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Concentration-dependent stimulation of cholinergic motor nerves or smooth muscle by [Nle^{13}]motilin in the isolated rabbit gastric antrum

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Abstract

In man, rabbit and cat, the effects of motilin and motilides are neurally mediated in vivo, whereas in vitro binding and contractility studies suggest the presence of a smooth muscular receptor. The aim of this study was to investigate in vitro interactions of motilin with the enteric excitatory neurotransmission in the gastric antrum of the rabbit. Circular muscle strips from the pre-pyloric antrum were subjected to electrical field stimulation (1 ms, 1–32 Hz, 10 s train) and muscle twitch responses were recorded isometrically. Induced twitch responses were frequency dependent (1–32 Hz) and entirely neurogenic (tetrodotoxin sensitive). [Nle^{13}]motilin dose-dependently (10^{-9}–10^{-8} M) enhanced the amplitude of, atropine sensitive, evoked contractions. At 4 Hz the response, expressed as a % of the response to 32 Hz, increased from 15.5 ± 4.1% (control) to 28.1 ± 5.8% (motilin 10^{-9} M), and to 45.8 ± 3.6% (motilin $10^{-8.5}$ M) ($P < 0.05$). This effect was not inhibited by hexamethonium ($10^{-3.3}$ M) but was abolished by the motilin receptor antagonist GM-109 (10^{-5} M). In unstimulated strips, motilin induced phasic-tonic contractions with a threshold concentration of 10^{-8} M and an pEC_{50} of 7.48, which were also inhibited by GM-109 (10^{-5} M) but not by tetrodotoxin ($10^{-5.5}$ M). The maximal tension, frequency and dose-dependency of carbachol-induced contractions were not influenced by motilin (pEC_{50}, carbachol: 6.48 ± 0.06 (control), 6.49 ± 0.07 (motilin)). In conclusion, motilin enhances contractions induced by electrical field stimulation in the rabbit antrum by a post-ganglionic interaction with the cholinergic neurotransmission in vitro at low doses and interacts directly with antral smooth muscle at high doses. This model is an accurate reflection of the in vivo effects of motilin and provides a tool to study neurogenic and myogenic actions of motilin and motilides in vitro. © 1997 Elsevier Science B.V.

Keywords: Motilin; Motilin receptor; Gastric motility; Enteric nervous system

1. Introduction

The 22 amino acid peptide motilin is able to induce gastrointestinal motor activity and has been implied in the regulation of phase III of the migrating motility complex in the mammalian gastrointestinal tract. Motilin is secreted by neuro-endocrine cells in the duodenal mucosa and is thought to have a hormonal action on the contractility of the gastric antrum and the proximal intestine. For a recent review see Poitras (1994). In vitro organ bath studies in man, rabbit and cat demonstrated that the contractile effects of motilin are mediated through a direct action on a smooth muscle receptor, since they are not antagonized by muscarinic blockade or by inhibition of axonal conductance with tetrodotoxin (Strunz et al., 1975; Adachi et al., 1981; Lüdtke et al., 1989; Depoortere et al., 1993, Kitazawa et al., 1994). Specific binding sites for motilin have been identified in homogenates of the antral smooth muscle layers of man, rabbit and cat (Bormans et al., 1986; Peeters et al., 1988; Depoortere et al., 1993) and biotinylated motilin binds specifically to isolated rabbit smooth muscle cells (Sakai et al., 1994).

In vivo studies, however, have demonstrated that motilin exerts its effects on gut motility predominantly by activating excitatory neuronal pathways. In the dog, a species in which motilin has no effects in vitro, motilin-induced contractions in vivo are mediated by cholinergic nerves in the antrum and by cholinergic and non-cholinergic nerves in the duodenum (Fox et al., 1983; Itoh, 1990). However, also in man and rabbit, species with a smooth muscle motilin-receptor, the effect of motilin in the gastric antrum in vivo, is blocked by atropine (Boivin et al., 1995; Kitazawa et al., 1994). Therefore, a discrepancy exists between the in vivo and in vitro mechanisms of motilin action in man and rabbit. Furthermore, this discrepancy

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also exists for the macrolide antibiotic erythromycin, and for its more potent derivatives, the motilides. In vitro erythromycin and the motilides EM-523 and EM-574, act in rabbit and man on a smooth muscle motilin receptor (Peeters et al., 1989; Depoortere et al., 1989; Satoh et al., 1990, 1994) as was definitively demonstrated by the development of the first motilin antagonist (Peeters et al., 1994; Depoortere et al., 1995). However, in dog and man the induction of gastric activity fronts by erythromycin and motilides is inhibited by atropine (Sarna et al., 1991; Shiba et al., 1995; Coulie et al., 1996).

Recently, neurally mediated effects were also detected in vitro. In the duodenum of the rabbit the tonic response to motilin appears to have a cholinergic component and motilin enhances the release of acetylcholine (Kitazawa et al., 1993). In the same species erythromycin in low doses has neurally mediated, chronotropic effects on betanechol and substance-P induced contractions in the gastric antrum and stimulates inhibitory neurotransmission to the pyloric muscle (Parkman et al., 1995, 1996). On the other hand, in the chicken proventriculus motilin enhanced the response to electrical field stimulation (Kitazawa et al., 1995). However, these effects required concentrations higher than those needed to stimulate smooth muscle tissue directly.

The aim of the present study was to investigate whether in vitro, motilin influences the excitatory neurotransmission in the upper gastrointestinal tract. Since the gastric antrum is the main target for therapeutic use of motilides, modulation of excitatory enteric nerve function by motilin was studied in the rabbit antrum. This species was chosen, since previous studies from our and other groups have shown that contractile effects of motilin demonstrated in the rabbit in vitro, have a high degree of correlation with findings in man.

2. Materials and methods

2.1. Animals

Adult New Zealand white rabbits of either sex (2.5 to 3.0 kg) were killed by cervical dislocation and bleeding. After midline laparotomy, the stomach was quickly removed and washed with saline. All experiments were approved by the Animal Ethics Committee of the University of Leuven.

2.2. Materials

The norleucine^{13} analogue of porcine motilin ([Nle^{13}]motilin) was obtained from Novabiochem (Laüfelfingen, Switzerland) and tetrodotoxin from Serva (Heidelberg, Germany). This analogue has been shown to be equipotent to porcine motilin in contraction studies and to have the same affinity as porcine motilin for the rabbit antral motilin receptor (Peeters et al., 1986). Moreover, recent studies have demonstrated that the pharmacophore of motilin resides in the residues 1,2,4 and 7 of the N-terminal portion of the peptide (Peeters et al., 1992). Furthermore, porcine motilin is equipotent to rabbit motilin in the rabbit (unpublished data).

Hexamethonium bromide, atropine sulfate and carbachol were purchased from Sigma (St. Louis, MO, USA). The motilin antagonist GM-109 was a gift from Dr. H. Takanashi (Chugai Pharmaceutical, Gotemba, Japan). All other chemicals were of analytical grade.

2.3. Tissue preparation and contractility studies

Circularly oriented whole thickness strips (20×0.2 mm) from the antral *muscularis externa* were prepared in Krebs solution (NaCl: 120.9 mM; NaH_2PO_4: 2.0 mM; $NaHCO_3$: 15.5 mM; KCl: 5.9 mM; $CaCl_2$: 1.25 mM, $MgCl_2$: 1.2 mM) at room temperature, after removal of the mucosa and submucosa using a dissection microscope. Strips were suspended in 10 ml of Krebs solution gassed with O_2 95%/CO_2 5% in a water jacketed organ bath solution at 37°C. Muscle contractions were measured using an isometric force transducer/amplifier (Harvard Apparatus, South Natick, MA, USA), recorded with an ink writer and sampled for digital analysis using a Labmaster A/D converter (Scientific Solutions, Dolon, OH, USA). Tissues were allowed to equilibrate for at least 90 min and bath solutions were changed every 30 min. Resting tension was set at 1 g. Electrical field stimulation was applied via two parallel platinum rod electrodes using a Grass S88 stimulator (Grass, Quincy, MA, USA). Frequency spectra (1, 2, 4, 8, 16 and 32 Hz) or single frequency pulses (4 Hz) were administered in pulse trains (pulse 1 ms, train 10 s, 60 V). Each consecutive pulse train was followed by a 90 s interval. 60 V was chosen as stimulus parameter because it induced submaximal twitch responses ($\pm 50\%$) as determined by voltage–response curves. At this voltage the actual current delivered to the strips was 250 mA. When recording frequency spectra, bath solutions were changed twice after the completion of each spectrum and strips were allowed to equilibrate for 10 min. When a stable amplitude of twitch responses was obtained at all frequencies, 100 μl aliquots of test agents were added to the bath solution.

In a separate set of experiments cumulative motilin and carbachol dose response curves were determined in unstimulated muscle strips in identical organ bath conditions using the same isometric force transducer and registrating equipment.

2.4. Data analysis

For field stimulation-experiments peak tension generation by the muscle twitch responses was measured. When recording frequency spectra, the muscle twitch response was expressed relative to the maximal response observed

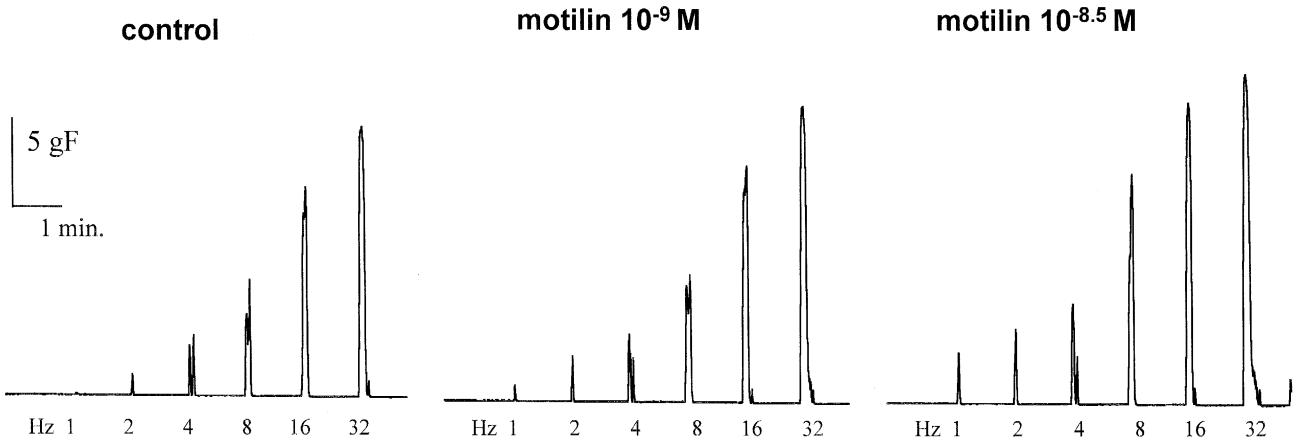

Fig. 1. Effect of two doses of [Nle[13]]motilin on electrically induced muscle contractions in a representative experiment. Note that at low stimulation frequencies (1–4 Hz) the effects of motilin are most pronounced.

at 32 Hz. The modulation of the response to electrical field stimulation by pharmacological agents in single frequency experiments was expressed relative to the control response. Statistical analysis of these data was performed using a Student's paired *t*-test when comparing two means and one way analysis of variance when comparing more than two means. Contractions induced by carbachol and [Nle[13]]motilin at different doses were averaged and expressed as a percentage of the response obtained with 10^{-5} M carbachol. Results obtained for the carbachol dose–response curves in control conditions and with [Nle[13]]motilin added were analyzed using a Student's *t*-test. Statistical significance was inferred at a P-value < 0.05.

3. Results

3.1. Characterization of the response to electrical field stimulation

In preliminary experiments performed under conditions normally used in our laboratory with duodenal preparations, spontaneous phasic contractions of the antral neuromuscular strips interfered with induced twitch responses. The spontaneous activity was not present in every preparation and was tetrodotoxin and atropine insensitive (data not shown). Spontaneous activity was suppressed by lowering the Ca^{2+} concentration from 2.50 to 1.25 mM and all subsequent experiments were therefore performed under these conditions.

Electrically-evoked muscle twitch responses were frequency dependent and increased in amplitude with increasing frequency reaching a maximum at 16 to 32 Hz. Both on- and off-responses were observed, although off-responses appeared generally at higher stimulation frequencies (8–32 Hz). Inhibition of axonal conduction with tetrodotoxin ($10^{-5.5}$ M) abolished all contractions in the entire frequency spectrum (1–32 Hz), demonstrating that all responses were neurogenic. The muscarinic antagonist atropine ($10^{-5.3}$ M) abolished all responses at stimulation frequencies lower than 4 Hz. At 4 and 8 Hz twitch responses were abolished in 4/8 experiments and in 4/8 reduced by 93.2 ± 7.5 and $77.4 \pm 5.5\%$, respectively, whereas at 16 and 32 Hz atropine reduced the amplitude by only 55.5 ± 7.5 (16) and $42.4 \pm 7.8\%$ (32) suggesting that both cholinergic and non-cholinergic pathways were involved in the generation of the twitch responses.

3.2. Modulation of the neurogenic response and induction of direct smooth muscle response by [Nle[13]]motilin

To investigate the actions of motilin on the electrically induced twitch responses, frequency spectra (1, 2, 4, 8, 16 and 32 Hz) were recorded. When a stable response was

Table 1
Dose- and frequency-dependent effects of [Nle[13]]motilin on field stimulation induced contractions of rabbit antrum

Frequency (Hz)	Control	Motilin			
		$10^{-9.5}$ M	10^{-9} M	$10^{-8.5}$ M	10^{-8} M
2	6.8 ± 2.6	5.3 ± 3.0	11.4 ± 4.2	40.0 ± 10.1 [a]	48.5 ± 12.2 [b]
4	15.5 ± 4.1	19.1 ± 4.3	28.1 ± 5.8 [a]	45.8 ± 3.6 [b]	53.6 ± 10.4 [b]
8	38.3 ± 8.5	29.7 ± 4.3	51.6 ± 9.4 [a]	69.7 ± 13.8 [a]	74.1 ± 16.3 [b]
16	65.2 ± 8.5	84.0 ± 9.6	84.8 ± 8.8 [a]	97.9 ± 10.7 [a]	110.8 ± 13.2 [a]
32	100	108.1 ± 5.2	111.6 ± 5.6	120.1 ± 5.2 [a]	125.7 ± 13.6 [a]

Note: Data are expressed as means $\pm$ SE of at least 4 independent experiments and as a percentage of the peak contraction force observed at 32 Hz in baseline conditions ([a] $P < 0.05$, [b] $P < 0.02$, compared to control).

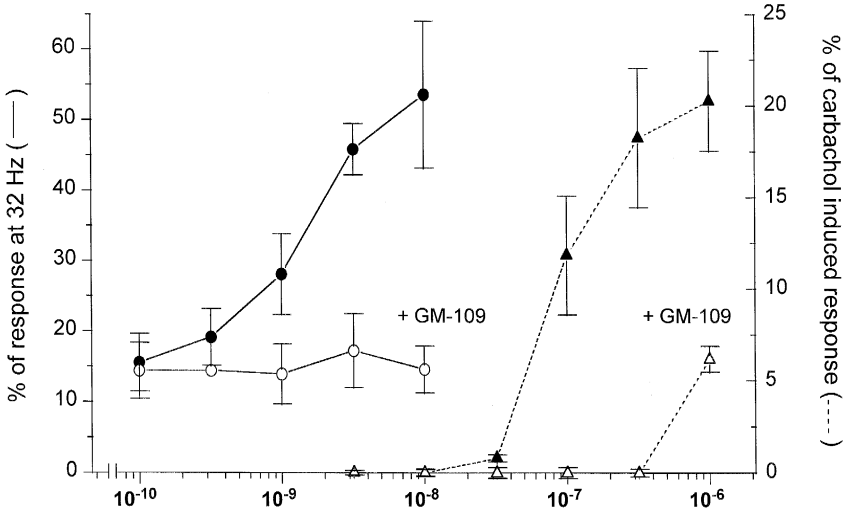

Fig. 2. Comparison between the effects of [Nle[13]]motilin on strips stimulated at 4 Hz (circles, full lines) and strips without stimulation (triangles, dashed lines). Values are expressed as a percentage of the response to 32 Hz for stimulated (left axis) and as a percentage of the contractile response induced by carbachol (10^{-5} M) for unstimulated contractions (right axis).

reached at all frequencies, different doses of [Nle[13]]motilin were added so as to achieve concentrations of $10^{-9.5}$, 10^{-9}, $10^{-8.5}$ and 10^{-8} M in the tissue bath. As can be seen in the tracings of a representative experiment, [Nle[13]]motilin induced a significant increase in the amplitude of the twitch response (Fig. 1). At 10^{-9} M the increase in amplitude reached statistical significance at 4, 8 and 16 Hz and at $10^{-8.5}$ M in the entire frequency spectrum (Table 1). Tetrodotoxin ($10^{-5.5}$ M) abolished all twitch responses to field stimulation in the control and motilin-treated preparations.

However in the absence of electrical stimulation, higher doses of [Nle[13]]motilin ($> 10^{-8}$ M) still induced a phasic and tonic contraction of the antral muscle preparations, indicating the preservation of the direct action of motilin on antral smooth muscle contractility. No effects of motilin were observed in preparations at doses of $10^{-8.5}$ M or lower when no electrical stimulation was applied. In tetrodotoxin treated, unstimulated preparations, [Nle[13]]motilin (10^{-6} M) still induced a contractile response.

The dose–response curves for unstimulated strips and for strips stimulated at 4 Hz are shown in Fig. 2. While the pEC_{50} for motilin to induce a response of unstimulated

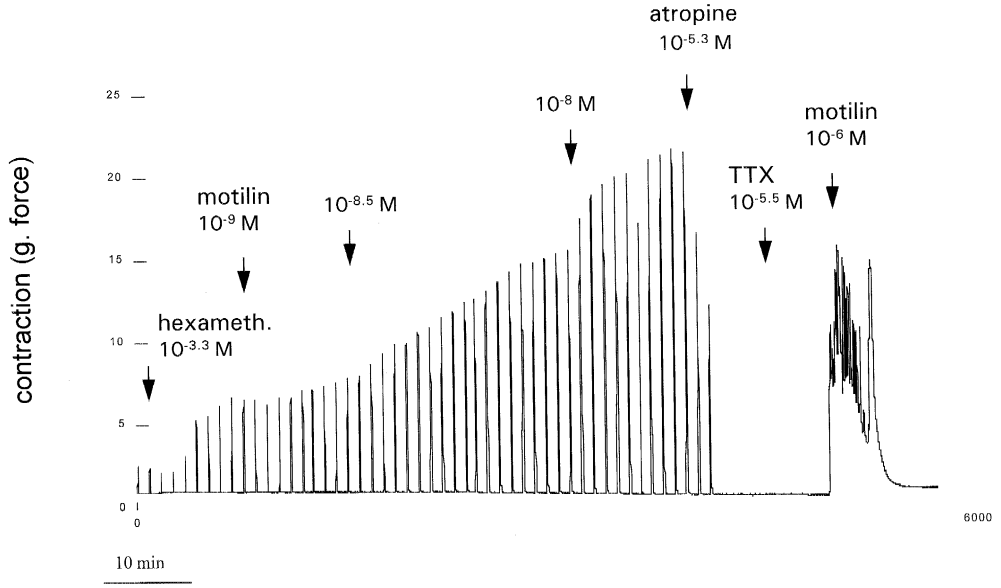

Fig. 3. Effect of hexamethonium (hexameth.) alone and in combination with increasing concentrations of [Nle[13]]po-motilin on electrically induced contractions (4 Hz, 60 V, 1 ms, 10 s train) in one representative experiment. After recording the effect of 10^{-8} M motilin, atropine followed by tetrodotoxin were added to the bath and the motilin concentration was raised to 10^{-6} M.

strips is 7.48 ± 0.11 (32.3 ± 8.5 nM), it is obvious that motilin is more potent in enhancing the effect of electrical stimulation. The pEC_{50} may be estimated at 8.73 ± 0.03 (Fig. 2). The motilin receptor antagonist GM-109 (10^{-5} M) did not alter the amplitude of twitch responses and was also without effect in unstimulated strips. However, the enhancement of the amplitude of electrically evoked contractions by [Nle^{13}]motilin was abolished in strips preincubated with GM-109 (10^{-5} M) for 10 min (Fig. 2). For example, at the highest concentration of motilin used (10^{-8} M) the response to EFS at 4 Hz was 56.3 ± 10.4 (% of the response at 32 Hz) in the absence and 14.6 ± 3.3 (% of the response at 32 Hz) in the presence of GM-109 (10^{-5} M) ($P < 0.02$). The latter value was not different from the electrically evoked responses in the absence of motilin. Similarly, the response of unstimulated strips to motilin was also blocked by GM-109 (Fig. 2).

3.3. Effect of cholinergic blockade on the neural motilin action

When the ganglion blocking agent hexamethonium ($10^{-3.3}$ M) was added to the bath solution, the response to electrical stimulation at 4 Hz increased to 2.9 ± 0.7 times the control value ($P < 0.05$). When [Nle^{13}]motilin was added in increasing concentrations (10^{-9}–10^{-8} M) a further dose-dependent increase in the amplitude of the response was observed (10^{-9} M: 1.7 ± 0.3, $10^{-8.5}$ M: 3.3 ± 0.7, 10^{-8} M: 5.4 ± 1.5 times the maximal contraction in the presence of hexamethonium, $P < 0.05$, $n = 6$). A representative tracing is shown in Fig. 3. Atropine ($10^{-5.3}$ M) administered after hexamethonium virtually abolished twitch responses. Prevailing low amplitude contractions were totally inhibited by tetrodotoxin ($10^{-5.5}$ M). However when high doses of [Nle^{13}]motilin (10^{-6} M) were added after atropine and tetrodotoxin incubation, a residual phasic and tonic direct smooth muscle effect was observed.

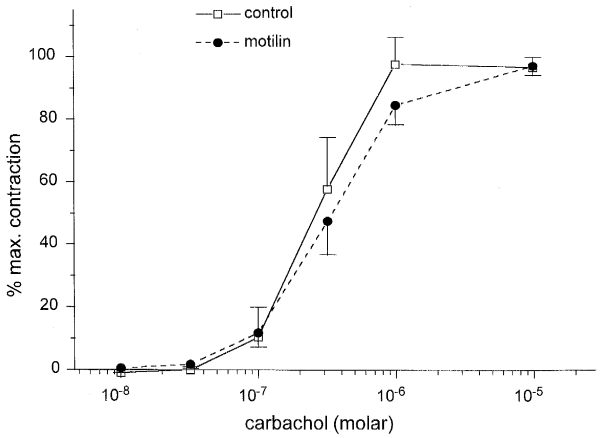

Fig. 4. Dose–response curve for the carbachol-induced contractions of rabbit antral muscle strips in the presence (●) and in the absence (□) of $10^{-8.5}$ M [Nle^{13}]po-motilin. The tension generated was averaged over a 75 s period prior to the administration of the subsequent dose and expressed as a percentage of the maximal contraction (at 10^{-5} M carbachol).

Table 2
Effect of motilin on the contractile parameters of carbachol induced contractility

	Control	Motilin ($10^{-8.5}$ M)
pEC_{50}	6.48 ± 0.06	6.49 ± 0.07
Max. contract. (gF)	11.6 ± 1.6	11.8 ± 0.9
Frequency (cpm)	4.6 ± 0.6	4.1 ± 0.8

Note: Data are shown as means ± SE of 6 independent experiments.

3.4. Effects of motilin on the response to carbachol

To investigate whether the stimulatory action of [Nle^{13}]motilin on electrically induced antral contractions in vitro could be due to a postsynaptic interaction with muscarinic receptors, antral strips were exposed to increasing doses of the muscarinic agonist carbachol, in the absence and in the presence of $10^{-8.5}$ M [Nle^{13}-]motilin (Fig. 4). Carbachol induced dose-dependent phasic and tonic contractions of the antral strips. The pEC_{50} for carbachol and the maximal isometric contraction force in response to 10^{-5} M carbachol was not affected by motilin (Table 2).

At a concentration of $10^{-6.5}$ M, carbachol-induced contractions were predominantly phasic whereas higher concentrations resulted in a mixed tonic and phasic contractile response. The phasic contractions induced by $10^{-6.5}$ M carbachol occurred at a frequency of 4.6 ± 0.6 cpm in control conditions. In the presence of motilin, the contraction frequency was not different from control conditions (4.1 ± 0.8, N.S., Table 2).

4. Discussion

This is the first in vitro study of the biological activity of motilin, providing evidence for high affinity motilin receptors on neural pathways. Our data demonstrate that motilin influences the contractility of the gastric antrum in the rabbit in vitro through the interaction with both postganglionic cholinergic nerves and gastric smooth muscle cells. However, the action of motilin on cholinergic nerves occurs at low concentrations (10^{-9}–10^{-8} M), which are unable to induce the phasic-tonic response of the muscle strips observed at higher concentrations. The neurogenic effects appear to be specifically mediated through motilin receptors, since they were abolished by the motilin receptor antagonist GM-109, which has been shown to selectively inhibit motilin induced contractions in the rabbit duodenum (Takanashi et al., 1995). Although the motilin receptor antagonist GM-109 also blocked the direct smooth muscle response, the difference in potency for the neuro-

genic and myogenic effect may indicate the existence of two different receptors.

Until recently it was generally accepted that all in vitro contractile effects of motilin were due to a direct interaction with smooth muscle motilin receptors and, although during the past few years evidence emerged for neurally mediated effects, these were observed at concentrations higher than those required for smooth muscle effects. Thus the potency of motilin to induce contractions via a direct effect on smooth muscle in duodenal preparations was 2.5 nM (EC_{50}), while in the same preparation the potency for the motilin-evoked release of acetylcholine was about 300 nM (Kitazawa et al., 1993). In a study of the effect of motilin on the response to electrical field stimulation of strips prepared from the proventriculus of the chicken, half-maximum enhancement was also observed at about 300 nM (Kitazawa et al., 1995) and the inhibitory effect of motilin on the rabbit pylorus, which appears to be mediated via nitrergic pathways, required 1 μM (Parkman et al., 1996). In our study the potency to induce contractions in unstimulated strips was 32.3 nM (pEC_{50} 7.48 ± 0.11), which corresponds well with the potency found by Kitazawa et al. (1994) in the same preparation: 28 nM (pEC_{50} 7.55). It was shown in this study and confirmed in ours, that this response is mediated via smooth muscle receptors as it is unaffected by TTX and atropine. However, the effect on the response to electrical field stimulation, which is neurally mediated, starts already at a threshold concentration of 10^{-9} M and with a pEC_{50} of 8.73. Thus, motilin is about ten times more potent in inducing the neurogenic effect.

As summarized above, in vivo studies suggest that motilin acts via neural pathways, most probably localized in the intrinsic nervous system of the upper gastrointestinal tract, since the effect of motilin is preserved in preparations devoid of extrinsic innervation in both dogs and rabbits (Van Lier Ribbink et al., 1989; Marzio et al., 1994). The electrical field stimulation used in the present experiments only stimulates intrinsic nerves and not smooth muscle, because all responses were blocked by tetrodotoxin. However, the fact that the excitatory effect of motilin was more pronounced at lower stimulation frequencies, suggests a differential sensitivity of enteric nerve subtypes for electrical stimulation. It is generally accepted that recruitment of non-cholinergic (atropine-resistant) neuro-muscular transmission in the enteric nervous system occurs at higher stimulation frequencies (Ambache and Freeman, 1968) and the effect of atropine in our preparation confirms this. Therefore, cholinergic excitatory pathways are probably the main target for neuronal effects of motilin in vitro, which is in keeping with the motilin induced excitatory response in the rabbit antrum in vivo (Kitazawa et al., 1994).

The ganglion blocking agent, hexamethonium, induced a significant rise in the amplitude of muscle twitch responses to field stimulation, suggesting that a tonic presynaptic inhibition is present in isolated rabbit antrum. Nevertheless, motilin induced an additional enhancement of the twitch responses in the presence of hexamethonium suggesting that the peptide acts at a postganglionic site. Earlier studies have shown that motilin enhances the acetylcholine induced contractions in isolated rabbit pyloric muscle suggesting a postsynaptic interaction of motilin with the muscarinic pathway (Strunz et al., 1976). However, we found that motilin at concentrations sufficient to enhance electrically induced contractions, did not alter the sensitivity nor the maximal response of the antral smooth muscle to carbachol. Therefore, motilin most probably facilitates antral neurotransmission by acting directly on the cholinergic nerves. In the central nervous system motilin in nanomolar concentrations causes a slow depolarization of neurons (Phillis and Kirkpatrick, 1979) and in the guinea pig antrum, electrophysiological evidence has been provided to support a role for erythromycin and motilin in modulating the excitability of myenteric nerves (Tack et al., 1991). Probably the same holds true for the rabbit gastric antrum.

To avoid interference by spontaneous phasic contractions with the twitch response, extracellular Ca^{2+} levels were reduced from 2.50 to 1.25 mM in all experiments. Previous work from our laboratory has demonstrated that the muscular contractions induced by motilin and erythromycin in the rabbit duodenum are dependent on extracellular Ca^{2+} (Peeters et al., 1991; Matthijs et al., 1988, 1989) and that the motilin induced muscular response is more sensitive to Ca^{2+}-entry-blockers as compared to the cholinergic response. Therefore, the pronounced effects of motilin on electrically induced contractions in a 1.25 mM [Ca^{2+}] solution observed in the present study, provides additional evidence to support a neural action of low motilin doses in vitro rather than a postsynaptic facilitation of the muscle contraction.

The shift between the dose–response curves for the neurally mediated, versus the direct smooth muscle effects, may explain the discrepancy between the in vivo and the in vitro data for motilin. If these findings can be extrapolated to other species, they may also explain why the motilin-receptor agonist erythromycin influences human motility in vivo through both neurogenic and myogenic mechanisms depending on the dose administered (Tack et al., 1992; Coulie et al., 1996). In this respect, a recent study found that erythromycin, at low doses, modulates the frequency of betanechol induced antral contractions in vitro (Parkman et al., 1995). This chronotropic effect was not abolished by ganglionic inhibition, but no further data were obtained on the identity of the neuronal pathway involved. In the present study we applied electrical field stimulation to directly stimulate the excitatory neurotransmission rather than applying exogenous agents. Our results indicate that motilin has a marked stimulatory effect on the amplitude of twitch responses, unlike the chronotropic effects observed with erythromycin. Interestingly, we did

not observe a chronotropic effect of motilin on the contractions induced by carbachol ($10^{-6.5}$ M). However in our study carbachol was applied in increasing concentrations contrary to the single dose experiments performed by Parkman et al. (1995).

In conclusion, this study demonstrates the interaction of motilin with cholinergic motor-nerves in the rabbit gastric antrum. Moreover, our results support the hypothesis that in vitro the distinction between neurogenic and myogenic actions of motilin is dose dependent, which reflects the in vivo effects of the motilin receptor agonist erythromycin and its analogues. Whether this distinction is due to motilin receptor subtypes remains to be determined.

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