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Guanylin-, heat-stable enterotoxin of *Escherichia coli*- and vasoactive intestinal peptide-induced water and ion secretion in the rat intestine in vivo

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Abstract

The heat-stable enterotoxin of *Escherichia coli* binds to an intestinal receptor, guanylyl cyclase-C, and produces cGMP to induce diarrhea. Guanylin is an endogenous ligand of this receptor. In the present in vivo study, the intestinal water and ion secretion induced by mucosal application of 2 nmol/ml guanylin or 5 or 10 units/ml heat-stable enterotoxin into closed loops was compared in the rat. The characteristics of secretion induced by cAMP following intravenous perfusion of 1.2 nmol/100 g per h vasoactive intestinal peptide were compared to those induced by cGMP. Unidirectional Na^+ and Cl^- fluxes were estimated by addition of ^{22}Na into the loop and i.v. injection of ^{36}Cl. Guanylin induced less water and ion secretion than that produced by heat-stable enterotoxin in the colon, confirming the results of in vitro studies, and also in duodenum and ileum. The cAMP- or cGMP-mediated response had a similar pattern, i.e., an inhibition of Na^+ absorption and an increase in anion secretion. © 1997 Elsevier Science B.V.

Keywords: Guanylin; Intestine; Ion transport; Na^+ and Cl^- unidirectional flux; STa (heat-stable enterotoxin of *Escherichia coli*); VIP (vasoactive intestinal peptide)

1. Introduction

The heat-stable enterotoxin, produced by enterotoxigenic *Escherichia coli*, induces secretory diarrhea as a result of stimulation of intestinal guanylate cyclase (Field et al., 1978; Hugues et al., 1978). Heat-stable enterotoxin binds to a specific receptor, guanylyl cyclase-C, present on the apical surface of enterocytes of rat small and large intestine, and induces intestinal secretion through an increase in cyclic guanosine monophosphate (cGMP) (Giannella et al., 1983; Guarino et al., 1987; Mezoff et al., 1992).

Recently, an endogenous ligand of the heat-stable enterotoxin receptor, the 15-amino-acid peptide guanylin, has been isolated and sequenced from the rat jejunum (Currie et al., 1992). The nucleotide sequences of the rat and human cDNAs (Wiegand et al., 1992a,b) and the human gene (2.6 kb) (Hill et al., 1995) have been published. The cDNA sequence permitted the search for the cellular source of the peptide. Guanylin mRNA has been found in human and rat intestine, with levels increasing from duodenum to colon (Wiegand et al., 1992a,b). Anti-proguanylin antibodies have revealed the presence of guanylin-like immunoreactivity in goblet cells in the rat duodenum, jejunum, ileum and the superficial epithelium of rat colon (Cohen et al., 1995; Li et al., 1995). Other antibodies raised against the N-terminal and C-terminals of the guanylin prohormone and tested on guinea pig intestine have demonstrated the expression of guanylin in enterochromaffin cells exclusively (Cetin et al., 1994; Kämpf et al., 1996).

The secretory effect of guanylin has been demonstrated in in vitro studies using Ussing chambers at the colonic level. In T84 cells, a cell line obtained from a colonic human cancer (Forte et al., 1993), as well as in tissues prepared from rat

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proximal colon (Wiegand et al., 1992a) or from mouse distal colon (Cuthbert et al., 1994), the secretory effect was related to activation of the cystic fibrosis transmembrane conductance regulator Cl^- channel. In human tissues, the response was additive from 1 nM to 1 μM guanylin, and greater in colon than in jejunum (Kühn et al., 1994). These investigations of guanylin activity were performed in vitro in the colon. The purpose of the present study was to describe the effect of several doses of guanylin on net water and ion fluxes, at four different levels of the rat intestine, in vivo. Use of radiolabeled Na^+ and Cl^- allowed us to thoroughly explore the activities of guanylin by calculating unidirectional fluxes. The experiments were conducted in parallel with studies on the secretory effect of STa. The characteristics of the water and ion movements induced by heat-stable enterotoxin were compared with those induced by vasoactive intestinal peptide, which acts through a cyclic adenosine monophosphate (cAMP)-dependent pathway.

2. Materials and methods

2.1. Water and ion transport

Experiments were conducted in agreement with the European Union decree for animal experiments (L358, 12-18-1986, user numbers 187 and 191).

The technique of closed loops in situ has been previously described (Hervieu et al., 1996). Briefly, about 160 male Sprague-Dawley rats, weighing 200–220 g, were fasted 48 h with free access to water. They were anesthetized with i.p. administration of pentobarbital (4 mg/100 g body weight), and a catheter was inserted into the jugular vein. All rats were perfused intravenously, via the jugular vein, with 0.9% NaCl solution at a rate of 3 ml/h for 30 min. Then, the abdomen was opened by a midline incision and ligated loops were prepared in each rat: duodenum and proximal jejunum (about 10 cm long) or distal ileum (about 10 cm long) and ascending colon (about 5 cm long from the caecum). The hepatobiliary canal was tied off. One milliliter of the test solution was instilled in all loops with a calibrated syringe, and an additional ligature was placed on the injection site. The loops were replaced in the abdomen, and the abdominal wall was sutured. The test solution contained 70 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 10 mM $NaHCO_3$, 136 mM mannitol, 5 g/l PEG 4000 (polyethylene glycol) with 1 kBq/ml [^{3}H]PEG 4000 (NEN, France) as an unabsorbable marker, and 0.17 kBq/ml ^{22}Na (Amersham, Les Ulis, France) for evaluation of unidirectional fluxes of Na^+, pH 8. The bolus injection of ^{36}Cl (16.8 kBq, New England Nuclear, Les Ulis, France) in the jugular vein permitted the evaluation of unidirectional Cl^- fluxes.

Guanylin and heat-stable enterotoxin were administered by the luminal route: the test solution contained either heat-stable *E. coli* enterotoxin (Sigma, St-Quentin-Fallavier, France) or rat guanylin (Bachem Biochimie, Voisins-Le-Bretonneux, France). The doses used were 5 units/ml and 10 units/ml (corresponding approximately to 0.025 nmol/ml and 0.05 nmol/ml) for heat-stable enterotoxin studies, and 0.125 nmol/ml, 0.5 nmol/ml or 2 nmol/ml for guanylin studies.

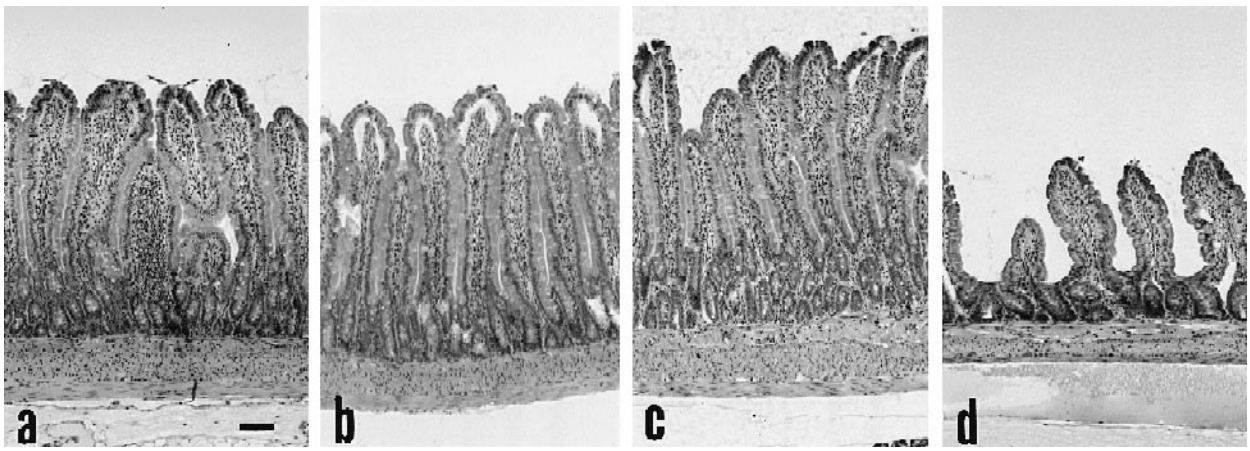

Fig. 1. Aspect of the duodenal wall after preparation of the loop and a 15-min incubation in the presence of 1 ml of test solution. Bouin's liquid fixation, paraffin embedding, sections parallel to the longitudinal axis of the villi. Hematoxylin, eosin, saffron. Bar = 100 μm. (a) Control loop. The general structure is maintained. The epithelium is continuous, thin lacunae underlie the cells at the apex of the villi. (b) Loop incubated in the presence of 2 nmol/ml guanylin. The structure and height of the wall are maintained. The epithelial layer is continuous, rare desquamated cells can be seen in the lumen. Large lacunae underlie the epithelium. (c) Loop incubated in the presence of 5 units/ml heat-stable enterotoxin of *E. coli* (STa). The height of the wall is maintained. Rare desquamated cells. (d) Loop incubated in the presence of 10 units/ml STa. The decrease in the height of the wall is secondary to the erosion of the villus tips and to the distension induced by the secreted water.

Vasoactive intestinal peptide was administered intravenously: rats received, via the jugular vein, 1.2 nmol/100 g body weight · h vasoactive intestinal peptide (Sigma) in NaCl solution, for 30 min.

The content of all loops was collected after an incubation with the test solution of 15 min for duodenal and jejunal loops, and 30 min for ileal and colonic loops. Blood was taken from the vena cava. The pH of the loop content was measured. Na^+ and K^+ concentrations were determined by flame spectrophotometry, Cl^- concentration by coulometric titration and HCO_3^- concentration by back-titration of an excess of 0.1 M HCl. ^{36}Cl, ^{22}Na and ^{3}H were assayed by liquid scintillimetry (Tricarb 1600 CA, Packard), and by direct γ counting (Cobra Auto-Gamma 5005, Packard) for ^{22}Na to determine the contribution of β emission, by making use of the correlation between γ and β discharges of the same sample.

The percentage of [^{3}H]PEG 4000 recovery was determined. When it was lower than 80%, the sample was discarded. The maintenance of the K^+ concentration at 5 mM in the small intestine, and at 8 mM in the proximal colon, indicated the

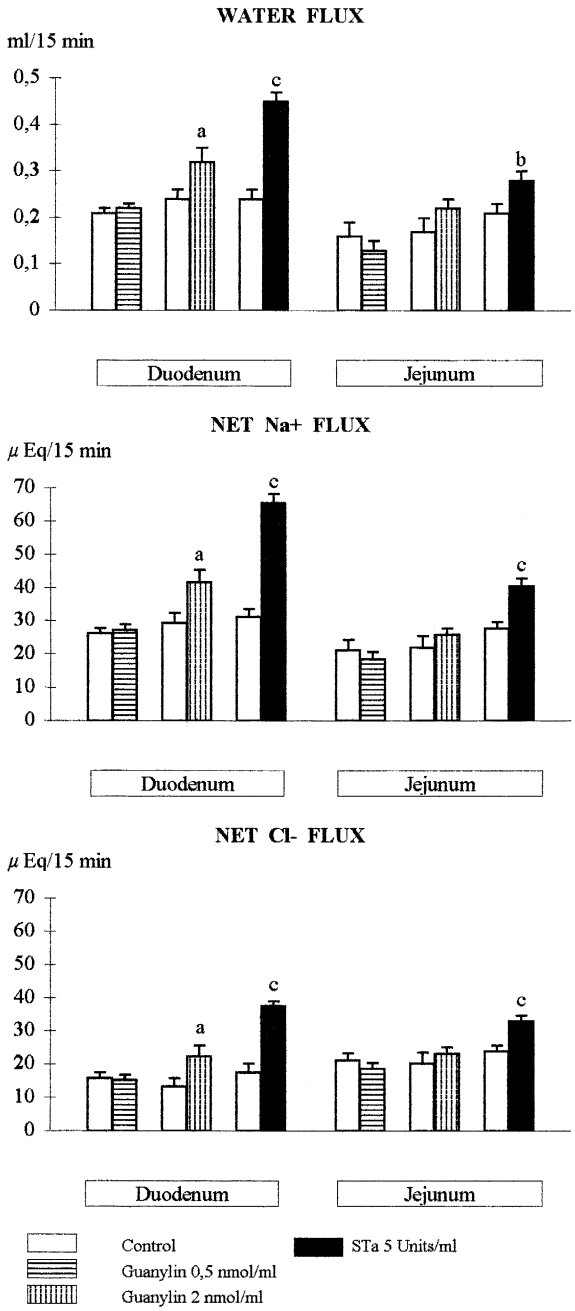

Fig. 2. Net water, Na^+ and Cl^- fluxes after an incubation of 15 min in control loops ($n = 8–23$), or in loops instilled with heat-stable enterotoxin of *E. coli* (STa) or guanylin ($n = 12–27$) in duodenum and proximal jejunum. Each column represents the mean and S.E.M. The letters indicate the statistical difference between control and treated rats ($^a P < 0.05$; $^b P < 0.01$; $^c P < 0.001$).

absence of K^+ extrusion from epithelial dead cells. The net water flux was measured gravimetrically as the weight difference between a full loop and an empty loop, and the ionic net flux from the changes in luminal concentration and the water volume (described in Appendix A). Na^+ influx and Cl^- efflux were calculated using the kinetic model of Berger and Steele (1958). In this system, the amount of material transported was calculated using measurements of specific activities in the lumen and blood (see Appendix A). Na^+ efflux was calculated as the difference between net Na^+ flux and Na^+ influx, and Cl^- influx was calculated as the difference between net Cl^- flux and Cl^- efflux. Negative values of net flux indicate net absorption and positive values indicate net secretion. The influxes represent the lumen-to-blood fluxes and are negative; the effluxes represent the blood-to-lumen fluxes and are positive. Water flux is expressed as ml/15 min for duodenum and jejunum and as ml/30 min for ileum and colon. Na^+ and Cl^- fluxes are expressed as μEq/15 min or as μEq/30 min. Statistical analysis of differences between control ($n = 6–30$) and treated rats ($n = 11–27$) was performed with Student's *t*-test for non-paired values.

2.2. Histological procedures

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, phloxin, and saffron. Sections were observed under light microscopy and photographed.

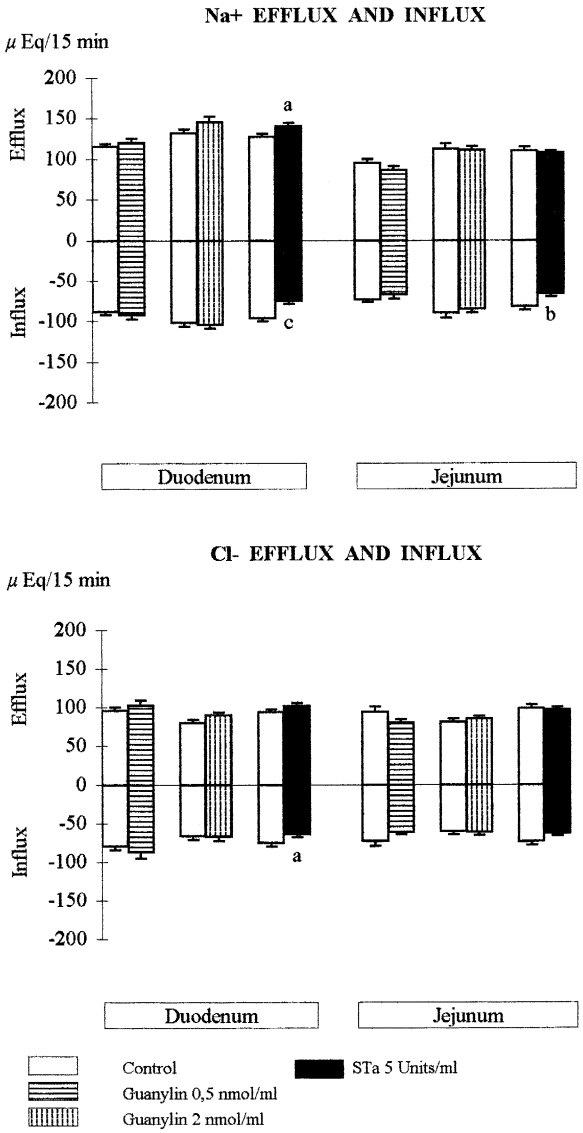

Fig. 3. Unidirectional Na^+ and Cl^- fluxes after an incubation of 15 min in control loops ($n = 8–23$) or in loops instilled with heat-stable enterotoxin of *E. coli* (STa) or guanylin ($n = 12–27$) in duodenum and proximal jejunum. Each column represents the mean and S.E.M. The letters indicate the statistical difference between control and treated rats ($^a P < 0.05$; $^b P < 0.01$; $^c P < 0.001$).

3. Results

3.1. Histological observation

Light microscopy provided no evidence of distension, desquamation or rupture of the epithelial continuity in control loops at any level of the intestine. In the presence of guanylin, at each dose from 0.125 nmol/ml to 2 nmol/ml, the tissue appeared intact and in a state of secretion with the presence of lacunae in the lamina propria underlying the villus epithelium. Instillation of 5 units/ml heat-stable enterotoxin caused no modification of the height of the wall, but some desquamation began to appear. The concentration of 10 units/ml heat-stable enterotoxin induced lesions in the upper intestine: distension of the tissue, decrease in the thickness of the wall, appearance of desquamated cells and hemorrhagic suffusion (Fig. 1). For this reason, the dose of 5 units/ml was chosen for heat-stable enterotoxin studies in duodenum and jejunum. The 10 units/ml dose of the enterotoxin did not damage the ileum or colon and was used in these two segments.

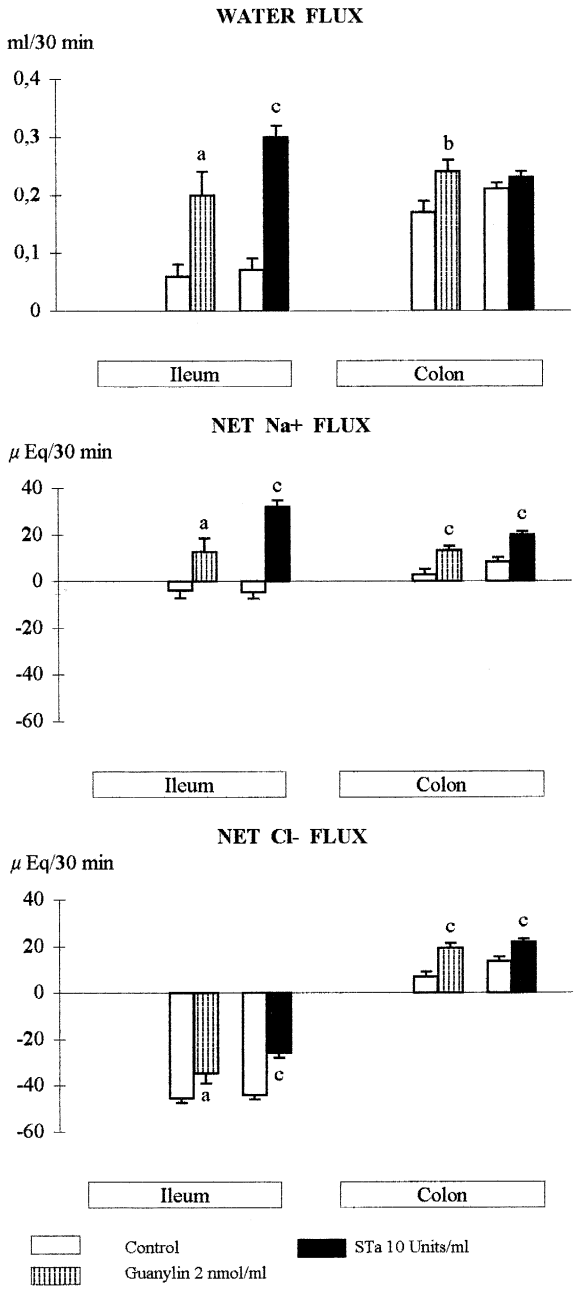

Fig. 4. Net water, Na^+ and Cl^- fluxes after an incubation of 30 min in control loops ($n = 10–30$) or in loops instilled with heat-stable enterotoxin of *E. coli* (STa) or guanylin ($n = 11–24$) in distal ileum and proximal colon. Each column represents the mean and S.E.M. The letters indicate the statistical difference between control and treated rats ($^a P < 0.05$; $^b P < 0.01$; $^c P < 0.001$).

3.2. Guanylin studies

At all levels of the intestine, the concentrations of 0.125 (data not shown) and 0.5 nmol/ml (data not shown for ileum and colon) of guanylin did not significantly change any net and unidirectional fluxes. The concentration of 2 nmol/ml increased ion and water secretion in the duodenum, distal ileum and ascending colon, but not in the proximal jejunum in comparison with that of corresponding control rats.

In the duodenum, guanylin induced a significant enhancement of water, Na^+ and Cl^- secretion. The increase in Na^+ and Cl^- secretions was equivalent ($+12.3 \pm 6.9$ and $+9.1 \pm 5.9$ μEq/15 min, respectively) (Fig. 2). With regard to unidirectional fluxes, guanylin induced no significant increase in Na^+ and Cl^- blood-to-lumen fluxes (Fig. 3).

At the ileal level, guanylin generated an increase in the net secretion of water, induced Na^+ secretion and decreased Cl^- absorption (Fig. 4). The apparent net secretion of Na^+ amounted to the sum of a non-significant enhancement of efflux ($P > 0.05$) and a non-significant decrease in influx ($P > 0.05$). The decline in net Cl^- absorption was attributable to an increase in blood-to-lumen Cl^- flux (Fig. 5).

In the colon, guanylin application resulted in an enhanced net secretion of water, Na^+ and Cl^- (Fig. 4). Na^+ secretion was enhanced via a decrease in influx (Fig. 5). The increase in net Cl^- flux was related to a decline in influx (Fig. 5).

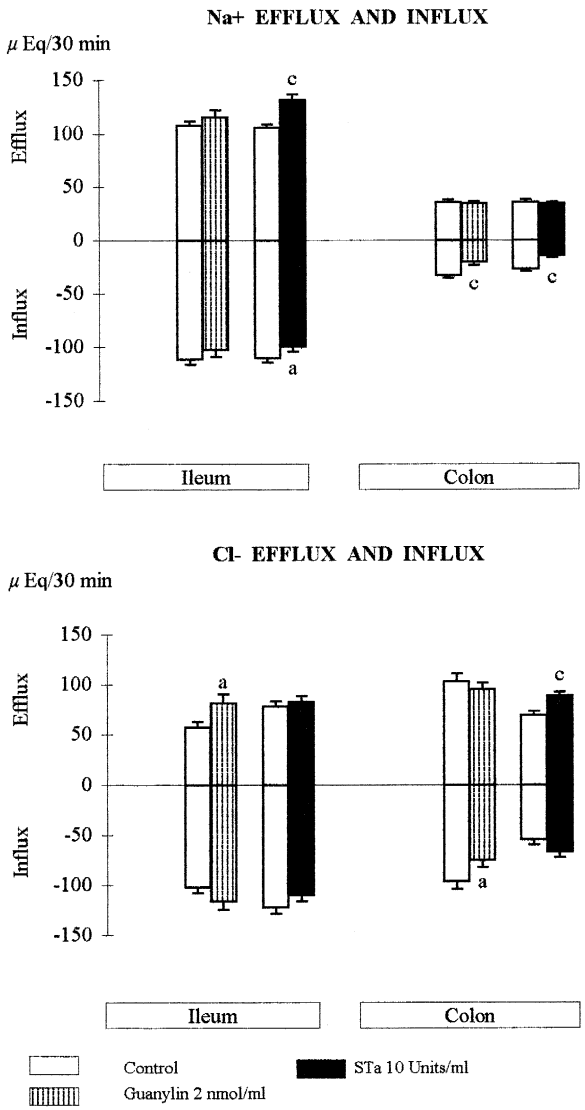

Fig. 5. Unidirectional Na^+ and Cl^- fluxes after an incubation of 30 min in control loops ($n = 10–30$) or in loops instilled with heat-stable enterotoxin of *E. coli* (STa) or guanylin ($n = 11–24$) in distal ileum and proximal colon. Each column represents the mean + or − S.E.M. The letters indicate the statistical difference between control and treated rats ($^a P < 0.05$; $^c P < 0.001$).

Table 1
Secretion induced by vasoactive intestinal peptide (VIP) in duodenum, proximal jejunum, distal ileum and proximal colon

Incubation time with the test solution and n	Variations in intestinal water and ion movements induced by intravenous perfusion of 1.2 nmol/100 g·h VIP for 30 min			
	Duodenum	Jejunum	Ileum	Colon
	15 min ($n = 13$)	15 min ($n = 13$–14)	30 min ($n = 15$–19)	30 min ($n = 6$–19)
Water (ml)	+0.46 [c]	+0.21 [c]	+0.34 [c]	+0.14 [c]
Net Na^+ flux (μEq)	+75 [c]	+36 [c]	+57 [c]	+26 [c]
Na^+ influx (μEq)	−63 [c]	−33 [c]	−29 [c]	−16 [c]
Na^+ efflux (μEq)	+12	+3	+28 [c]	+10
Net Cl^- flux (μEq)	+55 [c]	+30 [c]	−36 [c] (↓ absorption)	+20 [c]
Cl^- influx (μEq)	−41 [c]	−16	−37 [c]	+5
Cl^- efflux (μEq)	+14	+14	−1	+25 [b]
Net HCO_3^- flux (μEq)	+23 [c]	−4 [b] (↓ absorption)	+22 [c]	−5 [a] (↓ absorption)

Values represent the difference (increase (+) or decrease (−)) between treated animals and corresponding controls. The letters indicate the statistical difference between control and treated rats ([a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$).

3.3. Heat-stable enterotoxin studies

Heat-stable enterotoxin provoked a larger net secretion of water and ions throughout the intestine than did guanylin.

An increase in the net secretion of water, Na^+ and Cl^- was observed in the duodenum and even in the jejunum, contrary to the guanylin study (Fig. 2), accompanied by an increase in HCO_3^- secretion in the duodenum ($+11 \pm 2$ μEq/15 min; $P < 0.001$). These increases were larger at the duodenal level. The increase in Na^+ secretion was related to a reduction in Na^+ influx in both segments, associated with an increase in Na^+ efflux in the duodenum, but not in the jejunum (Fig. 3). With regard to unidirectional Cl^- fluxes, the enterotoxin induced a decrease in Cl^- influx which was significant at the duodenal level (Fig. 3).

In the ileum, heat-stable enterotoxin activity resulted in a larger increase than observed with guanylin in the net secretion of water and Na^+ particularly and in a decrease in Cl^- absorption (Fig. 4). As in the duodenum, an enhancement of HCO_3^- secretion was observed ($+13.5 \pm 4.1$ μEq/30 min; $P < 0.001$). The secretion of Na^+ was related to a combination of an increase in Na^+ efflux and a more moderate decrease in Na^+ influx (Fig. 5). The decrease in net Cl^- absorption was induced by the sum of a non-significant increase in efflux ($P > 0.05$) and a non-significant decrease in influx ($P > 0.05$) (Fig. 5).

In the ascending colon, Na^+ and Cl^- secretions were stimulated, as observed with guanylin, but the water flux was not significantly altered, in contrast to the secretion induced by guanylin (Fig. 4). The enterotoxin had less effect in the colon than in the ileum. Analysis of the unidirectional flux rates (Fig. 5) indicated that the major changes were a decrease in Na^+ influx, as with guanylin, and a rise in blood-to-lumen Cl^- flux, unlike guanylin.

3.4. Vasoactive intestinal peptide studies (Table 1)

Vasoactive intestinal peptide induced a strong secretion of water and ions, at all levels of the intestine. In the duodenum, vasoactive intestinal peptide increased the secretion of water, Na^+, Cl^- and HCO_3^-, and decreased Na^+ and Cl^- influxes. In the proximal jejunum, vasoactive intestinal peptide induced a secretion of net water, Na^+ and Cl^- and a decrease in Na^+ influx. In the distal ileum, vasoactive intestinal peptide induced a secretion of water and Na^+, abolished the net absorption of Cl^- and increased the secretion of HCO_3^-. The peptide increased Na^+ efflux and reduced Na^+ and Cl^- influxes. In the ascending colon, vasoactive intestinal peptide increased water, Na^+ and Cl^- secretion, reduced lumen-to-blood Na^+ flux and increased blood-to-lumen Cl^- flux.

4. Discussion

The present investigation shows for the first time that guanylin stimulates water and ion secretion in the duodenum, distal ileum and ascending colon of the rat, in vivo. The purpose of the study was to compare this secretory response with that of heat-stable enterotoxin of *E. coli*, and to compare the qualitative effect on unidirectional fluxes of enterotoxin, which acts via cGMP, with that of vasoactive intestinal peptide, which acts via cAMP, using the same ligated loop model.

4.1. Validation of the method

The closed loop method gives a direct measurement of net fluxes. In previous studies, the disappearance of ^{22}Na from the lumen and the appearance of ^{36}Cl in the lumen were used directly to evaluate unidirectional fluxes. However, these estimations overlooked the fact that the system was not in a steady state. In the present study, the calculation was performed according to the equations of Berger and Steele (1958) (see Appendix A), which give a better quantitative approach to influxes and effluxes than other calculations. The decrease in ^{22}Na luminal specific activity, and the effectiveness of Cl^- absorption and ^{36}Cl reabsorption are taken into account in the equations of Berger and Steele. The calculated Na^+ and Cl^- influxes and effluxes were particularly increased in the small intestine. In the proximal colon, the low permeability decreases passive diffusion and thus the disappearance of labelled molecules from the luminal colonic compartment. Inspection of the Na^+ lumen-to-blood flux showed that the speed of Na^+ disappearance decreased throughout the intestine, in agreement with the aboral decline in intestinal permeability. The estimates were 6.5 μEq/min in the duodenum, 5.6 μEq/min at the proximal jejunal level, 3.4 μEq/min in the distal ileum and 1.0 μEq/min in the ascending colon.

This ligated loop in vivo model, in which radioactivity is used to evaluate unidirectional fluxes, does not permit one to identify the transporters involved in the action of the peptides: the calculated influxes and effluxes represent the sum of all processes occurring via the cellular and the paracellular pathway. However, this method gives information about the effect of the peptides on Na^+ and Cl^- lumen-to-blood or blood-to-lumen fluxes, which might be stimulated and/or inhibited, suggesting a possible participation of a transporter.

Guanylin and heat-stable enterotoxin were applied intraluminally. The common receptor, guanylyl cyclase-C, of heat-stable enterotoxin, guanylin and uroguanylin is found in purified jejunal brush-border membranes (Guarino et al., 1987) and appears to be specifically localized in all enterocytes in every segment of the gut, as determined by immunohistochemistry (Kämpf et al., 1996). In vitro studies with Ussing chambers demonstrated the secretory response only when guanylin was applied to the mucosal site (Forte et al., 1993; Cuthbert et al., 1994). Endogenous guanylin was found to be released into the apical bath solution of isolated human intestinal mucosa mounted in Ussing chambers (Kühn et al., 1996) and into the lumen of the isolated perfused ileum in the rat: guanylin output increased from a basal value of 0.08 pmol/min to 0.6 pmol/min after cholinergic stimulation (Moro et al., 1996).

The action of the heat-stable enterotoxin of *E. coli* is rapid: cGMP concentration and short-circuit current (I_{sc}) are maximally increased in the rabbit ileum mounted in Ussing chambers within 5 min of exposure to heat-stable enterotoxin (Field et al., 1978). So the incubation time was chosen to be short enough to maintain some radioactivity in the lumen.

4.2. Effect of guanylin on water and ion transport

Guanylin, at the dose of 2 nmol/ml, (1) produced an increase in water, Na^+ and Cl^- secretion in the duodenum after an incubation of 15 min, (2) did not modify the exchanges in the jejunum, (3) induced water and Na^+ secretion and decreased Cl^- absorption by increasing Cl^- efflux in the ileum, and (4) increased water, Na^+ and Cl^- secretion in the colon, by decreasing Na^+ and Cl^- influxes. In vitro studies have demonstrated a secretory effect in the colon when guanylin was added to the mucosal side of Ussing chambers at a concentration of 1 μM (Wiegand et al., 1992b; Kühn et al., 1994). Specific activation of the cystic fibrosis transmembrane conductance regulator Cl^- channel was demonstrated by patch-clamp measurements and by the release of ^{125}I from preloaded T84 cells (Chao et al., 1994). The absence of a secretory effect in colonic epithelium from transgenic cystic fibrosis mice (Cuthbert et al., 1994) confirmed the cystic fibrosis transmembrane conductance regulator Cl^- channel as a specific target of guanylin. The present study demonstrated that guanylin produced approximately the same magnitude of water and ion secretion in the duodenum, distal ileum and ascending colon; this secretion was reached after an incubation time of 15 min at the duodenal level, and 30 min at ileal and colonic levels. No secretory response was observed in the jejunum. The difference might be related to a difference in the number and affinity of the receptor (Krause et al., 1994), or to a specific functional response of each segment.

4.3. Heat-stable enterotoxin study

In comparison with guanylin, whose doses were respectively 80 times and 40 times higher, the enterotoxin induced a water and ion secretion which was two times greater in the duodenum and ileum, and equivalent in the ascending colon. Our investigations illustrated that guanylin was a less potent stimulant of water and ion secretion than heat-stable enterotoxin of *E. coli* (confirming prior in vitro colonic researches), because it has been shown that these two peptides possess a similar affinity for the receptor guanylyl cyclase-C (Hamra et al., 1993).

A greater secretion permitted a better analysis of the functional response at each level: heat-stable enterotoxin induced: (1) in the duodenum, a stimulation of Na^+ blood-to-lumen flux and of HCO_3^- secretion, and an inhibition of Na^+ and Cl^- influxes; (2) in the jejunum, an inhibition of Na^+ influx; (3) in the distal ileum, a stimulation of Na^+ efflux and of HCO_3^-

secretion with an inhibition of Na^+ influx; and (4) in the ascending colon, a stimulation of Cl^- efflux, and an inhibition of Na^+ influx. The effect of heat-stable enterotoxin on unidirectional fluxes was described earlier in vivo and in vitro. For unidirectional Cl^- fluxes, in the rabbit ileum, the increase in I_{sc} was found to be associated with an increase in Cl^- efflux, and a decrease in Cl^- influx (Field et al., 1978). The activation of a Cl^- channel was then confirmed in rabbit ileum (Guandalini et al., 1982), and on T84 colonic cell line (Huott et al., 1988), before it was demonstrated to involve the specific cystic fibrosis transmembrane conductance regulator Cl^- channel (Chao et al., 1994). For unidirectional Na^+ fluxes, a decline in Na^+ influx was observed in vivo in porcine ligated jejunum (Hamilton et al., 1977), and in vitro in ascending colon of pigs and rats (Argenzio and Whipp, 1981; Nobles et al., 1991), confirming our measurements of unidirectional fluxes.

Our analysis of unidirectional fluxes provides indications about the targets of heat-stable enterotoxin of *E. coli*. The decrease in Na^+ influx at each level is in favor of an inhibition of the Na^+/H^- exchanger (De Jonge, 1984), a regulator of the intracellular pH and participant in Na^+ absorption. The Na^+/H^+ exchanger is present in the jejunum, ileum and colon of the rat intestine (Bookstein et al., 1994). In our model, dimethyl-amiloride, an inhibitor of this exchanger, produced an inhibition of Na^+ influx in all segments of the intestine (unpublished results). The decrease in Cl^- influx in duodenum and ileum argues in favor of an inhibition of Cl^-/HCO_3^- exchange, which acts in parallel with Na^+/H^+ exchange to permit electroneutral NaCl transport. However, Na^+/HCO_3^- cotransport might participate in HCO_3^- secretion in both segments, whereas the Cl^- channel is activated in the ascending colon. The evaluation of unidirectional fluxes favors the model of maximal secretion elicited by the combination of activation of secretory processes and inhibition of absorptive processes.

4.4. Comparison of intestinal secretion induced by vasoactive intestinal peptide and heat-stable enterotoxin of E. coli

We previously showed, using the same experimental model, that vasoactive intestinal peptide, perfused at the dose of 1.2 nmol/100 g · h into the jugular vein, produced water and ion secretion into the lumen of the duodenum, jejunum and ileum (Chikh Issa et al., 1992; Marquet et al., 1994; Grishina et al., 1995). In the present study, we confirmed that vasoactive intestinal peptide, perfused at the same dose for 30 min, induced water and ion secretion throughout the intestine. Heat-stable enterotoxin does not increase cAMP level and the heat-stable enterotoxin-mediated effect resembles that of cAMP-mediated mechanisms (Guandalini et al., 1982; Huott et al., 1988). In T84 cells, heat-stable enterotoxin induces Cl^- secretion, by activating the cAMP-dependent protein kinase A by an increase in cGMP (Forte et al., 1992; Chao et al., 1994). Thus, the effects of heat-stable enterotoxin on intestinal secretion might occur via cAMP- and cGMP-dependent protein kinases (i.e., cNuc protein kinases). We have demonstrated that heat-stable enterotoxin, which acts through cGMP, and vasoactive intestinal peptide, which acts through cAMP, have the same target not only in the colon, but also in the duodenum, proximal jejunum and distal ileum.

4.5. Conclusion

The present research illustrates that guanylin induced secretion in the rat intestine in vivo, not only in the colon, confirming in vitro studies, but also in the duodenum and ileum. The water and ion secretion mediated by vasoactive intestinal peptide, via cAMP, and by heat-stable enterotoxin of *E. coli*, via cGMP, was large and led to a similar qualitative action on net and unidirectional fluxes at each segment of the intestine: an inhibition of Na^+ influx, and an increase in anion secretion. The secretory effect of guanylin was minor in comparison to that induced by the heat-stable enterotoxin. Moreover, guanylin may be a part of a system controlling the epithelial electrolyte balance at the intestinal and renal level. Guanylyl cyclase-C receptor has been found in the proximal tubule (Krause et al., 1990) and guanylin in the rat kidney (Currie et al., 1992). Uroguanylin, a peptide which has 53% homology with guanylin and which has been isolated from urine (Hamra et al., 1993), is also found in opossum colonic mucosa (Hamra et al., 1996) and its mRNA has been detected throughout the human intestine (Hill et al., 1996). Uroguanylin elicits a cGMP-dependent secretory response in T84 cells (Hamra et al., 1993) and natriuresis in the perfused kidney (Forte and Hamra, 1996). We have demonstrated, in the same experimental model, that atrial natriuretic factor (ANF), a cGMP-dependent peptide which is diuretic and natriuretic, elicits water, Na^+ and Cl^- secretion in the duodenum and colon (Hervieu et al., 1996). These similarities suggest that guanylin and/or uroguanylin may be involved, like atrial natriuretic factor, in the regulation of fluid and electrolyte homeostasis by acting on the intestine and the kidney.

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Appendix A

● **Net ionic fluxes**

Net Na^+ flux (μEq/15 or 30 min) = $([Na^+]_f \times V_f) - ([Na^+]_i \times V_i)$.

Net Cl^- flux (μEq/15 or 30 min) = $([Cl^-]_f \times V_f) - ([Cl^-]_i \times V_i)$.

V_i: initial luminal volume (1 ml); V_f: final luminal volume.

$[Na^+]_{i,f}$ and $[Cl^-]_{i,f}$: initial, final luminal Na^+ and Cl^- concentration (μEq/ml).

● **Specific activities**

$$\text{Specific activity (SA) of } {}^{22}\text{Na or } {}^{36}\text{Cl in luminal fluid} = \frac{{}^{22}\text{Na or } {}^{36}\text{Cl (cpm/ml)}}{\text{Na}^+ \text{ or Cl}^- \text{ (}\mu\text{Eq/ml)}}$$

$$\text{Specific activity (SA) of } {}^{36}\text{Cl in plasma} = \frac{{}^{36}\text{Cl (cpm/ml)}}{\text{Cl}^- \text{ (}\mu\text{Eq/ml)}}$$

● **Unidirectional ionic fluxes**

$$Na^+ \text{ influx} = \text{net } Na^+ \text{ flux} \times \left[\frac{\ln\left[\left(\text{luminal SA}_f \text{ of } Na^+\right) \div \left(\text{luminal SA}_i \text{ of } Na^+\right)\right]}{-\ln\left([Na^+]_f \times V_f\right) \div \left([Na^+]_i \times V_i\right)} - 1 \right]$$

$$Na^+ \text{ efflux} = \text{net } Na^+ \text{ flux} - Na^+ \text{ influx}$$

$$Cl^- \text{ efflux} = \text{net } Cl^- \text{ flux} \times \left[\frac{\ln\left[\left(\text{luminal SA}_f \text{ of } Cl^- - \text{plasmatic SA}_f \text{ of } Cl^-\right) \div \left(-\text{plasmatic SA}_i \text{ of } Cl^-\right)\right]}{-\ln\left([Cl^-]_f \times V_f\right) \div \left([Cl^-]_i \times V_i\right)} \right]$$

$$Cl^- \text{ influx} = \text{net } Cl^- \text{ flux} - Cl^- \text{ efflux}$$

SA_i: initial specific activity; SA_f: final specific activity.

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