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Differential Sensitivities of Morphine and Motilin to Initiate Migrating Motor Complex in Isolated Intestinal Segments

Regeneration of Intrinsic Nerves

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The effect of morphine and motilin on surgically isolated segments of small intestine was studied in 8 dogs. In 4 dogs, the small intestine was divided into four segments by simple transection and reanastomosis (group 1); in 4 others, a 2–4-cm colonic segment was interposed at each of the transection sites (group 2). The migrating motor complex initially cycled independently in each segment in group 1 dogs; after that the migration of the migrating motor complex across transection and reanastomosis began to recover and the recovery was complete 100 days after surgery. In contrast, in group 2 dogs the migration of the migrating motor complex from one segment to the next did not recover even 180 days after surgery. Morphine bolus or infusion initiated premature phase III activity in all segments but the sensitivity to morphine decreased distally. Motilin bolus or infusion initiated premature phase III activity only in the first three segments. The sensitivity to motilin also decreased distally. We concluded that (a) the interposition of a foreign segment severely impedes or prevents the regeneration of enteric nerves; (b) the sensitivity of morphine and motilin in initiating premature phase III activity decreases distally in small intestine; and (c) motilin does not initiate premature phase III activity in the ileum, but morphine may initiate premature phase III activity at all sites in the small intestine.

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In the small intestine, the migrating motor complex (MMC) is a cyclic motor activity that normally begins in the duodenum and migrates distally (1). The underlying mechanism of cycling of this motor activity is an intrinsic neural oscillator (2,3). In the small intestine, these MMC oscillators are arranged in the form of a chain and are coupled in the forward direction by intrinsic nerves. Beginning with the end of phase III activity, the initial 20% of the MMC cycle represents the absolutely refractory state during which an external stimulus does not stimulate a premature MMC cycle. The remainder of the cycle represents the relatively refractory state during which premature phase III activity can be initiated by an external stimulus. The minimum strength of stimulus required to initiate premature phase III activity decreases progressively during the relatively refractory state of the MMC cycle (2).

Both morphine and motilin, when injected during the relatively refractory state, have been reported to initiate premature MMC cycles in the intact small intestine (2,4–9). A premature phase III activity induced by morphine or motilin always starts in the proximal duodenum, regardless of the location of current phase III activity along the bowel or the period in the relatively refractory state when the drug is injected. Does this mean that the receptor sites at which morphine and motilin act to initiate premature phase III activity are present only in the proximal small intestine? This study was undertaken to answer this question by administration of morphine and motilin in isolated intestinal segments that had independent MMC cycles. Previous in vitro studies have indicated that the ability of

Abbreviation used in this paper: MMC, migrating motor complex.

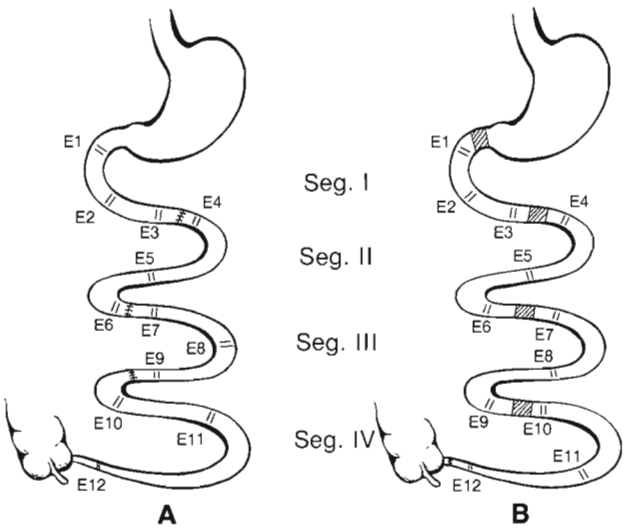

Figure 1. Surgical procedures and arrangement of electrodes in group 1 (*A*) and group 2 (*B*) dogs.

motilin to initiate contractions in muscle strips decreases distally in the alimentary tract (10). We wondered, therefore, if there is a similar decrease in the sensitivity of motilin to initiate premature phase III activity in conscious dogs.

We reported earlier that a transection and reanastomosis in the small intestine temporarily uncouples the MMC oscillators on each side of the junction. However, about 100 days after surgery the intrinsic nerves regenerate and the MMC oscillators are recoupled. Another objective of this study was to obtain further evidence of regeneration of intrinsic nerves by interposing colonic segments at the transection and anastomosis sites and to see if the intrinsic nerves regenerate through foreign neural structures. An abstract of this work has appeared elsewhere (11).

Materials and Methods

Experiments were done on 8 healthy conscious dogs of either sex weighing 20–30 kg. A midventral laparotomy was made under general anesthesia to expose the abdominal cavity. Atropine (100 μg/kg) was administered to minimize the shortening of small intestine due to handling. The entire length of small intestine was measured, divided into four segments of equal length, and marked with sutures. Twelve bipolar electrodes were implanted on the seromuscular layer as shown in Figure 1. Each segment had three electrodes: one was 10 cm proximal to the distal margin of the segment, one was 10 cm distal to the proximal margin of the segment, and the third was halfway between the two. The electrode leads were brought out through a stainless steel cannula as described previously (12). The dogs were allowed to recover for 10 days before making control recordings of morphine and motilin administrations. The dogs were kept on the same diet (equal volumes of canned food and water mixed in a blender) throughout the study period.

The dogs were then divided into two groups. In the 4 dogs of group 1, the small intestine was divided into four segments by simple transection and end-to-end reanastomosis at the three sites marked previously by sutures (Figure 1*A*). In the 4 dogs of group 2, the small intestine was transected just distal to the gastroduodenal junction, just proximal to the ileocecal junction, and at the three sites marked previously with sutures. Colonic segments (2–4 cm long) were interposed at the sites of transection, except for the most distal site near the ileocecal junction; this site was reanastomosed by an end-to-end procedure (Figure 1*B*). The dogs in group 2 were kept on hyperalimentation through the superior vena cava for 1 wk after surgery. A transitory pyloric stenosis occurred due to the interposition of a colonic segment in early postoperative days.

The dogs were trained to lie unrestrained during recording sessions. The recordings were made on a 12-channel Grass recorder (model 7, Grass Instrument Co., Quincy, Mass.) with lower and upper cutoff frequencies set at 0.1 and 15 Hz, respectively. The signals from 8 of 12 electrodes, comprising the proximal and distal electrodes of each segment, were recorded on an FM magnetic tape recorder (model 3968A, Hewlett–Packard Co., Palo Alto, Calif.) for later condensing of the records in time and electronic filtering. The signals were bandpass filtered in the frequency range of 5–10 Hz to separate out electrical control and response activities.

The dogs were fasted for 14–18 h before each recording session. At least two spontaneous phase III activities were recorded at the most proximal electrode in the small intestine during control recordings, or at the most proximal electrode of each isolated segment in subsequent recording sessions. The MMC period was determined in each case and taken as 100%. The end of phase III activity marked the beginning of the next MMC cycle. A bolus intravenous injection of morphine (100 and 200 μg/kg) or motilin (0.1 μg/kg) was then given at ~40% of the MMC cycle in the intact small intestine or in individual isolated segments, and the recording was continued for 3 h. In other experiments, morphine (50 and 200 μg/kg · h) or motilin (0.3 μg/kg · h) infusions were started at 20% of the MMC cycle in the intact small intestine or the first isolated segment, and the infusions were continued for 3 h. All morphine and motilin experiments were done during the first 40 days after surgery when each isolated segment was reported to have independent MMC cycles (3).

A premature phase III activity was defined as one that occurred in response to bolus administration (within 10 min) and the period of the premature MMC cycle was <80% of the previous spontaneous MMC cycle. The recurrence of premature MMC cycles was further tested by using the paired *t*-test in which the period of the spontaneous MMC cycle was compared with the period of the MMC cycle during which bolus was administered or infusion was started. A p value of ≤0.05 was considered statistically significant.

Table 1. Effect of Morphine and Motilin on the Migrating Motor Complex Period in the Intact Small Intestine[a]

	Spontaneous MMC cycle period (*min*)	Premature MMC cycle period (*min*)
Morphine, 100 μg/kg	106 ± 7	50 ± 3
Motilin, 0.1 μg/kg	105 ± 7	52 ± 3
Morphine infusion, 50 μg/kg · h	100 ± 7	55 ± 5
Motilin infusion, 0.3 μg/kg · h	100 ± 7	58 ± 5

MMC, migrating motor complex. [a] n = 24.

Results

Effect of Motilin and Morphine on the Migrating Motor Complex in Intact Small Intestine and Isolated Segments

In the intact small intestine a morphine or a motilin bolus injection or an infusion always initiated premature phase III activity in the duodenum, and this premature phase III activity migrated distally as reported earlier (4–9). The mean period of the premature MMC cycle was significantly shorter than that of the spontaneous MMC cycle before morphine and motilin bolus injections and infusions (Table 1).

Morphine bolus injection, administered at 40% of the MMC cycle in any of the isolated intestinal segments, initiated a premature phase III activity in that segment and in all others that were in their relatively refractory state at the time of injection. In Figure 2, a 100-μg/kg bolus of morphine was given at 40% of the MMC cycle in the first segment. All other segments were in their relatively refractory state at this time. The morphine bolus initiated premature phase III activity concurrently in all four segments and within each segment the phase III activity migrated caudad. The incidence of premature initiation of phase III activity in response to the 100-μg/kg morphine bolus was 100% in the first two segments and it decreased distally as shown in Figure 3*A*. The incidence of premature phase III activity was 100% in all four segments when 200 μg/kg morphine was administered (Figure 3*A*). The incidence of premature phase III activity decreased distally for infusion rates of 50 and 200 μg/kg · h but in the distal two segments the incidence was higher with 200 μg/kg · h than with 50 μg/kg · h (Figure 3*B*). The mean period of the MMC cycle during which morphine was administered was significantly shorter in all four segments (Table 2) in response to 100 μg/kg, 200 μg/kg, and 200 μg/kg · h morphine, and only in the first two segments in response to 50 μg/kg · h morphine (Table 2).

Motilin bolus injections (0.1 μg/kg) or motilin infusions (0.3 μg/kg · h) initiated premature phase III activity only in the first three segments. In Figure 4 a motilin bolus was given at 40% of the MMC cycle in the second segment. All other segments were also in their relatively refractory state at this time. The motilin bolus, however, initiated a premature phase III only in the first and second segments. The per-

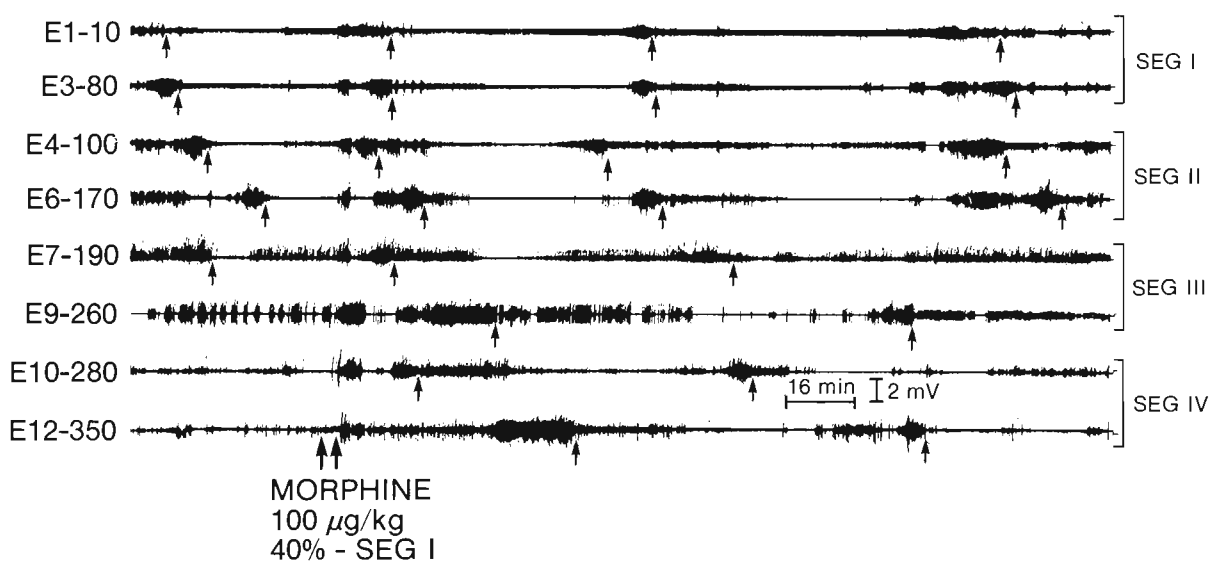

Figure 2. Morphine bolus was given at 40% of the migrating motor complex cycle in the first isolated segment. *Single vertical arrows* indicate the end of phase III activity at the corresponding recording site. *Double vertical arrows* indicate the time of morphine injection. Morphine initiated premature phase III activity concurrently in all four segments that were in their relatively refractory state at the time of morphine injection. Morphine also caused a burst of response activity (spikes) simultaneously throughout the small intestine before it initiated premature phase III activities.

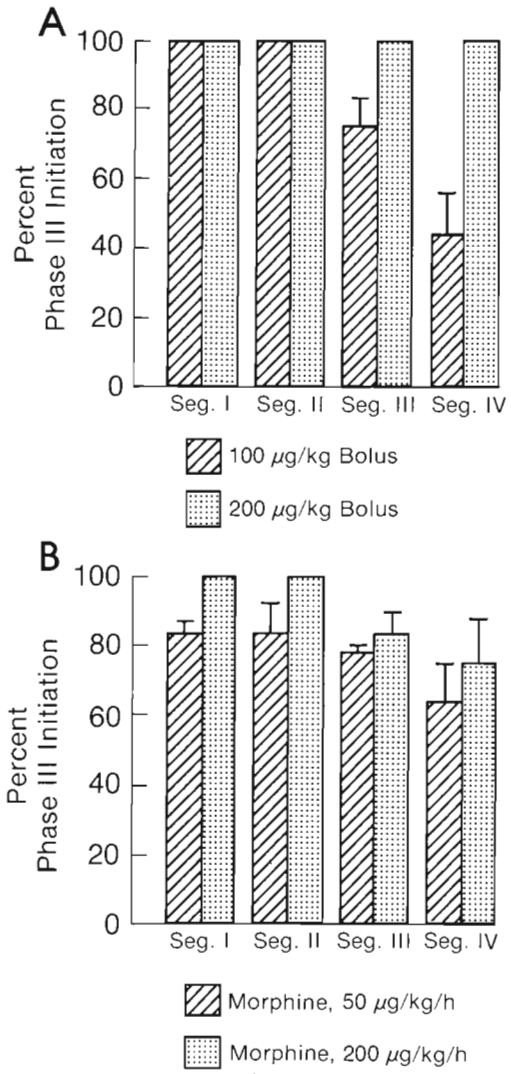

Figure 3. Graph showing percentage initiation of premature phase III activity in response to morphine in all four segments. Percentage initiation decreased distally and was less at lower doses.

centage initiation for both bolus and infusions in the first segment was close to 100%, like that for morphine, but the percentage initiation decreased dramatically and progressively in the second and third segments (Figure 5). The mean period of the MMC cycle during which motilin was administered was significantly shorter than that of the spontaneous MMC cycle only in the first two segments (Table 3).

Regeneration of Enteric Nerves

Initially, the MMC cycled independently in all isolated segments. In group 1 dogs, the MMC coupling between adjacent segments began to recover 40–60 days after surgery and at about 100 days after surgery, the MMCs were coupled in all segments as reported previously (3). In contrast, the MMC coupling never recovered in group 2 dogs with interposed colonic segments even 180 days after surgery. Figure 6 shows independent MMC cycles in all four segments in a dog in group 2, 112 days after surgery.

We and others reported earlier that the intrinsic MMC period of the first isolated intestinal segment was longer than that of the second segment (3,13). One of the reasons for this finding may have been that the first segment was connected to the stomach and its intrinsic period may be affected by that of the stomach. However, we found no significant difference in the gradient of the intrinsic MMC period in group 1 and group 2 dogs as illustrated in Figure 7. In group 2 dogs, the first segment was isolated from the stomach by an interposed colonic segment. In both cases, the mean intrinsic MMC period of the second segment was shorter than that of the first segment. Figure 7 shows the mean periods of the MMC cycle in the intact small intestine and in isolated intestinal segments. The intact and intrinsic MMC periods were not significantly different from each other in the two groups in any of the isolated segments.

Discussion

In the intact small intestine, morphine and motilin always initiated caudad-migrating phase III activity in the proximal duodenum. In the isolated intestinal segments, however, phase III activities were initiated concurrently in all segments that were in their relatively refractory state at the time of bolus

Table 2. Effect of Morphine on the Migrating Motor Complex Period in Isolated Segments[a]

Dose	MMC period (*min*)			
	Segment I	Segment II	Segment III	Segment IV
100 μg/kg				
Spontaneous	96 ± 9	88 ± 11	131 ± 21	94 ± 12
1st cycle post-morphine	47 ± 5[b]	42 ± 3[c]	68 ± 14[d]	77 ± 10[d]
200 μg/kg				
Spontaneous	182 ± 30	108 ± 14	120 ± 14	104 ± 11
1st cycle post-morphine	39 ± 8[b]	43 ± 6[b]	58 ± 6[b]	59 ± 9[c]
50 μg/kg · h				
Spontaneous	128 ± 9	94 ± 7	108 ± 9	123 ± 11
1st cycle post-morphine	55 ± 6[c]	58 ± 9[d]	93 ± 24	92 ± 19
200 μg/kg · h				
Spontaneous	116 ± 7	109 ± 11	132 ± 20	157 ± 17
1st cycle post-morphine	49 ± 5[b]	54 ± 9[c]	82 ± 19[c]	84 ± 10[d]

MMC, migrating motor complex. [a] $n = 24$. [b] $p < 0.001$. [c] $p < 0.01$. [d] $p < 0.05$.

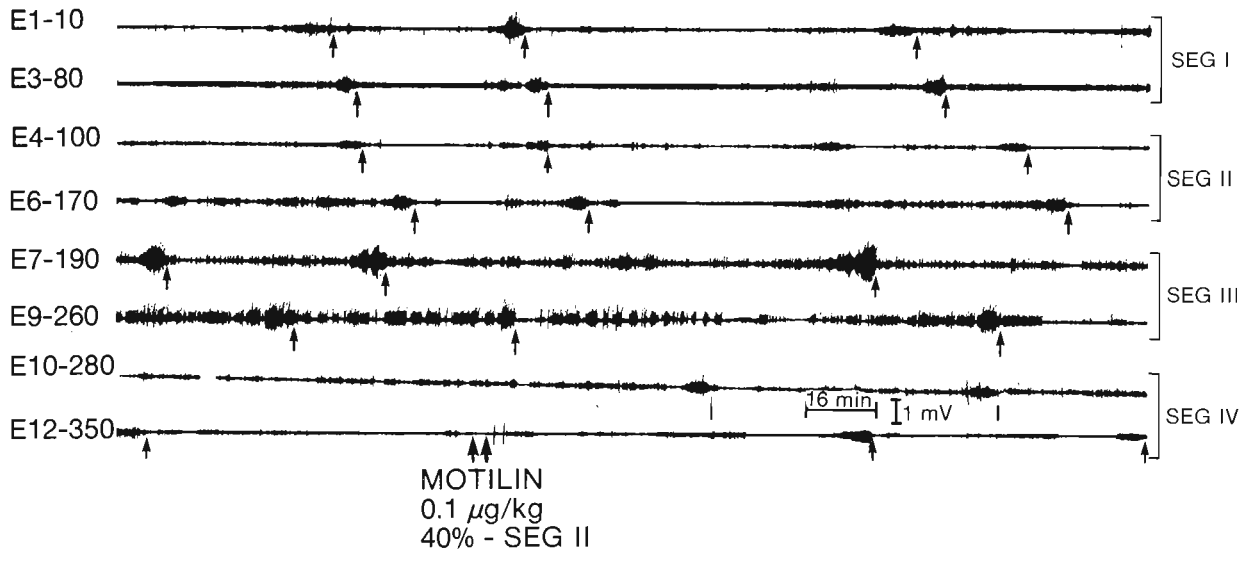

Figure 4. Motilin bolus was given at 40% of the migrating motor complex cycle in the second segment. All segments were in their relatively refractory state at this time, but premature phase III activity was initiated only in the first two segments. *Single vertical arrows* indicate the end of phase III activity; *double vertical arrows* indicate the time of motilin injection.

injection, except in the distal segment where motilin did not initiate phase III activity. In response to bolus injection and infusion of morphine and motilin, the incidence of premature phase III activities decreased distally in isolated segments. This observation implied that the sensitivity of receptor sites or the number of receptor sites to both morphine and motilin decreases distally. In fact, the distal small intestine may not even have receptors through which motilin acts to initiate premature phase III activity. This finding confirms our earlier hypothesis that the receptor sites to initiate premature phase III activities are different for morphine and motilin (8). The morphine receptor sites to initiate premature phase III activity are present in the ileum, but the motilin receptor sites are absent or rare in the ileum.

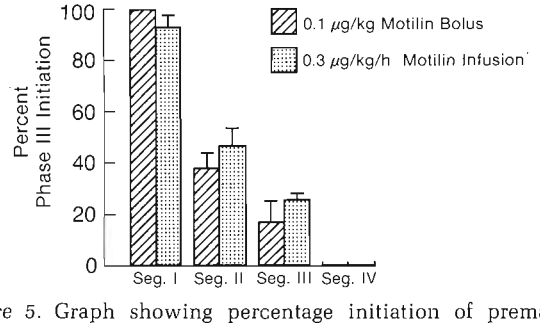

Figure 5. Graph showing percentage initiation of premature phase III activity in response to motilin in all four segments. The response to motilin decreased distally and was absent in the fourth segment.

The results of this study further confirm that the MMC phenomenon in the small intestine can be explained by a chain of MMC relaxation oscillators. When these MMC oscillators, representing small intestinal segments, were uncoupled from each other, each oscillator could be stimulated to concurrently initiate a premature phase III activity. It remains unexplained, however, why the distal oscillators are not stimulated to initiate premature phase III activity when the small intestine is intact. The same stimulus that initiated a premature phase III activity in isolated segments stimulated phase III activity only in the most proximal segment in the intact small intestine. Descending inhibition by phase III activity initiated in the proximal segment through the enteric nervous system may be involved. Interruption of neural continuity may eliminate this

Table 3. Effect of Motilin on the Migrating Motor Complex Period in Isolated Intestinal Segments[a]

Dose	MMC period (*min*)			
	Segment I	Segment II	Segment III	Segment IV
0.1 μg/kg				
Spontaneous	100 ± 7	83 ± 7	95 ± 10	97 ± 10
1st cycle post-morphine	52 ± 3[b]	67 ± 9[c]	104 ± 15	115 ± 8
0.3 μg/kg · h				
Spontaneous	117 ± 11	97 ± 8	101 ± 5	113 ± 4
1st cycle post-morphine	57 ± 8[b]	69 ± 11[c]	96 ± 12	138 ± 11

MMC, migrating motor complex. [a] $n = 24$. [b] $p < 0.001$. [c] $p < 0.05$.

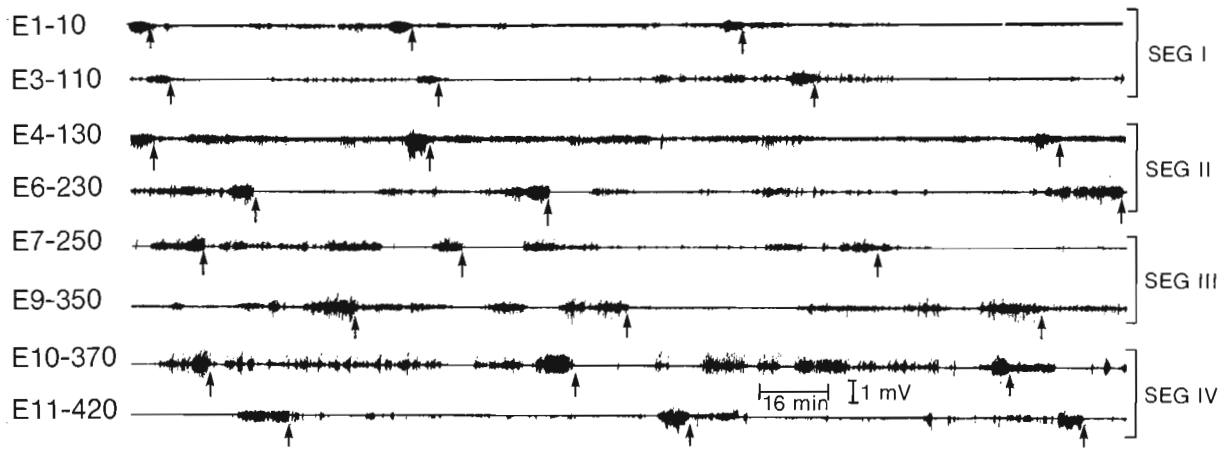

Figure 6. Tracing of independent migrating motor complex cycles in all four segments 112 days after surgery in a dog from group 2. The migrating motor complex did not recouple even 180 days after interposition of colonic segments at the site of transection. *Single arrows* indicate the end of phase III activities.

descending inhibition and enable the distal segments to be stimulated concurrently with the most proximal segment.

The gradient of the intrinsic MMC frequency in the small intestine was the same whether colonic segments were interposed or not. Further, the intrinsic MMC period of the proximal segment was the same whether it was separated from the stomach by a colonic segment or not, and in both cases it was slightly longer than that of the second segment. This implies that the higher intrinsic MMC frequency of the second segment is an inherent characteristic of small intestine; it is not an artifact due to the stomach pulling down the frequency of the first segment when it is uncoupled from the rest of the small intestine (14). The MMC frequency is the reciprocal of the MMC period. This finding is consistent with the results of Heppell et al. (14) and Bueno et al. (15) who found that gastrectomy does not affect the frequency of the MMC oscillation in the intact small intestine.

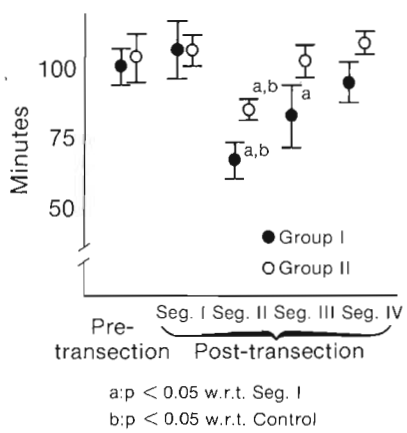

Figure 7. The intrinsic migrating motor complex periods in all four isolated segments and in the intact small intestine. The second segment has a shorter intrinsic migrating motor complex period than that of the first segment.

Another factor that may contribute to a slightly higher intrinsic MMC frequency of the second segment than of the first segment is the effect of circulating endogenous motilin released by MMC contractions in the first segment. Plasma motilin concentration begins to increase with the onset of phase II activity in the duodenum and peaks in a few minutes after the start of duodenal phase III activity (16,17). Our present study shows that the MMC in the second segment can be driven by motilin. The plasma motilin released from the first segment may advance the MMC cycle in the second segment, provided that the plasma motilin concentration is greater than the threshold level at that time in the relatively refractory state of the second segment (2,18). The plasma motilin concentration, however, may not be enough to strongly couple the two segments. Conversely, very little or no motilin may be released in association with the MMC contractions in the second segment and, hence, the MMC cycling in the second segment will not affect the frequency of the MMC cycling in the first segment. Motilin-containing enterochromaffin cells are present largely in the duodenum and proximal jejunum, which was the first segment in our study (19,20). The last two segments respond poorly or not at all to motilin and hence their frequencies will not be affected by circulating plasma motilin.

This study confirms that the recoupling of the MMC across an end-to-end reanastomosis is due to the regeneration of intrinsic nerves (3). The interposition of a foreign segment prevented or considerably

delayed this regeneration. These results indicate that nerves may regenerate where previous neuronal structures do not exist (e.g., in the gap at the site of simple reanastomosis), but once the regenerated nerve fibers encounter an intact neural structure such as that in the colonic segment, the ability or the urge to regenerate may stop, particularly if the intact neural structure is different from that of their own. In group 2 dogs, it is possible that the nerves may have regenerated up to the proximal end of the colonic segment. The other possibility is that the rate of regeneration is so small that it may take a long time to regenerate across a 2–4-cm-long colonic segment. For example, it took nearly 3 mo for nerves to regenerate completely across a 1–2-mm reanastomosis. These possibilities remain to be explored by immunocytochemical studies.

Strunz et al. (10) reported a gradient of sensitivity to motilin down the small intestine in in vitro studies. Our studies in conscious dogs show that there is not only a gradient of motilin sensitivity to initiate contractions but also to initiate premature MMCs.

In conclusion, the effectiveness of motilin and morphine in initiating a premature phase III activity decreases distally in the small intestine. Motilin does not initiate premature phase III activity in the ileum. The gastric cyclic motor activity has no effect on the frequency of MMCs in the duodenum. The intrinsic nerves either do not regenerate through a foreign neuronal structure or take a very long time to regenerate.

References

1. Szurszewski JH. A migrating electric complex of the canine small intestine. Am J Physiol 1969;217:1757–63.
2. Sarna S, Northcott P, Belbeck L. Mechanism of cycling of migrating myoelectric complexes: effect of morphine. Am J Physiol 1982;242(Gastrointest Liver Physiol 5):G588–95.
3. Sarna S, Condon RE, Cowles V. Enteric mechanisms of initiation of migrating myoelectric complexes in dogs. Gastroenterology 1983;84:814–22.
4. Itoh Z, Honda R, Hiwatashi K, et al. Motilin-induced mechanical activity in the canine alimentary tract. Scand J Gastroenterol 1976;11(Suppl 39):93–110.
5. Chey WY, Lee KY. Actions of motilin on gastrointestinal motility and plasma immunoreactive motilin concentration in interdigestive and postprandial states. Endocrinol Jpn 1980;(Suppl 1):173–7.
6. Vantrappen GR, Janssens J, Peeters TL, Bloom SR, Christofides ND, Hellemans J. Motilin and the interdigestive migrating motor complex in man. Dig Dis Sci 1979;24:497–500.
7. Wingate DL, Ruppin H, Green WER, et al. Motilin-induced electrical activity in the canine gastrointestinal tract. Scand J Gastroenterol 1976;11(Suppl 39):111–8.
8. Sarna S, Condon RE, Cowles V. Morphine versus motilin in the initiation of migrating myoelectric complexes. Am J Physiol 1983;245(Gastrointest Liver Physiol 8):G217–20.
9. Sarna SK, Lang IM. Dose- and time-dependent biphasic response of morphine on migrating myoelectric complex cycling. J Pharm Exp Ther (in press).
10. Strunz U, Domschke W, Mitznegg P, et al. Analysis of the motor effects of 13-norleucine motilin on the rabbit, guinea pig, rat, and human alimentary tract in vitro. Gastroenterology 1975;68:1485–91.
11. Matsumoto T, Frantzides CT, Sarna SK, Condon RE, Cowles VE. Differential sensitivity of morphine and motilin in isolated small intestinal segments (abstr). Gastroenterology 1984;85:1177.
12. Sarna SK, Stoddard C, Belbeck L, McWade D. Intrinsic nervous control of migrating myoelectric complexes (MMC's). Am J Physiol 1981;241(Gastroenterol Liver Physiol):G16–23.
13. Sarr MG, Kelly KA. Myoelectric activity of the autotransplanted canine jejunoileum. Gastroenterology 1981;81:303–10.
14. Heppell J, Taylor BM, Kelly KA. Gastric influences on canine small intestinal myoelectric activity. Dig Dis Sci 1984;29:849–52.
15. Bueno L, Rayner V, Ruckebusch Y. Initiation of the migrating myoelectric complex in dogs. J Physiol (Lond) 1981;316:309–18.
16. Sarna S, Chey WY, Condon RE, Dodds WJ, Myers T, Chang TM. The cause-and-effect relationship between motilin and migrating myoelectric complexes. Am J Physiol 1983;245(Gastroenterol Liver Physiol):G277–84.
17. Sarna SK, Chey W, Condon RE, Dodds W, Myers T, Chang TM. Motilin release and the migrating myoelectric complexes. In: Roman C, ed. Gastrointestinal motility. Lancaster, England: MTP Press, 1984:223–30.
18. Sarna S, Condon RE. Morphine-initiated migrating myoelectric complexes in the fed state in dogs. Gastroenterology 1984;86:662–9.
19. Pearse AGE, Polak JM, Bloom SR, Adams C, Dryburgh JR, Brown JC. Enterochromaffin cells of the mammalian small intestine as the source of motilin. Virchows Arch [Cell Pathol] 1974;16:111–20.
20. Polak JM, Pearse AGE, Heath CM. Complete identification of endocrine cells in the gastrointestinal tract using semithin-thin sections to identify motilin cells in human and animal intestine. Gut 1975;16:224–9.