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Research Article

The myoelectric response of the rabbit ileum was studied in response to live Vibrio cholerae culture, a whole cell lysate of cholera, and the purified enterotoxin. Each cholera preparation produced a series of highly organized migrating action potential complexes (MAPC). An MAPC was defined as action potential discharge with a duration of 2.5 s or longer, followed by similar activity on at least one other consecutive electrode site. The mean and modal onset time of MAPC activity occurred 4 h after the infection with live Vibrio cholerae culture, the freeze-dried whole cell lysate preparation, or the purified enterotoxin. After the onset of activity this pattern persisted for the duration of the recording period (up to 12 h). The MAPC had a mean propagation velocity of 0.85+/-0.07 cm/s (mean+/-SEM), which remained constant with time. Direct visual observation of the loop revealed that the MAPC's resulted in contractions that propelled intraluminal contents in an aborad direction. The mean fluid output from the 12-cm ileal loops was 6.4+/-1.1 ml/h (mean+/-SEM). Control experiments consisted of recordings from: (a) a ligated ileal loop into which nothing was placed; (b) a ligated ileal loop into which either uninfected culture broth or 0.9% NaCl solution was injected; (c) a ligated ileal loop infused with 0.9% NaCl solution at a rate of 11.2 ml/h, and (d) rapid injection […]

Intestinal Myoelectric Activity in Response to Live *Vibrio cholerae* and Cholera Enterotoxin

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ABSTRACT The myoelectric response of the rabbit ileum was studied in response to live *Vibrio cholerae* culture, a whole cell lysate of cholera, and the purified enterotoxin. Each cholera preparation produced a series of highly organized migrating action potential complexes (MAPC). An MAPC was defined as action potential discharge with a duration of 2.5 s or longer, followed by similar activity on at least one other consecutive electrode site. The mean and modal onset time of MAPC activity occurred 4 h after the infection with live *Vibrio cholerae* culture, the freeze-dried whole cell lysate preparation, or the purified enterotoxin. After the onset of activity this pattern persisted for the duration of the recording period (up to 12 h). The MAPC had a mean propagation velocity of 0.85±0.07 cm/s (mean ±SEM), which remained constant with time. Direct visual observation of the loop revealed that the MAPC's resulted in contractions that propelled intraluminal contents in an aborad direction. The mean fluid output from the 12-cm ileal loops was 6.4±1.1 ml/h (mean ±SEM). Control experiments consisted of recordings from: (*a*) a ligated ileal loop into which nothing was placed; (*b*) a ligated ileal loop into which either uninfected culture broth or 0.9% NaCl solution was injected; (*c*) a ligated ileal loop infused with 0.9% NaCl solution at a rate of 11.2 ml/h, and (*d*) rapid injection of 1.0, 2.5, 5.0, or 10.0-ml boluses of 0.9% NaCl into the proximal catheter. MAPC activity was not observed in any of the control experiments. These studies indicate that in addition to a secretory component to cholera, there exists a highly organized MAPC that results in contractions that propel intraluminal contents in an aborad direction.

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INTRODUCTION

The mechanism by which cholera produces diarrhea has been of considerable interest in recent years (1–5). The current concept is that cholera diarrhea results from an active secretory process. The secretions have been shown to be predominantly of small bowel origin (6, 7). The diarrhea is caused without invasion of the organism into the mucosa and there are no observed changes in intestinal morphology (8, 9). The active secretory process (10, 11) accounts for fluid loss, dehydration, and electrolyte imbalance. Recently, it has been shown that the enterotoxin produces the above effects by altering the concentration of intracellular 3′,5′ cyclic AMP (12, 13). The rise in 3′,5′ cyclic AMP results from activation of adenylate cyclase (14, 15), but the exact mechanism of activation is unknown at present.

To date, only the secretory disturbances of cholera diarrhea have been investigated in depth. Clinically, cholera diarrhea has been described as painless once the dehydration and electrolyte deficiencies have been corrected (16). Early investigators described the intestinal loop as flaccid when exposed to live *Vibrio* or the enterotoxin (1). The term "open pipe phenomenon" evolved to describe this observation, that is, a segment of bowel offering little resistance to the flow of intraluminal contents. An alternative explanation may be that an increase in organized contractile activity develops that contributes to aborad fluid movement. Studies using sulfobromophalein dye placed in the stomach of infant rabbits infected with cholera demonstrated a shortened transit time (17). However, other studies using an indicator dilution technique revealed normal to decreased values during segmental perfusion (18). There-

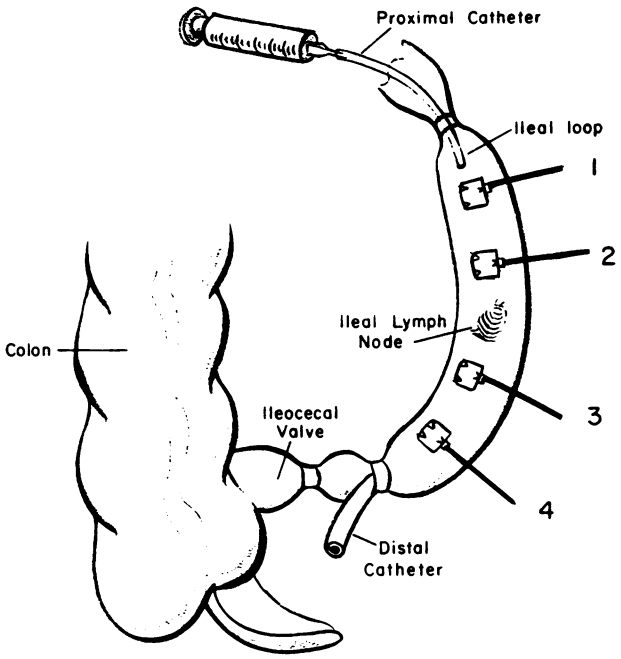

FIGURE 1 Schematic illustrations of a 12-cm distal ileal loop model. Four monopolar silver-silver chloride electrodes are illustrated in their approximate position, with the ileal lymph node as an anatomic landmark. A small catheter was inserted proximally for the administration of the enterotoxin, A larger catheter was inserted distally for the outflow of intraluminal contents.

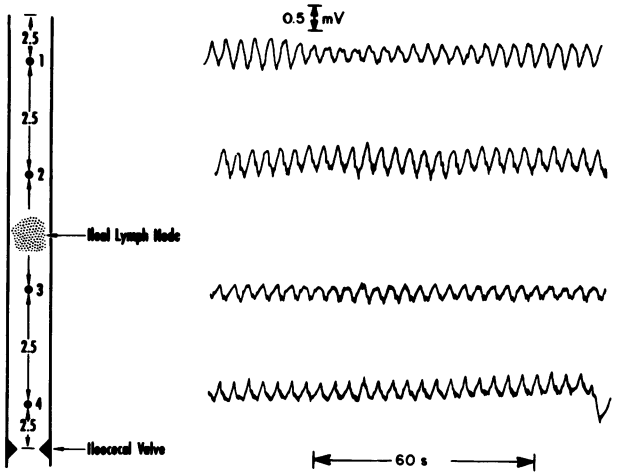

FIGURE 2 A control tracing from the distal ileal segment. The electrode placement is illustrated schematically at the left. The slow wave rhythm is illustrated on all four electrode sites. There is action potential discharge activity on electrode 2.

fore, the evidence for the presence or absence of a motility component in cholera diarrhea is at best questionable.

Myoelectric recording techniques may be used to assess the electrical events regulating intestinal contractile activity (19–22). The purpose of this study was to investigate the myoelectric events in the ileum of New Zealand white rabbits exposed to cultures of live *Vibrio cholerae,* freeze-dried whole cell lysate preparation, or the purified enterotoxin.

METHODS

All studies were performed in New Zealand white rabbits of either sex weighing between 1.5 and 3.0 kg. The rabbits were anesthetized initially with pentobarbitol sodium (30 mg/kg) through an ear vein. Additional anesthesia was administered through a catheter placed in the external jugular vein. A tracheostomy was performed in each animal. The distal ileum was located through a midline abdominal incision.

Fig. 1 illustrates the ileal preparation used in all studies. The preparation consisted of the distal 12 cm of small bowel. Four monopolar silver-silver chloride electrodes (23) were sewn to the serosal surface at 2.5-cm intervals. Each electrode was connected to a rectilinear recorder (Beckman RM Dynograph, Beckman Instruments, Fullerton, Calif.) through AC couplers (9806 A). Studies were performed with a time constant of 1.0 s, a high-frequency filter cutoff of 22 Hz and a sensitivity of 0.5 mV/cm. An indifferent electrode was placed in the subcutaneous tissue of a hind limb. Respirations were recorded by a pneumograph placed around the chest and attached to a pressure transducer (Statham P32, venous, Statham Instruments Div., Gould Inc., Oxnard, Calif.). The intra-abdominal temperature was recorded with a standard centrigrade thermometer upon opening of the peritoneal cavity and maintained at this level throughout the experiment by means of a 60-W incandescent heating light.

The proximal end of the ileal loop was catheterized with a polyethylene tube (inside diameter, 0.030 inch; outside diameter 0.048 inch), inserted through a serosal puncture and secured by a purse-string suture. The distal end of the loop was catheterized by a second polyethylene tube (inside diameter, 0.187 inch; outside diameter, 0.250 inch) and secured by a distal ligature. The proximal catheter was used to administer materials into the ileal loop. The distal catheter was used to collect ileal output. All animals were allowed to stabilize for at least 1 h after surgical preparation. After this period, one of the following materials was administered into the proximal end of the loop: (*a*) 1.0 ml of live *Vibrio cholerae*[1] culture containing $1.8–4.6 \times 10^8$ organisms/ml; (*b*) 1.0 ml of the freeze-dried whole cell lysate preparation of *Vibrio cholerae*[2] (100 mg/ml); and (*c*) 1.0 ml of the purified enterotoxin of *Vibrio cholerae*[3] (100 μg/ml). Animals were studied for 8–12 h after the administration of compounds into the ileal loop.

Control experiments consisted of: (*a*) no material administered into the ileal loop; (*b*) 1.0 ml of the sterile culture broth administered into the ileal loop, (*c*) 1.0 ml of 0.9% NaCl solution administered into the ileal loop; (*d*)

[1] Calcutta strain, supplied by the Clinical Disease Center, Atlanta, Ga.

[2] The cholera toxin employed was prepared under contract for the National Institute of Allergy and Infectious Disease, National Institutes of Health. *J. Infect. Dis.* 121: S63, 1970.

[3] Purified protein obtained from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.

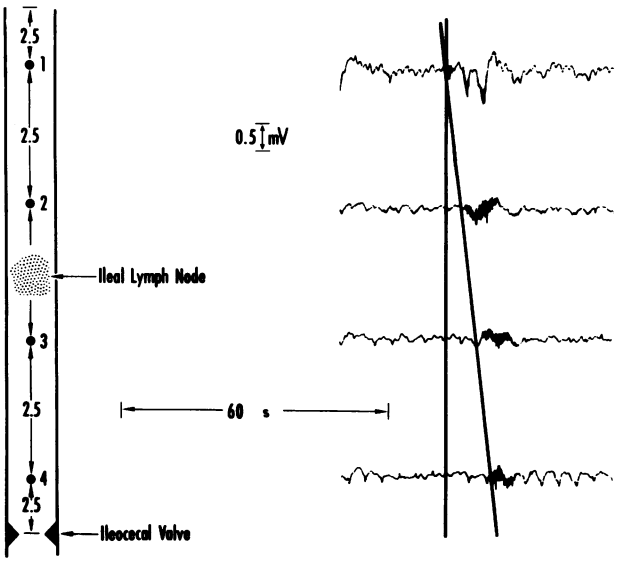

FIGURE 3 The MAPC. The electrode placement is illustrated schematically on the left. The slope of the line as compared to the vertical reference line represents the propagation velocity of the MAPC. The propagation velocity of this complex was 0.85 cm/s.

0.9% NaCl solution perfused into the loop at a rate of 11.2 ml/h (7.2–8.4 being the expected output for a 12-cm loop, as calculated from Grady and Keusch [2]); and (*e*) 1.0, 2.5, 5.0, and 10.0-ml boluses of 0.9% NaCl rapidly injected into the proximal catheter.

All myoelectric recordings were evaluated for the following parameters: slow wave frequency, slow wave propagation velocity, migrating action potential complex (MAPC) [4] frequency, the MAPC propagation velocity, the MAPC onset time, and ileal fluid output from the distal catheter in milliliters per hour. The results were evaluated by a Student *t* test.

RESULTS

Fig. 2 illustrates normal slow wave activity in a control recording. Action potential discharge activity can be seen on several slow waves in lead 2.

Fig. 3 illustrates the ileal myoelectric response observed after the administration of a live *Vibrio cholerae* culture. Identical patterns were observed with the freeze-dried whole cell lysate or the purified enterotoxin. A highly organized MAPC occurred and propagated through the four leads of the myoelectric recording. An MAPC was defined as action potential discharge of 2.5 s or longer, occurring on at least two consecutive electrode sites. The action potential discharge activity often lasted as long as 8–10 s over a distance of several slow waves. The duration of the action potential discharge activity, however, varied from complex to complex. In this recording the MAPC had a propagation velocity

[4] *Abbreviation used in this paper:* MAPC, migrating action potential complex.

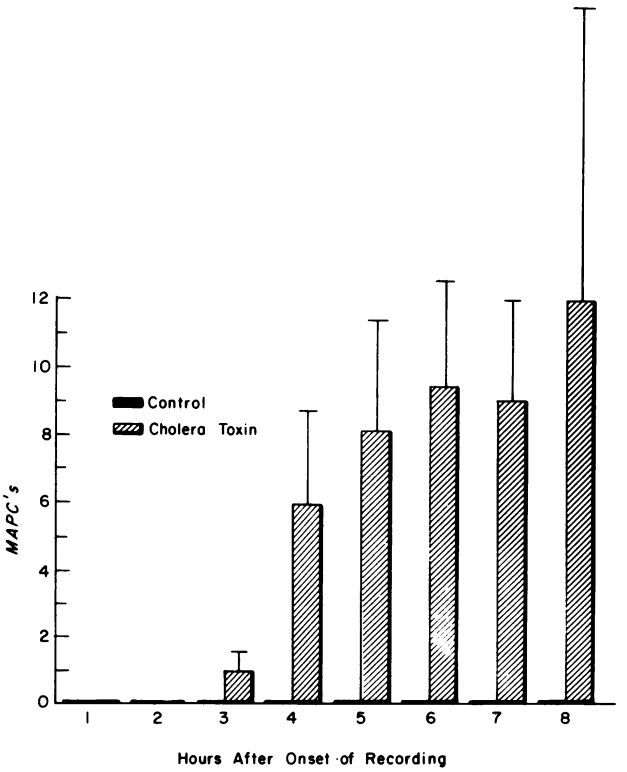

FIGURE 4 The onset time of MAPC's for the 8-h recording period. The vertical axis represents the mean number of MAPC's per hour. The horizontal axis represents hours after the onset of recording of the infected ileal loops. The solid bars represent controls; the hatched bars represent cholera-infected loops. The line above each bar represents the SEM.

of 0.85 cm/s. Direct visual observation of the loop through a polyethylene covering placed over the abdominal incision revealed a propagating ring contraction occurring simultaneously with MAPC activity. The contractions resulted in the propulsion of intraluminal contents out through the distal catheter.

13 animals were studied. 3 of the 13 animals did not react to the cholera toxin. There was an absence of both fluid accumulation and motility in the nonreactors. This nonreactor rate for motility was similar to the nonreactor rate observed by Leitch and Burrows for secretion (24).

Fig. 4 illustrates the onset time of MAPC activity for an 8-h recording period after the administration of live *Vibrio cholerae* culture ($n = 3$), the whole cell lysate ($n = 3$), the purified enterotoxin ($n = 3$), or a double loop preparation ($n = 1$). The mean and modal onset time for the MAPC was 4 h. In one experiment, MAPC activity occurred as early as 3 h. The MAPC activity continued for the duration of the recording period (up to 8 h after infection). In two of the experiments the recording period was extended up to 12 h.

TABLE I

The Myoelectric Parameters of Control and Cholera-Infected Ileal Loops

	Control (n = 3)	Broth (n = 3)	NaCl infusion (11.2 ml/h) (n = 3)	Live *Vibrio* culture (n = 3)	Whole cell lysate (n = 4)	Enterotoxin (n = 3)
Slow wave frequency, *cycles/min*	17.3±2.9	17.0±0.6	13.6±0.7*	15.7±1.2	18.2±0.4	15.5±2.7
Slow wave propagation‡ velocity *cm/s*	1.14±0.14	1.58±0.35	1.44±0.56	1.12±0.1	1.32±0.03	1.17±0.12
Number of MAPC, h^{-1}	None	None	None	7.12±3.12	8.72±2.47	5.4±1.08
MAPC onset time, *h after infection*§	—	—	—	4.0±0.57	3.5±0.50	5.0±1.00
MAPC propagation velocity, *cm/s*§	—	—	—	0.85±0.13	0.84±0.01	1.50±0.30

Resuts are means±SEM.

* ($P < 0.01$) highly significant over control value of 17.3±2.9, P values for the broth, live *Vibrio* culture, whole cell lysate, or the enterotoxin are all >0.05.

‡ ($P > 0.05$) for the broth, infusion, *Vibrio*, whole cell lysate, or enterotoxin as compared to the control.

§ ($P > 0.05$) for whole cell lysate or enterotoxin as compared to the live *Vibrio* culture.

MAPC activity persisted for the duration of the recording period.

Table I summarizes the parameters of myoelectric activity seen in all control and cholera studies. In all cholera studies no significant alteration in slow wave frequency or slow wave propagation velocity occurred when compared to control preparations. The three types of cholera preparations produced no significant differences in the number of MAPC's over time. Fig. 5 illustrates the 10 ileal loops infected with live *Vibrio cholerae,* the whole cell lysate, or the purified enterotoxin on the right, and the six control loops on the left. The number of MAPC's per hour is represented on the vertical axis. The horizontal axis represents a 3-h recording period after onset of MAPC activity. A random occurrence of MAPC's was observed in the cholera-infected loops. No MAPC's were observed in the control experiments. The number of MAPC's per hour and the time of onset were similar for the culture, the whole cell lysate, and the purified enterotoxin. The MAPC propagation velocity was similar for the live *Vibrio cholerae* culture and the whole cell lysate, but was increased with the purified enterotoxin. The difference in the propagation velocity for the purified enterotoxin, however, was not statistically significant ($P > 0.05$).

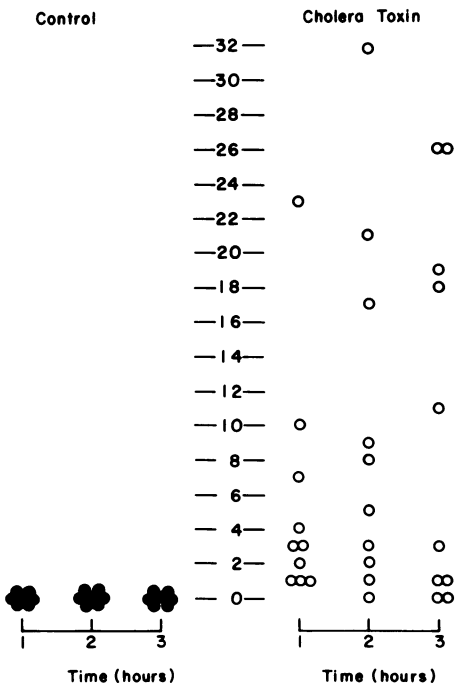

FIGURE 5 The number of MAPC's per hour for the 10 loops infected with the live *Vibrio* culture, the whole cell lysate, or its purified enterotoxin is shown on the vertical axis. The horizontal axis represents a 3-h recording period after the onset of MAPC activity. The left column illustrates no MAPC activity in the six control recordings. The right column illustrates a random occurrence of MAPC's for the 10-cholera-infected preparations.

Ileal fluid output was 6.4±1.1 ml/h (mean±SEM) for the three types of cholera preparation. In contrast, administration of 1.0 ml of the sterile culture broth or 1.0 ml of 0.9% NaCl solution in the ileal loop resulted in no fluid output. Constant infusion of 0.9% NaCl solution at a rate of 11.2 ml/h into the ileal loop resulted in a collection of 11.2±0.3 ml/h from the distal catheter.

Bolus injections of 1.0, 2.5, 5.0, and 10.0 ml were studied for the effect of sudden distention on the myoelectric activity of the ileal loop. Action potential discharge activity occurred but did not fulfill the criteria of the MAPC. In one of the cholera-infected loops, the distal catheter became occluded by a bolus of mucus. The loop had gradually distended from the secretion produced by the toxin. All action potential discharge activity had ceased and flattening of the slow waves was noted. Once the mucous plug had been removed and the distention of the loop relieved, the slow wave amplitude

returned to normal and the MAPC activity soon developed. It appears that distention is not related to the onset of the complex activity.

Fluid accumulation in response to cholera toxin was observed in the distal catheter as early as 2 h after infection. This fluid accumulation occurred without the presence of action potential discharge activity. However, propulsion of the intraluminal contents did not occur until the onset of the MAPC.

The effect of cholera enterotoxin was also studied in one double-loop preparation. This preparation consisted of two adjacent ileal loops in the same animal. 1 ml of cholera enterotoxin was administered into the distal loop and 1.0 ml of sterile 0.9% NaCl was administered into the proximal loop. MAPC activity and fluid output occurred only in the infected loop. In 10 additional experiments designed to evaluate the mechanism of the MAPC, scanning electrodes were placed beyond the distal ligature. No MAPC activity occurred. If the distal ligature was not secured, MAPC activity was noted at the site of the most distally placed electrode, as far as the transverse colon. Thus, this observation suggested a local and specific effect of the toxin on the motility component of cholera. Similar values were obtained in these additional experiments on MAPC onset time, 3.77±0.73 (mean±SEM), and MAPC propagation velocity, 1.17±0.15 (mean±SEM). These values were not significantly different ($P > 0.05$) from the values of live *Vibrio* cultures, the whole cell lysate, or the purified enterotoxin.

DISCUSSION

These studies indicated that when rabbit ileal loops were exposed to live *Vibrio cholerae* cultures, the freeze-dried whole cell lysate preparation, or the purified enterotoxin, a highly organized myoelectric pattern occurred. This pattern can best be described as an MAPC. These MAPC's were observed only in the cholera-treated loops.

The MAPC as seen in these experiments represented a new observation. No similar types of myoelectric recordings in response to infection with *Vibrio cholerae* or its enterotoxin have been previously reported. The MAPC activity seemed to be a specific localized effect of cholera enterotoxin. The MAPC could be produced only in the ileal loop exposed to the cholera or to its enterotoxin. Adjacent loops of bowel in cholera-treated animals did not show the altered myoelectric pattern. Additionally, MAPC activity could not be induced by sterile broth or by ileal perfusion with saline. The latter study used a perfusion rate of 11.2 ml/h, in excess of the output as calculated from a previously determined secretory rate of 600–700 μl/cm per h (7.2–8.4 ml/h) (2). In addition, rapid control injections of 1.0, 2.5, 5.0, and 10.0 ml of 0.9% NaCl produced action potential discharge activity but did not meet the criteria for the definition of the MAPC. These studies suggested that the MAPC was not simply a result of fluid transport in the ileal loop.

Certain characteristics of the MAPC are of interest. The onset of activity (4 h) was beyond the time of the reported observations on intestinal secretion as measured by a change in concentration of cyclic 3′,5′ AMP (30–120 min) (2, 15). After the onset of MAPC activity, the number of MAPC's for each hour of recording varied widely (Fig. 5), and occurred at random. However, the MAPC activity was noted to persist for the duration of the recording period (up to 12 h) after exposure to the cholera or its toxin. Although no alterations in slow wave frequency or slow wave propagation velocity were noted, some distortion in slow wave configuration was observed just before the onset of MAPC activity. These changes consisted of a widening of the base of the slow wave and an increase in slow wave amplitude. The propagation velocity of the MAPC differed for the live *Vibrio* (0.85 cm/s) and whole cell lysate (0.84 cm/s) as compared to the purified enterotoxin (1.50 cm/s). This difference in propagation velocity of the MAPC between the purified enterotoxin and the live *Vibrio* or whole cell lysate was not statistically significant and remains unexplained. These propagation velocities, however, are in the range of the control slow wave propagation velocity (1.14 cm/s), suggesting that MAPC's are propagated with the slow waves.

Although the present studies were designed to measure changes in myoelectric activity in response to cholera, direct visual observation of the loop revealed that the MAPC resulted in propagating ring contractions that propelled intraluminal contents through the distal catheter, emptying the loop. Minimal fluid movement occurred if MAPC activity was absent.

A motility component in response to cholera enterotoxin has not been previously noted, despite multiple studies using bowel loop preparations. Several factors may explain the previous failure to observe contractile responses to cholera or its toxin. First, early experiments consisted of closed or ligated ileal loops. We have observed that moderate loop distention may result in a flattening of the slow wave and an absence of action potential activity (unpublished observations). One may speculate that the distention of the loop from impairment of fluid output may interfere with electrical communication between slow wave activity in the longitudinal smooth muscle layer and the action potential discharge activity in the circular smooth muscle layer of the ileum (25, 26). Second, the small bowel secretory process occurs at 30–120 min, before the onset of MAPC

activity. Most secretory studies were not long enough to observe the changes in motility. Third, myoelectric recordings have not been previously made in cholera-treated animals.

In summary, we have demonstrated a highly organized MAPC in rabbit ileal loops exposed to live *Vibrio cholerae,* its whole cell lysate, or the purified enterotoxin. The MAPC resulted in propagating ring contractions that propelled intraluminal contents in an aborad direction. The MAPC activity appeared to be a localized phenomenon that did not occur under control conditions. If the MAPC activity observed in the ligated loops is present throughout the entire length of small intestine, then the motility component would appear to contribute significantly to cholera diarrhea. These studies provide initial evidence to suggest that in New Zealand white rabbits, a change in small intestinal motor activity may contribute to the movement of intraluminal contents occurring from cholera infection.

ACKNOWLEDGMENTS

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