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Fluid Secretory Responses to Enterotoxin STa and 8-bromo-cyclic GMP in Fed and Nutrionally-Deprived Gerbils: Jejunum, Ileum and Colon *in vivo*

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Summary

Fluid transport was measured gravimetrically *in vivo* in the jejunum, ileum and colon of fed, fasting (four days) and undernourished (50 % of control food intake for 21 days) gerbils (*Gerbillus cheesmani*). The effects of luminal enterotoxin *Escherichia coli* STa (50 ng/ml) and luminal 8-bromo-cyclic GMP (cGMP 1 mM) on fluid transport across jejunum, ileum and colon were also assessed. Fasting and undernourishment reversed the normal basal fluid absorption measured in fed ileum and colon into secretion. Neither fasting nor undernourishment had any effect on jejunal basal fluid absorption. In jejuna, ilea and colons of fed animals as well as in jejuna from fasting and undernourished gerbils STa (50 ng) reversed the normal absorptive "tone" to secretion but it had no significant effects on fluid secretion in either the ileum or colon from fasted gerbils. STa increased significantly the fluid secretion in ileum from undernourished gerbils. Luminal cGMP had no effect on basal absorptive tone in the jejunum of fed and fasted gerbils, but reversed absorption into secretion in the jejuna from undernourished gerbils. In the ilea taken from fed animals the small basal absorption was reversed to secretion by luminal cGMP. Although cGMP produced no significant changes in fluid secretion in the ilea taken from fasted gerbils, yet it caused a significant increase in those from undernourished gerbils. In the colon taken from fed animals cGMP decreased the basal fluid absorption significantly, but it had no significant effect on fluid secretion in the colon of fasted or undernourished gerbils. We conclude that fasting and undernourishment have no significant effects on fluid transport across the gerbil jejunum but reversed basal absorption in the fed ileum and colon into secretion. cGMP mimic the effects of STa in the jejunum taken from undernourished gerbils, in the ileum obtained under the three nutritional states and in the colon taken from fasting animals.

Key words
Gerbil • Ileum • Jejunum • Starvation • Undernourishment

Introduction

Fasting and malnutrition increase intestinal secretion of ions and fluid both under basal conditions and in response to secretory agonists. Nzegwu and Levin (1994 a,b) suggested that the marked effects of fasting on intestinal epithelial function partially depend on regulation of the intestinal epithelium by the enteric nervous system. Hayden and Carey (2000) also concluded that fasting enhanced enteric neural control of

basal ion transport and increased paracellular permeability in piglet jejunum. In rats, the basal fluid movement in the small intestine measured *in vivo* showed strong similarities with the electrogenic secretion obtained *in vitro* (Young and Levin 1990a,b). Al-Balool (2002) reported that starvation increased the basal short circuit current (Isc) across the proximal and middle colon, but had no effect on that across the distal colon. In their review on intestinal transport during fasting and malnutrition, Ferraris and Carey (2000) reported that fasting and malnutrition had been shown to induce a shift in basal ion transport in the small intestine from a neutral state or net absorptive flux to a more secretory state.

The enterotoxin *E. coli* STa induces fluid secretion in rat small (Nzegwu and Levin 1994c, Mourad and Nassar 2000) and large intestine (Nzegwu and Levin 1996). STa binds to a guanylin receptor and causes an elevation in cGMP (Huott *et al*. 1988, Cohen *et al*. 1993). In the present study the effects of fasting and undernourishment on fluid transport across the gerbil large intestine were investigated to find whether the fluid movement in large intestine of gerbil match the electrogenic secretion responses, as in the rat. In addition, we also tried to characterize the fluid absorptive and secretory behavior of the jejunum, ileum and colon in fasted and undernourished gerbil *in vivo* under basal conditions and after stimulation with the bacterial toxin *E. coli* STa and cyclic nucleotide GMP.

Methods

Animals and diet

Gerbils (*Gerbillus cheesmani*) of both sexes, body weight 36-40 g, were captured from the desert and kept in an animal house for at least three weeks before use. Three nutritional groups were used. The fed group gerbils had free access to water and food (SDS Rodent maintenance diet, Essex, England) and were held in rooms maintained at 27±2 °C. The lights were on from 05:00 h until 17:00 h at 50 % humidity. In the fasted group, water was given *ad libitum*, but food was removed for 4 days before the experiment. A chronically undernourished group was housed in individual cages and was fed 50 % of the control food intake for 21 days. The animals were housed routinely in plastic cages with wire-mesh floors to reduce coprophagy.

Chemicals

All the chemicals were obtained from Sigma.

Methods

Fluid movements were measured in anesthetized gerbils (thiopentone sodium (30 mg/kg body weight, i.p) *in vivo* using the gravimetric technique as described previously by Young and Levin (1992). Approximately 0.25-0.5 ml of 0.9 % NaCl was instilled into the ligated loops (6-7 cm length) from a weighed, fluid filled syringe (A g). The syringe was then reweighed (B g) and the weight of fluid instilled, just sufficient to distend the loop, was calculated by difference the A-B g. Fluid movement into and out of the loops was left for 30 min. At the end of the period the loop was excised, blotted free of excess fluid and weighed (C g). It was then cut, opened, drained, blotted and reweighed (D g). The amount of fluid recovered was obtained by subtraction (C-D g). The net movement of fluid into or from the loop was calculated as [(C-D)-(A-B)] g. If absorption had taken place, there was a net loss of fluid from the loop and the sign of fluid movement is given as negative, but if secretion had occurred there was a net gain of fluid into the loop and the sign of the fluid movement is given as positive

The experiments to assess the effects of luminal STa or cGMP were conducted by instilling 0.25-0.5 ml of 0.9 % NaCl containing either STa (50 ng/ml) or 1 mM cGMP into the lumen of the closed loop for 30 min.

The results are expressed as mg/cm/30 min, and are given as the mean ± S.E.M. Statistical significance was assessed using Student's unpaired t-test and the data of the different groups were compared by the one-way ANOVA test (SPSS program Windows Version 7). Differences were considered significant when $P<0.05$.

Results

Basal fluid transport measured in jejunum, ileum and colon.

In order to confirm that the increases in electrogenic transport activity in the fasted colon (Al-Balool 2002) were a valid index of fluid secretion, a series of experiments were undertaken to measure fluid movements across the colon. In addition, the basal fluid transport was also studied in the jejuna and ilea taken from fed, fasted and undernourished animals. The effects of starvation and undernourishment on basal fluid transport measured in the jejunum, ileum and colon are shown in Table 1.

Table 1. Basal fluid transport measured in vivo in the jejunum, ileum and colon of fed, fasted and undernourished gerbils.

	Basal fluid transport (mg/cm/30 min)		
	Jejunum	**Ileum**	**Colon**
Fed	–12.7±1.6 [6]	–2.7±1.8 [8][a]	–14.0±0.6 [6][d]
Fasted	–12.4±1.9 [5]	+11.9±0.6 [5][b]	+7.0±0.6 [6][e]
Under-nourished	–11.3±1.6 [4]	+9.1±0.7 [5][c]	+11.3±1.4 [5][f]

Results are given as mean ± S.E.M. with the number of animals used in square brackets. Absorption from the lumen is assigned a minus sign and secretion into the lumen a plus sign. Significant differences: a *vs* b, d *vs* e, d *vs* f < 0.001; a *vs* c <0.01

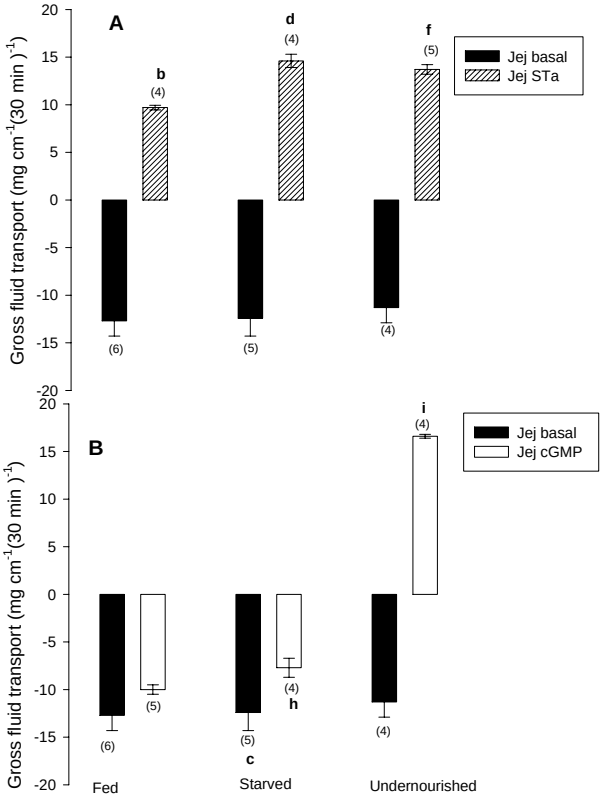

Fig. 1. Effects of luminal STa (A) or 8-bromo-cyclic GMP (B) on fluid transport in jejunum. Gross fluid transport (mg cm^{-1} (30 min) $^{-1}$) measured in the jejunum from fed, fasted and chronically undernourished gerbils. Data are shown as the mean ± S.E.M. with number of animals in brackets. STa (50 ng in 1 ml 0.9 % NaCl); cGMP (1 mM). Positive values represent secretion, whereas negative values indicate absorption. Significant differences: b *vs* d, b *vs* f p<0.001, c *vs* h p<0.05.

Fasting and undernourishment did not change the absorption of fluid in the jejunum significantly. However, fasting dramatically reversed the normal basal absorptive tone in the ileum and colon measured in fed animals into a secretory one. Similarly, the basal absorptive tone of fed animals reversed to secretion in the ileum and colon from undernourished gerbils.

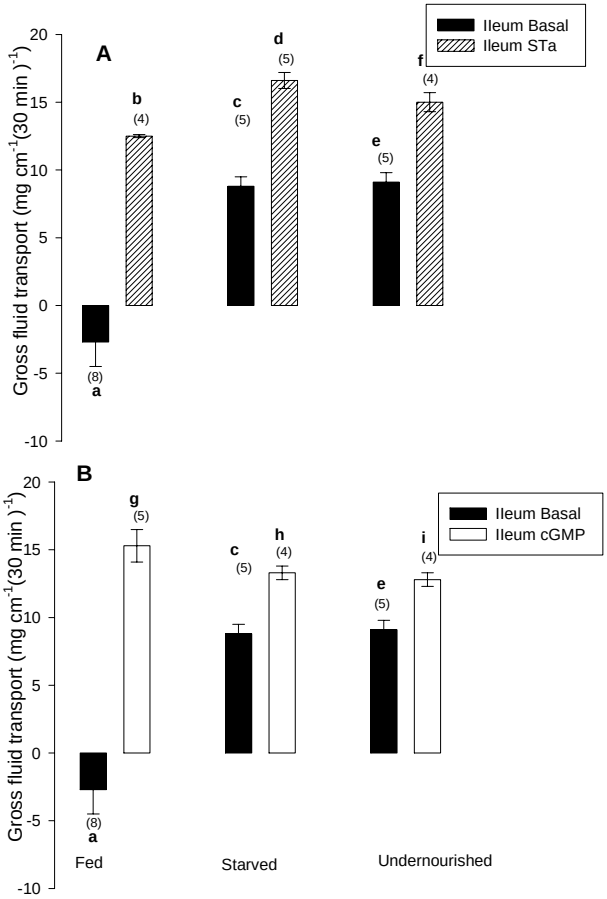

Fig. 2. Effects of luminal STa (A) or 8-bromo-cyclic GMP (B) on fluid transport in the ileum. Gross fluid transport (mg cm^{-1} (30 min) $^{-1}$) measured in the ileum from fed, fasted and chronically undernourished gerbils. Data are shown as the mean ± S.E.M. with number of animals in brackets. STa (50 ng in 1ml 0.9 % NaCl); cGMP (1 mM). Positive values represent secretion, whereas negative values indicate absorption. Significant differences: b *vs* d, e *vs* f p<0.001, b *vs* f, c *vs* h, c *vs* d, e *vs* i, p<0.05.

Effects of enterotoxin STa on fluid transport across jejunum, ileum and colon from fed, fasted and undernourished gerbils.

The effects of STa on fluid transport across jejunum, ileum and colon from fed, fasted and undernourished gerbils are shown in Figs. 1A, 2A and 3A, respectively. In jejuna taken from gerbils with the three feeding conditions (Fig. 1A) STa reversed the normal absorption into secretion and the amount of fluid secreted as a result of the presence of STa in the jejuna taken from fasted and undernourished animals were significantly higher than in the jejuna of fed gerbils. In the ileum (Fig. 2A), the basal fluid absorption in fed

animals was converted to secretion. Fluid secretion in the presence of STa was enhanced in the ilea taken from fasted and undernourished gerbils. Ilea taken from fasted and undernourished gerbils showed a significant increase in fluid secretion in the presence of STa.

In the colon taken from fed gerbils (Fig. 3A) STa produced similar effects as in the jejunum and ileum, i.e. absorption changed into secretion. In the colon taken from fasted animals, STa did not produced any significant change in the fluid from that observed in its absence.

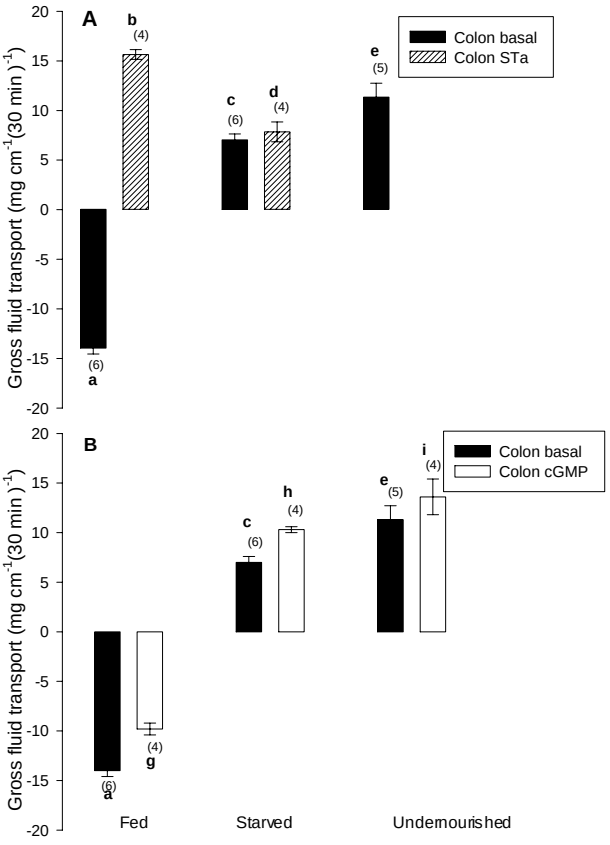

Fig. 3. Effects of luminal STa (A) or 8-bromo-cyclic GMP (B) on fluid transport in the colon. Gross fluid transport (mg cm^{-1} (30 $min)^{-1}$) measured in the jejunum from fed, fasted and chronically undernourished gerbils. Data are shown as the mean ± S.E.M. with number of animals in brackets. STa (50 ng in 1ml 0.9 % NaCl); cGMP (1 mM). Positive values represent secretion, whereas negative values indicate absorption. Significant differences: a *vs* g, $p<0.005$, c *vs* h, $p<0.05$.

Effects of luminal 8-bromo-cyclic GMP on fluid transport across jejunum, ileum and colon in fed, fasted and undernourished gerbils.

One action of the enterotoxin STa is to activate the secretion by raising cGMP concentrations in the enterocytes (Field *et al.* 1978). This induces electrogenic Cl^- secretion and inhibits electroneutral NaCl absorption (Guandalini *et al.* 1982). Luminal instillation of a lipid-soluble derivative of cyclic GMP also induces fluid secretion in the rat (Eklund *et al.* 1986, Nzegwu and Levin 1994c). This secretogogue allows to activate the Sta-dependent secretory mechanisms in the intestine without the presence of STa.

The effects of 1 mM cGMP on fluid transport across jejunum ileum and colon in fed, fasted and undernourished gerbils are shown in Figs. 1B, 2B and 3B. In jejunum of fed animals, cGMP produces no significant changes but in the jejunum taken from fasted gerbils, cGMP significantly decreased fluid absorption. Therefore, in the jejunum taken from fed and fasted animals cGMP failed to mimic the effects of STa. However, cGMP converted absorption into secretion in the jejunum taken from undernourished gerbils. This is an effect similar to that of STa.

In the ileum, cGMP produces the same effects as STa in the three feeding conditions, i.e. it changed fluid absorption into secretion in the ilea taken from fed gerbils. It produced no significant effects on fluid secretion in fasted ileum, but significantly increased fluid secretion across the ileum taken from undernourished gerbils. In the colon, cGMP failed to convert normal absorption into secretion in fed animals as STa, but it decreased absorption in this region. Similar to the action of STa, cGMP did not change fluid secretion in the colon taken from fasted animals.

Discussion

The results of the present study have shown clearly that fasting and undernourishment have different actions on fluid transport in the jejunum, ileum and colon. In the absence of any secretory stimulant, the jejuna from fed controls, fasted and undernourished gerbils displayed a basal absorptive tone which was not significantly different. However, measurement of ileal fluid movement *in vivo* showed that in fed gerbils the basal, unstimulated "tone" of the ileum was absorption which changed to secretion after four days of fasting. These results agree with those in rats where Young and Levin (1990a,b, 1992) found that when compared with fed animals fasting for three days caused no change in the jejunum fluid transport, but changed the absorptive tone into net secretion in the ileum.

Fasting increased the basal Isc of proximal and mid colon only while undernourishment increased the

basal Isc of the three regions of the colon (Al-Balool 2002). One of the objectives of the present study was to see whether electrogenic and fluid transport in the colon match one another. The colon from the fasted gerbils behaves similarly to that of the ileum, i.e. the basal fluid absorption is changed after 4 days of fasting to secretion. Both the ileum and colon from undernourished animals showed a greatly enhanced secretion when compared with the fed ones. Therefore, fluid movements in the large intestine of gerbils measured *in vivo* showed strong similarities with the electrogenic secretion. In gerbils, there were differences between partially nutritionally deprived animals (undernourished) and totally nutritionally deprived (fasted) animals; four days of fasting caused a small but significant increase in basal Isc of jejunum and ileum, while undernourishment caused a greater increase in Isc (unpublished data).

It may be of interest to mention that Cooke and Carey (1990) reviewing the level of enteric tonic neural activity influencing basal ion transfer *in vitro* (measured as the short circuit-current) and basal ion and fluid transfer measured *in vivo*, reported that the conflicting results were probably caused by factors such as luminal content and stretch of the gut wall affecting the neural activity at the time of removal or measurement.

In both small (jejunum and ileum) and large intestine (colon) of fed gerbils, STa (50 ng/ml) changed the normal basal absorption into secretion. In fasted animals, STa reversed the fluid absorption into secretion in the jejunum and increased fluid secretion in the ileum. STa converted basal fluid absorption into secretion in jejuna and produced a significant increase in fluid secretion in ilea from undernourished gerbils.

Therefore, STa increased fluid transport across the three regions of the intestine in the three feeding conditions. It converted fluid absorption into secretion in jejuna from fed, fasted and undernourished gerbils as well as in the ilea and colon taken from fed animals. STa also increased fluid secretion in fasted and undernourished ilea. Using the same dose STa as in the present paper, Nzegwu and Levin (1994c) found that only the jejunum from undernourished rats showed a significant increase in fluid secretion. Thus in the gerbil ileum, like in the rat jejunum, undernourishment may induce or uncover a neural pathway in the enteric nervous system that activates intestinal secretion.

STa is known to directly activate the production of cGMP in enterocytes causing both electrogenic ion and fluid secretion (Jacewicz *et al.* 1990, Rolfe and Levin 1999). In the present study, cGMP succeeded in mimicking the effects of STa under the following conditions: (i) jejuna taken from undernourished gerbils, (ii) in ilea in the three feeding conditions, and (iii) in fasted colons (both STa and cGMP did not produce any significant changes in fluid secretion).

Volant *et al.* (1997) showed that guanylin (8-bromocyclic GMP) was a less potent stimulant of water and ion secretion than heat-stable enterotoxin of *E. coli*. They also found that in rats STa induced (i) inhibition of Na^+ influx in the jejunum, (ii) stimulation of Na^+ efflux and of HCO_3^- secretion in the distal ileum with inhibition of Na^+ influx, and (iii) stimulation of Cl^- efflux and an inhibition of Na^+ influx in the ascending colon.

Analysis of binding data of STa in rat intestinal membranes suggests the presence of high and low-affinity receptors (Hugues *et al.* 1991). High-affinity sites, unlike low-affinity ones may not be coupled to the activation of guanylate cyclase (Ieda *et al.* 1999). Therefore, the results of the present study confirm the suggestion made earlier, that guanylin was less potent in stimulating water and ion secretion than the heat-stable enterotoxin of *E. coli*.

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References

AL-BALOOL FY: Functional activities of the colon of the desert gerbil (*Gerbillus cheesmani*). *Comp Biochem Physiol C* **132:** 153-160, 2002.

COHEN MB, JENSEN NJ, HAWKINS JA, MANN EA, THOMPSON MR LENTZE MJ, GIANNELLA RA: Receptors for *Escherichia coli* heat-stable enterotoxin in human intestine and in human intestine cell line (Caco-2) *J Cell Physiol* **156**: 138-144, 1993.

COOKE HJ, CAREY HV: Neural regulation of intestinal ion transport. In: *Textbook of Secretory Diarrhea*. LEBENTHAL E, DUFFEY ME (eds), Raven Press, New York, 1990, pp 1-14.

EKLUND S, JODAL M, LUNDGREN O: The net fluid secretion caused by cyclic 3'5'- guanosine monophosphate in rat jejunum in vivo is mediated by a local nervous reflex. *Acta Physiol Scand* **128:** 57-63, 1986.

FERRARIS RP, CAREY HV: Intestinal transport during fasting and malnutrition. *Annu Rev Nutr* **20**: 195-219, 2000.

FIELD M, GRAF LH, Jr. LAIRED WJ, SMITH PL: Heat-stable enterotoxin of *Escherichia coli: in vitro* effects on guanylate cyclase, cyclic GMP concentration, and ion transport in small intestine. *Proc Natl Acad Sci USA* **75:** 2800-2804, 1978.

GUANDALINI S, RAO MC, SMITH PL, FIELD M: cGMP modulation of ileal ion transport: in vitro effects of *Escherichia coli* heat-stable enterotoxin.. *Am J Physiol* **243:** G36-G41, 1982.

HAYDEN UL, CAREY HV: Neural control of intestinal ion transport and paracellular permeability is altered by nutritional status. *Am J Physiol* **278**: R1589-R1594, 2000.

HUGUES M, CRANE M, HAKKI S, O'HANLEY P, WALDMAN SA: Identification and characterization of a new family of high-affinity receptors for *Escherichia coli* heat-stable enterotoxin in rat intestinal membranes. *Biochemistry* **30:** 10738-10745, 1991.

HUOTT PA, LIU W, MCROBERTS GA, GIARNELLA RA, DHARMSATHAPHORN K: The mechanism of *E. coli* heat-stable enterotoxin in a human colonic cell. *J Clin Invest* **82**: 514-523, 1988.

IEDA H, NARUSE S, KITAGAWA M, ISHIGURO H, HAYAKAWA T: Effects of guanylin and uroguanylin on rat jejunal fluid and electrolyte transport: comparison with heat-stable enterotoxin. *Regul Pept* **79**: 165-171, 1999.

JACEWICZ M, HULL AE, KEUSCH GT: Enterotoxin receptors. In: *Textbook of Secretory Diarrhea*. LEBENTHAL E, DUFEY ME (eds), Raven Press, New York, 1990, pp 139-162.

MOURAD FH, NASSAR CF: Effects of vasoactive intestinal polypeptide (VIP) antagonism on rat jejunal fluid and electrolyte secretion induced by cholera and *Escherichia coli* enterotoxins. *Gut* **47**: 382-386, 2000.

NZEGWU HC, LEVIN RJ: Neurally maintained hypersecretion in undernourished rat intestine activated by *E. coli* STa enterotoxin and cyclic nucleotides in vitro. *J Physiol Lond* **479**: 159-169, 1994a.

NZEGWU HC, LEVIN RJ: Role of the enteric nervous system in the maintained hypersecretion induced by enterotoxin STa in the nutritionally deprived intestine *Gut* **35**: 1237-1243, 1994b.

NZEGWU HC, LEVIN RJ: Fluid hypersecretion induced by enterotoxin STa in nutritionally deprived rats: jejunal and ileal dynamic in vivo. *Exp Physiol* **79**: 546-560, 1994c.

NZEGWU HC, LEVIN RJ: Luminal capsaicin inhibits fluid secretion induced by enterotoxin *E. coli* STa but not by carbachol, in vivo in rat small and large intestine. *Exp Physiol* **81:** 313-315, 1996.

ROLFE VE, LEVIN RJ: Vagotomy inhibits the jejunal fluid secretion activated by luminal *Escherichia coli* STa in the rat in vivo. *Gut* **44**: 615-619, 1999.

VOLANT K, GRISHINA O, DESCROID-VAGNE M, PANSU D: Guanylin heat-stable enterotoxin of *Escherichia coli* and vasoactive intestinal peptide-induced water and ion secretion in the rat intestine. *Eur J Pharmacol* **328**: 217-227, 1997.

YOUNG A, LEVIN RJ: Diarrhoea of famine and malnutrition: investigations using a rat model. 1- Jejunal hypersecretion induced by starvation. *Gut* **31**: 43-53, 1990a.

YOUNG A, LEVIN RJ: Diarrhoea of famine and malnutrition: investigations using a rat model. 2- Ileal hypersecretion induced by starvation. *Gut* **31**: 162-169, 1990b.

YOUNG A, LEVIN RJ: Intestinal hypersecretion of the refed-fasted rat: a model for alimentary diarrhoea. *Gut* **33**: 1050-1056, 1992.

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