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Role of 5-hydroxytryptamine type 3 receptors in rat intestinal fluid and electrolyte secretion induced by cholera and *Escherichia coli* enterotoxins

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Abstract

Cholera toxin and *Escherichia coli* heat labile toxin (LT) induced intestinal secretion has in the past been attributed exclusively to an increase in intracellular cAMP whereas *E coli* heat stable toxin (ST) induced secretion is mediated through cGMP. Evidence is accumulating on the importance of 5-hydroxytryptamine (5-HT) in cholera toxin induced secretion, but its role in LT and ST is not well established. This study therefore investigated in vivo the effect of 5-HT_3 receptor antagonist, granisetron, on intestinal fluid and electrolyte secretion induced by cholera toxin, LT, and ST. Granisetron (30, 75, 150, or 300 μg/kg) was given subcutaneously to adult male Wistar rats 90 minutes before instillation of 75 μg cholera toxin or 50 μg LT in isolated whole small intestine. In situ small intestinal perfusion was performed with an iso-osmotic plasma electrolyte solution (PES) to assess fluid movement. In a second group of animals, granisetron (300 μg/kg) was given subcutaneously and two hours later small intestinal perfusion with PES containing 200 μg/l ST was performed. Cholera toxin induced net fluid secretion (median −50·1 μl/min/g (interquartile range −59·5 to −29·8)) was found to be dose dependently decreased or abolished by granisetron (plateau effect at 75 μg/kg: 18 (−7·8 to 28), $p<0·01$). Granisetron in high dose (300 μg/kg), however, failed to prevent LT or ST induced secretion (−52 (−121 to −71) *v* −31 (−44 to −18), and (−39 (−49 to 17) *v* (−22 (−39 to −3)), respectively). Sodium and chloride movement paralleled that of fluid. In conclusion, these data show that 5-HT and 5-HT_3 receptors play an important part in cholera toxin induced secretion but are not involved in *E coli* heat stable or heat labile toxin induced secretion

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Keywords: 5-hydroxytryptamine, cholera toxin, *E coli*.

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Intestinal secretion induced by cholera toxin and the structurally related enterotoxin, *Escherichia coli* heat labile toxin (LT) has been in the past, attributed solely to the activation of adenylate cyclase with the corresponding increase in intracellular cAMP. Evidence has been accumulating, however, on the importance of 5-hydroxytryptamine (5-HT) present in enterochromaffin cells and of the enteric nervous system in the pathophysiology of cholera toxin induced secretion.[1–3] Nilsson *et al* have shown that enterochromaffin cells in the cat small intestine discharge their contents of 5-HT after exposure to cholera toxin.[1] Plasma and gut lumen 5-HT is increased in human volunteers exposed to a subclinical dose of cholera toxin.[4 5] In addition, inhibition of cholera toxin induced secretion by neuronal blockade using tetrodotoxin and lidocaine has unveiled the importance of the enteric nervous system in the secretory process.[6] On the basis of these findings, it has been proposed that cholera toxin promotes cAMP mediated release of 5-HT from enterochromaffin cells, which then stimulates dendrites immediately adjacent to the intestinal epithelium.[7 8] Beubler *et al* have found that pre-treatment with 5-HT_3 antagonists partially prevents cholera toxin induced fluid secretion in rat jejunum.[3] LT is structurally, immunologically, and functionally related to cholera toxin.[9 10] LT, like cholera toxin, possesses five β subunits and a single α subunit and binds to the same receptor on enterocytes (GM1 ganglioside) leading to activation of adenylate cyclase and increase in intracellular cAMP.[9 11] Whether 5-HT participates in the secretory process induced by LT has not been previously studied.

The *E coli* heat stable toxin (ST) binds to a specific receptor on the apical membrane of the enterocyte and exhibits a very rapid onset of action by inhibiting electroneutral Na^+Cl^- absorption and inducing Cl^- secretion, a process that occurs in parallel with increased values of intracellular cGMP.[12–14] Although the enteric nervous system has been shown to play an important part in ST induced secretion,[15] the neurotransmitters involved are not yet fully characterised.[14 16–19] As 5-HT is a neurotransmitter and as 5-HT_3 receptors are present exclusively on neurons,[20 21] it would be interesting therefore to discover if 5-HT plays a part in the secretory process induced by ST.

The aim of our study was to investigate the effect of 5-HT type 3 receptor antagonist granisetron on cholera toxin induced secretion and the role of 5-HT and 5-HT_3 receptors in

the pathophysiology of cholera toxin, LT, and ST induced intestinal secretion using an animal model in vivo.

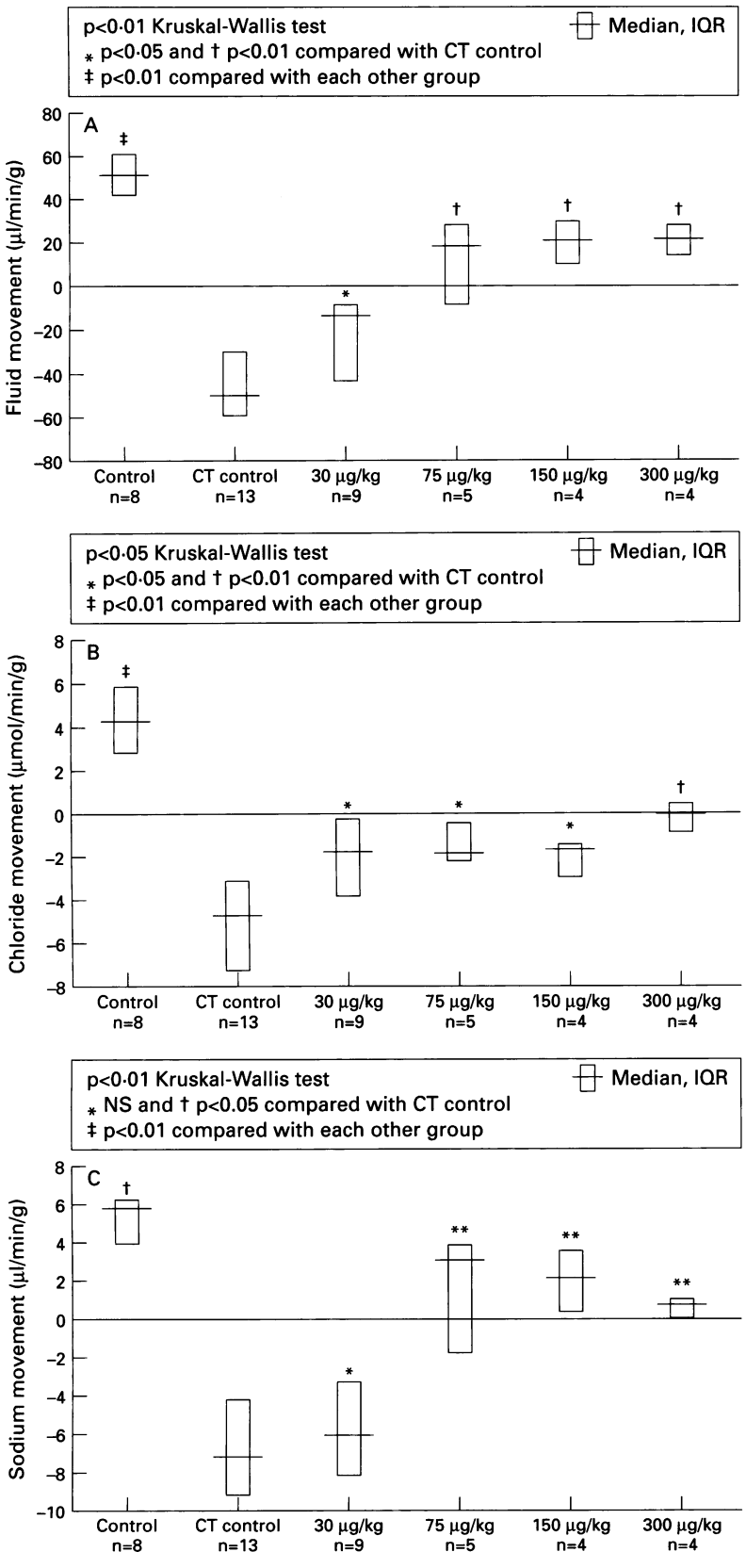

Figure 1: Effect of cholera toxin 75 µg on (A) fluid, (B) chloride, and (C) sodium movement in control and in rats pre-treated with subcutaneous granisetron 30, 75, 150, and 300 µg/kg. Fluid movement is expressed in µl/min/g, chloride and sodium in µmol/min/g dry intestinal weight. Results are expressed as median and interquartile range (IQR); positive values denote absorption and negative values denote secretion. Kruskal-Wallis test is used to study the effect of different doses of granisetron on fluid and electrolyte movement, and Wilcoxon rank sum test is used to test the differences between pairs.

Methods

Cholera toxin and LT experiments

Male adult Wistar rats (180–220 g body weight) were fasted for 18 hours with free access to water. The rats were anaesthetised with intraperitoneal injection of sodium pentobarbitone (60 mg/kg) and maintained throughout the experiments by interval intraperitoneal injections (15–30 mg/kg) as necessary. The abdomen was opened through a midline incision and cannulas were inserted into the small intestine proximally (5 cm distal to the duodenojejunal junction) and distally in the terminal ileum (1–2 cm proximal to ileocaecal junction), and fixed by ligation as described previously.[22] The isolated intestinal segment was gently flushed with isotonic saline (37°C) and then air was injected to clear the small intestine of residual content before the instillation of 75 µg cholera toxin in 6 ml of isotonic saline or 6 ml isotonic saline alone (controls) and clamping both proximal and distal cannulas. The intestine was returned to the abdominal cavity and the abdomen was closed. After two hours, the clamps were removed and the intestine was perfused at a rate of 0·5 ml/min with plasma electrolyte solution (PES) containing Na 140, K 4, Cl 104, HCO_3 40 mmol/l to which 5 g polyethylene glycol 4000 (PEG) and 4 µCi/l of [^{14}C]-PEG were added. Thirty minutes were allowed to elapse to ensure establishment of a steady state after which three consecutive 10 minute collections of the effluent were obtained from distal cannula. This dose of cholera toxin was used as it has previously been shown to cause maximum fluid secretion in this model.[23] Similarly, LT was given in a dose of 50 µg or 75 µg to get the same level of fluid secretion as cholera toxin. The 5-HT$_3$ antagonist, granisetron, was given subcutaneously as a dose of 30, 75, 150, or 300 µg/kg in 0·3 ml saline (for the cholera toxin experiments) and 300 µg/kg (for the LT experiments) at the same time as the anaesthetic. Control animals were given subcutaneously saline without granisetron. Animals were kept at 37°C using a heat pad and an overhead lamp. At the end of the experiments the rats were killed by an overdose of pentobarbitone and the perfused intestinal segment was removed, rinsed, blotted, and desiccated in an oven at 100°C to obtain the dry weight. The samples of effluent were analysed immediately or kept frozen at −20°C and analysed within two weeks.

ST experiments

In experiments with ST, a 25 cm segment of jejunum starting 5 cm distal to the duodenojejunal junction was perfused with PES containing [^{14}C]-PEG to which 200 µg/l ST (equivalent to 50 000 mouse units)[24] was added. After 30 minutes' perfusion to establish steady state, three consecutive 10 minute collections of the effluent were obtained. Granisetron 300 µg/kg or saline was given subcutaneously with the anaesthetic. Steady state

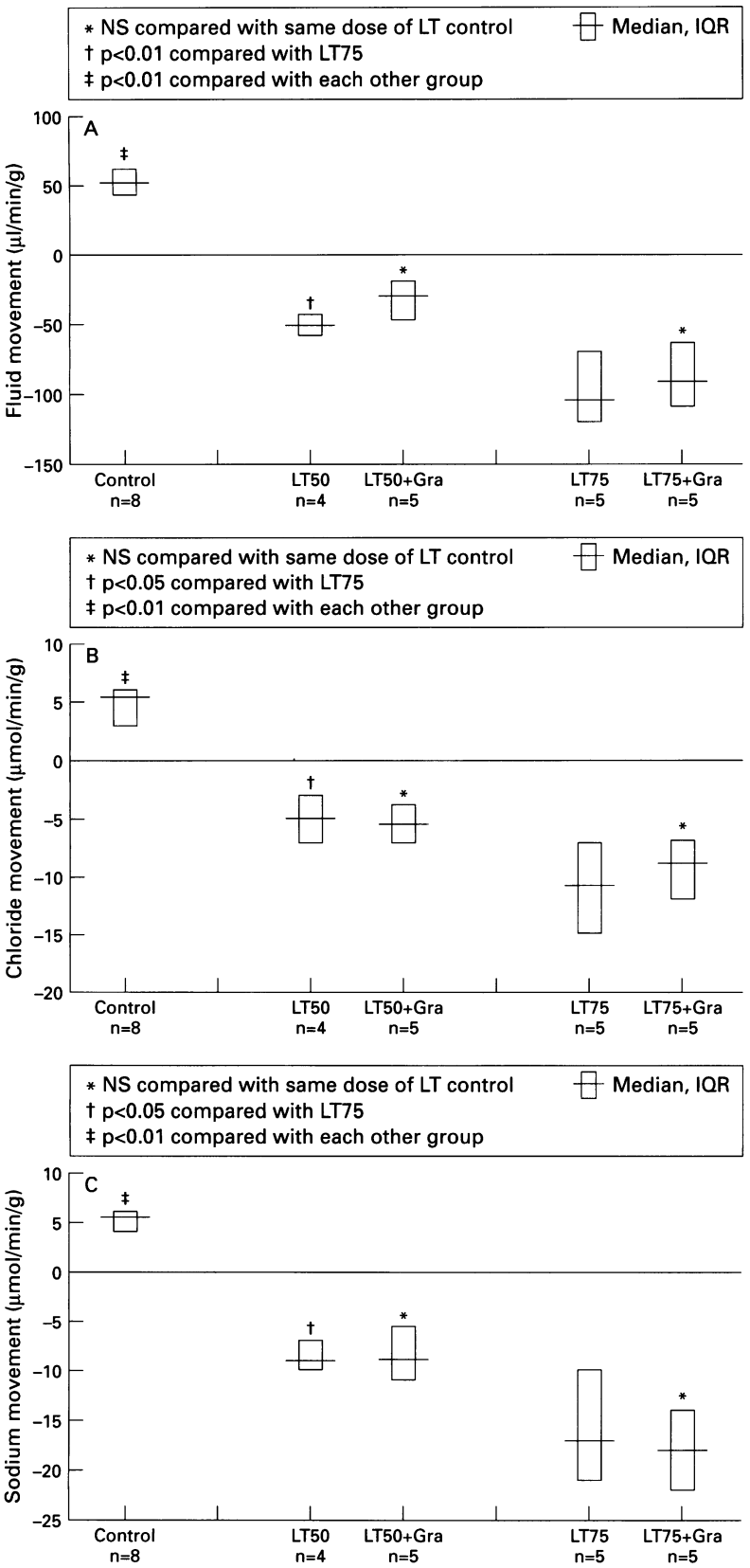

Figure 2: Effect of E coli *heat labile toxin 50 µg (LT50) and 75 µg (LT75) on (A) fluid, (B) chloride, and (C) sodium movement in control and in rats pre-treated with subcutaneous granisetron (Gra) 300 µg/kg. Fluid movement is expressed in µl/min/g, chloride and sodium in µmol/min/g dry intestinal weight. Results are expressed as median and interquartile range (IQR); positive values denote absorption and negative values denote secretion. Wilcoxon rank sum test is used to test the differences between pairs.*

condition was shown by less than 5% variation in water movement between consecutive 10 minute collections and also the values were accepted only if recovery of radioactive PEG fell between 95 and 105%.[22 23]

Analytical methods

[^{14}C]-PEG concentrations in the effluent were measured in triplicate by liquid scintillation spectroscopy in LKB Wallac Ultra-beta 1210 scintillation counter. Sodium and potassium concentrations were determined by a flame photometer (Instrument Laboratories 943), and chloride concentrations by Chemlab (CMMI chloridemeter).

The mean of the net fluid and solute movement of the three consecutive effluent samples was calculated and expressed respectively as µl/min/g and µmol/min/g of dry intestinal weight. Positive values denote net absorption and negative values net secretion.

Materials

Cholera toxin, ST, and LT were obtained from Sigma Chemical Company. The 5-HT$_3$ receptor antagonist, granisetron was supplied by SmithKline Beecham, UK. Granisetron is considered a specific 5-HT$_3$ receptor antagonist in the gut.[25] Radiolabelled polyethylene glycol ([^{14}C]-PEG 4000) was obtained from Amersham International and all other chemicals were supplied by British Drug House (BDH Chemicals).

Statistics

Results are expressed as median and interquartile range in each group of animals studied. Differences in fluid and solute movement with different doses of granisetron were examined using the Kruskal-Wallis test for non-parametric multiple comparisons, and differences between pairs in all other experiments were tested using the Wilcoxon rank sum test.

Results

Cholera toxin and LT experiments

Net fluid secretion occurred in all the animals receiving cholera toxin (median −50·1 µl/min/g (interquartile range −59·5 to −29·8); n=13) (Fig 1A). Chloride movement paralleled that of fluid (−4·7 µmol/min/g (−7·1 to −2·2)) (Fig 1B). Granisetron dose dependently reduced fluid and electrolyte secretion ($p<0·01$, Kruskal-Wallis test). Granisetron at 30 µg/kg significantly decreased cholera toxin induced fluid secretion (−13·5 (−43·1 to −8·7), n=9; $p<0·03$) and at 75 µg/kg reversed fluid secretion to absorption (18 (7·8 to 28), n=5; $p<0·01$) but fluid absorption was still less than that in normal non-secreting controls (51 (42 to 61), n=8; $p<0·01$) (Fig 1A). Higher doses of granisetron (150 and 300 µg/kg) did not increase fluid absorption further (21 (10 to 30), n=4 and 21 (15 to 28), n=4, respectively). Chloride secretion was also significantly reduced in a dose dependent manner by granisetron (Fig 1B). Sodium secretion was unaffected by the low dose of granisetron, however, it was reversed to absorption by a dose of 75 µg/kg or higher (Fig 1C). Granisetron had no effect on fluid movement in normal non-secreting intestine (49 (29 to 56), n=9; NS).

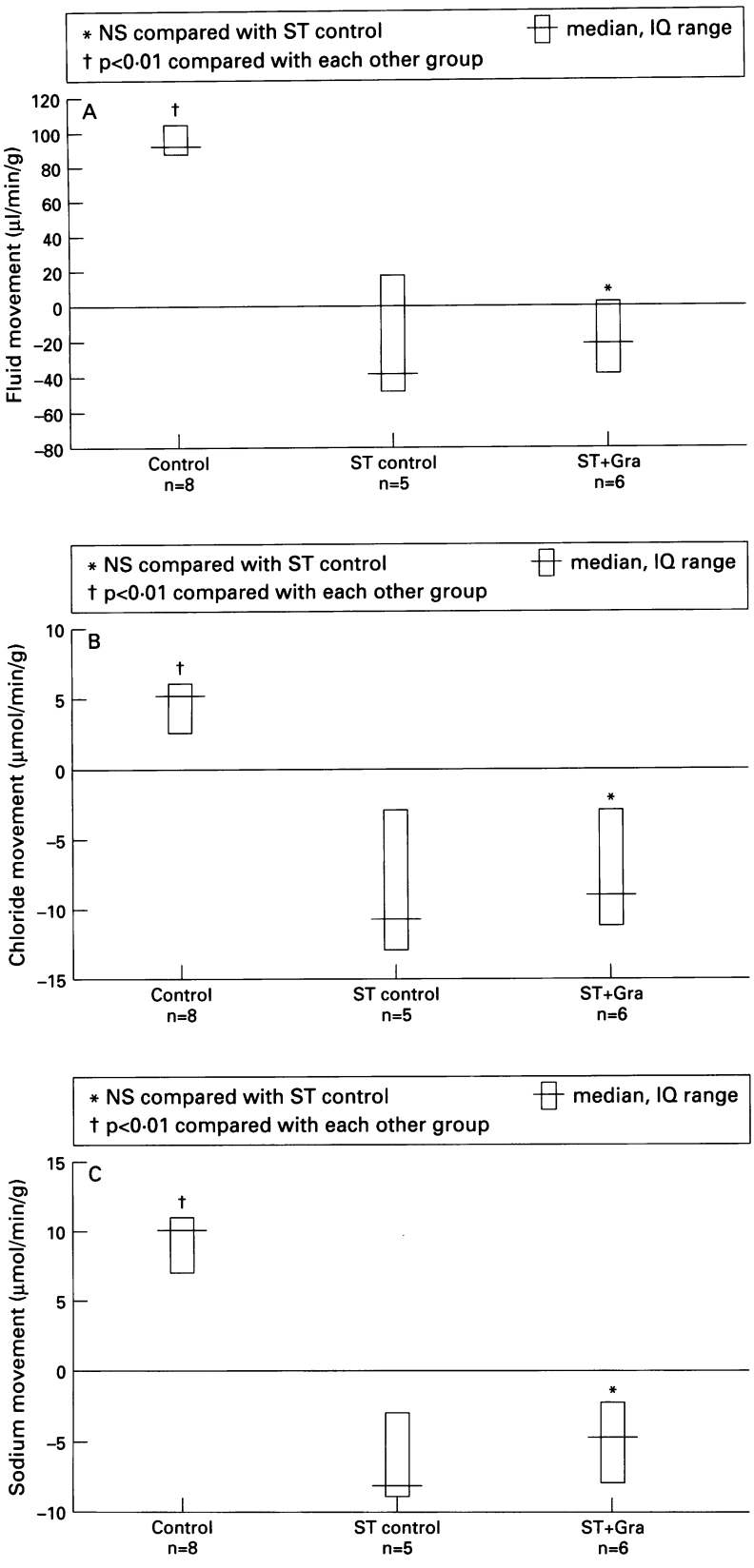

Figure 3: Effect of E coli *heat stable toxin on (A) fluid, (B) chloride, and (C) sodium movement in control and in rats pre-treated with subcutaneous granisetron (Gra) 300 µg/kg. Fluid movement is expressed in µl/min/g, chloride and sodium in µmol/min/g dry intestinal weight. Results are expressed as median and interquartile range (IQR); positive values denote absorption and negative values denote secretion. Wilcoxon rank sum test is used to test the differences between pairs.*

LT at a dose of 75 µg caused marked fluid secretion (−106 (−121 to −71)) and at 50 µg the secretion was similar to that seen with 75 µg cholera toxin (−52 (−59 to −44)). At both doses of LT, and in contrast with cholera toxin, granisetron (300 µg/kg) had no effect on fluid and electrolyte secretion (Fig 2).

ST experiments

Perfusing the small intestine with a solution containing ST caused appreciable fluid, chloride, and sodium secretion (−39 (−49 to 17) µl/min/g, −10·7 (−13 to −3) µmol/min/g, and −6 (−9 to −3), respectively; n=5) (Fig 3). Granisetron in a dose of 300 µg/kg had no effect on fluid and electrolyte secretion (Fig 3).

Discussion

We have shown that the 5-HT$_3$ receptor antagonist granisetron can dose dependently prevent cholera toxin induced fluid and electrolyte secretion. Our experiments further support the importance of 5-HT in cholera toxin induced fluid secretion. It is known that cholera toxin simulates adenylate cyclase and increases intracellular cAMP not only in enterocytes but also in many other cell types. In enterochromaffin cells, this increase in cAMP leads to their degranulation and subsequent 5-HT release,[25] which closely correlates with changes in fluid movement.[3] 5-HT induced secretion, in contrast with cholera toxin, does not use cAMP as a mediator[26] but seems to increase calcium gating in epithelial cells[26 27] or activate intestinal neuronal reflexes, or both.[28] Cholera toxin has also been shown to activate nerve reflexes as part of its secretory action,[6] which can be reversed by neuronal blockade. In our experiments, as well as in a previous study,[3] cholera toxin induced secretion was reversed by 5-HT type 3 receptor antagonism. As 5-HT$_3$ receptors are present exclusively on neurons,[20 21] our findings provide further evidence for an important role of 5-HT in cholera toxin induced fluid secretion as a result of a stimulatory effect on neuronal structures.

The pathophysiology of diarrhoea caused by other enterotoxins has not been as extensively investigated as that caused by cholera toxin. LT, which is structurally similar to cholera toxin, binds to the same receptor on enterocytes (GM1 ganglioside) and has been shown to stimulate adenylate cyclase with the corresponding increase in cAMP production.[9 11] The two toxins are not completely identical in their amino acid composition,[9–11 30] however, and their binding affinity is different.[10 30] Griffith *et al*[30] have shown that intestinal brush borders from Wistar rats bind 20–30 times more LT than cholera toxin and that LT binds to a variety of brush border galactoproteins only weakly recognised by cholera toxin. In our experiments, the differential effect of 5-HT$_3$ receptor antagonism on cholera toxin and LT suggest that there are other fundamental differences in the pathophysiology of diarrhoea caused by these enterotoxins. Although a dose of LT that causes the same amount of secretion as cholera toxin was used, 5-HT$_3$ antagonism had no effect on fluid and electrolyte secretion. Whereas 5-HT plays an important part in cholera toxin induced secretion, it seems it had no role in LT induced secretion.

It is likely that binding of LT to additional receptors on enterocytes plays a pathophysiologic part in the secretory process. Holmgren *et al*[31] have shown in vivo that blocking cholera toxin receptors by the inactive B subunits (CT_B) does not prevent LT induced secretion; however, blocking LT receptors by LT_B totally prevent cholera toxin induced secretion showing that LT binds to receptors not recognised by cholera toxin and induces the same amount of secretion. Although LT causes an increase in cAMP, it is not known if this leads to enterochromaffin cells degranulation and consequently 5-HT release; measurement of enterochromaffin cell degranulation or luminal 5-HT would clarify this point.

The role of cGMP in ST induced intestinal secretion is well established.[13 14 32] Whether cGMP alone or other mediators are also involved is not known. It has been shown that, like in cholera toxin, the enteric nervous system is also involved in ST induced secretion as shown by the inhibitory effect of hexamethonium, lidocaine, and tetrodotoxin.[15 17 18] The involvement of prostaglandins is not well established and there is controversy about the effect of indomethacin on ST induced secretion.[33 34] Beubler *et al*[35–37] have recently suggested that ST induces fluid secretion predominantly by local 5-HT release. 5-HT_3 antagonism failed to prevent ST induced secretion in vivo in our experiments, however, although it was effective in reversing cholera toxin induced secretion. Our results are against a role for 5-HT in the pathophysiology of ST induced intestinal secretion and are in accordance with two previous findings. Forsberg *et al*[38] have shown in vitro that cGMP, in contrast with cAMP, does not lead to an increase in serotonin release from enterochromaffin cells. Secondly, Rolfe *et al*[17 18] using intestinal segments mounted in Ussing chambers failed to find any difference in the change in short circuit current between 5-HT desensitised rat terminal ileal tissues and controls, after exposure to ST; in addition cGMP induced a change in short circuit current in 5-HT desensitised muscle stripped sheets. It was concluded that both ST *E coli* and cGMP can both activate intestinal electrogenic secretion in vitro without the mediation of 5-HT.

In conclusion, our findings support the view that 5-HT participates in cholera toxin induced secretion, presumably as a result of adenylate cyclase activation and 5-HT release from enterochromaffin cells, but not in LT or ST secretion.

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