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Racecadotril demonstrates intestinal antisecretory activity in vivo

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SUMMARY

Background Racecadotril (acetorphan), a potent enkephalinase inhibitor, protects endogenous enkephalins from degradation. Racecadotril exhibits experimental and clinical antidiarrhoeal activity without any effect on intestinal motility, suggesting selective antisecretory activity. The antisecretory effect of racecadotril was directly assessed in the present study.
Methods A 1 m, jejunal, Thiry–Vella loop was created in six mongrel dogs, and water and ionic fluxes were evaluated during infusion (2 mL/min) of Tyrode solution labelled with ^{14}C-polyethylene glycol. Fluxes were determined both in the basal state and 5–6 h after commencement of a 2-h infusion of cholera toxin (0.4 μg/mL). Racecadotril (10 mg/kg) or vehicle was given orally with and without prior intravenous administration of naloxone (0.1 mg/kg) or phentolamine (0.2 mg/kg).
Results Basal absorption remained unchanged following racecadotril administration; however, racecadotril significantly decreased ($P = 0.01$) cholera toxin-induced water, sodium, and potassium hypersecretion, from 0.73 ± 0.15 to 0.37 ± 0.13 mL/min; from 125.0 ± 16.1 to 14.7 ± 9.5 μMol/min; and from 3.41 ± 0.66 to 1.66 ± 0.61 μMol/min, respectively. This antisecretory activity of racecadotril was suppressed by naloxone but not by phentolamine.
Conclusions This study directly demonstrates the antisecretory activity of racecadotril in relation to the protection of endogenous enkephalins.

INTRODUCTION

An improved understanding of the pathophysiology of diarrhoea has provided the basis for a more rational therapeutic approach. Although the antidiarrhoeal properties of the opioids have been known for some time, the mechanism of action and the existence of different populations of opioid receptors has only been established recently.[1]

The μ-receptors are primarily involved in the regulation of intestinal motility, and their activation leads to disruption of normal intestinal peristalsis and increased tone of the rectal sphincter. Activation of the δ-receptors leads to reduced secretion of water and electrolytes through a reduction in cyclic AMP.[2] The antidiarrhoeal effects of μ-receptor agonists such as morphine, codeine, diphenoxylate, and loperamide result mainly from their antitransit activity. In contrast, δ-receptor agonists such as the enkephalins display their antidiarrhoeal effects via antisecretory activity; analogues of methionine–enkephalin (metenkephalin) have been shown to enhance intestinal absorption both *in vitro*[3–7] and *in vivo*.[8,9]

However, enkephalin-like pentapeptides have not been found to be effective therapeutically. A more physiological approach therefore comes from inhibition of the metalloendopeptidase enzyme, enkephalinase (neprilysin, EC 3.4.24.11). Inhibition of this enzyme delays the inactivation of endogenous enkephalins and reinforces their antisecretory activity.

Racecadotril [racecadotril is the official international non-proprietary name (INN); the drug was known as

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acetorphan in early studies], a potent inhibitor of enkephalinase, inhibits castor oil-induced hypersecretory diarrhoea in both rodents[10] and healthy subjects.[11] No effect of racecadotril on intestinal motility has been detected during experimental studies[10,12] or clinical trials in acute diarrhoea.[11,13,14]

Moreover, in contrast to drugs such as loperamide, racecadotril does not induce bacterial overgrowth in the small bowel,[15] nor does it produce constipation in healthy subjects[12] or in patients with acute diarrhoea.[11,13,14]

The purpose of the present study was to make a direct assessment of the antisecretory effect of racecadotril on a validated model of secretory diarrhoea in the dog. This model involves infusion of cholera enterotoxin after creation of a jejunal Thiry–Vella loop.

MATERIALS AND METHODS

Animal preparation

Six adult mongrel dogs weighing 15–20 kg were used in this study. Under halothane anaesthesia, a 1m, jejunal, Thiry–Vella loop was created beginning about 50 cm from the duodenojejunal junction. Intestinal continuity was restored by end-to-end anastomosis. Both ends of the loop were exteriorized on the left flank and sutured to the skin.

The animals were placed in metabolic cages and allowed to recover for 3 weeks before the experiments began. During this period, the loops were infused daily with 100 mL of an isotonic electrolyte solution.

Infusion procedure

Twice a week, after the dogs had been fasted for 12 h, a Foley catheter (0.5 cm in diameter) was introduced 10 cm into each end of the Thiry–Vella loop and the distal balloon distended with 5 mL of tap water. An isotonic electrolyte solution (Tyrode solution) containing (in mM): Na^+, 140; K^+, 5; Cl^-, 110; HCO_3^-, 35; glucose, 26; and polyethylene glycol (PEG) (2 g, 5 μCi ^{14}C per litre) was then infused at a rate of 2 mL/min over 10 h.

After an equilibration period of 100 min, the outflow of the Thiry–Vella loop was drained by gravity and collected continuously into flasks during consecutive 20-min periods over the total infusion period (8 h). Two hours after the start of outflow collection, the original solution was replaced for 2 h by a test solution containing 0.4 μg/mL of cholera toxin (Sigma, St Louis, MO, USA), but otherwise identical in composition to the control fluid. The control solution was then replaced and infused again for an additional 6 h.

Experimental design

Two series of experiments were performed. In the first series, jejunal infusions were performed with and without (control group) oral administration of 10 mg/kg of racecadotril 1 h before the beginning of the cholera toxin infusion. Each of these experiments was carried out three times in each dog.

In the second series, the same experimental model and procedure were used to determine the physiological mechanism of the effects. Racecadotril (10 mg/kg) or matched placebo were administered orally with or without intravenous injection of the opioid antagonist naloxone (0.1 mg/kg) or the α-receptor antagonist phentolamine (0.2 mg/kg). Naloxone or phentolamine were administered 10 min prior to racecadotril and 120 min after the cholera toxin infusion began. Each experiment was carried out three times in each dog.

Measurements of ^{14}C radioactivity, corresponding to PEG concentrations, were made by liquid scintillation (SL 20, Intertechnique, Plaisir, France), and sodium and potassium concentrations were measured by flame photometry (Eppendorf, Hamburg, Germany).

Water, sodium, and potassium net fluxes were determined from PEG concentrations using the following formulae:

$$\text{Water absorption (mL/min)} = F_\text{p} - \left[F_\text{p} \times \frac{[\text{PEG}]_i}{[\text{PEG}]_o}\right] \quad (1)$$

$$\text{Electrolyte absorption (μMol/min)} = [E]_i F_\text{p} - \left[[E]_o F_\text{p} \times \frac{[\text{PEG}]_i}{[\text{PEG}]_o}\right] \quad (2)$$

in which i = inflow, o = outflow, Fp is the rate of infusion expressed in mL/min and E is the electrolyte concentration in μMol/min.

Mean hourly values for absorption were calculated at different intervals during the infusion period, and expressed as means ± s.d., the values for each hourly interval being the mean of three 20-min samples. A plus sign signifies absorption from the lumen, while a minus sign denotes secretion in the results from both series of experiments.

Statistical analysis

Statistical analysis was performed using a Wilcoxon matched-pairs signed-rank test and differences were considered significant for $P < 0.05$.

RESULTS

Series 1

The variations in the PEG concentration in the perfusate showed that steady state was reached between 100 and 120 min after the experiment began. Thus, 'basal values' were the mean values obtained from 60 to 0 min before the beginning of the cholera toxin infusion. Under basal conditions ($n = 9$), there was net absorption of water ($+ 0.74 \pm 0.14$ mL/min), sodium ($+ 75.0 \pm 13.9$ μMol/min), and potassium ($+ 1.16 \pm 0.39$ μMol/min) (Figure 1).

Changes in net water and ion transport were observed after the second hour of cholera toxin infusion, the maximal effect occurring between 300 and 360 min after the beginning of the cholera toxin infusion. 'Cholera toxin' values were the mean values obtained during this maximal period. Figure 1 shows that net water, sodium, and potassium transport changed to secretion (-0.73 ± 0.15 mL/min; -125.0 ± 16.1 μMol/min; and -3.41 ± 0.66 μMol/min, respectively).

When racecadotril was administered orally, basal absorption of water and electrolytes did not change (Figure 1). After cholera toxin infusion, net fluxes of water, sodium, and potassium were significantly less ($P = 0.01$) with racecadotril compared with cholera toxin control values. The respective figures for net water, sodium, and potassium fluxes were -0.37 ± 0.13 mL/min; -14.7 ± 9.5 μMol/min; and -1.66 ± 0.61 μMol/min (Figure 1).

Series 2

The basal and cholera toxin values for water flux calculated from experiments carried out in three different dogs were similar to those obtained from the first series of experiments. After oral administration of racecadotril, similar effects to those seen in series 1 were obtained. These effects were antagonized after intravenous injection of the opioid antagonist naloxone, but remained unchanged after administration of phentolamine (Figure 2).

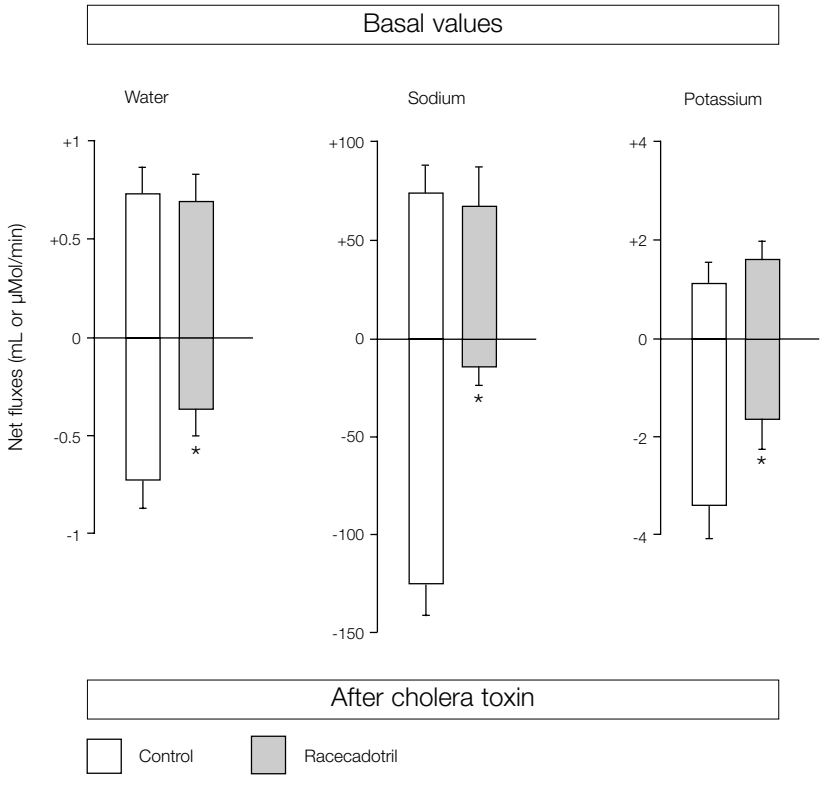

Figure 1. Effects of oral racecadotril (10 mg/kg) on water, sodium, and potassium fluxes in dogs (mean ± s.d.; $n = 9$) before and after cholera toxin infusion (0.4 μg/mL for 2 h). *$P = 0.01$ vs. control measurements. A positive value denotes net absorption and a negative value net secretion. 'Basal values' and 'after cholera toxin' values are mean values obtained 60–0 min before and 300–360 min after the beginning of cholera toxin infusion, respectively.

DISCUSSION

This study demonstrates that the enkephalinase inhibitor, racecadotril, administered orally, does not affect absorption in the basal state but inhibits cholera toxin-induced hypersecretion in a jejunal Thiry–Vella loop in dogs. Furthermore, it confirms the mechanism of action of racecadotril as its antisecretory effect was antagonized by the opioid antagonist naloxone, but not by the α-receptor antagonist phentolamine.

Our previous studies have suggested that analogues of metenkephalin can act centrally to affect intestinal transport of water and electrolyes in dogs;[16] this study demonstrates the effect of endogenous enkephalins on hydroelectrolytic absorption. The Thiry–Vella loop experimental model is based upon a neurovascularly intact isolated jejunal segment out of normal gastrointestinal continuity. Thus, the action of orally administered racecadotril is, at least in part, mediated through a neurovascular pathway. The endogenous enkephalins act on the δ-receptors of the submucosal plexus, a level of action that does not exclude an associated action on the intestinal mucosa. However, they act peripherally rather than centrally, as racecadotril is readily hydrolysed after oral administration to the active compound thiorphan,[17] which does not cross the blood–brain barrier.[18,19]

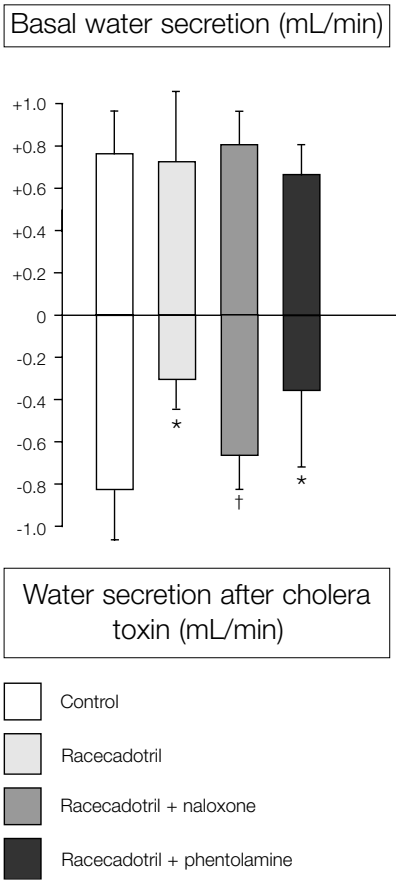

Figure 2. Effects of intravenous naloxone (0.1 mg/kg) and phentolamine (0.2 mg/kg) on water secretion in dogs (mean ± s.d.; $n = 9$) before and after cholera toxin infusion (0.4 μg/mL for 2 h). Values are given without (control) and with prior administration of oral racecadotril (10 mg/kg). *$P < 0.05$ vs. control measurements; †$P < 0.05$ vs. racecadotril measurements. A positive value denotes net absorption and a negative value net secretion. 'Basal values' and 'after cholera toxin' values are mean values obtained 60–0 min before and 300–360 min after the beginning of cholera toxin infusion, respectively.

Although there is evidence that the release of enkephalins is involved in both the physiological change in absorption[20,21] and in prostaglandin-induced secretion,[22] our studies suggest that the role of endogenous enkephalins revealed by the effects of racecadotril is more marked under pathological than physiological conditions. In agreement with this is the finding that the drug did not modify electrolytes under basal conditions, but significantly reduced cholera toxin-induced changes in a naloxone-reversible manner.

Cholera toxin is believed to cause hypersecretory diarrhoea via its specific action on the cyclic AMP-adenylate cyclase system. Cholera toxin activates the adenyl cyclase signalling system, producing intracellular accumulation of high levels of cyclic AMP, which results in the accumulation of sodium chloride and water in the intestinal lumen. Hence the effect of racecadotril on cholera toxin-induced secretion provides further evidence that endogenous enkephalin activity is mediated via cyclic AMP.[2]

The intestinal antisecretory effects of racecadotril against cholera toxin-induced hypersecretion in dogs are in agreement with results from both experimental and clinical studies. Castor oil-induced hypersecretion was inhibited when racecadotril was administered orally in both rodents[10] and healthy human subjects.[11] Moreover, the lack of effect of racecadotril on gastrointestinal transit time has been demonstrated by measurements of the transit of a charcoal meal in rodents,[10] of *Escherichia coli* strains in gnotobiotic piglets,[15] and of radiopaque markers in healthy human subjects.[12] Taken together, these data indicate that the antidiarrhoeal activity of racecadotril derives from the drug's pure antisecretory action, which has been demonstrated in this study.

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