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Anticholeraic Effect of Methylated Casein in Rat Jejunum

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ABSTRACT. To explore the antisecretory effect of methylated casein (MC) cholera toxin was placed in isolated jejunal loops, and in vivo water fluxes were measured 3 h later in the presence or absence of MC. Secretion was observed in the loops filled with Ringer's solution only, but net absorption was observed in all 10 loops to which MC was added. Its actions was evident within 20 min, and was exerted directly on the luminal side of the epithelium. This response was dose-dependent and the antisecretory effect vanished after boiling MC and after ultrafiltration. In vitro, the antisecretory effect of MC consisted of reversing net Na and Cl fluxes from secretion to absorption (ΔJ_{net}^{Na} = 6.18 ± 1.25 and ΔJ_{net}^{Cl} = 5.10 ± 1.66 μEq·h^{-1}·cm^{-2}). This change was due to the enhancement of mucosal to serosal flux. Transepithelial potential difference and tissue conductance did not alter. Interestingly, MC did not interfere with intestinal function in the absence of stimulation by cholera toxin. In the presence of cholera toxin, MC and glucose both stimulated ionic absorption by different mechanisms, MC stimulating neutral NaCl absorption, and glucose stimulating electrogenic Na absorption. MC did not alter basal adenylate cyclase activity but it inhibited the cholera toxin-stimulated increase in activity. The present results indicate that methylated casein inhibits water and electrolyte secretion induced by cholera toxin in rat jejunum. Its availability, low cost, and curative effect from the luminal side constitute compelling indications for further investigation. (Pediatr Res 18:1075–1079, 1984)

Abbreviations

CT, cholera toxin
I_{sc}, short circuit current (μEq·h^{-1}·cm^{-2})
PD, transepithelial potential difference (mV)
G, tissue conductance (mS·cm^{-2})
J_{ms}, mucosal to serosal flux (μEq·h^{-1}·cm^{-2})
J_{sm}, serosal to mucosal flux (μEq·h^{-1}·cm^{-2})
MC, methylated casein

The mechanism of the diarrhea induced by purified cholera toxin has been extensively studied over the last 15 years (for recent review, see Refs. 6 and 12). Cholera toxin is known to induce intestinal secretion through the epithelium by a mechanism involving four sequential events: 1) specific binding of the B subunit to the luminal membrane; 2) stimulation of adenylate cyclase at the basolateral membrane by the A subunit; 3) stimulation of electrolyte secretion, with preservation of glucose-Na absorption, as a consequence (at least in part), of increased intracellular cAMP; and 4) water secretion into the intestinal lumen as a result of electrolyte secretion.

As our knowledge of cholera toxin-induced secretion improved, the concept that intestinal secretion could be inhibited developed. In fact, a wide variety of agents which alter electrolyte transport and are thus potentially antidiarrheal have been identified; they include propranolol, anti-inflammatory drugs, somatostatin, phenothazides, opiates, colchicine, and others (for recent review, see Refs. 8 and 19).

While physiologists were studying secretion, clinicians were looking for an effective therapy for acute diarrhea. These combined efforts resulted in the striking finding that oral glucose electrolyte solutions reduced dehydration in cholera (9, 17). It was then hoped that the use of antisecretory agents would constitute a further therapeutic advance. However, many of these drugs may have serious drawbacks when used in humans, including 1) systemic effects related to high blood concentration and to the fact these effects are not specific to the intestine, 2) delayed action, 3) IV route of administration, and 4) high cost.

Another approach to the pharmacological treatment of acute diarrhea would be to examine the antisecretory effect of the drugs traditionally used in diarrhea. Methylated lactoproteins are commonly used with success (5, 15) in treating acute diarrhea in France. They are cheap, widely available, and administered by mouth. This paper deals with the anticholeraic effect of methylated casein in rat jejunum.

MATERIALS AND METHODS

Wistar rats weighing 200–250 g after an overnight fast were anesthetized with intraperitoneal pentobarbital (1.5% solution, 0.1 ml/100 g of body weight). After tracheotomy, they were placed under a heated hood. Two to four isolated jejunal loops of 10 cm each were prepared. The first one started 5 cm from the pylorus; the loops were separated by a distance of 4 to 5 cm. Each loop was filled with 1.5 ml Ringer's solution at 37° C, with or without 10 μg purified CT (Sigma), and incubated for 3 h (Period I) to allow intestinal secretion to develop.

Transport Studies. In vivo. At the end of the 3-h incubation period, the intestinal fluid was collected from each loop. Loops were rinsed and shortened by 1 cm to exclude the opening made by the first fluid collection. They were then filled with 1.5 ml Ringer's solution with or without the compound to be tested. After 20 min (Period II), loops were removed, opened, and emptied, and the entire luminal contents were collected. Water fluxes (J_v) were measured either by diluting mol wt 4000 [^{3}H] polyethylene glycol (New England Nuclear) (50 μCi/loop) or by weighing the fluid collected. The two methods gave essentially the same results (see below); J_v are expressed in μl of fluid absorbed (positive values) or secreted (negative values) per min and per cm^2 of loop area (measured with a ruler at the end of the experiment).

In vitro. In a second group of rats, the unstripped jejunum was mounted as a flat sheet between two Lucite half-chambers, after the 30-min incubation period, as previously described for rat and rabbit ileum (3, 18, 20, 21). The loops prior to mounting in the chambers were handled exactly as in the *in vivo* studies. Transepithelial unidirectional Na and Cl fluxes from mucosa to serosa ($J_{m\rightarrow s}$) and from serosa to mucosa ($J_{s\rightarrow m}$) were measured under short circuit conditions using ^{22}Na and ^{36}Cl as isotopic tracers. Isc, PD, and G were measured with an automatic voltage clamp

that corrects for fluid resistance. The mean values for the fluxes and electrical parameters measured between 40 and 130 min after the tissue was mounted in the chambers are reported. Both sides of the tissue were bathed with the following Ringer's solution (in mM): NaCl, 115; NaHCO$_3$, 25; K$_2$HPO$_4$, 2.4; KH$_2$PO$_4$, 0.4; MgCl$_2$, 1.2; CaCl$_2$, 1.2. In some experiments, 10 mM D-glucose was added on both sides. No methylated casein was added *in vitro*.

Enzyme assays. At the end of the 20-min *in vivo* incubation, mucosal scrapings were obtained with a glass slide. The whole mucosal homogenate was then assayed for adenylate cyclase activity by the method of Krishna *et al.* (10, 22), and expressed in pmol cAMP formed/mg protein/5 min. Protein concentrations were determined using bovine serum albumin as standard.

Chemicals. Methylated casein was obtained from Clin-Midy (Paris). It was prepared by precipitating casein from skimmed milk with lactic acid and methylating it with formaldehyde (14). This crude preparation was then diluted in Ringer's solution (10 mg/ml) and centrifuged at 10,000 rpm for 15 min. The supernatant was filtered through Millipore Immersible CX that retains molecules whose mol wt exceeds 10,000. The fraction exceeding 10,000 is referred to here as MC.

MC was further characterized by chemical tests with a total nitrogen of 15.1 g/100 g (Kjeldahl method) and with formaldehyde of near 3 g/100 g dry matter (extraction by steam distillation after phosphoric acid hydrolysis) (1). The amino acid composition of MC was detemined with an autoanalyzer after 6N hydrochloric acid for 24 h at 110° C.

No free amino acids were detected in MC. Its amino acid composition was very similar to that of purified casein, except for decreased lysine and tyrosine. In addition, two new compounds, resistant to acid hydrolysis, were detected in MC: an N'-methyllysine derivative, and a product eluted in the aminogram beween histidine and arginine; its formation is attributed to a cross-linkage between a methylene bridge and the lysine and tyrosine side chains (Lys-CH$_2$-Tyr) (1). Purified casein (crystallized Na caseinate) was obtained from Nestlé (Vevey, Switzerland).

Statistical analysis. Means and ranges were compared by Student's or paired t test and nonparametric tests (Mann-Whitney U test or Wilcoxon one-sample test).

RESULTS

Effects of MC on water fluxes in vivo. The results for water fluxes in the presence and abence of 10 mg/ml MC in jejunum previously treated with cholera toxin are shown in Table 1. While secretion was observed in the loops containing Ringer's solution only (even after removal of CT), net absorption was observed in all 10 loops to which MC was added ($p < 0.01$). As the antisecretory effect might have been due to several factors, the following were examined: 1) the method of water flux measurement used. Comparison of results for the water weight and [^{3}H]polyethylene glycol dilution techniques showed no significant difference between them: for example, in loop 1, filled with Ringer's solution only, ΔJ_v, the difference between the J_v obtained during the two periods, was -0.44 ± 0.39 μl·min^{-1}·cm^{-2} with the water weight technique, and -0.56 ± 0.45 μl·min^{-1}·cm^{-2} with [^{3}H] polyethylene glycol, and in loop 2 containing MC, ΔJ_v values for the two techniques were 1.54 ± 0.17 and 1.68 ± 0.22, respectively. Therefore, the water weight technique was used in the next experiments.

2) The presence of free amino acid associated with MC might have stimulated Na and water absorption, but this possibility is unlikely for three reasons: no free amino acid was detected in the MC solution, MC was the fraction retained by the Millipore filter (moreover, the ultrafiltrate fraction had no effect on J_v), and the antisecretory effect vanished after boiling the MC solution for 10 min. The individual ΔJ_v values obtained with fresh and boiled MC are given in Figure 1. In all 10 rats studied, the

Table 1. *Effects of methylated casein on water fluxes (J_v) in cholera toxin-treated rat jejunum in vivo**

	J_v (μl·min^{-1}·cm^{-2})	
Conditions	Loop 1	Loop 2
Period I		
Ringer's	−0.70	−0.87
+Cholera toxin	±0.10	±0.09
Period II		
Ringer's only	−1.15	
	±0.44	
+Methylated casein		+0.67
		±0.21
ΔJ_v (Period II − Period I)	−0.44	+1.54
	±0.39	±0.17
p	NS	<0.001

* Values are means ± SE of results for 10 rats. Two adjacent loops were filled with 1.5 ml Ringer's solution + 10 μg cholera toxin for a period of 3 h (Period I). Fluid was then collected and the same loops were refilled with either Ringer's or Ringer's + methylated casein (10 mg/ml) for 20 min (Period II). Positive values indicate absorption and negative values, secretion.
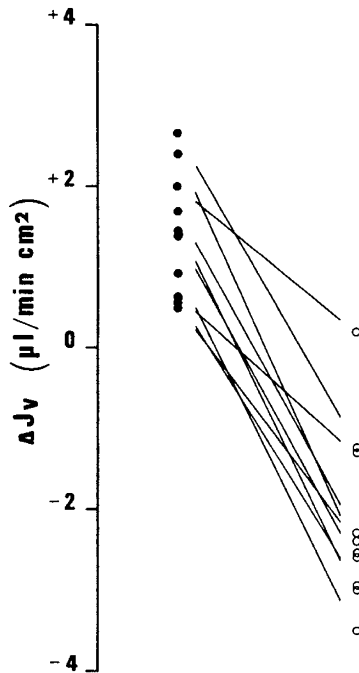

Fig. 1. Effects of heat on the antisecretory action of methylated casein. Two adjacent loops from the same rats were filled with Ringer's solution + cholera toxin (Period I); after 3 h, the same loops were emptied and then filled for 20 min (Period II) with Ringer's to which fresh (●) or boiled (○) methylated casein had been added. ΔJ_v represents the difference between the water fluxes for the two periods.

antisecretory effect of MC was only present in the loops filled with fresh MC.

Stimulation by free amino acid was also unlikely in view of the *in vitro* experiments, in which MC exhibited antisecretory effects despite no addition of MC to the Ussing chambers.

3) A dose-effect relationship was established for the contents of three loops incubated with CT for 3 h (Period I) in 10 rats (see "Materials and Methods"). During Period II, when loops contained either 1.5 ml Ringer's solution only, or 1.5 ml Ringer's with 7.5 or 15 mg of MC, ΔJ_v values displayed highly significant differences, since they were respectively −0.81 ± 0.21, +0.31 ± 0.16, and +1.01 ± 0.2 μl·min^{-1}·cm^{-2} ($p < 0.001$).

4) Purified (nonmethylated) casein did not interfere with the water secretion induced by cholera toxin in 13 rats (ΔJ_v = 1.67 ± 1.17 and −1.59 ± 0.91 μl·min^{-1}·cm^{-2} in the absence and presence of casein, respectively).

Effects of MC on basal ion fluxes in vivo and in vitro. In another group of 10 rats, loops were filled with 1.5 ml Ringer's solution with or without 15 mg MC, but with no cholera toxin in either case. After 20 min, the water absorption rate was not statistically different in the two kinds of loops: J_v = 0.42 ± 0.12 μl·min^{-1}·cm^{-2} without MC and 0.21 ± 0.15 μl·min^{-1}·cm^{-2} in the presence of MC. The observation that MC had no effect in the absence of cholera toxin was further substantiated by the *in vitro* ion flux measurements on the same pieces of tissue (Table 2). In the control mucosa (Ringer's solution without MC), I_{sc} and net chloride secretion values were not statistically different, while no net Na flux was observed. In the presence of MC, no change was seen in any of the electrical parameters or ion fluxes studied.

Effects of MC on ion secretion induced by CT. The upper part of Table 3 shows the effect of CT on ion transport. It increased I_{sc} and PD and reduced G. It also stimulated net Na and Cl secretion. These results are very similar to those for guinea pig ileum and in certain rabbit ileum preparations (3, 18, 22). In another group of 10 rats, a 3-h stimulation period with cholera toxin was followed by 20-min incubation with Ringer's solution with or without MC. Two pieces from each loop were then mounted in Ussing chambers filled exclusively with Ringer's solution. The results are shown in the lower part of Table 3. MC reversed net Na and Cl fluxes from secretion to absorption. This change was due to an increase in the $J_{m \to s}$ and a decrease in $J_{s \to m}$ (although not statistically significant for $J^{Cl}_{s \to m}$). I_{sc} was not stimulated but J^{Na}_{net} and J^{Cl}_{net} rose by 6.2 and 5.1, respectively. Finally, net Na and Cl absorption in the presence of cholera toxin and MC was greater than the control values obtained with Ringer's solution only.

Effects of glucose in jejunum treated with MC. In a first series of seven rats, the jejunum was filled with MC for 20 min *in vivo*. Two pieces from each loop were then mounted in Ussing chambers containing Ringer's solution with and without 10 mM glucose (no MC was added *in vitro*). The results are shown in the upper part of Table 4. Glucose increased I_{sc}, PD, G, $J^{Na}_{m \to s}$ and J^{Na}_{net}. These results are consistent with the stimulation of electrogenic Na absorption typical of the effects of glucose on small intestine epithelium. In another series of seven rats, two loops of jejunum were treated *in vivo* first with CT and then with

Table 2. *Effects of MC on ion transport properties in control rat jejunum**

	I_{sc}	PD	G	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}
Ringer's	1.27 ± 0.18	1.90 ± 0.31	18.2 ± 1.81	8.86 ± 1.45	8.29 ± 0.88	0.57 ± 0.94	7.76 ± 0.82	8.42 ± 1.10	−0.66 ± 1.04
MC	1.26 ± 0.14	2.05 ± 0.13	16.24 ± 1.15	7.52 ± 0.84	7.44 ± 0.51	0.08 ± 0.70	9.02 ± 1.48	10.38 ± 1.36	−1.36 ± 1.26
Δ	−0.01 ± 0.16	+0.15 ± 0.30	−1.96 ± 1.18	−1.34 ± 1.99	−0.85 ± 1.16	−0.49 ± 0.81	+0.74 ± 1.35	+1.96 ± 0.60	−0.70 ± 1.73
p	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Values are means ± SE of results for 10 rats. Units for I_{sc} and ion fluxes are μEq·H^{-1}·cm^{-2}; units for PD are mV, and units for G are mS·cm^{-2}. Student's two-tailed t test for paired variates was used in determining the statistical significance of the difference due to MC. Prior to *in vitro* measurements, loops were filed with Ringer's solution with or without MC, but with no cholera toxin in either case.

Table 3. Effects of MC on ion transport properties in cholera toxin-treated tissues*

	I_{sc}	PD	G	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}
Ringer's	1.15 ± 0.13	1.81 ± 0.23	18.15 ± 2.20	7.72 ± 1.10	7.68 ± 0.64	0.04 ± 0.86	6.69 ± 0.75	8.32 ± 0.98	−1.63 ± 0.97
CT	1.58 ± 0.16	2.76 ± 0.34	15.96 ± 0.77	5.92 ± 0.49	8.82 ± 0.53	−2.83 ± 0.54	6.70 ± 0.52	9.78 ± 0.87	−3.14 ± 0.81
p	<0.05	<0.05	<0.001	NS	NS	<0.01	NS	NS	<0.05
CT	1.78 ± 0.18	3.25 ± 0.38	15.10 ± 0.79	5.53 ± 0.40	8.01 ± 0.50	−2.48 ± 0.42	6.85 ± 0.58	9.08 ± 0.80	−2.23 ± 0.71
CT + MC	2.07 ± 0.15	3.20 ± 0.24	17.65 ± 1.18	9.64 ± 0.91	5.95 ± 0.79	3.69 ± 1.12	11.51 ± 1.51	8.63 ± 0.83	2.88 ± 1.44
Δ	+0.29 ± 0.19	−0.05 ± 0.34	+2.55 ± 1.11	+4.11 ± 0.78	−2.07 ± 0.9	+6.18 ± 1.25	+4.66 ± 1.20	−0.45 ± 0.89	+5.10 ± 1.66
p	NS	NS	NS	<0.001	<0.05	<0.001	<0.005	NS	<0.005

* The upper and lower parts of the table, respectively, give the values (mean ± SE) for two different groups of 10 rats each. See footnote to Table 2 for additional information. Loops were filled with Ringer's solution with or without CT (upper part). In another group, a 3-h stimulation period with CT was followed by 20-min incubation with or without MC (lower part).

Table 4. Effects of 10 mM glucose on ion transport properties in rat jejunum treated with MC

	I_{sc}	PD	G	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}
MC	1.24 ± 0.13	2.33 ± 0.07	14.43 ± 1.14	7.86 ± 1.28	6.92 ± 0.72	0.94 ± 0.64	9.25 ± 1.56	11.15 ± 1.28	−1.90 ± 0.59
MC + glucose	2.47 ± 0.23	3.60 ± 0.16	21.53 ± 1.85	11.48 ± 1.13	8.07 ± 1.32	3.41 ± 0.46	10.79 ± 1.28	13.22 ± 0.75	−2.43 ± 1.26
Δ	+1.23 ± 0.18	+1.27 ± 0.12	+7.10 ± 1.56	+3.62 ± 1.21	+1.15 ± 1.07	+2.47 ± 0.95	+1.54 ± 1.42	+2.07 ± 1.94	−0.53 ± 1.43
p	<0.001	<0.001	<0.005	<0.05	NS	<0.005	NS	NS	NS
CT + MC	1.94 ± 0.13	3.16 ± 0.37	17.78 ± 2.38	10.73 ± 1.35	7.71 ± 1.36	3.02 ± 1.74	10.59 ± 1.32	9.80 ± 1.38	0.79 ± 0.53
CT + MC + glucose	4.41 ± 0.5	4.16 ± 0.12	28.75 ± 3.61	15.57 ± 1.79	11.35 ± 1.72	4.22 ± 1.21	12.52 ± 1.32	9.53 ± 0.95	3.00 ± 1.61
Δ	+2.47 ± 0.47	+1.01 ± 0.36	+10.97 ± 2.55	4.83 ± 2.02	3.64 ± 1.12	+1.20 ± 1.70	1.94 ± 1.74	−0.27 ± 1.92	+2.21 ± 1.63
p	<0.001	<0.002	<0.002	<0.05	NS	NS	NS	NS	NS

* The upper and lower parts of the table, respectively, give the values (mean ± SE) for two different groups of seven rats each. See footnote to Table 2 for additional information. Loops were filled with MC with or without glucose, but with no cholera toxin in either case (upper part). In another group, a 3-h stimulation period with CT was followed by a 20-min incubation with MC, and with and without glucose (lower part).
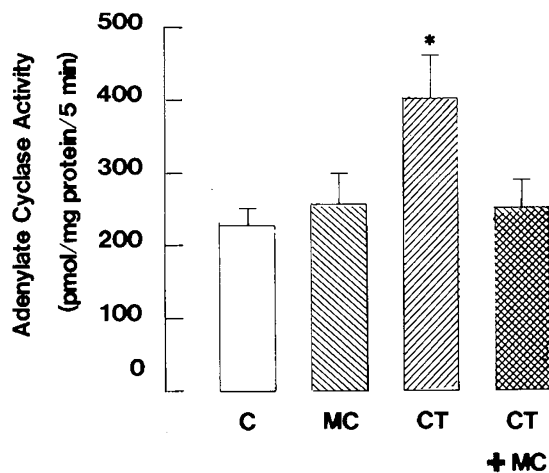

Fig. 2. Mucosal adenylate cyclase activity in the control (C) and cholera toxin-treated tissues with and without methylated casein. *, statistically different from control values ($p < 0.02$). Values are means + SE of six animals.

MC. The tissue use then mounted in Ussing chambers with and without 10 mM glucose. The results are shown in the lower part of Table 4. Glucose increased I_{sc}, PD, G and J^{Na}_{ms}. Net Na and Cl fluxes both rose but not enough to reach statistical significance. Whatever the precise mechanism involved in this complex situation, these results clearly indicate that glucose and MC both stimulate ionic absorption by different mechanisms. They are, at least in part, additive.

Effect of MC on mucosal adenylate cyclase activity. The results for adenylate cyclase activity in the presence and absence of MC previously treated with cholera toxin are shown in Figure 2, MC did not alter basal adenylate cyclase activity but inhibited the cholera toxin-stimulated increase in activity.

DISCUSSION

This study indicates that methylated casein inhibits water and electrolyte secretion induced by cholera toxin in rat jejunum. Interestingly, MC does not interfere with intestinal functions in the absence of stimulation by cholera toxin. Its effects are visible within 20 min and it acts directly on the luminal side of the epithelium. The anticholeraic action observed in rat jejunum might have a clinical counterpart. However, the efficacy of MC in humans has only been demonstrated in types of acute diarrhea which are not of choleraic origin (5, 15).

The present *in vitro* experiments provide some insight into the mechanism of the antisecretory effect of MC. Firstly, it inhibits the secretion induced by cholera toxin by interfering with electrolyte transport. Under basal (Ringer's solution) conditions, comparison of I_{sc} and net electrolyte fluxes suggests the presence of either electrogenic Cl secretion with only minimal Na absorption or of a combination of active electrogenic Na absorption and neutral NaCl secretion, as previously discussed (13, 22). Here MC did not alter the basal rate of electrolyte transport. When cholera toxin alone was added to the basal solution, net secretion of both Na and Cl was observed with a minimal increase in I_{sc}. This suggests that cholera toxin stimulates both a neutral NaCl transport process and electrogenic secretion of Cl.

In rat ileum (22), CT has similar consequences, although its effect on net Na flux is essentially one of reducing Na absorption instead of generating net secretion. Although controversies persist about the significance of Na secretion in small intestine, cholera toxin clearly alters both neutral NaCl and electrogenic Cl fluxes (3, 18). The effect of MC is essentially exerted through powerful stimulation of neutral NaCl absorption. Comparison of the fluxes obtained in the presence of MC with and without cholera toxin suggests that MC does not only inhibit the electrolyte secretion produced by cholera toxin but also stimulates asorption in the toxin's presence.

Glucose is known to stimulate electrogenic Na absorption (4, 7, 13, 23) and thereby to counterbalance the electrolyte secretion induced by cholera toxin. It was therefore of interest to compare

the effects of glucose and MC on electrolyte absorption. Table 4 clearly indicates that glucose stimulates electrogenic Na absorption in the presence of MC, and both in the presence and absence of cholera toxin. These results show that the antisecretory effects of MC and glucose are complementary since MC stimulates neutral NaCl absorption and glucose stimulates electrogenic Na absorption.

MC only inhibits secretion after the latter's stimulation by cholera toxin. This might indicate that MC interferes with cholera toxin at a point presumably located beyond the binding site at the luminal membrane (11, 12). Another possibility would be that methylation strengthens the lipophilic properties of casein, that would then be incorporated into the brush border membrane and would thus directly interfere with ionic transport.

Many pharmacological agents inhibit the *in vivo* and *in vitro* effects of cholera toxin in animal models. In humans, some have proved efficient but most have serious drawbacks (19). It was therefore of interest to consider the antisecretory effects of drugs already in use for empirical treatment of human diarrhea. Berberine is a good example of such an approach; it is conventionally used as a treatment of human diarrhea. It has been found to inhibit the active secretion produced by cholera toxin and other secretagogues (22).

MC represents another drug, belonging to the group of agents inhibiting secretion induced by cholera toxin. MC possesses particularly advantageous characteristics. 1) Its antisecretory action is fast and takes effect within 20 min of its entering the intestinal lumen. 2) Its antisecretory effect lasts for at least 3 h. *In vitro*, this effect persists when cholera toxin and MC are absent from the mucosal solution. 3) The antisecretory effect is only present after intestinal secretion has been stimulated. MC does not affect electrolyte transport in control intestine. 4) MC exerts its antisecretory effect by stimulating neutral absorption of NaCl, without interfering with the Na electrogenic absorption stimulated by glucose. The effect of MC on electrogenic Cl secretion is not clear in this preparation of rat jejunum. 5) An interesting point concerns the target organ. The effects of placing MC on the luminal side only were studied. Because of MC's low solubility in water, it was not placed in the bathing solution *in vitro*, either on the mucosal or serosal sides. Because of its protein structure, it is probable that little or no MC reached the blood side of the intestine by the end of the 20-min exposure period. Probably, too, when MC was placed in the intestinal lumen, it exerted its effect on the intestine alone; no systemic effect was expected and, in fact, none has so far been reported. 6) Finally, it is of interest to note that MC is a cheap and easily available product.

Recently, it has been suggested that milk proteins of different origin may also interfere with the secretory effect of cholera toxin (2, 16). Obviously, many questions concerning the antisecretory effect of MC and related milk protein remain unanswered. However, its availability, low cost, and curative effect from the luminal side constitute compelling indications for further investigation.

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