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THE EFFECT OF CHLORPROMAZINE ON THE FUNCTION OF COLONIC AND ILEAL MUCOSA IN THE ANAESTHETIZED RAT

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1 The effect of chlorpromazine (Cpz) on the net fluxes of water and sodium in the ileum and colon has been studied in the anaesthetized rat.

2 Under control conditions both the ileum and colon absorbed water and sodium. Cpz (100 μmol/kg, s.c.) had no significant effect on these basal rates of absorption.

3 Intestinal secretion was stimulated by a combination of intra-arterial prostaglandin E_1 (PGE_1; 10 nmol kg^{-1} min^{-1}) and intraluminal theophylline (25 mM). A marked potentiation occurred between PGE_1 and theophylline in the stimulation of colonic and ileal secretion.

4 In the ileum, a dose-related inhibition of the PGE_1/theophylline-induced changes in the net fluxes of water and sodium was produced by Cpz (0.1 to 100 μmol/kg, s.c.). Under these conditions the inhibition of the secretagogue-induced changes was significant using Cpz at 1 μmol/kg (s.c.) and a dose of Cpz of 100 μmol/kg (s.c.) returned the net fluxes of both water and sodium to basal levels.

5 In the colon, Cpz, at doses of 0.1 and 1 μmol/kg subcutaneously did not inhibit the PGE_1/theophylline-induced changes in the net fluxes of water and sodium, and, in contrast with the ileum, significant changes were only obtained with Cpz at 10 μmol/kg. Increasing the dose of Cpz to 100 μmol/kg further inhibited the net flux of sodium, but not that of water in the colon, and in each case the secretagogue-induced changes were not returned to basal levels.

Introduction

Chlorpromazine (Cpz) has been used successfully to reduce the secretory diarrhoea caused by intestinal infection with *Vibrio cholerae* (cholera) in man and *E. coli* in swine (Holmgren & Greenough, 1980; Holmgren, 1981). In addition, experiments in mice have confirmed that Cpz inhibits secretion induced by a variety of secretagogues in the small intestine (Holmgren, Lange & Lönnroth, 1978; Robins-Browne & Levine, 1981).

In contrast, no information is available about the effect of Cpz on water and electrolyte fluxes in the large intestine, although Binder (1979) has suggested that 'fluid transport in the colon is critical to the regulation of intestinal fluid and electrolyte balance and is the ultimate determinant of diarrhoea'. Thus in the present experiments the inhibition by Cpz of secretagogue-induced net fluid and electrolyte transport has been investigated in the colon of the anaesthetized rat, and a comparison made with the effects of Cpz in the ileum.

Methods

In the present experiments the net fluxes of fluid and electrolyte were measured essentially according to Beubler & Lembeck (1980). However, there were practical difficulties associated with measuring the intraluminal volume of the intestine using a high molecular weight volume marker, and in this section our resolution of these problems is described.

Operative procedure

Male Wistar rats (approx. 200 g) were allowed free access to food and water. The rats were anaesthetized with dialurethane (a mixture of allobarbitone, 100 mg/kg s.c., and urethane, 400 mg/kg s.c.) and then dosed with indomethacin (10 mg/kg s.c.) to inhibit endogenous prostanoid formation. The trachea was intubated and the left carotid artery was cannulated such that the tip of the catheter was close to the aorta. The abdomen was opened by a midline incision, and the entire colon and approximately 15 cm of terminal ileum (adjacent to the caecum) rinsed out with warm isotonic mannitol solution. These segments of intestine were emptied by blowing air through the lumen followed by gentle squeezing, and then ligated at one end. On completing the operation the rats were given a subcutaneous injection of chlorpromazine (Cpz) or saline, and then left for a 30 min waiting period.

The scheme for sample instillation and collection is

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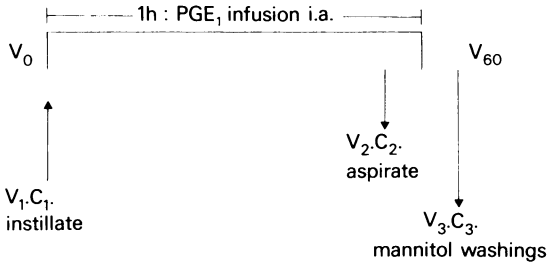

Figure 1 Scheme of sample instillation and collection: where

↑ indicates instillation of solution into intestine
↓ indicates collection of solution from intestine
V_0 = initial intraluminal volume (ml)
V_1 = volume of instillate (1.5 ml)
†C_1 = [^{14}C]-PEG activity of instillate
*V_2 = volume of aspirate (ml)
†C_2 = [^{14}C]-PEG activity of aspirate
V_3 = volume of mannitol washings (ml)
†C_3 = [^{14}C]-PEG activity of mannitol washings
V_{60} = final intraluminal volume (ml)

*the volume drained from the lumen, V_2, is not necessarily identical with the calculated intraluminal volume, V_{60}; see text.
†the activity of [^{14}C]-PEG in the samples was determined by liquid scintillation spectrometry.

shown in Figure 1. After 30 min each segment of intestine was filled with 1.5 ml of Tyrode solution ($V_1.C_1$) and tied off with a second ligature.

The Tyrode solution contained (mM): NaCl 137, KCl 2.7, $MgCl_2$ 0.8, $CaCl_2$ 1.8, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5.6 and [^{14}C]-polyethylene glycol-4000 ([^{14}C]-PEG, 5 g/l and 20 μCi/l) as a volume marker. In some experiments the solution also contained theophylline 25 mM. On instillation of the intraluminal solution a retrograde infusion of prostaglandin E_1 (PGE_1) into the left carotid artery was started and continued for 1 h. The segments were then removed, their lengths measured and the contents collected ($V_2.C_2$). The lumen was then rinsed through with 5 ml of isotonic mannitol solution to wash out any residual [^{14}C]-PEG ($V_3.C_3$). Preliminary experiments showed that washing the segments through with a further 2.5 ml of mannitol solution did not improve the recovery of [^{14}C]-PEG.

Measurement of intraluminal volume

In preliminary control experiments the percentage recoveries of [^{14}C]-PEG from the ileum and colon were respectively 93.6 ± 1.9% and 96.2 ± 1.6% ($n = 6$), and none of the drug treatments significantly affected these recoveries. Since the recovery of [^{14}C]-PEG from the intestine was incomplete it was not possible to calculate the intraluminal volume from the total [^{14}C]-PEG instilled. Therefore V_{60} was calculated from the total [^{14}C]-PEG recovered at the end of the experiment since it is this material which was available for dilution.

$$\text{Thus, } V_{60} = \frac{\text{total [}^{14}\text{C]-PEG recovered}}{\text{activity of [}^{14}\text{C]-PEG}} = \frac{(V_2.C_2) + (V_3.C_3)}{C_2} \quad \mu l$$

In a series of 20 experiments, V_2, the intraluminal volume collected by drainage after 1 h and measured gravimetrically, was compared with V_{60}, the calculated intraluminal volume. The mean values for V_{60} and V_2 were 1400 ± 90 μl and 1350 ± 90 μl respectively in the ileum, and 1400 ± 100 μl and 1370 ± 100 μl respectively in the colon. Although the mean values of V_{60} and V_2 were in close agreement, analysis of the paired data revealed that in both segments of intestine the mean V_{60} value was significantly greater than the corresponding mean V_2 value ($P < 0.01$ for the paired comparison in both ileum and colon; Wilcoxon matched-pairs signed-ranks test). In addition, residual radioactivity (C_3) could be washed from all segments of intestine studied, indicating that the direct measurement of intraluminal volume (V_2) would provide an underestimate of the true value. Thus, in subsequent experiments intraluminal volume was determined by calculation (V_{60}) as described above.

Measurement of residual volume

Although considerable care was taken in draining each segment of intestine before instilling the Tyrode solution (V_1; 1.5 ml), some residual solution did remain in the lumen, and account of this had to be taken in calculating the initial intraluminal volume, V_0. The residual volume (V_R) was determined in a series of twelve experiments in the following way. After the operative procedure, following the 30 min waiting period, the segments of the intestine were filled with 1.5 ml of isotonic mannitol solution which contained [^{14}C]-PEG (5 g/l and 20 μCi/l) as a volume marker ($V_1.C_1$). The segments were tied off with a second ligature and immediately removed, their lengths measured and the contents collected ($V_2.C_2$). Each segment was then rinsed through with 5 ml of isotonic mannitol solution as described previously ($V_3.C_3$). The residual volume (V_R) was calculated from the total recoverable [^{14}C]-PEG as described above.

$$\text{Thus, } V_R = \frac{\frac{(V_2.C_2) + (V_3.C_3)}{C_2} - V_1}{L} \quad \mu l/cm$$

where L is the length of intestine (cm).

In these experiments the mean residual volumes were 12.5 ± 1.3 μl/cm in the ileum and 18.9 ± 2.1 μl/cm in the colon. These mean values were then used to calculate the predicted initial intraluminal volume, V_0, in all subsequent experiments.

$$\text{Thus, } V_0 = V_1 + (V_R.L)\ \mu l$$

and for example, in the ileum

$$V_0 = 1500 + (12.5.L)\ \mu l$$

when L is the length of that segment (cm).

Changes in intraluminal volume

The net water flux (ΔV) was calculated as the difference between the initial and final intraluminal volumes.

$$\text{Thus, } \Delta V = \frac{V_{60} - V_0}{L} \quad \mu l\, cm^{-1}\, h^{-1}$$

where a negative sign (−) indicates a net absorption and a positive sign (+) indicates a net secretion.

Sodium fluxes

The concentration of sodium in the solutions was measured with a Radiometer FLM3 flame photometer, and the net flux of sodium (ΔNa^+) calculated as described above for ΔV. The residual sodium content (Na^+_R) was 1.7 ± 0.1 μEq/cm in the ileum and 1.2 ± 0.1 μEq/cm in the colon ($n = 12$). These mean values were used to calculate the initial intraluminal sodium content (Na^+_0).

Materials

Allobarbitone (5,5-diallylbarbituric acid), theophylline, indomethacin and chlorpromazine hydrochloride (Sigma), urethane (Koch-Light), polyethylene glycol-4000 (BDH), [^{14}C]-polyethylene glycol-4000 (Radiochemical Centre, Amersham) and prostaglandin E_1 (Cambrian) were used.

Analysis of results

Results are expressed as mean ± s.e.mean. The difference between two means was examined statistically using the Mann Whitney U test for unpaired data, and the Wilcoxon matched-pairs signed ranks test for paired data. The statistical tests are described by Siegel (1956). Two-tailed tests were used and a P value of less than 0.05 was considered to be significant.

Results

Stimulation of intestinal secretion

Under control conditions water and sodium absorption occurred in both the ileum and the colon. (Figure 2).

When administered separately, both PGE_1 (10 nmol kg^{-1} min^{-1}, i.a.) and theophylline (25 mM intraluminally) produced relatively small changes in the net rates of water and sodium absorption. However, a combination of theophylline (25 mM) plus PGE_1 at 10 nmol kg^{-1} min^{-1} i.a. produced a net secretion of water in both segments of intestine. A net secretion of sodium was elicited in the ileum but not in the colon, although in the latter tissue the net absorption of sodium was markedly reduced. Increasing the dose of PGE_1 to 30 nmol kg^{-1} min^{-1} i.a. (plus 25 mM theophylline) caused a net secretion of water and sodium in both the ileum and colon. The results are summarized in Figure 2.

Since a submaximal response was obtained with theophylline (25 mM) plus PGE_1 at 10 nmol kg^{-1} min^{-1} i.a., this combination of doses was used in experiments on the effects of Cpz.

The effect of chlorpromazine on basal intestinal function

Before studying the effects of Cpz (always given subcutaneously) on the changes in intestinal function induced by PGE_1 plus theophylline, the effect of Cpz on the absorption of water and sodium was determined under control conditions. The results are shown in Figure 3. The changes in basal intestinal function following Cpz (100 μmol/kg) were small and statistically insignificant in both the ileum and the colon.

The effect of chlorpromazine on prostaglandin/theophylline-induced intestinal secretion

The results of these experiments are shown in Figure 4. In the ileum, doses of Cpz in the range 0.1 to 100 μmol/kg inhibited the drug-induced fluxes of water and sodium in a dose-related manner. This inhibition was statistically significant using Cpz at a dose of 1 μmol/kg, and increasing the dose of Cpz to 100 μg/kg returned the net fluxes of both water and sodium to control levels.

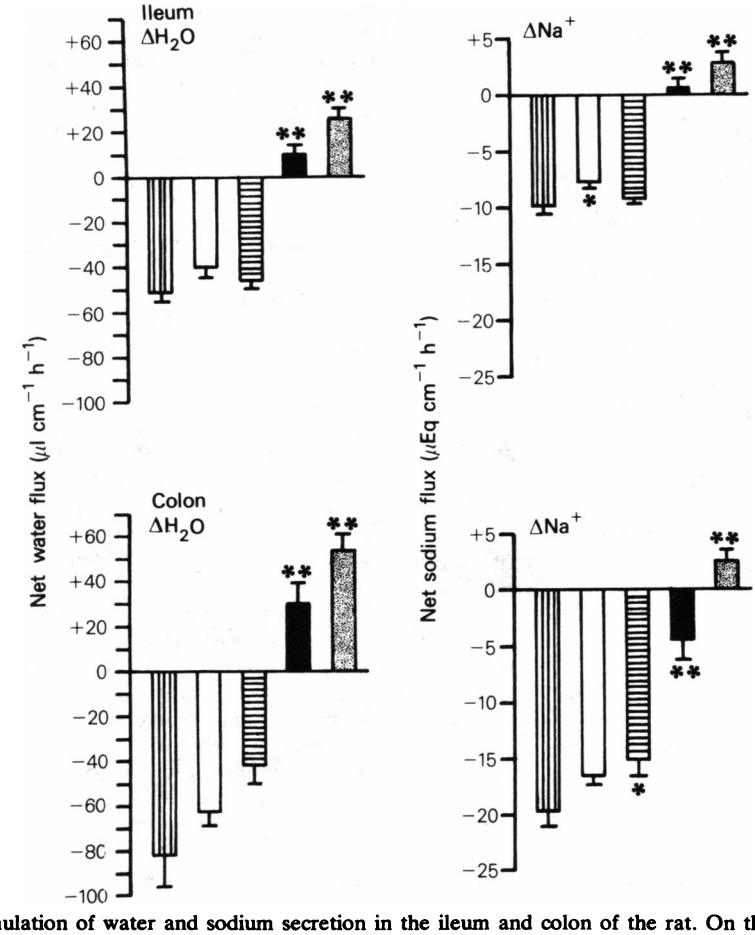

Figure 2 Stimulation of water and sodium secretion in the ileum and colon of the rat. On the ordinate axes, + indicates a net secretion; − indicates a net absorption. Vertically striped columns = control ($n = 6$); open columns = theophylline 25 mM ($n = 6$); horizontally striped columns = prostaglandin E_1 (PGE_1) 10 nmol kg^{-1} min^{-1} (i.a.) ($n = 7$); solid columns = PGE_1 10 nmol kg^{-1} min^{-1} (i.a.) plus theophylline 25 mM ($n = 6$); stippled columns = PGE_1 30 nmol kg^{-1} min^{-1} (i.a.) plus theophylline 25 mM ($n = 5$). Significant difference between test group and control: $P < 0.05$; $^{**}P < 0.01$. Vertical lines show s.e.mean.

The colon was less sensitive to Cpz than the ileum, and doses of the compound of 0.1 and 1 μmol/kg failed to affect significantly the net fluxes of water and sodium. A higher dose of Cpz, 10 μmol/kg, did produce a significant inhibition of the secretagogue-induced changes and increasing the dose of Cpz to 100 μmol/kg further inhibited the net flux of sodium, but not that of water in the colon. Also, in contrast with the ileum, the highest dose of Cpz (100 μmol/kg) did not return the PGE_1/theophylline-induced changes in the colon to control levels.

Discussion

It has been pointed out (Racusen & Binder, 1980) that compared with the small intestine, relatively little information is available about the effects of prostaglandins on fluid and electrolyte transport in the colon. Indeed, in 1975, Milton-Thompson and his co-workers (Milton-Thompson, Cummings, Newman, Billings & Misiewicz, 1975) reported that parenteral infusion of PGE_2 in man did not affect colonic absorptive function. However, studies on experimental animals have shown that prostaglandins are active in the colon. Intraluminal application of PGE_1 to rabbit colon induced net fluid secretion (Taub, Coyne, Bonorris, Chung, Coyne & Schoenfield, 1978), although similar administration of E prostaglandins to rat colon merely reduced the rate of fluid absorption; a net secretion was not stimulated (Beubler & Juan, 1978; Rampton, Breuer, Vaja, Sladen & Dowling, 1980). To date, the only known evidence that colonic function is affected by paren-

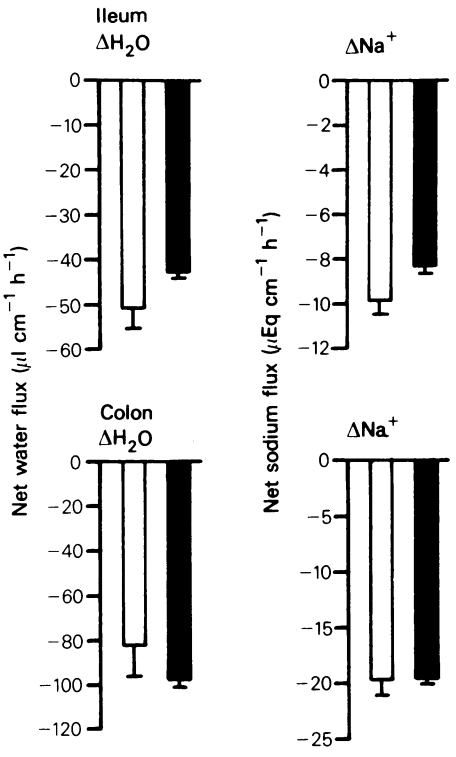

Figure 3 The effect of chlorpromazine (Cpz, 100 μmol/kg, s.c.) on the basal levels of water and sodium absorption in the ileum and colon of the anaesthetized rat. Open columns = control ($n = 6$); solid columns = Cpz 100 μmol/kg, s.c. ($n = 4$). The sign given to the values on the ordinate axes are explained in Figure 2. The vertical lines show s.e.mean.

teral prostaglandins is that of Hardcastle, Hardcastle & Redfern (1980) where intravenous injection of PGE_2 increased transepithelial potential difference in the rat, and this work has been corroborated by experiments on isolated colonic mucosa where the serosal application of E prostaglandins affected ion fluxes and electrical measurements (Frizzell, Heintze & Stewart, 1980; Racusen & Binder, 1980). In the present experiments the effects of parenterally administered PGE_1 on fluid and electrolyte fluxes in the colon has been examined *in vivo*. Intra-arterial infusion of PGE_1 only slightly reduced the net absorption of fluid and sodium, but a combination of PGE_1 plus intraluminal theophylline stimulated a marked colonic secretion. Such an interaction between PGE_1 and a phosphodiesterase inhibitor has been recorded previously by Beubler & Lembeck (1980) in rat jejunum, and, indeed, this is an expected result since it is well documented that prostaglandins of the E series increase mucosal cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels in the small intestine and large intestine of the rat (Beubler & Lembeck, 1980; hardcastle *et al.*, 1980; Racusen & Binder, 1980). In addition, experiments in other isolated intestinal epithelia have shown that the effects of PGE_2 on ion fluxes and electrical parameters mimic precisely those of cyclic AMP (Frizzell *et al.*, 1980).

In the present experiments it was found that Cpz did not affect the basal function of the ileum and colon of the rat, and similar observations have been made using Cpz in mouse small intestine *in vivo* (Holmgren *et al.*, 1978) and another phenothiazine compound, trifluoperazine, in rabbit ileum *in vitro* (Ilundain & Naftalin, 1979; Smith & Field, 1980). However, Cpz is very effective as an inhibitor of drug-induced intestinal secretion. In the ileum, the present work shows that Cpz produced a dose-dependent inhibition of PGE_1/theophylline-induced secretion in the rat, and that a dose of Cpz of 100 μmol/kg returned the fluid and sodium response to basal levels. A similar effect of Cpz has been reported in mouse small intestine against fluid secretion induced by bacterial toxins, cyclic nucleotides and PGE_1 (Holmgren *et al.*, 1978; Robins-Browne & Levine, 1981), and in an *in vitro* preparation of rabbit ileal mucosa trifluoperazine inhibited the changes in ion fluxes and electrical parameters induced by cyclic nucleotides, bacterial toxins and the calcium ionophore, A23187 (Ilundain & Naftalin, 1979; Smith & Field, 1980).

The effect of Cpz on the function of the large intestine *in vivo*, determined in the present study, has not been reported previously in any species. Cpz exhibited a higher threshold for antisecretory activity in the colon than in the ileum; in the latter tissue a dose of Cpz of 1 μmol/kg produced a significant inhibition of the secretagogue-induced changes, but this had to be increased to 10 μmol/kg before a significant effect was observed in the colon. Also, in contrast with the ileum, the highest dose of Cpz (100 μmol/kg) failed to return the net fluxes of water and sodium in the colon to control levels.

An explanation for the differences between the ileum and the colon in their responses to Cpz *in vivo* cannot be found at present. Indeed, the present experiments do not reveal the mechanism by which Cpz inhibits intestinal secretion, although this action of the phenothiazines may have a multifactorial origin since these compounds possess a wide spectrum of pharmacological activity (Byck, 1975). However, one possibility of particular interest is that Cpz might inhibit colonic secretion through an interaction with the Ca^{2+}-calmodulin complex, because Ilundain & Naftalin (1979) have reported that trifluoperazine exerts such an effect in the ileum.

It is known that Cpz inhibits secretion in the small intestine in both experimental animals and man

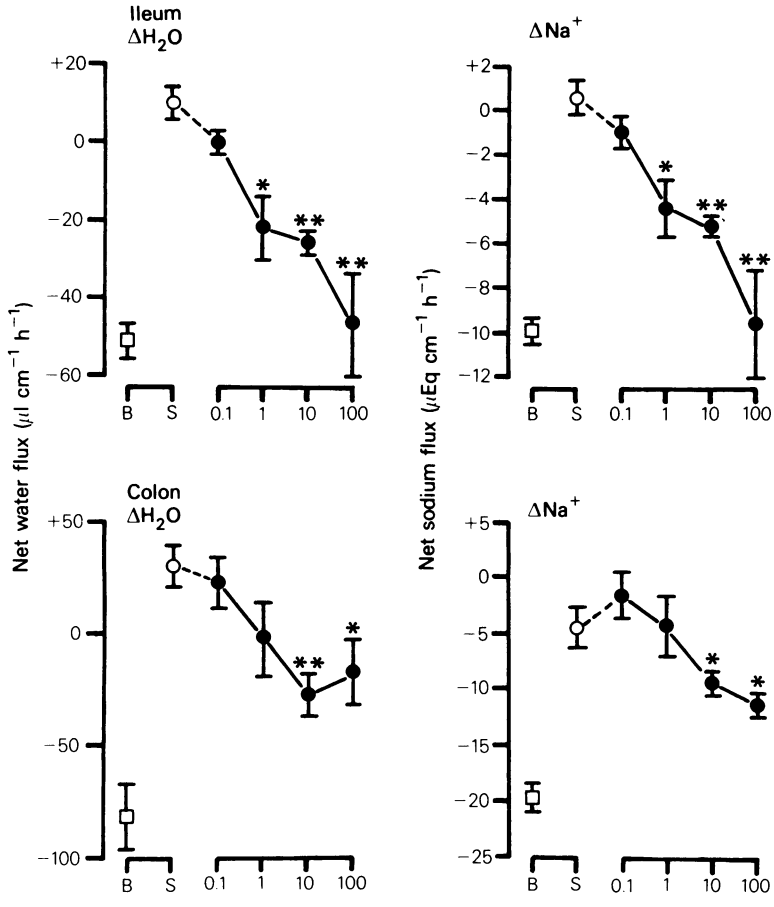

Figure 4 The effect of chlorpromazine (Cpz) on the net fluxes of water and sodium induced by prostaglandin E_1 (PGE_1, 10 nmol kg^{-1} min^{-1}, i.a.) plus theophylline (intraluminal, 25 mM) in the ileum and colon of the anaesthetized rat. The signs given to the values on the ordinate axes are explained in Figure 2. Basal fluxes (B, □). Fluxes induced by PGE_1 plus theophylline (S, ○). The effect of Cpz on the secretagogue-induced changes (●). Significant difference between the Cpz-treated groups and PGE_1 plus theophylline; $*P<0.05$; $**P<0.01$. Vertical lines show s.e.mean. Each point represents the mean of 5 to 9 experiments.

(Holmgren, 1981) and since the present study has also shown that Cpz inhibits PGE_1/theophylline-induced secretion in rat colon it is possible that the phenothiazines may inhibit colonic secretion in man. Indeed, phenothiazine compounds might ameliorate the diarrhoea associated with inflammatory bowel disease, particularly since in these patients it is likely that the mucosal secretion (Edmonds & Pilcher, 1973; Hawker, McKay & Turnberg, 1980) is mediated, at least in part, by endogenous prostaglandin formation (Rampton, Sladen & Youlten, 1980; Hawkey & Truelove, 1981).

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