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Prostaglandin- and Theophylline-Induced Cl Secretion in Rat Distal Colon Is Inhibited by Microtubule Inhibitors

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The aim of the present study was to examine the possible role of microtubules in chloride secretion by distal rat colon stimulated by prostaglandin (PGE_2) and theophylline. Distal colonic tissue from male rats was mounted in Ussing chambers, and short-circuit current (I_{sc}) was measured to assess chloride secretion. Three microtubule inhibitors, colchicine, nocodazole, and taxol, all inhibited the stimulated I_{sc} and reduced the 60-min integrated secretory response to PGE_2 and theophylline ($\int I_{sc}dt$) by 39–52%, whereas the inactive colchicine analog lumicolchicine did not. Atropine and tetrodotoxin had no effect on stimulated chloride secretion. To confirm the source of I_{sc}, unidirectional $^{22}Na^+$ and $^{36}Cl^-$ fluxes were measured in tissues exposed to lumicolchicine (control) or colchicine. Control tissues absorbed both chloride [5.0 (1.1–8.6) (median and 95% confidence interval) $\mu eq/cm^2/hr$] and sodium [2.8 (0.9–7.2) $\mu eq/cm^2/hr$], and this net absorption was reduced by 96% and 79%, respectively, by treatment with PGE_2 and theophylline due to an increase in serosal-to-mucosal chloride and sodium movement. Colchicine-treated tissues exhibited similar net basal chloride and sodium absorption that was reduced by 71% and 75%, respectively, by treatment with PGE_2 and theophylline. Thus the PGE_2- and theophylline-induced increase in chloride secretion was significantly reduced by colchicine ($P < 0.05$ by Wilcoxon rank-sum test), whereas colchicine had no effect on PGE_2- and theophylline-induced changes in sodium fluxes. Furthermore, the colchicine-related changes in stimulated chloride secretion were numerically similar to colchicine-related changes in stimulated I_{sc}. These findings indicate that microtubules are required for normal PGE_2- and theophylline-induced chloride secretion in distal rat colon and suggest that induced chloride secretion may involve vesicular insertion of ion transporters into the plasma membrane or other microtubule-dependent regulatory processes.

KEY WORDS: theophylline; intestinal ion transport; colchicine; Ussing chamber; radioisotopes; chloride secretion.

Intestinal chloride secretion is an active process similar to chloride secretion in other epithelial tissues (1–5). In a currently accepted model (Figure 1), chloride is transported across the basolateral cell membrane by an electroneutral step (Na, K, 2 Cl cotransport) driven by the sodium gradient, main-

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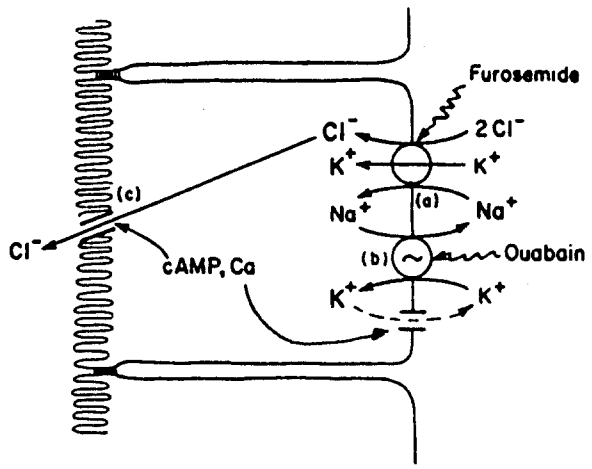

Fig 1. Model of cyclic-AMP- and Ca^{2+}-stimulated active Cl secretion by epithelia (Adapted from Binder HJ, Sandle GI: Electrolyte absorption and secretion in the mammalian colon. *In* Physiology of the Gastrointestinal tract, Second Edition, LR Johnson (ed). New York, Raven Press, 1987, pp 1389–1418). For details, see text.

tained by Na,K-ATPase, and by potassium recycling through a basolateral potassium conductance. Chloride is thought to subsequently traverse the apical membrane through a chloride channel, driven by a favorable electrochemical gradient. Upon stimulation of chloride secretion, the rates of all these transport mechanisms are thought to increase; however, the rate-limiting step may be the number and open state of apical chloride channels (6). Potential mechanisms for regulation of chloride secretion include insertion and/or removal of membrane transporters, for example, chloride channels in the apical membrane, and/or Na, K, 2 Cl cotransporters in the basolateral membrane. Indeed, insertion and removal of plasma membrane transporters via endo- or exocytosis of intracellular vesicles is an important mechanism for regulation of electrolyte transport in several epithelia, such as stomach (7), urinary bladder (8, 9), and distal nephron (10). An intact microtubular system appears necessary for directed and efficient movement of at least some vesicles containing transporters (11).

Three observations suggest that microtubule-directed movement of vesicles might also constitute an important mechanism for regulation of chloride transport in intestine. First, in small intestine the microtubule-inhibitors colchicine and vinblastine inhibit chloride secretion induced by cholera toxin and prostaglandin by approximately 50% (12). Second, a preliminary report demonstrated that apical vesicles in the non-goblet cells of the rabbit colon disappeared following stimulation of chloride secretion by PGE_2 (13). Third, chloride secretion in T_{84} cells, stimulated by cAMP or Ca-dependent agonists, is associated with the appearance of vesicles, labeled with antibodies directed to disulfonic stilbene binding sites, at the plasma membrane, and the appearance of these vesicles was inhibited by microtubule inhibitors (14).

These results suggest a role for microtubules in chloride secretion by the intestine, possibly in facilitating insertion/removal of ion transporters. In the present study this hypothesis was tested by measuring the effect of the microtubule-inhibitors colchicine, nocodazole and taxol on chloride secretion in rat distal colon mounted in Ussing chambers assessed by measuring short-circuit current and $^{22}Na^+$ and $^{36}Cl^-$ fluxes.

MATERIALS AND METHODS

Materials. Male 250 to 300-g Sprague-Dawley rats from Bantin-Kingman, (Fremont, California) had free access to food and water prior to the studies. Ussing chambers [Research & Development Unit, University of California San Francisco, or World Precision Instruments, (WPI), New Haven, Connecticut] were used with a voltage clamp (model 742C, Department of Bioengineering, University of Iowa, Ames, Iowa, or WPI), gas-lift perfusion chambers (MRA Corporation, Clearwater, Florida), and calomel half-cells (No. K401, Radiometer, Copenhagen, Denmark). All drugs were obtained from Sigma Chemicals (St. Louis, Missouri) except taxol which was a kind gift from Dr. Matthew Suffness (NIH, Bethesda, Maryland). Radioisotopes were obtained from New England Nuclear (Boston, Massachusetts).

Short-Circuit Current. For most experiments rats were divided into five experimental groups (I, II, III, IV, V) as shown in Table 1. Two hours prior to sacrifice animals were given intravenously: solvent (0.1 ml DMSO, groups I, IV, V), colchicine (5 mg/kg, group III), or lumicolchicine (an inactive colchicine analog) (5 mg/kg, group II). Animals were pretreated *in vivo* with colchicine (or lumicolchicine) to assure sufficient time for specific anti-microtubule action of colchicine (11, 12). Rats were sacrificed, and distal colons were harvested and washed in ice-cold saline. The tissues were prepared in oxygenated HEPES-buffered saline and unstripped tissues were mounted in Ussing chambers (bath volume 10 ml, surface area 0.5 cm^2). Bathing solutions [Krebs bicarbonate buffer containing (in millimoles per liter) Na^+ 137, Cl^- 120, K^+ 5.9, HCO_3^- 25, HPO_4^- 1.2, SO_3^{2-} 1.2, Mg^{2+} 1.2, Ca^{2+} 2.5, glucose 11.5] contained indomethacin (10^{-5} M) to inhibit endogenous prostaglandin formation and drugs (in groups II–V) or solvent (group I) as shown in Table 1. Tissues were exposed to nocodazole or taxol only during *in vitro* incubation as the specific effect of these compounds on microtubules occurs relatively rapidly (15–17). Tissues were short-circuited by a voltage

TABLE 1. EFFECT OF VARIOUS DRUGS ON ELECTRICAL PARAMETERS IN RAT DISTAL COLON*

Group and drug (N)	*Exposure prior to PG (hr)*	*Drug conc*	*Basal R* (Ω/cm^2)	*Basal* I_{sc} ($\mu A/cm^2$)	*Stim R* (Ω/cm^2)	ΔI_{sc}*/5 min* ($\mu A/cm^2$)	$\int_{t_0}^{t_{60}} I_{sc}\,dt$ (mC/cm^2)
I DMSO (7)	2.5	3 μl/ml	152 (100–239)	30 (21–48)	125[c] (86–170)	56 (46–93)	292 (251–444)
II Lumicolchicine (7)	2.5	1×10^{-4} M	156 (118–167)	24 (14–33)	122[c] (97–125)	57 (49–86)	317 (266–375)
III Colchicine (8)	2.5	1×10^{-4} M	118 (89–155)	20 (2–37)	98[c] (79–146)	37[a] (29–49)	179[a] (106–225)
IV Nocodazole (8)	0.5	3×10^{-5} M	134 (115–192)	35 (24–46)	125[c] (111–165)	34[a] (24–45)	155[a] (107–262)
V Taxol (8)	0.5	2×10^{-5} M	99[b] (70–128)	36 (21–40)	91[c] (62–120)	33[a] (20–60)	140[a] (88–289)

*Values represent median and 95% confidence intervals. R indicates transepithelial resistance under basal conditions (basal R) and 10 min after PGE_2 and theophylline stimulation (stim R). The ΔI_{sc}/5 min indicates the change in I_{sc} 5 min after addition of PGE_2 and theophylline. The integrated I_{sc} response ($\int I_{sc}\,dt$) was calculated after subtraction of basal I_{sc}. a, $P < 0.01$, and b, $P < 0.05$ compared to DMSO or lumicolchicine (Kruskal-Wallis test); c, $P < 0.05$ compared to the corresponding basal R (Wilcoxon signed-rank test).

clamp. I_{sc} was measured continuously throughout the experiment. Every 20 sec current pulses were passed across the epithelium to shift the PD by 1 mV, the current was recorded, and transepithelial resistance (R) was calculated. If R was below 50 Ω/cm^2, the tissue was considered as damaged and discarded. After a 30-min stabilization period, chloride secretion was stimulated by adding both PGE_2 (5×10^{-5} M) to the serosal bath and theophylline (10^{-2} M) to both baths of all tissues, concentrations shown to produce a maximum I_{sc} response (data not shown). I_{sc} was measured for a further 60 min after addition of PGE_2 and theophylline. I_{sc} at 5 min after exposure to PGE_2 and theophylline was recorded as one measure of the secretory response. In order to get a more reliable estimate of the overall secretory response, an array of I_{sc} data points was used to calculate the integrated secretory response over 60 mins. A polynomial was fitted to I_{sc} data points obtained every 24 sec during the first 4 min after addition of PGE_2 and theophylline, then at 5, 6, 7, 8, and 10 mins, and subsequently every 5 min until 60 min was reached. Area under the curve ($\int I_{sc}\,dt$) was calculated by a numerical integration procedure after subtraction of baseline I_{sc} and was used as a measure of the integrated secretory response induced by PGE_2 and theophylline.

In order to compare the initial I_{sc} response to PGE_2 and theophylline (assessed as the initial rate of change of I_{sc}) between the different experimental groups, a different curve-fitting procedure was employed. Values of I_{sc} over the first 10 min after addition of PGE_2 and theophylline were obtained from the continuous tracings at the intervals given above. Plots of these I_{sc} data points versus time were analyzed on a Hewlett-Packard 9852B computer using a nonlinear least-squares procedure utilizing the Euler method for numerical solutions of the differential equations. The best fit function, defined as the simplest (ie, smallest number of parameters) function, was a monoexponential function of the form:

$$y(t) = P_1[1 - e^{-P_2(t-P_3)}]$$

where y represents the I_{sc} response as a function of time (t); P_1 is the maximum increase in I_{sc}; P_2 is the time constant of the monoexponential rise in I_{sc}; P_3 is the time lag between addition of the drug and the beginning of the I_{sc} response. $P_1 \times P_2$ thus indicates the initial slope of the I_{sc} response, and this value was compared between the different groups.

In selected studies, three further groups of rats were studied (Table 2). Rats were sacrificed, and distal colons were harvested, washed, and mounted in Ussing chambers as described above. Two adjacent pieces from each colon were treated as a pair. One piece was exposed to serosal atropine (10^{-5} M) or tetrodotoxin (TTX, 10^{-7} M) for 20 min while its corresponding pair was treated with vehicle. After a 30-min stabilization period, chloride secretion by all tissues was stimulated by adding PGE_2 (5×10^{-5} M) to the serosal bath and theophylline (10^{-2} M) to both baths. I_{sc}, PD, and tissue resistance were monitored as described above for 20 min after addition of PGE_2 and theophylline. The peak I_{sc} response obtained at about 5 min (after subtraction of baseline I_{sc}) was used as a measure of the secretory response induced by PGE_2 and theophylline.

Radioisotope Flux Studies. Rats were pretreated with an intravenous injection of lumicolchicine (control group) or colchicine (experimental group) 2 hr before sacrifice as described above. Distal rat colon tissues were mounted in Ussing chambers containing Krebs bicarbonate buffer and indomethacin as well as either lumicolchicine or colchicine as described above. Tissues were paired by matching resistances, and sodium and chloride fluxes and I_{sc} were measured while the tissues were short-circuited. Three microCuries of the isotopes $^{22}Na^+$ and $^{36}Cl^-$ were added to the bath on the mucosal side of one tissue and to the serosal side of its pair. The opposite bath was sampled (0.5 ml) immediately (baseline value) and every 15 min for 30 min before and for 60 min after the addition of PGE_2 and theophylline, and buffer was added to maintain bath volume. Specific activity measurements and double-label counting was performed as previously described (18, 19). Fluxes were calculated according to the conventional formula (19):

TABLE 2. EFFECTS OF ATROPINE AND TTX ON ELECTRICAL PARAMETERS IN RAT DISTAL COLON*

	Control	*Atropine*	P	*Control*	*TTX*	P
Basal I_{sc} ($\mu A/cm^2$)	23 (12–63)	26 (1–50)	NS	39 (19–67)	13 (8–23)	<0.05
Basal PD (mV)	−4.0 (−6.8–−2.0)	−4.4 (−7.0–0.1)	NS	−5.2 (−11.0–−3.0)	−2.7 (−3.8–−0.9)	<0.05
Basal R (Ω/cm^2)	90 (70–106)	92 (68–106)	NS	99 (77–128)	106 (68–158)	NS‡
ΔI_{sc}/5 min ($\mu A/cm^2$)	80 (55–116)	84 (46–112)	NS	90 (82–122)	82 (60–110)	NS
Stim PD (mV)	−5.3† (−8.6–−3.2)	−5.9† (−8.5–−4.7)	NS	−7.9† (−13.3–−4.9)	−4.3† (−8.3–−1.9)	<0.05
Stim R (Ω/cm^2)	70† (60–93)	71† (61–92)	NS	80† (53–99)	85† (55–106)	NS

*Values represent median and 95% confidence interval for values obtained from a total of 14 paired tissues, seven each for atropine and TTX. *P* value indicates statistical significance when comparing pairwise control and drug values.
†Statistical significance when stimulated PD and R values are compared with their corresponding basal values.
‡NS indicates $P > 0.05$.

$$J\ (\mu eq/cm^2/hr) = \frac{(\text{cpm } B - f \text{ cpm } A) \times [\text{ion}] \times V}{\text{cpm/ml (hot side)} \times A \times t}$$

where *A* and *B* refer to two successive 0.5-ml samples from the cold side.

For each tissue, two 15-min control flux periods were averaged. Values from the first 15-min period after addition of PGE_2 and theophylline (when I_{sc} was changing) were discarded. The differences in the unidirectional ion fluxes are reported as net movement for each tissue pair. Residual ion flux (J^R_{net}) was calculated from the equation:

$$J^R_{net} = I_{sc} - (J^{Na}_{net} - J^{Cl}_{net})$$

Statistics. Nonparametric (distribution-free) statistics were chosen. Our small data set does not allow verification of the assumptions underlying the parametric tests, ie, normally distributed data with equal variance among groups. Formal tests for normality require at least 15–20 observations to yield meaningful results (20). Values, therefore, are given as median and 95% confidence intervals, calculated by the method of Walsh averages (21). Wilcoxon rank-sum test and Wilcoxon signed-rank test were used when appropriate to evaluate differences between experimental groups. The null hypothesis of no difference was always tested against a two-sided alternative. When several groups were compared, a nonparametric one way analysis of variance was employed (Kruskal-Wallis test). If the null hypothesis of no difference was rejected, the procedure for multiple comparison suggested by Conover (22) was used to determine which groups tended to differ. $P < 0.05$ was considered statistically significant.

RESULTS

Short-Circuit Current. Figure 2A illustrates the time course of I_{sc} by distal rat colon after stimulation by PGE_2 and theophylline in the absence and presence of microtubule-inhibitors. Median values for I_{sc} are shown after subtraction of basal I_{sc}, which did not differ significantly between the various groups (Table 1). The overall shapes of the curves were similar, although steady-state I_{sc} responses occurred earlier (5–10 min) in tissues exposed to microtubule inhibitors compared to control tissues (10–20 min), and the maximum I_{sc}

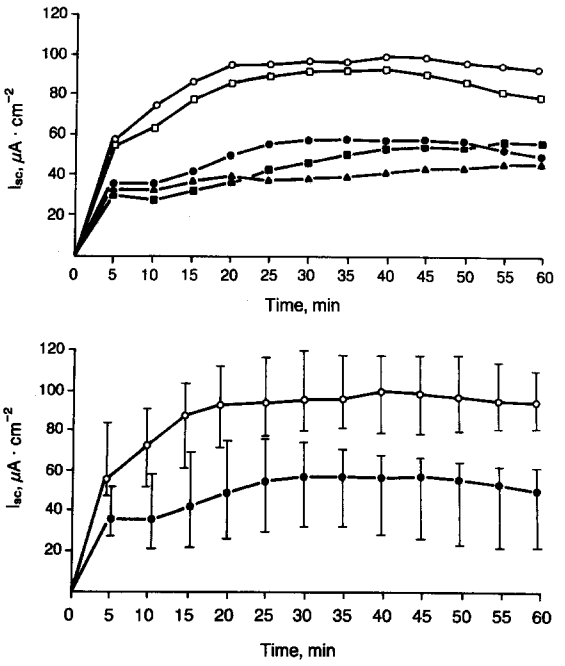

Fig 2. (A) Median I_{sc} response following PGE_2 and theophylline in five groups of rats: □, DMSO (group I, $N = 8$); ○, lumicolchicine (group II, $N = 8$); ●, colchicine (group III, $N = 7$); ■, nocodazole (group IV, $N = 7$); ▲, taxol (group V, $N = 7$). Data points were obtained at 5-min intervals from the continuous experimental tracings. (B) Median I_{sc} response to PGE_2 and theophylline: ○, lumicolchicine ($N = 8$); ●, colchicine ($N = 7$). Bars represent 95% confidence intervals.

attained was less for tissues treated with colchicine, nocodazole, and taxol. To give a perspective of the uncertainty of the data, the median response together with 95% confidence intervals are given for lumicolchicine and colchicine in Figure 2B. The confidence intervals for the remaining groups are in the same range, but for clarity are not depicted.

Median (and 95% confidence interval) values for basal and stimulated I_{sc} and for tissue resistance for all treatment groups are given in Table 1. Basal I_{sc} was similar in all groups although basal tissue resistance was lower in taxol-treated tissues but not in those treated with colchicine or nocodazole. After addition of PGE_2 and theophylline, I_{sc} rose in all groups, but the increase in I_{sc} at 5 min (chosen as representative of initial secretory response) was reduced significantly by the active microtubule inhibitors (Table 1). After stimulation, transepithelial resistance (R) fell significantly in all experimental groups, probably due primarily to changes in paracellular conductance in this relatively leaky epithelium.

The integrated secretory response, $\int I_{sc}\, dt$, also was calculated for all experiments (as described in Materials and Methods) to give a broad perspective over the entire secretory response. The median values for $\int I_{sc}\, dt$ for groups I–V were 292, 317, 179, 155, and 140 mC/cm^2, respectively, and are shown in Table 1. The microtubule inhibitors colchicine, nocodazole, and taxol reduced $\int I_{sc}\, dt$ significantly by 39, 47, and 52%, respectively, compared with DMSO, whereas lumicolchicine did not.

Figure 3 shows I_{sc} over the first 10 min after stimulation by PGE_2 and theophylline in two typical experiments in which tissues were treated with lumicolchicine or colchicine. The initial slopes (rate of change of I_{sc}), estimated as described in Materials and Methods, did not differ significantly among the various treatment groups (data not shown).

Since colchicine might alter release of neurotransmitters, additional studies were performed to examine a role for enteric neurons in mediating PGE_2- and theophylline-induced chloride secretion. Tissues were exposed to either atropine (10^{-5} M) or tetrodotoxin (TTX, 10^{-7} M) or vehicle prior to treatment with PGE_2 and theophylline. As shown in Table 2, atropine had no effect on either basal or stimulated PD, I_{sc}, or tissue resistance. TTX treatment significantly decreased basal I_{sc} and PD but did not affect PGE_2 and theophylline stimulation of chloride secretion.

Radioisotope Flux Studies. To confirm the mechanism for PGE_2- and theophylline-stimulated I_{sc} in this tissue, flux studies were performed. Unstimulated control (lumicolchicine-treated) tissues exhibited net absorption of both chloride and sodium at median rates of 5.0 and 2.8 $\mu eq/cm^2/hr$, respectively (Table 3). Colchicine-treated tissues exhibited similar basal rates of net absorption of chloride and sodium, 4.2 and 3.2 $\mu eq/cm^2/hr$, respectively, and these values did not differ significantly from those in control tissues. Since net absorption of chloride and sodium were both greater than I_{sc}, these ion fluxes can be interpreted to represent net electroneutral absorption of sodium and chloride through parallel electroneutral exchangers, as has been previously reported (23). Calculated residual ion fluxes in control and colchicine-treated tissues averaged 1.9 and 1.4 $\mu eq/cm^2/hr$. These values did not differ significantly from each other and they may reflect secretion of anions such as bicarbonate (possibly via Cl^-/HCO_3^- exchange) or absorption of cations such as potassium. Administration of PGE_2 and theophylline to both control and colchicine-treated tissues caused an increase in the unidirectional serosa-to-mucosa fluxes of both chloride and sodium, whereas mucosa-to-serosa fluxes of both ions were essentially unaffected. Concommitantly both I_{sc} and tissue conductance increased. Thus in control tissues net absorption of both sodium and chloride decreased due to increased serosal-to-mucosal fluxes of both ions, and stimulated serosal-to-mucosal chloride flux (5.7 $\mu eq/cm^2/hr$) was numerically greater than stimulated serosal-to-

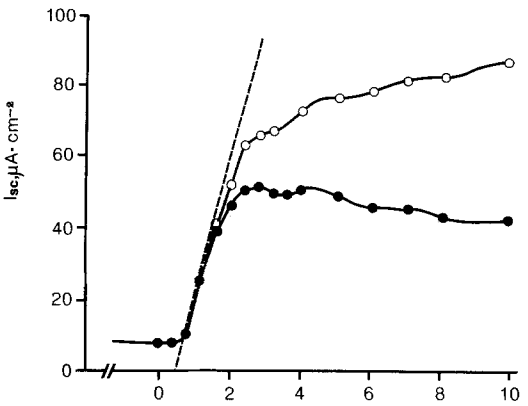

Fig 3. Typical 10-min I_{sc} responses of two experiments after stimulation (at time 0) by PGE_2 and theophylline, the tissues being pretreated with lumicolchicine (○) and colchicine (●), respectively. The curves were obtained by a polynomial curve fitting procedure as described in Materials and Methods based on data points as indicated. The common initial slope was drawn by eye.

TABLE 3. EFFECT OF ADMINISTRATION OF PGE_2 AND THEOPHYLLINE ON NET NA AND CL TRANSPORT IN DISTAL RAT COLON*

	$J^{Na}_{s\to m}$	$J^{Na}_{m\to s}$	J^{Na}_{net}	$J^{Cl}_{s\to m}$	$J^{Cl}_{m\to s}$	J^{Cl}_{net}	I_{sc}	J^{R}_{net}
Control (N = 8 pairs)								
Basal	3.3 (1.5–4.0)	6.1 (4.0–9.0)	2.8 (0.9–7.2)	6.3 (5.3–9.9)	11.3 (10.5–14.1)	5.0 (1.1–8.6)	0.5 (0.3–1.3)	1.9 (−1.9–4.5)
PGE_2/ theophylline	6.4 (4.3–9.2)	7.0 (5.6–10.1)	0.6 (−0.4–4.5)	12.0 (10.1–17.0)	12.2 (11.4–13.9)	0.2 (−2.7–2.5)	3.6 (3.0–4.1)	1.5 (0.8–2.9)
PGE_2/theoph.— basal	3.1 (2.2–5.1)	0.9 (−1.5–3.7)	−2.2 (−5.2–−0.1)	5.7 (3.4–8.1)	0.9 (−1.9–2.7)	−4.8 (−7.6–−3.1)	3.1 (2.2–3.6)	−0.4 −3.8–3.4)
Colchicine (N = 8 pairs)								
Basal	3.6 (2.3–4.4)	6.8 (6.2–8.6)	3.2 (2.6–5.6)	6.2 (5.8–6.6)	10.4 (8.7–13.6)	4.2 (2.3–8.0)	0.5 (0.1–1.1)	1.4 (0.0–3.5)
PGE_2/ theophylline	5.6 (4.5–6.7)	6.4 (4.7–10.4)	0.8 (0.2–4.6)	9.9 (8.4–10.6)	11.1 (8.5–15.5)	1.2 (−0.5–5.8)	2.6 (2.0–3.1)	2.6 (1.6–4.6)
PGE_2/theoph.— basal	2.0 (−0.2–4.0)	−0.4 (−2.8–3.1)	−2.4 (−4.4–−0.2)	3.7 (2.2–4.4)	0.7 (−0.9–1.7)	−3.0 (−4.4–−0.5)	2.1 (1.6–2.5)	1.2 (−0.1–3.8)
P value	NS	NS	NS	<0.05	NS	<0.01	<0.05	NS

*Sixteen pairs of tissues were pretreated with lumicolchicine (control) or with colchicine. ^{22}Na and ^{36}Cl were used as markers of transepithelial Na and Cl transport, respectively. Ion fluxes and short-circuit current (I_{sc}) are given as median with 95% confidence interval in μeq/cm²/hr. $J^{ion}_{s\to m}$ and $J^{ion}_{m\to s}$ indicate unidirectional ion fluxes to the mucosal and serosal side of the tissue, respectively. J^{ion}_{net} indicates calculated net ion fluxes where positive and negative values indicate net absorption and secretion, respectively. I_{sc} was measured as described in Materials and Methods and is given as the average of the two paired unidirectional ion flux periods. Residual ion flux (J^{R}_{net}) was calculated according to the equation: $J^{R}_{net} = I_{sc} - (J^{Na}_{net} - J^{Cl}_{net})$. Wilcoxon signed-rank test was used for paired comparisons between colchicine and control group ion fluxes (PGE_2/theophylline—basal), and NS indicates $P > 0.05$.

mucosal sodium flux (3.1 μeq/cm²/hr). The difference between PGE_2- and theophylline-induced serosal-to-mucosal fluxes of chloride and sodium (2.6 μeq/cm²/hr) was similar to the absolute rise in I_{sc} after treatment with PGE_2 and theophylline (3.1 μeq/cm²/hr), suggesting that these agents indeed stimulated electrogenic chloride secretion, as has been reported previously (23). Of note, PGE_2- and/or theophylline-induced serosal-to-mucosal sodium flux has been observed by previous authors, although the mechanism for this effect is unknown (23, 24). Collectively, these PGE_2- and theophylline-induced changes in ion fluxes may reflect: (1) a combination of electroneutral sodium and chloride secretion (2.2 μeq/cm²/hr) and electrogenic chloride secretion (2.6 μeq/cm²/hr); (2) electrogenic secretion of both sodium and chloride with the latter predominating; (3) increased paracellular flux of both ions with decreased equivalent electroneutral absorption of sodium and chloride in conjunction with electrogenic chloride secretion; or (4) electrogenic chloride secretion with increased paracellular serosal-to-mucosal sodium flux due to solvent drag in the secreting crypts of this complex epithelia. The latter two explanations are the most likely, based on current concepts of colonic ion transport.

In colchicine-treated tissues, a similar pattern of responses was observed in that basal net absorption of both sodium and chloride was reduced following stimulation with PGE_2 and theophylline due to stimulated chloride, and to a lesser extent, sodium serosal-to-mucosal fluxes, and the difference between PGE_2- and theophylline-induced chloride and sodium serosal-to-mucosal fluxes of 1.7 μeq/cm²/hr was also similar to the 2.1 μeq/cm²/hr increase in I_{sc}, suggesting stimulation of electrogenic chloride secretion (Table 3). Basal fluxes and PGE_2- plus theophylline-induced sodium fluxes were not affected by colchicine; however, colchicine decreased stimulated chloride secretion (3.0 vs 4.8 μeq/cm²/hr) as well as stimulated I_{sc} (2.1 vs 3.1 μeq/cm²/hr). Collectively these observations suggest that colchicine partially inhibited PGE_2- and theophylline-induced electrogenic chloride secretion, but had no effect on basal ion fluxes, tissue conductance, or stimulated sodium transport.

DISCUSSION

Apical chloride channels have been characterized in colonic tissue from several species (25–28); some may be regulated by cAMP and Ca^{2+}, and opening/

closing of these channels may play an important role in regulating chloride secretion in colon. A potential further level of regulation involves controlled insertion or removal of additional preformed chloride channels, Na, K, 2 Cl cotransporters or others into or from the cell membrane. Indeed, a role for membrane fusion in regulating solute transport has been clearly demonstrated in several cell types (7–10, 29–33). In these and other tissues, an intact microtubule system may be necessary for directed and efficient transport and insertion of vesicles into the plasma membrane (11, 17, 32, 33, 35–37). Drugs such as colchicine can be used to examine the role of microtubules in this and other cellular processes (38). Colchicine and the antifungal drug nocodazole bind to tubulin and block the polymerization of tubulin into microtubules (15, 17), whereas taxol promotes assembly of inactive microtubules (16). In our study, these drugs were used at concentrations known to be specific for these effects in other cell types (15, 16).

The principal finding of the present study was that three chemically unrelated microtubule-inhibitors reduced PGE_2- and theophylline-stimulated I_{sc} and chloride secretion in distal rat colon by approximately 40%. PGE_2 has been demonstrated to induce a marked increase in colonic mucosal cAMP level (39), and the combination of PGE_2 and theophylline has been used by ourselves and others to stimulated colonic chloride secretion (40). Chloride secretion was assessed by previously well-characterized techniques, that is, I_{sc} in colonic tissue mounted in Ussing chambers (41). Measured either as I_{sc} 5 min after addition of PGE_2 and theophylline or as the 60-min integrated response, all three unrelated microtubule inhibitors decreased chloride secretion by about 40%.

In order to confirm that I_{sc} indeed reflects changes in chloride secretion, transepithelial fluxes of ^{22}Na and ^{36}Cl also were measured using conventional techniques with one microtubule inhibitor (colchicine) and its inactive analog, lumicolchicine. Under basal conditions, colonic tissue absorbed both sodium and chloride in an electroneutral manner, probably via paired exchangers. Stimulation by PGE_2 and theophylline increased serosal-to-mucosal chloride flux, and this increase approximated the increase in I_{sc}, reflecting chloride secretion. Similar to its effects on I_{sc}, colchicine decreased stimulated chloride secretion measured as $^{36}Cl^-$ flux by about 35% but had no effect on chloride absorption or sodium fluxes. In contrast, PGE_2 and theophylline did not alter mucosal-to-serosal chloride flux. Indeed net chloride absorption did not convert to net chloride secretion following stimulation by PGE_2 and theophylline due to a substantial basal mucosal-to-serosal chloride flux that was not reduced by these agents (Table 3).

We and others (23, 24) also observed PGE_2 and/or theophylline stimulation of serosal-to-mucosal sodium flux, via unknown mechanisms. In experiments of this type, ion fluxes may occur through cellular and paracellular routes, and it is generally assumed that paracellular fluxes are symmetric. The simplest explanation for the observed PGE_2- and theophylline-induced increase in serosal-to-mucosal sodium flux is a combination of increased bidirectional paracellular fluxes and equivalent decreased sodium absorption. This mechanism is, in fact, consistent with prior observations of cAMP-induced decreases in sodium and chloride absorption (42–45). Increased serosal-to-mucosal sodium flux could also be due to convection and solvent drag in chloride-secreting crypts. Finally, although less likely, PGE_2-induced cellular sodium secretion by some as yet unidentified transport mechanism could be present.

Others have suggested (46, 47) that prostaglandins might alter colonic ion transport by two pathways: direct effects on colonocytes via cAMP formation and indirect effects via stimulation of enteric neurons. It is possible that colchicine might alter release of neurotransmitters from enteric neurons and thereby affect PGE_2-induced chloride secretion. Studies shown in Table 2, however, indicate this is unlikely. PGE_2- and theophylline-induced increases in PD and I_{sc} were not affected by pretreatment with either atropine or TTX, observations similar to those recently published by Karayalcin et al (48).

Collectively, the I_{sc} and isotope flux studies indicate that: (1) the principal source of stimulated I_{sc} in this tissue is electrogenic chloride secretion; (2) the change in I_{sc} induced by PGE_2 and theophylline correlate well with the observed change in chloride fluxes, suggesting that the microtubule inhibitors directly alter chloride secretion; and (3) PGE_2 and theophylline have little effect on other electrogenic transport mechanisms. In view of the model of intestinal chloride secretion shown in Figure 1, these findings suggest that microtubule inhibitors may interfere with the regulated insertion of new transporters into the apical and/or basolateral membranes of secretory cells in distal rat colon, thus reducing the maximal rate of stimulated chloride secretion.

If PGE_2- and theophylline-induced chloride secretion in rat colon were regulated, at least in part,

by insertion of additional membrane transporters, then it might be expected that the initial rate of increase of I_{sc} (possibly due to function of existent transporters) would be little affected by microtubule inhibitors, whereas the maximal rate of secretion (due to flux through both existent and newly inserted transporters) would be significantly reduced. Indeed, in inhibitor-treated tissues, the shape of the I_{sc} secretory response curve evidenced no change in initial slope, but rather a marked reduction in the sustained part of the response (Figure 3).

Several points merit emphasis regarding the specificity of microtubule inhibitors, as other effects of these agents, such as inhibition of intestinal Na,K-ATPase activity (49) by colchicine, have been noted. It is unlikely, however, that other effects could account for our findings. First, colchicine in concentrations up to 10^{-3} mol/liter does not affect Na,K-ATPase activity in renal medullary tissue (50). Second, lumicolchicine, which is thought to have all the nonspecific properties of colchicine but does not inhibit mitosis or bind to tubulin (51), was used as a control in this as well as previous studies. Third, Notis et al (12) previously noted that intestinal glucose-stimulated sodium transport was not affected by concentrations of colchicine or vinblastine that markedly altered chloride secretion, nor did these drugs themselves affect intestinal adenylate cyclase activity or cAMP concentration. Fourth, the microtubule inhibitors had no effect, in this study, on either mucosal-to-serosal or serosal-to-mucosal sodium transport, suggesting that their effects on chloride secretion were specific and not due to a generalized inhibitory effects on colonocyte ion transport. Finally, the observation that three chemically unrelated microtubule-inhibiting drugs reduced chloride secretion to a similar degree suggests that the effect of the drugs was due to effects on microtubules. Whether the inhibitory effects reflect changes in microtubule-mediated transport of chloride channels or other transporters such as the basolateral Na, K, 2 Cl symporter cannot be answered directly from these studies. Indeed it is possible that microtubules are required for insertion and/or removal of regulatory proteins rather than preformed transporters *per se*. Microtubule inhibition might affect ion transport through other mechanisms such as changes in tight junction structure and/or function and thus solute transport; however, in this study neither transepithelial resistance nor mucosal-to-serosal ion fluxes were significantly altered by the drugs employed. Finally, correlations between colonocyte endo- and exocytosis and changes in chloride secretion would be helpful in supporting our hypothesis; however, the complex architecture of the colon and lack of agreement on the cell or cells responsible for chloride secretion make such studies difficult to perform.

In conclusion, the results of these studies suggest that in rat distal colon, approximately 40% of chloride secretion stimulated by PGE_2 and theophylline may involve microtubule-dependent processes, possibly vesicle-mediated insertion of ion transporters. The relevance of these findings to the clinical observations of diarrhea in patients ingesting colchicine remains to be demonstrated.

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