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Racecadotril: a new approach to the treatment of diarrhoea

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Abstract

Since enkephalins were discovered in 1975, it has become clear that they play an antisecretory role in the gastrointestinal tract. Hence a rational research programme was directed at the development of a drug that would preserve these neurotransmitter peptides in the gut by preventing their inactivation. This research programme has resulted in the development of the enkephalinase inhibitor, racecadotril. Preclinical studies have demonstrated the efficacy of racecadotril in two models of hypersecretory diarrhoea: infusion of cholera toxin and castor oil-induced diarrhoea. Moreover, unlike loperamide, racecadotril did not prolong transit time in the small intestine or colon. Further experiments have shown that racecadotril does not promote bacterial overgrowth in the small intestine. Racecadotril lacks any potential for neurotoxicity, and radiolabelled studies have demonstrated that the drug does not enter the brain after oral administration. No potential for abuse or physical dependence has been seen. It is concluded that racecadotril demonstrates specificity of antisecretory action on the gastrointestinal tract without any adverse effect on gastrointestinal motility, and that the results of the preclinical studies indicate the potential usefulness in the treatment of hypersecretory diarrhoea in man.

Keywords: Antisecretory agents; Enkephalins; Hypersecretory diarrhoea; Racecadotril

1. Introduction

There are three types of endogenous opioid peptides: the enkephalins, beta-endorphins, and the dynorphins (Table 1). Each of these neurotransmitter peptides acts preferentially on a different subtype of opiate receptor; enkephalins act at the δ-receptor, beta-endorphins at the μ-receptor, and dynorphins at the κ-receptor. However, these opioid peptides are not confined to the central nervous system, but are also found in the enterochromaffin cells and neurons of the myenteric and submucosal plexus of the stomach and small intestine [1].

2. The role of enkephalins in the gut

Opioid agonists act on μ- and δ-receptors to alter gut motility and secretion. Drugs that activate the μ-receptor prolong oro-caecal and colonic transit times by disrupting the gut's electrical activity, increasing gut capacity, and delaying the passage of fluids through the small intestine; they have no direct effect on absorption [2–5]. Drugs that activate the δ-receptor should reduce secretory activity in the gut [6]. Since the endogenous opioid peptides were discovered over 20 years ago, a number of drugs have been developed that act at the μ-receptor, but until recently researchers have not been able to develop a drug that acted specifically at the δ-receptor.

The enkephalins were discovered in 1975, and act as neurotransmitters along the gastrointestinal tract where they are found in high levels in the mucosal cells. When the enkephalins bind to the δ-receptor, the level of cyclic AMP decreases, leading to reduced secretion of water and electrolytes. Hence, in order to develop a drug that would prolong the antisecretory action of the enkephalins, our research was aimed at identifying a peptidase that could cleave these peptides and, subsequently, a peptidase inhibitor. It was in this way that we identified an enzyme which we termed 'enkephalinase'. The official number of this membrane-bound metalloendopeptidase is EC 3.4.24.11, and its official

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name is neprilysin. Enkephalinase is mainly found in the enterocytes [7], the cells responsible for the exchange of water and electrolytes between the body and the lumen of the gut.

Once the enkephalinase enzyme had been identified, we carried out further research to develop molecules that would act as potent, selective, and well tolerated inhibitors of this enzyme. This research led to the development of the enkephalinase inhibitor racecadotril (acetorphan was the name given to the drug in early studies; racecadotril is the official INN).

3. Racecadotril is effective in models of hypersecretory diarrhoea

We carried out a number of preclinical studies to examine the mode of action of racecadotril. Fig. 1 shows the results of an experiment carried out on an isolated portion of the dog jejunum to evaluate the effect of racecadotril in a hypersecretory model of diarrhoea [8]. A 1-m, jejunal, Thiry–Vella loop was created and basal fluxes of water, sodium, and potassium were measured. As Fig. 1 shows, the net flux was in the direction of absorption, and racecadotril (10 ng/kg) had no effect on basal fluxes. Cholera toxin (0.4 mg/l) was then infused for 2 h and racecadotril or vehicle were given orally 1 h before the infusion.

Infusion of cholera toxin reversed the net fluxes of water, sodium, and potassium, resulting in net hypersecretion (Fig. 1). However, prior administration of racecadotril significantly ($P = 0.01$) reduced the effect of cholera toxin, demonstrating the antisecretory activity of the enkephalinase inhibitor. Moreover, the effect of racecadotril on cholera toxin-induced hypersecretion was completely blocked by prior administration of the opioid antagonist naloxone (0.1 mg/kg) but not by the α-receptor blocker phentolamine (0.2 mg/kg), indicating that racecadotril acted specifically via protection of the released enkephalins against inactivation by enkephalinase.

The antisecretory activity of racecadotril was confirmed in another animal model of hypersecretory diarrhoea (castor oil-induced diarrhoea) [9]. Racecadotril was given intravenously to starved rats 30 min prior to oral administration of a standard dose of castor oil (1.0 ml), and the onset of diarrhoea noted over a 4-h observation period. Fig. 2 shows that racecadotril delayed the onset of the diarrhoea and also reduced the stool weight in these rats ($P < 0.01$). This effect was dose dependent and was abolished by co-administration of subcutaneous naloxone (2×2 mg/kg), once again demonstrating the specific action of racecadotril on enkephalins. In this model, racecadotril was shown to be equivalent in potency to the μ-receptor antagonist, loperamide (2 mg/kg po) (Fig. 2).

Table 1

Three types of endogenous opioid peptides and their receptors

Opioid peptides	Opioid receptor subtypes	Selective agonists	Biological responses
Enkephalins (Tyr-Gly-Gly-Phe-Met Tyr-Gly-Gly-Phe-Leu)	Delta	DPDE	Gastrointestinal secretion, cognition, and central cardiovascular functions
Endomorphins and ß-endorphin	Mu	Morphine Loperamide	Gastrointestinal motility, respiration, nociception, hormonal secretions
Dynorphins	Kappa	U69593	Nociception, diuresis, hormonal secretions

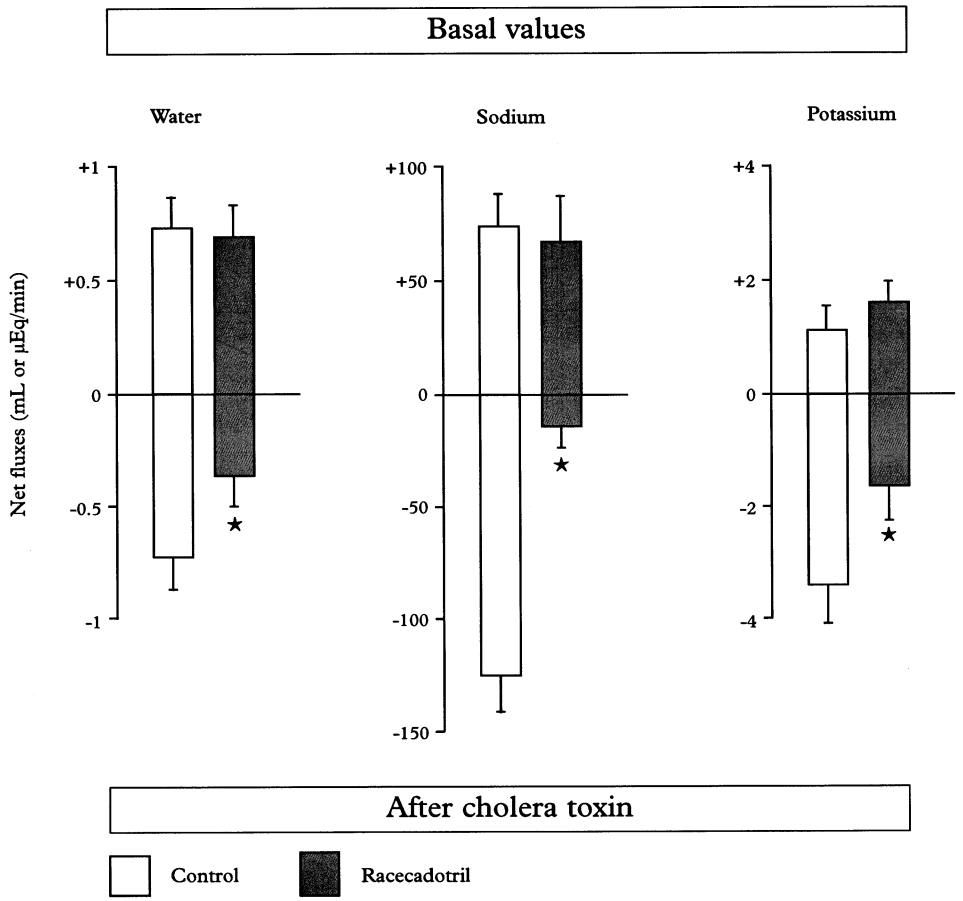

Fig. 1. Effects of oral racecadotril (10 mg/kg) on water, sodium, and potassium fluxes in dogs (mean ± SD; $n = 9$) before and after cholera toxin infusion (0.4 mg/l for 2 h). $*P = 0.01$ *v* control measurements. A positive value denotes net absorption and a negative value net secretion. 'Basal values' 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. (After Primi et al., 1999 [8]).

In summary, agents such as enterotoxins, vasoactive intestinal peptide, or prostaglandin E_2 trigger an increase in cyclic AMP levels, leading to hypersecretion of water and electrolytes. Enkephalins are released in an attempt to reduce the levels of cyclic AMP and thus counteract this hypersecretion. The enkephalins exert their effect via the δ-receptor. However, their inactivation by enkephalinase limits their antisecretory activity. The enkephalinase inhibitor, racecadotril, prevents the breakdown of enkephalins and prolongs their antisecretory effect.

4. Racecadotril does not prolong gastrointestinal transit time

In contrast to drugs that act via the μ-receptor, racecadotril does not prolong gastrointestinal transit time. This has been demonstrated in a number of preclinical studies.

Rats were pretreated with racecadotril (40 mg/kg po) or the μ-receptor agonist loperamide (2 mg/kg po) [9]. A charcoal suspension (1 ml) was given orally by gavage 20 min later. The animals were killed after a further 20 min and their intestines examined to see how far the charcoal had travelled along the small intestine. Loperamide reduced gastrointestinal transit by 27% compared with control animals ($P < 0.05$), whereas racecadotril had no significant effect on transit.

The same experiment was carried out in rats who had received castor oil 60 min before racecadotril and 80 min before the charcoal suspension [9]. Once again, loperamide produced a significant reduction in gastrointestinal transit compared with control animals ($P < 0.01$), while racecadotril had no significant effect.

The effect of racecadotril on colonic transit was assessed in rats and mice who received the drug (40 mg/kg po) 60 min after castor oil and 20 min after charcoal suspension [9]. The results showed that racecadotril had no significant effect on colonic transit.

4.1. No promotion of bacterial overgrowth

An important consequence of racecadotril's lack of effect on gastrointestinal transit is that the drug will not

promote overgrowth of bacteria in the small intestine, a side-effect that can lead to serious complications such as toxic megacolon or 'blind-loop syndrome', which have been observed with antimotility drugs [10,11].

The effects of racecadotril and loperamide on the intestinal overgrowth of a non-pathogenic strain of *Escherichia coli* (E 404) were compared in newborn gnotobiotic piglets [12]. Piglets were orally inoculated with this bacterium on day 3 after birth, 1 h before oral treatment with racecadotril (20 mg/kg bid) or loperamide (1 mg/kg bid). Both drugs were given for 4 days, at the end of which time the animals were sacrificed using chloroform. The *E. coli* content of the proximal jejunum and the *E. coli* ratio of stomach:proximal jejunum were similar in racecadotril-treated and in control animals. In contrast, loperamide-treated animals had a significantly higher *E. coli* content in the proximal jejunum than the racecadotril or control groups ($P = 0.04$ and 0.005, respectively). Similar results were obtained for the *E. coli* ratio of stomach: proximal jejunum ($P = 0.05$ and 0.03, respectively, versus racecadotril and control animals). The results of this study are summarized in Table 2.

5. Racecadotril is well tolerated centrally

When both drugs were given bid for 4 days at high oral doses in the above experiment (130 mg/kg bid racecadotril and 5 mg/kg loperamide bid), three out of four piglets on loperamide exhibited neurotoxicity followed by death. In contrast, animals receiving racecadotril showed no signs of clinical neurotoxicity and no deaths occurred. The reason for this difference lies in the fact that these newborn piglets have an immature blood–brain barrier, allowing both drugs to enter the central nervous system. Hence this study demonstrates the good neurological tolerability of racecadotril compared with loperamide, which is

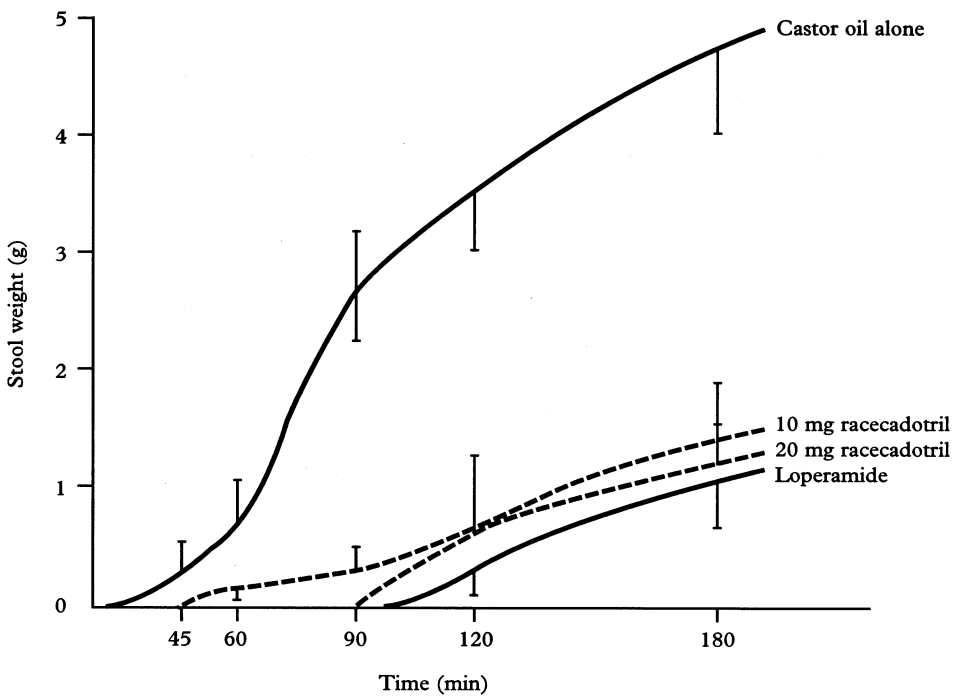

Fig. 2. Mean (± SEM) cumulative stool weights in rats who received racecadotril (10 or 20 mg/kg iv) or loperamide (2 mg/kg po) 30 min before oral administration of castor oil. $P < 0.01$ Castor oil versus active treatment. (After Marçais-Collado et al., 1987 [9]).

Table 2
E. coli content of the proximal jejunum (S_1) in gnotobiotic piglets: comparison of bacterial proliferation after administration of racecadotril or loperamide (after Duval-lflah et al., 1999 [12])

Number of *E. coli*/g content (median)	Racecadotril (20 mg/kg bid)	Loperamide (1 mg/kg bid)	Control	P^a
Log_{10}	6.0	8.1	6.6	0.03
10^6	1	120	4	
P[b]	0.04	0.005		
	NS ($P^b = 0.86$)			

[a] Kruskal–Wallis test.
[b] Newman–Keuls test.

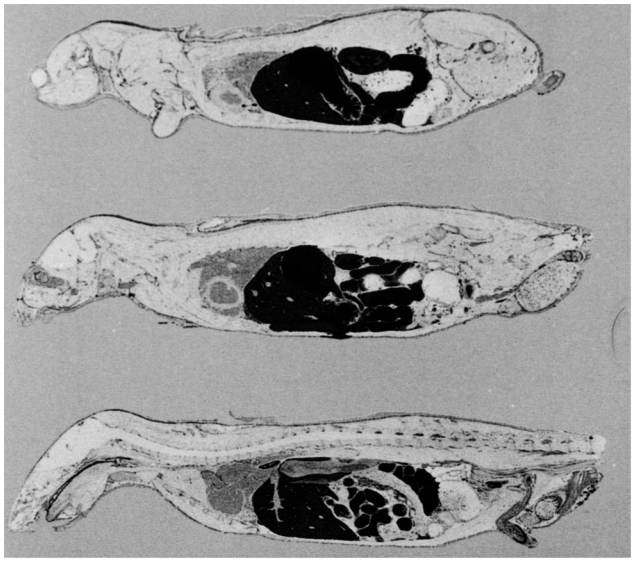

Fig. 3. Whole body autoradiography of a rat which received [^{14}C] racecadotril (10 mg/kg po) 1 h before sacrifice.

known to cause central toxicity. Because of this toxicity, loperamide is not recommended for children aged less than 2 years [13].

A further study was carried out to examine the distribution of radiolabelled racecadotril in the brain after oral administration in rats. [^{14}C] racecadotril (10 mg/kg) was given orally 1 h before the animals were sacrificed. Fig. 3 demonstrates that, although racecadotril is well distributed throughout the gastrointestinal tract, the drug does not enter the brain, once again demonstrating the selective action of racecadotril.

Studies were carried out in rats and monkeys to discover whether racecadotril demonstrated any potential for abuse or physical dependence. The results of these studies were negative [14]. Other studies in mice and rats have failed to demonstrate any central analgesic effect with racecadotril [15].

6. Conclusion

The results of the preclinical studies carried out with racecadotril show that this compound is a potent and specific inhibitor of enkephalinase, and thereby acts to prolong the antisecretory effect of the enkephalins in the gastrointestinal tract. Racecadotril has proven effective in models of hypersecretory diarrhoea, and does not increase intestinal transit time, again demonstrating the drug's specificity of action. Central tolerability is good, even in animals with an immature blood–brain barrier, and no abuse potential or physical dependence has been observed since the drug does not enter the brain after oral administration. The results of the preclinical studies indicate that racecadotril should prove effective and well tolerated in the treatment of hypersecretory diarrhoea in man.

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