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Short communication

The effects of cisapride on elemental content in neonatal mouse small intestine in vivo

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Abstract

The effects of cisapride on elemental content of enterocytes, smooth muscle cells and lamina propria in neonatal mouse jejunum were studied using electron probe X-ray microanalysis. One hour after injection with cisapride (1.7 or 0.017 mg/kg body weight i.p.), Cl content was significantly reduced in villus base, crypt and smooth muscle cells and Na content decreased in muscle cells. No changes were observed in Na or Cl within villus tip cells. Total Ca content did not change significantly in any cell type following treatment with cisapride. These results confirm that cisapride induces net Cl^- secretion in neonatal mouse jejunum.

Keywords: Cisapride; Electron probe X-ray microanalysis; Small intestine; (Mouse)

1. Introduction

Cisapride is a recently developed gastrointestinal prokinetic agent which is used for the treatment of gastrointestinal disorders, including gastro-oesophageal reflux, gastroparesis and chronic constipation (Tack et al., 1995). It is thought that cisapride increases gut motility and fluid secretion by acting as a 5-HT_4 receptor partial agonist (Meulemans and Schuurkes, 1992; Hansen and Jaffe, 1994), although differences between animal species and intestinal region exist (Deridder and Schuurkes, 1993; Franks et al., 1995), reflecting the complex nature of the action of 5-HT in the gut (Scott et al., 1992; Kellum et al., 1994). No data exist on the effects of cisapride on ion transport in the neontal gut. We have therefore used the technique of electron probe X-ray microanalysis (EPXMA), to measure elemental content in individual intestinal cells in neonatal mouse jejunum and confirm that cisapride causes increased Cl^- secretion without effect on NaCl absorption.

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2. Materials and methods

2.1. Administration of cisapride

Nine-day-old unfasted, suckling Balb/C mice were injected with cisapride (Janssen Pharmaceutica, Beerse, Belgium) i.p., at a dose of 1.7 or 0.017 mg/kg body weight. Age-matched controls were injected i.p with vehicle alone. Mice were observed for 1 h after drug administration for signs of defaecation or any side-effects. The mice were then killed by decapitation and samples distal jejunum, 1–3 mm in length dissected and rapidly plunge-frozen in liquid propane cooled by liquid nitrogen (Spencer et al., 1990).

2.2. Cryoultramicrotomy

Ultrathin cryosections (300 nm) were obtained with an FC4/Ultracut E cryoultramicrotome (Leica, UK), using dry glass knives at a sectioning temperature of 133 K. Sections were mounted on formvar/carbon-coated Ti grids, sandwiched under a second grid and freeze dried at a temperature of 193 K overnight. After warming to room temperature, the grids were separated, coated with a sec-

ond layer of carbon and stored under desiccating conditions before analysis.

2.3. Electron probe X-ray microanalysis

Sections were observed and analysed in a JEOL 100CX II electron microscope, fitted with a high resolution scanning attachment, LaB_6 filament and a Link 860 series 2, 30 mm^2 Si(Li) detector and multichannel analyser (Spencer et al., 1990). Data for villus tip, base and crypt cells and muscle cells were obtained from regions of cytoplasm free of mitochondria, using a scanning raster (0.10 μm^2) at $\times 40\,000$ magnification. Data for lamina propria were obtained from large areas (approximately 25 μm^2) which included both intra- and extracellular components.

Quantitation of elemental content for Na, Cl, K and Ca in terms of mmol/kg dry weight was performed with reference to gelatin/inorganic salt standards and spectra corrected for the contribution of the grid and film to the background continuum.

2.4. Statistics

Statistical comparisons were made by nested analysis of variance (ANOVA), using Dunnett's post-hoc procedure for comparing controls with cisapride-treated groups.

3. Results

One hour after drug administration, mice treated with 1.7 mg/kg body weight cisapride appeared drowsy and lethargic, when compared with control animals and mice treated with 0.017 mg/kg body weight cisapride. Mice in both cisapride-treated groups had clear signs of increased defaecation (2–3 and 2–4 faecal pellets during the 1 h period for 0.017 and 1.7 mg/kg body weight cisapride-treated groups, respectively) the pellets being of normal appearance. Control mice did not defaecate during the period of observation.

Fig. 1 shows an unstained freeze dried cryosection of the crypt region of mouse jejunum. No morphological differences were seen between control and cisapride-treated mouse intestine. The content of elements in cytoplasm of villus tip, villus base, crypt, and muscle cells and areas of submucosal lamina propria of control and cisapride-treated tissues is given in Table 1.

One hour after treatment with cisapride, significant differences in elemental content were observed after administration of both doses of cisapride (0.017 and 1.7 mg/kg). With only one exception, the changes described below relate to data from both cisapride-treated groups. Cisapride had no statistically significant effect on elemental content of villus tip cells. However, in crypt and villus base cells, cisapride caused a significant reduction in Cl content, without effect on Na or K content. In contrast, Cl

Fig. 1. Unstained freeze-dried cryosection of 9-day-old mouse small intestine, 1 h after treatment with cisapride, sectioned through the villus region. Brush border (arrowhead), nuclei (N), lumen (asterisk). Scale bar represents 5 μm.

Table 1
Elemental content in cytoplasm of villus tip, villus base and crypt cells, lamina propria and smooth muscle cells, from control and cisapride-treated mouse jejunum

	n	Content (mmol/kg dry weight)			
		Na	K	Cl	Ca
Villus tip cells					
Control	42	179 ± 8	729 ± 15	214 ± 5	6.6 ± 1.3
Cisapride (1.7 mg/kg)	84	173 ± 9	731 ± 18	229 ± 7	7.1 ± 1.2
Cisapride (0.017 mg/kg)	44	165 ± 10	695 ± 21	214 ± 7	3.6 ± 1.3
Villus base cells					
Control	53	112 ± 8	758 ± 17	232 ± 7	5.7 ± 1.1
Cisapride (1.7 mg/kg)	21	92 ± 8	702 ± 21	189 ± 4 [b]	6.7 ± 1.5
Cisapride (0.017 mg/kg)	29	86 ± 10	718 ± 28	192 ± 12 [b]	9.3 ± 3.1
Crypt cells					
Control	62	133 ± 8	892 ± 17	221 ± 7	4.6 ± 1.0
Cisapride (1.7 mg/kg)	48	124 ± 7	925 ± 21	180 ± 6 [b]	7.0 ± 2.0
Cisapride (0.017 mg/kg)	66	118 ± 10	844 ± 17	174 ± 9 [b]	2.0 ± 1.3
Lamina propria					
Control	16	291 ± 25	715 ± 35	280 ± 25	4.0 ± 2.6
Cisapride (1.7 mg/kg)	23	322 ± 25	709 ± 30	325 ± 9 [a]	2.4 ± 1.9
Cisapride (0.017 mg/kg)	24	277 ± 18	614 ± 18 [a]	377 ± 14 [b]	6.8 ± 1.1
Smooth muscle cells					
Control	48	132 ± 6	590 ± 12	218 ± 5	3.8 ± 1.0
Cisapride (1.7 mg/kg)	37	92 ± 5 [b]	550 ± 24	152 ± 10 [b]	5.1 ± 1.3
Cisapride (0.017 mg/kg)	41	94 ± 7 [b]	603 ± 19	131 ± 6 [b]	1.7 ± 1.2

Results are mean ± S.E.M. from three animals per group; n = number of cells analysed. Significant difference from control is indicated by [a] $P < 0.05$, [b] $P < 0.01$.

content was increased in the lamina propria. The K content was also significantly reduced in lamina propria (0.017 mg/kg group only).

In the cytoplasm of smooth muscle cells, Na and Cl content was significantly decreased. No statistically significant changes were observed in total Ca content in any compartment.

4. Discussion

Cisapride enhanced gastrointestinal motility in the neonatal mice used in this study, in agreement with previous studies in adult mice (Haga et al., 1994). The significant reduction in Na and Cl content of smooth muscle cells is consistent with the enhanced muscle contraction which occurs following treatment with cisapride (Tack et al., 1995). The content of Na and Cl in peripheral cytoplasm of vascular smooth muscle cells is similarly reduced after contraction (Bond et al., 1984). The higher dose of cisapride (1.7 mg/kg) resulted in the mice being lethargic. This side-effect has been reported to be mild or non-existent in humans (Tack et al., 1995). Mice given 0.017 mg/kg did not exhibit signs of lethargy, but showed changes in intracellular elemental content comparable to the mice given cisapride at 1.7 mg/kg.

Cisapride had a significant effect on elemental content of various compartments in neonatal mouse jejunum. Cisapride has no direct effect on enterocytes: addition of cisapride to in vitro intestinal preparations stripped of muscle and myenteric plexus did not change short circuit current in rat ileum (Moriarty et al., 1987). Cisapride blocks 5-HT-mediated secretion in vitro (Moriarty et al., 1987), but endogenous 5-HT does not contribute to the intrinsic transport properties of stripped intestinal preparations in vitro (Moriarty et al., 1987). It is possible that the differences in crypt and villus base cell cytoplasm of control and cisapride-treated mice represent inhibition of Cl^- transport normally mediated by tonic release of 5-HT in vivo, reducing neutral Cl^- entry into crypt and villus base cells across the basolateral membrane via the $NaCl_2K$ cotransport mechanism and therefore giving rise to the observed increase in Cl content in the lamina propria. However, changes in lamina propria are difficult to interpret given that measurements are taken over a large area, including contributions from both intracellular and extracellular sources. Other methods must therefore be used to measure Cl content in extracellular fluid as this is not possible in freeze dried cryosections.

In contrast, cisapride increases short circuit current in vitro in unstripped rat small intestine (Hardcastle et al., 1984) and stripped guinea pig ileum (Scott et al., 1992). Cisapride also induces secretion in rat small intestine in vivo (Hansen and Jaffe, 1994; Hardcastle et al., 1984), although the latter group has recently shown that low intravenous doses of cisapride do not change transintestinal potential difference in rat intestine in vivo (Franks et al., 1995). Stimulation of intestinal secretion in the adult mouse leads to a decrease in crypt cell Cl content, as determined by EPXMA, due to an increased flux of Cl^- across the cell (Von Zglinicki and Roomans, 1989). The observed decrease in crypt/villus base cell Cl content in the present study is therefore consistent with cisapride-induced net Cl^- secretion mediated via 5-HT_4 receptor activation, in agreement with studies in other species (Hansen and Jaffe, 1994; Scott et al., 1992). The location of the 5-HT_4 receptor is unclear, but may be non-neuronal in mice (Hegde et al., 1994) and other species (Scott et al., 1992; Kellum et al., 1994).

Cl content also decreased in villus base cells, following stimulation with cisapride, suggesting a potential secretory role for cells outside the crypt. Measurements of apical membrane potentials along the crypt villus axis in rat small intestine in response to secretagogues suggest that secretion can occur both in crypt and villus cells (Stewart and Turnberg, 1989). However, cisapride did not change Na, K or Cl content in villus tip cells. These data also suggest that 5-HT_4 receptor activation does not inhibit coupled NaCl absorption in mouse jejunum, in agreement with studies in human jejunum (Kellum et al., 1994).

No significant changes in Ca content were observed following treatment with cisapride. However, this does not preclude changes in cytoplasmic Ca^{2+} concentration, which modulate many cell processes including ion transport, since EPXMA measures total Ca content (i.e. free ion + bound), but where the majority is in the bound state.

In conclusion, cisapride causes increased motility and secretion in the small intestine of neonatal mice in agreement with previous studies. In addition, cisapride does not affect neutral NaCl uptake in the neonatal small intestine.

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