Gly resulted in complete loss of the effect, it may be said that these amino-acid sequences are at least necessary for the pharmacological effect of motilin.

The authors would like to thank Mr Y. Hoshino for his skilful technical assistance.

March 1, 1976

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