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Distant intestinal stimulation by cholera toxin in rat in vivo

Fabrice Marquet [a], Danielle Pansu [b], Monique Descroix-Vagne [c,*]

[a] *INSERM U 45, Hôpital Edouard Herriot, Pavillon H bis, 69437 Lyon Cedex 03, France*
[b] *Ecole Pratique des Hautes Etudes, Hôpital Edouard Herriot, Pavillon H bis, 69437 Lyon Cedex 03, France*
[c] *IUT A, Laboratoire de Nutrition, Université Claude Bernard Lyon I, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne, France*

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Abstract

Cholera toxin (16 μg/rat) locally administered in the jejunum of anesthetized rats stimulated jejunal secretion and also distant duodenal secretion, as determined with the ligated loop technique. The release of prostaglandin E_2 in both jejunal and duodenal secretions and in plasma was increased by cholera toxin, while the release of 5-hydroxytryptamine (5-HT) was unchanged in the early phase of secretion (2 h). The inhibitor of prostaglandin E_2 release, indomethacin (10 mg/kg, s.c.), and the 5-HT_3 subtype receptor antagonist, granisetron (30 μg/kg i.v.), inhibited the jejunal secretion but had no effect on distant duodenal secretion. However, indomethacin statistically significantly decreased prostaglandin E_2 release in both jejunal and duodenal secretions as well as in plasma. The vasoactive intestinal peptide antagonist (VIP-(6–28), 1.2 nmol/100 g h) did not modify jejunal and duodenal secretions. Our study confirmed the local involvement of 5-HT and prostaglandin E_2 in choleraic jejunal secretion but not in distant duodenal secretion.

Keywords: Duodenum; Jejunum; Cholera toxin; 5-HT (5-hydroxytryptamine, serotonin); Prostaglandin E_2; Sorbin; VIP (vasoactive intestinal peptide) receptor antagonist

1. Introduction

Cholera toxin induces hypersecretion by acting directly on the enterocyte (Field, 1979) and indirectly by the release of neurotransmitters (Cassuto et al., 1983; Autore et al., 1987; Beubler et al., 1989). We showed (Marquet et al., 1998) that C-terminal derivatives of sorbin, a 153-amino acid peptide isolated from porcine small intestine, were able to inhibit both effects of cholera toxin, inhibiting both duodenal and jejunal secretion when cholera toxin was locally administered in the jejunum. Sorbin has already been shown to inhibit basal duodenal secretion (Charpin et al., 1992) and vasoactive intestinal polypeptide (VIP)-stimulated secretion in different parts of the intestine, predominantly in the duodenum (Grishina et al., 1995), ileum (Marquet et al., 1994) and colon. In order to improve our knowledge of the mechanism of action of sorbin, we attempted to determine whether prostaglandin E_2, 5-hydroxytryptamine (5-HT) and VIP were involved in the distant duodenal response to cholera toxin, which is inhibited by sorbin derivatives. The use of specific inhibitors allowed us to determine the involvement of prostaglandin E_2, 5-HT and VIP, in the local and distant intestinal responses to cholera toxin. The role of 5-HT was of interest, because sorbin has been detected in some enterochromaffin cells containing the amine and the peptide (Abou el Fadil et al., 1997).

2. Materials and methods

2.1. Animals

About 130 male Sprague–Dawley rats (200 ± 20 g b.wt., IFFA CREDO, 69210 Saint Germain sur l'Arbresle, France) were used. They were deprived of food for 48 h before experiments, but had free access to water. The rats were anesthetized with pentobarbital sodium (55 mg/kg i.p.). Animal management followed the directives of the European Economic Community (user number 187 and 191).

* Corresponding author. Tel.: +33-4-72-69-20-65; Fax: +33-4-72-69-20-50; E-mail: descroix@cismsun.univ-lyon1.fr

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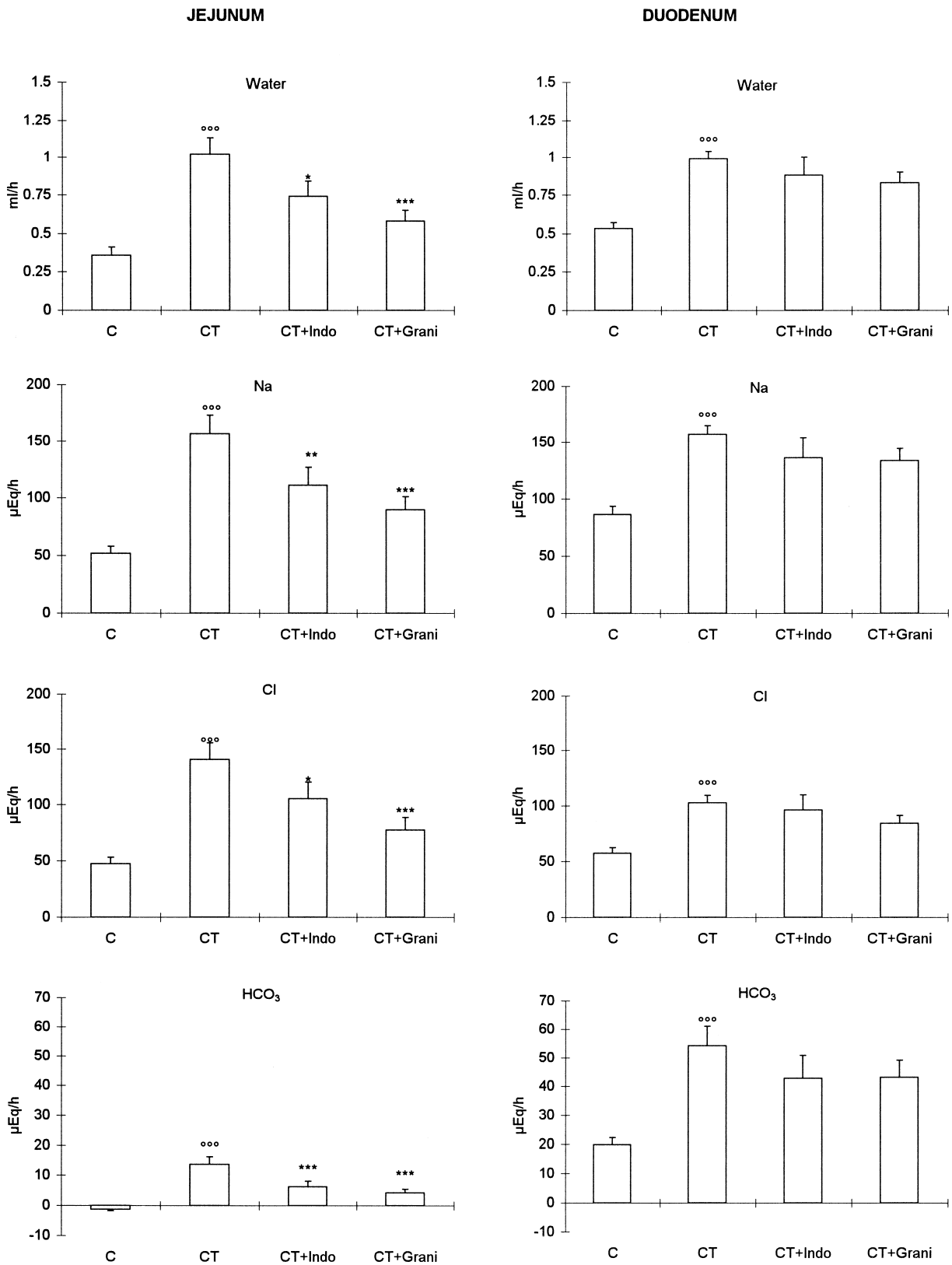


Fig. 1. Jejunal and duodenal water and electrolyte secretion in controls (C, 33 rats), and after intra-luminal administration in the jejunum of cholera toxin (16 μg/rat, CT, 18 rats). During cholera toxin administration, indomethacin was s.c. injected (CT + Indo, 10 mg/kg, 16 rats), or granisetron was i.v. infused (CT + Grani, 30 μg/kg, 16 rats). Cholera toxin and indomethacin injections were performed 2 h before death. Granisetron infusion was performed for 2 h. The test solution was present for 1 h in the intestinal loops. Mean ± S.E.M. $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$, significantly different from control group; $^{*}P < 0.05$, $^{***}P < 0.001$, significantly different from cholera toxin group (Student's *t*-test).

Table 1
Jejunal and duodenal water and electrolyte secretion under basal conditions (control) and after administration of indomethacin (10 mg/kg, s.c.) or granisetron (30 μg/kg, i.v.)

	Jejunum			Duodenum		
	Control	Indomethacin	Granisetron	Control	Indomethacin	Granisetron
N	33	15	14	33	15	15
Water flux (ml/h)	0.36 ± 0.05	0.28 ± 0.03	0.21 ± 0.02	0.53 ± 0.04	0.42 ± 0.05	0.40 ± 0.04
Na flux (μeq/h)	51.79 ± 5.95	43.15 ± 4.31	33.59 ± 2.36	86.30 ± 6.81	72.19 ± 7.29	68.61 ± 5.54
Cl flux (μeq/h)	47.36 ± 3.60	42.47 ± 4.48	30.94 ± 2.14	57.42 ± 4.87	50.62 ± 6.56	46.23 ± 5.50
HCO_3 flux (μeq/h)	−1.48 ± 0.38	−2.58 ± 0.46	−3.22 ± 0.63	19.94 ± 2.35	16.60 ± 2.36	13.52 ± 1.94

Positive values represent secretion; negative values represent absorption.
Analysis of variance: $F > 0.05$.

2.2. Experimental protocol

The protocol of ligated loops in situ has been described in detail (Chikh-Issa et al., 1993). It was modified by three changes: (1) jejunal stimulation was elicited by luminal flushing with cholera toxin followed by a 2-h contact time; (2) evaluation of the induced secretion at the jejunal and duodenal level was achieved by making two ligated loops, 1 h after flushing, the loops being maintained for 1 h before collection; (3) drug was administered by i.v. or s.c. routes, immediately before cholera toxin instillation.

2.2.1. Jejunal stimulation by cholera toxin

Anesthetized rats were equipped with a catheter in the jugular vein and submitted to laparotomy. The jejunum was injected with 16 μg of cholera toxin (Sigma, St. Louis, MO) in 1 ml of water (cholera toxin group) at about 4 cm below the Treitz ligament, with a finger pinch to avoid reflux in duodenum. The intestine was gently replaced in the abdomen; the toxin could migrate aborally. Control rats received 1 ml of water only. Simultaneously, rats were i.v. perfused with 9 g/l NaCl solution at a rate of 3 ml/h.

2.2.2. Drug administration

Thirty-three rats received water intraluminally and saline i.v. (control group); 18 rats received cholera toxin intraluminally and saline i.v. (cholera toxin group). In 32 rats, cholera toxin was administered in the jejunum with either an i.v. infusion of the 5-HT_3 subtype receptor antagonist granisetron (SmithKline Beecham Lab. Pharm., 30 μg/kg, 16 rats), or a s.c. injection of indomethacin (Sigma, 10 mg/kg, 16 rats). In 29 rats, water was injected in the jejunum together with either an i.v. infusion of the 5-HT_3 subtype receptor antagonist granisetron (30 μg/kg, 14 rats) or a s.c. injection of indomethacin (10 mg/kg, 15 rats).

In a complementary randomized experiment using a different batch of cholera toxin, eight rats received cholera toxin in the jejunum and an i.v. infusion of VIP receptor antagonist VIP-(6–28) (Sigma, 1.2 nmol/100 g.h), six rats received cholera toxin and six rats received only water.

2.2.3. Duodenal and jejunal ligated loop technique

One hour after cholera toxin or water administration, the abdominal wall of each rat was re-opened to make duodenal and jejunal loops. For the duodenum, the choledoco-pancreatic duct was ligated and the loop was delimited between two ligations, one at the level of the pylorus, the other at the level of the Treitz ligament, making the duodenal loop about 8 cm long (about 1 g wet weight). The proximal jejunal loop was delimited about 4 cm below the Treitz ligament and was 10 cm long (about 1 g wet weight). After instillation of one ml of the test solution in each segment, the abdominal wall was sutured. The test solution, chosen to suppress basal absorption, was charac-

Table 2
Jejunal and duodenal water and electrolyte secretion under control conditions and after intraluminal administration in the jejunum of cholera toxin (16 μg/rat) alone or combined with a 2-h i.v. infusion of VIP antagonist (CT + VIP-(6–28), 1.2 nmol/100 g h)

	Jejunum			Duodenum		
	Control	Cholera toxin	CT + VIP-(6-28)	Control	Cholera toxin	CT + VIP-(6–28)
N	6	6	8	6	6	8
Water flux (ml/h)	0.36 ± 0.10	0.98 ± 0.11[b]	1.06 ± 0.15	0.43 ± 0.08	0.79 ± 0.14	0.98 ± 0.12
Na flux (μeq/h)	45.30 ± 8.37	109.21 ± 7.47[c]	108.87 ± 12.22	52.45 ± 8.62	94.01 ± 15.16[a]	103.94 ± 10.98
Cl flux (μeq/h)	55.57 ± 17.11	133.93 ± 17.02[a]	145.53 ± 20.74	50.63 ± 13.18	80.42 ± 19.18	105.95 ± 8.16
HCO_3 flux (μeq/h)	0.21 ± 1.93	11.71 ± 2.71[a]	15.72 ± 4.64	21.21 ± 4.70	45.79 ± 5.54	45.53 ± 12.31

VIP: vasoactive intestinal peptide.
VIP-(6–28): VIP receptor antagonist.
CT: cholera toxin.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, cholera toxin group significantly different from control group. No significant difference between cholera toxin group and CT + VIP-(6–28) group.

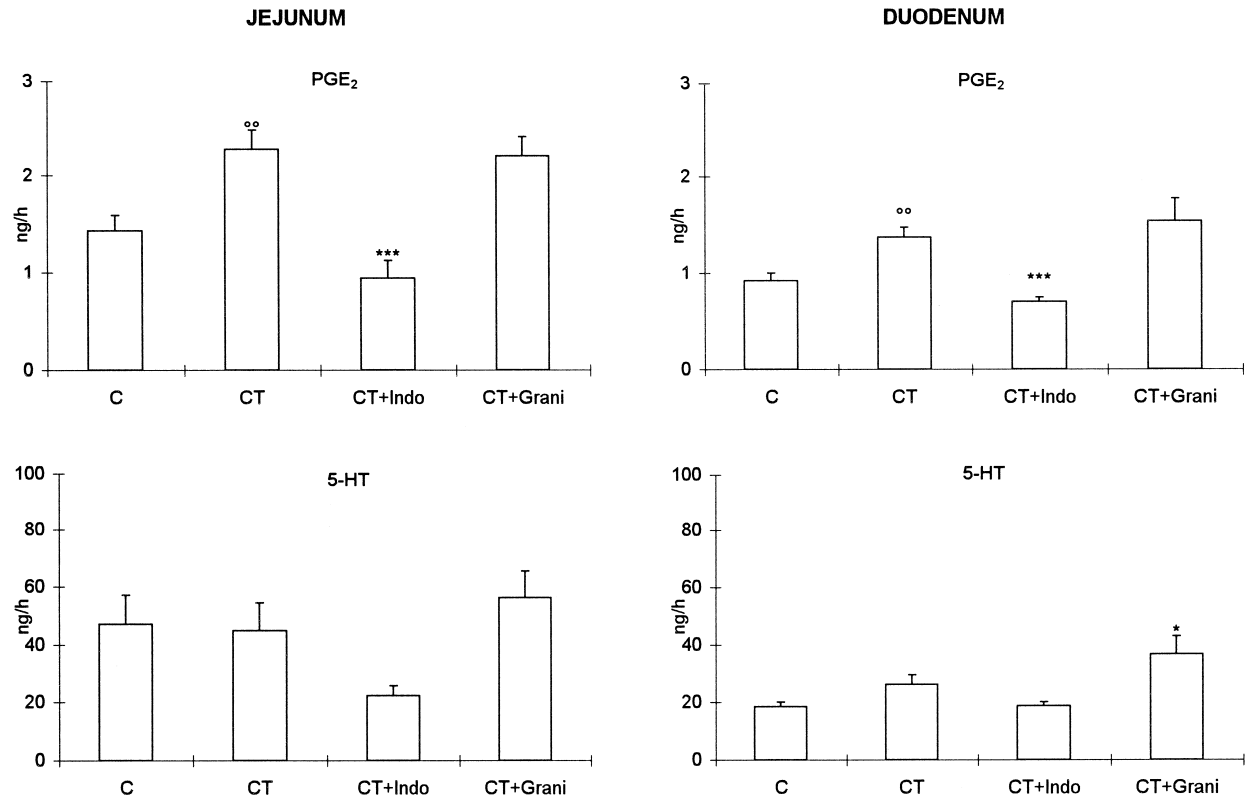


Fig. 2. Prostaglandin E_2 (top) and 5-HT (bottom) were determined in the duodenum and jejunum juices collected in the loops after 1 h, in control (C, 33 rats), cholera toxin alone (CT, 18 rats), or cholera toxin associated with s.c. injected indomethacin (CT + Indo, 10 mg/kg, 16 rats) or i.v. infused granisetron (CT + Grani, 30 μg/kg, 16 rats) groups. Mean ± S.E.M. $^{\circ\circ}P < 0.01$, significantly different from control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ significantly different from cholera toxin group (Student's *t*-test).

terized by a low salt concentration (Na^+ 80 mM, K^+ 5.2 mM, Ca^{2+} 1.2 mM, Cl^- 77.6 mM, HCO_3^- 10 mM, pH 8.2). It was made iso-osmotic with 136 mM of mannitol and contained 5 g/l of polyethylene glycol 4000 (PEG) and 1.01 kBq/ml of [^{3}H]PEG (Du Pont NEN®) used as nonabsorbable marker. One hour after test solution instillation (2 h after cholera toxin administration), the rats were killed. The loop content was collected, weighed, centrifuged and tested. Portal venous blood was collected into tubes containing heparin (2.5 IU/ml) and centrifuged for plasma prostaglandin E_2 and 5-HT determinations.

2.3. Determination of net fluid secretion

Net water secretion was determined gravimetrically 1 h after the instillation of the test solution and is expressed as ml/h.

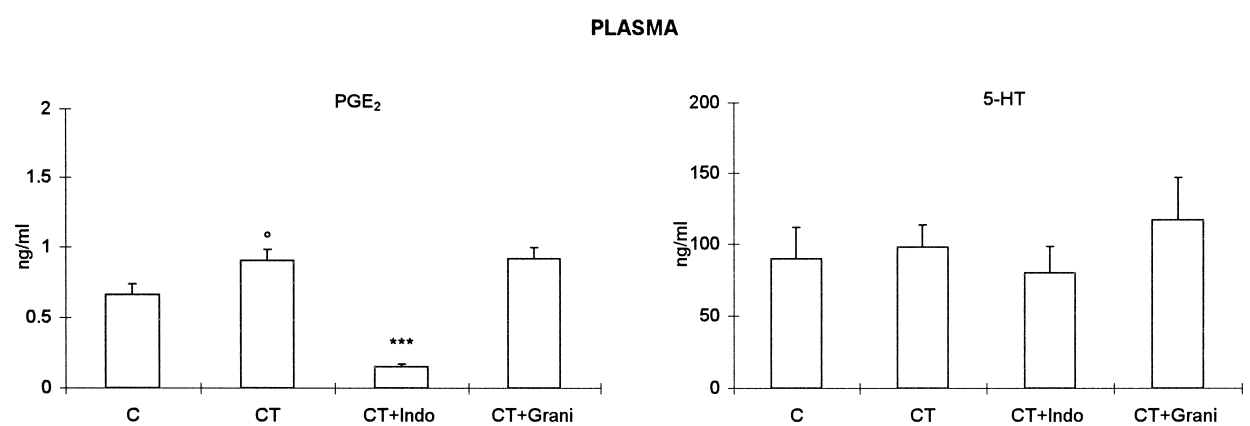


Fig. 3. Plasma prostaglandin E_2 (left) and 5-HT (right) concentrations in control (C, 33 rats), after intra-luminal administration in the jejunum of cholera toxin (16 μg/rat, CT, 18 rats), alone or with s.c. injection of indomethacin (CT + Indo, 10 mg/kg, 16 rats), or i.v. infusion of granisetron (CT + Grani, 30 μg/kg, 16 rats). Mean ± S.E.M. $^{\circ}P < 0.05$ significantly different from control group; $^{***}P < 0.001$ significantly different from cholera toxin group (Student's *t*-test).

2.4. Analytic procedures

The percentage of [^{3}H]PEG recovery was determined by liquid scintillimetry (Packard Tri-Carb 1600 CA). When it was lower than 70%, the sample was discarded. Bicarbonate was measured with the acido-alcalimetric method as described by Preshaw and Grossman (1965). Chloride content was monitored by coulometric titration (Cotlove et al., 1958). Na^+ and K^+ were determined by flame photometry. The electrolyte net fluxes were calculated per hour. Net absorption is indicated by a negative value and net secretion by a positive value. The samples for prostaglandin E_2 measurements were stored at −20°C and analyzed within 3 weeks with the prostaglandin E_2 [^{125}I] Biotrak™ radioimmunoassay system (RPA 530, Amersham International, Buckinghamshire, England). 5-HT was measured immediately with a reverse-phase liquid chromatography with amperometric detection with ClinRep® (Merck/Recipe, Munich, distributed in France by Precision Instruments, Marseille).

2.5. Histological control

At the end of some experiments, intestinal tissue was taken at each level for histological examination. Tissues were fixed in Bouin's solution, embedded in paraffin, sectioned and stained with hematoxylin, eosin and saffron. Sections were observed under light microscopy and photographed.

2.6. Statistics

The results are given as means ± S.E.M. The treated groups were compared to controls by using analysis of variance and Student's *t*-test with the common residual variance.

3. Results

3.1. Water and electrolyte secretion in jejunum and duodenum

Cholera toxin elicited water, Na^+, Cl^- and HCO_3^- net secretion (Fig. 1), both in the jejunum, which was directly in contact with cholera toxin, and in the duodenum, which was not in contact with cholera toxin. In the jejunum, the increase was +183% for water, +203% for Na^+ and +197% for Cl^-. A reversal from absorption to secretion was observed for HCO_3^-. In the duodenum, the increase was +87% for water, +82% for Na^+, +79% for Cl^- and +114% for HCO_3^-.

Indomethacin significantly reduced cholera toxin-induced secretion in the jejunum (−27%, −29%, −25% and −54% for water, Na^+, Cl^- and HCO_3^-, respectively) but had no significant effect on water and electrolyte secretion in the duodenum (Fig. 1). Indomethacin alone had no effect on basal fluid and electrolyte secretion in the jejunum and duodenum (Table 1).

The 5-HT_3 subtype receptor antagonist granisetron significantly reduced cholera toxin-induced water and electrolyte secretion in the jejunum (respectively −43%, −43%, −45% and −68% for water, Na^+, Cl^- and HCO_3^-), but did not significantly modify the fluxes in duodenal fluid (Fig. 1). Under basal conditions, granisetron alone produced a slight decrease of water and electrolyte secretion in the jejunum and duodenum (Table 1) which, however, did not reach statistical significance.

The VIP receptor antagonist VIP-(6–28) did not modify the water and electrolyte response to cholera toxin locally in the jejunum and distally in the duodenum. In this series of experiments, the stimulation by a new batch of cholera toxin was lower than that with the first batch (Na^+ increase was only 70% and 60% of the preceding values in the jejunum and duodenum, respectively) (Table 2).

3.2. Prostaglandin E_2 luminal release

Cholera toxin increased prostaglandin E_2 release in the fluid from the jejunum and the duodenum (+59% and

Table 4
Plasma concentration (ng/ml) of prostaglandin E_2 and 5-HT in the control group, the indomethacin-treated group (10 mg/kg, s.c.) and the granisetron-treated group (30 μg/kg, i.v.)

	Plasma		
	Control	Indomethacin	Granisetron
N	33	15	14
PGE_2 (ng/ml)	0.66 ± 0.08	0.11 ± 0.02[c]	0.33 ± 0.04[b]
5-HT (ng/ml)	89.71 ± 21.76	22.98 ± 2.88[a]	33.68 ± 5.10[a]

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, significantly different from control (analysis of variance and Student's *t*-test).

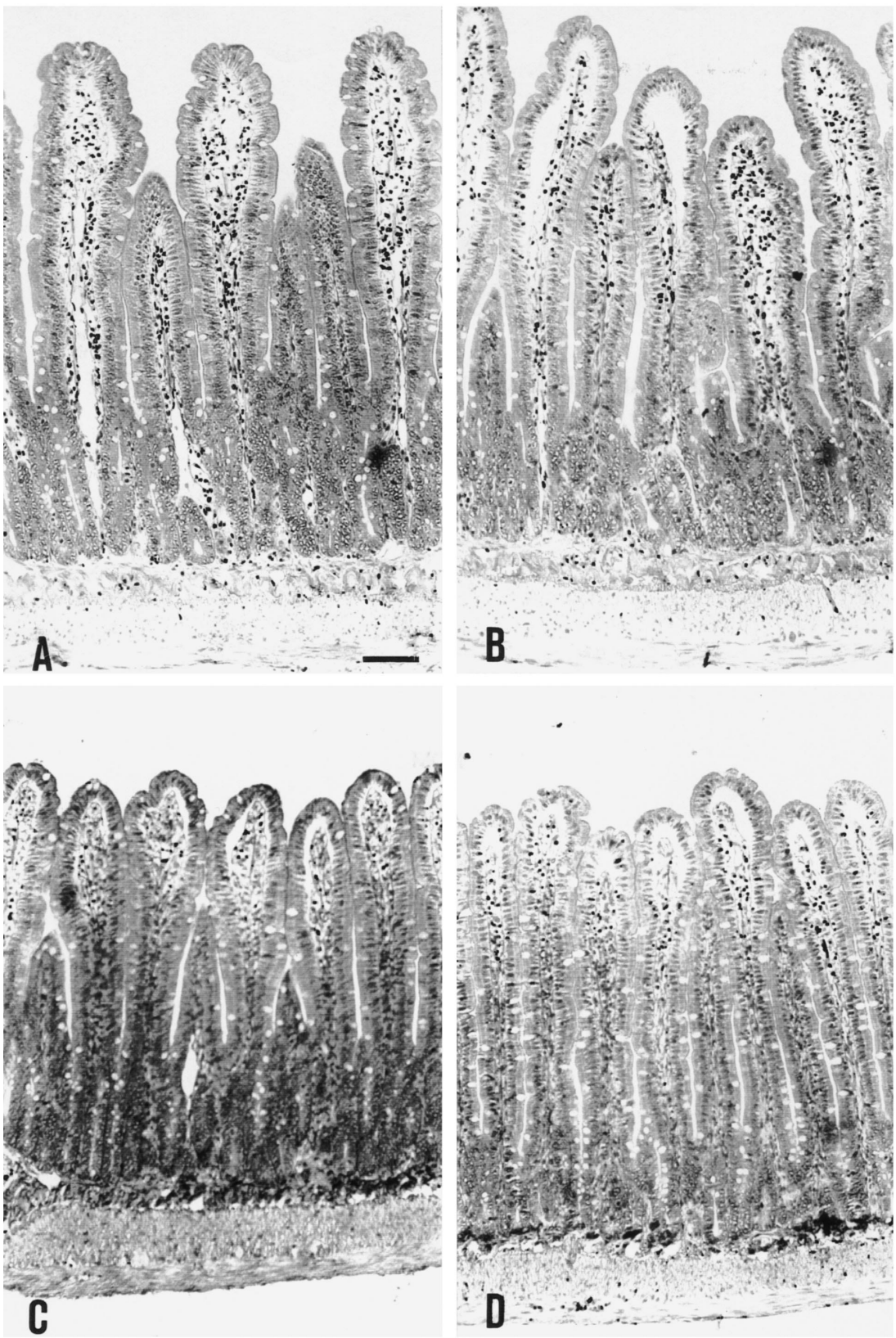
Table 3
Release of prostaglandin E_2 and 5-HT into jejunum and duodenum juices (ng/h), collected from the loops in the control group, the indomethacin-treated group (10 mg/kg, s.c.) and the granisetron-treated group (30 μg/kg, i.v.)

	Jejunum			Duodenum		
	Control	Indomethacin	Granisetron	Control	Indomethacin	Granisetron
N	33	15	14	33	15	15
PGE_2 (ng/h)	1.43 ± 0.16	0.65 ± 0.15[a]	1.08 ± 0.18	0.92 ± 0.08	0.35 ± 0.04[c]	1.16 ± 0.18
5-HT (ng/h)	47.01 ± 10.15	17.58 ± 2.67	40.89 ± 9.50	18.52 ± 1.58	14.43 ± 0.95	24.51 ± 2.61

[a] $P < 0.05$, [c] $P < 0.001$, significantly different from control (analysis of variance and Student's *t*-test).

+49% vs. control values, respectively) (Fig. 2), and also the prostaglandin E_2 concentration in plasma (+36%) (Fig. 3).

Indomethacin caused a significant decrease of cholera toxin-induced prostaglandin E_2 output both in jejunal and in duodenal fluids (respectively −58% and −49%)



(Fig. 2), and in plasma (−83%) (Fig. 3). Under basal conditions, indomethacin significantly decreased prostaglandin E_2 output in jejunal (−55%) and duodenal (−61%) fluids (Table 3) and plasma (−83%) (Table 4).

Granisetron did not modify the cholera toxin-induced prostaglandin E_2 release in the jejunum and duodenum (Fig. 2) and did not change the plasma concentration of prostaglandin E_2 (Fig. 3). Under basal conditions, granisetron did not modify prostaglandin E_2 release in the jejunum and duodenum (Table 3) but decreased the plasma concentration of prostaglandin E_2 (−50%) (Table 4).

3.3. 5-HT luminal release

Cholera toxin did not modify 5-HT release either in jejunal and in duodenal fluids (Fig. 2), or the 5-HT plasma concentration (Fig. 3).

Indomethacin slightly decreased 5-HT output in the jejunum and duodenum during cholera toxin administration (Fig. 2), and the 5-HT plasma concentration (Fig. 3); however, none of these decreases reached statistical significance. Under basal conditions, the same nonstatistically significant decreases were also observed in the jejunum and duodenum (Table 3), while the 5-HT plasma concentration was statistically decreased by indomethacin (Table 4).

Granisetron caused a slight increase of 5-HT release in the duodenal content ($P < 0.05$) and in the jejunal content ($P > 0.05$) (Fig. 2). A similar small increase was also observed in plasma ($P > 0.05$) (Fig. 3). Under basal conditions, granisetron did not modify significantly the 5-HT output in jejunal and duodenal fluids (Table 3), but induced a decrease of 5-HT plasma concentration ($P < 0.05$) (Table 4).

3.4. Histological results

Histological findings (Fig. 4) showed that the 60-min incubation with the saline-mannitol control solution maintained the normal structure of the duodenal and jejunal wall. After jejunal stimulation by luminal flushing with cholera toxin, the intestinal walls did not show epithelial desquamation. The enlargement of the sub-epithelial lacunae, a sign of the increased reabsorption of stimulated secretions (Marquet et al., 1994), was clearly visible.

4. Discussion

The present work is a detailed study of the early phase of cholera toxin stimulation. Under the same conditions, we previously characterized the inhibitory effect of sorbin on the secretion induced by cholera toxin in the locally flushed jejunum as well as in the distant duodenum which was not exposed to the toxin (Marquet et al., 1998). The purpose was to identify the effectors responsible for the distant secretion, in order to obtain information about the targets of sorbin and a better understanding of the action of sorbin.

The 2-h jejunal contact with cholera toxin induced jejunal and duodenal secretion, confirming our previous data (Marquet et al., 1998). A distant effect of cholera toxin has already been described by Nocerino et al. (1995), who stimulated rat colon secretion by installing cholera toxin in the jejunum. The transection of the intestine interrupted the distant secretory effect of cholera toxin, revealing the involvement of the enteric nervous system. That experiment used continuous perfusion of the colon and measured the increase in the volume collected; however, this method does not distinguish between a real increase in secretion and a decrease in absorption secondary to transit acceleration. The ligated loop model maintains the blood and nerve supply but creates an occlusion, and thus suppresses the effect of cholera toxin on motility. Thus, we consider that the increase in the collected volume represents the net secretion induced by cholera toxin.

Cholera toxin possesses a direct effect on enterocytes by binding to Gm1 receptors (Mathias and Clench, 1989) and increasing cAMP concentration (Field et al., 1972). Cholera toxin also elicits the release of 5-HT from enterochrommaffin cells into the lumen (Nilsson et al., 1983; Beubler et al., 1989). The direct secretory effect of 5-HT on epithelial cells is amplified by stimulating the release of tissue prostaglandins (Van Loon et al., 1992). Nocerino et al. (1995) demonstrated an inhibitory effect of aspirin on jejunal secretion and no effect on distant colon secretion. We obtained a similar effect by showing that indomethacin, a potent inhibitor of the release of prostaglandin E_2, like aspirin, inhibited jejunal secretion but was without effect on distant duodenal secretion. The effect of indomethacin was shown by the decrease in the luminal release of prostaglandin E_2 into the jejunal secretions and the duodenal secretions, demonstrating the dissociation between the decrease of prostaglandin E_2 release and the absence of inhibition of duodenal secretion. The present data confirmed previous findings that prostaglandin E_2 is involved, at least partly, in the local response to cholera toxin, but is not involved in the distant intestinal response, proximal (present study) or distal intestinal response

Fig. 4. Histological aspect of the duodenal and jejunal tissues of the loop after 60 min contact with the saline–mannitol test solution (2 h contact with cholera toxin). (A,B) Duodenum. (C,D) Jejunum. Cholera toxin was instilled in the jejunum 60 min before loop was made and diffused freely aborally, without reflux in the duodenum. (A) Control duodenal loop. (B) Duodenal loop after distant administration of cholera toxin. (C) Control jejunal loop. (D) Jejunal loop after local administration of cholera toxin. The integrity of the structure of the intestinal wall was maintained after cholera toxin stimulation. The sub-epithelial lacunae, larger after cholera toxin administration, are due to the reabsorption of the increased secretions. Bouin's liquid fixation, hematoxylin, eosin, saffron staining. Bar scale = 100 μm.

(Nocerino et al., 1995). Furthermore, the plasma prostaglandin E_2 concentration, which was increased by cholera toxin treatment, was strongly inhibited by indomethacin, as expected. Blood prostaglandin E_2 levels did not play a role in the distant cholera toxin-induced secretion.

Granisetron, a 5-HT_3 subtype receptor antagonist, strongly inhibited the jejunal secretion induced by the in situ injection of cholera toxin. The present data are in full agreement with the results of previous studies with the rat (Sjöqvist et al., 1992; Beubler et al., 1993; Mourad et al., 1995) and in pig jejunum (Hansen and Skadhauge, 1994; Hansen et al., 1994; Grondhal et al., 1996). Ketanserin, a 5-HT_2 subtype receptor antagonist, gave contradictory results (Beubler and Horina, 1990; Sjöqvist et al., 1992). The inhibitory effect of granisetron was observed in the locally injected jejunum but was not obtained in the distant duodenum. The concentration of 5-HT in the plasma and in the fluid secreted from the jejunum and the duodenum showed the same tendency to increase during granisetron treatment. Thus, there is a dissociation between the increase in 5-HT concentration in both duodenal and jejunal fluids and the decrease in fluid secretion in the jejunum and no effect in the duodenum. With the loops technique, which avoids motor interference on secretion and the use of labelled Cl^- and Na^+ to determine unidirectional in and out fluxes (Grishina et al., 1998), we showed that 5-HT intraluminally administered increased water and electrolyte secretion at all intestinal levels (duodenum, jejunum, ileum and colon). This increase in luminal content was secondary to a decrease of intestinal absorption, partly due to a decrease of Na^+/H^+ exchange and mostly due to a decrease of passive ion transport, while a stimulation of Cl^- secretion was only seen in the colon.

The VIP receptor antagonist VIP-(6–28) did not modify cholera toxin-induced secretion in the jejunum nor that of distant duodenum, showing that under our experimental conditions VIP is not involved in local or distant choleraic secretion. Some arguments have been presented for a neuronal response through VIP secretion, since VIP release was found in stool water of choleraic patients (Bloom et al., 1976) and in venous blood after 30 min of contact with cholera toxin in rat (Cassuto et al., 1980, 1981). Receptors for cholera toxin were demonstrated in VIP neurons in guinea pig submucosal plexus (Jiang et al., 1993) but they cannot explain the local and distant secretory response since cholera toxin is localized only at the brush borders of intestinal cells (Peterson et al., 1972). Under our conditions, the integrity of the epithelial barrier, which was verified histologically, is against any involvement of VIP secretion and is in agreement with the absence of effect of the VIP receptor antagonist.

Our study showed that the effect of cholera toxin on distant duodenal secretion did not involve 5-HT, prostaglandin E_2 or VIP, since specific inhibitors of the mediators were ineffective. Thus, the inhibitory effect induced by sorbin in the distant duodenum is not related to the control of these mediators, but is probably due to a direct effect of sorbin on the enterocytes.

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