

Direct Mucosal Targeting of Colonic Receptors by Prokinetic Drugs in an Experimental Model

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Abstract

Isolated perfused segments of pig ileum and sigmoid colon were used as an extrinsically denervated model of intestinal fluid propulsion to compare the effects of intraluminal (IL) with intraarterial (IA) administration of cisapride and mebeverine. The ileal segments had a spontaneous mean activity of 0.008 (SEM 0.003) ml Krebs propelled aborally min⁻¹, with propulsive waves at a mean frequency of 8.3 (1.6) min⁻¹. The sigmoid colon segments ejected a mean of 0.013 (0.009) ml Krebs min⁻¹, with propulsive waves at 3.9 (0.8) min⁻¹. IL cisapride produced a dose-dependent response in the dose range 1 x 10⁻⁹ M to 3 x 10⁻¹ & 1-3 x 10⁻⁷ M in the ileum, and 3 x 10⁻¹¹ to 3 x 10⁻⁹ M in the colonic segments, IL cisapride was significantly more effective than IA delivery of equivalent doses. IL instilled mebeverine (1 x 10⁻⁶ M) inhibited the carbachol dose response of the ileal and colonic segments more than an equivalent dose of mebeverine infused IA. We conclude that the isolated perfused pig intestine is an effective model for studying the pharmacological effects of drugs and their routes of delivery. Cisapride and mebeverine were more effective per given concentration, when delivered IL than IA in both the ileum and sigmoid colon preparations. The qualitative effects of either IL or IA drug delivery were not affected by extrinsic denervation.

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Introduction

The direct intraluminal (IL) administration of drugs into the intestine may have an important regional action and therefore clinical implications in the effective management of functional bowel disorders. We have previously published findings in the anaesthetised *in vivo* pig, that cisapride and mebeverine when infused IL into the sigmoid colon resulted in a pharmacological dose dependent responses, which was more efficacious and localised than systemic administration of these drugs.¹ The maximum effect achieved with IL administration was compared with and found greater than that achieved with intravenous delivery.

The purpose of the present study was to compare the effects of IL instilled drug with equivalent doses of intra-arterial (IA) drug in an appropriate *in vitro* model, where higher concentrations of IA infused drugs would be achieved without dilution into the body fluid volume. We also wanted to test whether the effects of IL drug administration were confined to the colon or occurred in

the small intestine because the mode of action of cisapride in the guinea pig ileum differs from that in the colon.²⁻⁴ Furthermore, as the response of pancreatic secretion to intraluminal amino acids and fatty acids depends upon the vagal reflexes,^{5,6} we were interested in the effects of extrinsic denervation upon the response of gastrointestinal motility to IL drug administration.

Weems et al⁷⁻⁹ developed an effective isolated perfused experimental model, for studying the effect of IA infused drugs on cat intestinal motility. A segment of intestine was kept viable by infusing the artery with oxygenated Krebs solution and immersion in a heated water bath. A constant volume of Krebs solution was maintained in the lumen of the isolated intestine. As the intestine segment propelled the intraluminal contents from its oral to aboral end, certain pressures were generated onto the intraluminal fluids. By measuring these pressures, the motility of the segment of intestine could then be deduced.¹⁰ We applied the pig intestine to this model, because the pig resembles man in its omnivorous

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diet and its colonic longitudinal muscle is concentrated into taenia coli.

Materials and Methods

Sixteen segments of intestine with intact vascular supply were freshly harvested from 9 abattoir pigs sacrificed by exsanguination and 7 anaesthetised laboratory animals. The laboratory animals were "large white pigs" in breed, bred specially by the University Department of Agriculture, University of Nottingham, United Kingdom. Anaesthesia was by intravenous metomidate (Janssen Pharmaceutica, Belgium) at a dose of 1.3 mg/kg animal body weight. There were 8 pieces of terminal ileum, of mean length 16.1 (0.7) cm and 8 segments of sigmoid colon of mean length 16.3 (0.8) cm. These intestinal segments were preserved in modified Krebs solution (Na^+ , 137 mM; K^+ , 5.9 mM; Ca^{2+} , 2.5 mM; Mg^{2+} , 1.2 mM; Cl^- , 134 mM; HCO_3^- , 15.5 mM; H_2PO_4^- , 1.2 mM; glucose, 11.5 mM, saturated with 95% oxygen and 5% carbon dioxide) and rapidly transported to the laboratory.

The supplying artery was cannulated and perfused with the oxygen saturated modified Krebs solution, containing Dextran 70 (30 mg ml^{-1}) to reduce oedema, at a rate of 5 to 6 ml min^{-1} . The perfusing solution was maintained at a temperature of 37°C by passing through a heating coil. An attached 3-way tap allowed for IA administration of drugs.

Placed at each cut end of the bowel was a minimal resistance, constant capacitance system consisting of a 75 cm long, 2 mm diameter glass tubing containing a vertical column of modified Krebs solution, open to the atmosphere at the top (Fig. 1). These were attached to the open end of the bowel by a 3 cm long portex tubing horizontal limb, over which the intestine was double tied with linen. Another horizontal limb was connected via a 3-way tap to pressure transducers (Statham, UK). This 3-way tap enabled the appropriate amount of fluid to be introduced into the system, and IL administration of drugs into the oral end of the bowel lumen. The intestinal segment ends were then allowed to lie about 8 cm apart, to form a small loop without tension.

Modified Krebs solution was then infused into the intestinal lumen to exclude any air, and until an equal column of fluid pressure was obtained at each end of the gut. In the ileal segments, the mean volume infused was 17.5 (1.8) ml with a mean pressure of 6.1 (0.4) cm H_2O , and in the sigmoid colon specimens, the mean volume infused was 13 (2.2) ml with a mean pressure of 6.8 (0.4) cm H_2O . The preparation was immersed in an organ bath containing 2 L modified Krebs solution saturated with 95% oxygen and 5% carbon dioxide, kept at a constant temperature of 37°C by a surrounding heated water bath. In addition, the organ bath temperature was monitored with a digital thermometer (Comark, USA).

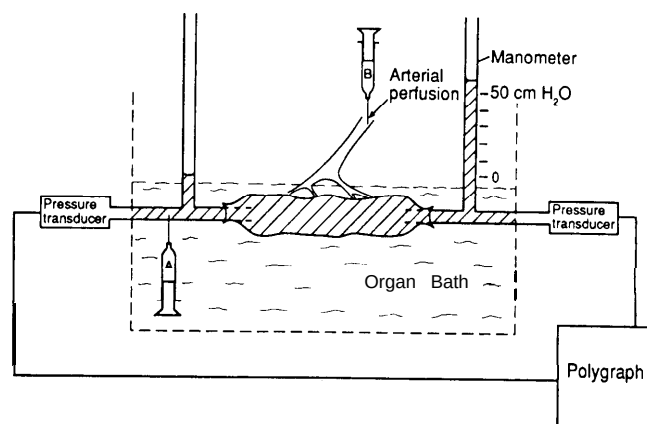


Fig. 1. Diagrammatic representation of experimental set-up. Drugs were delivered either intraluminally via 3-way tap (A; at arboreal end) or intra-arterially via 3-way tap (B).

The organ bath was replaced with fresh modified Krebs solution at 1 h intervals.

The first 15 minutes of recordings were discarded while the experimental set-up stabilised, and the next 30 minutes of observation were used as baseline. The drugs administered were IL and IA cisapride, IA carbachol, and IL and IA mebeverine. For IL instillation, 2 ml of intraluminal fluid was aspirated via the 3-way tap, the drug gently delivered and the difference in volume to make up the 2 ml replaced. This procedure was carefully performed to avoid physical stimulation of the intestine by distension. Adequate time was allowed for the bowel to recover to its baseline activity between various dosages of drugs.

The pressure transducer output was recorded via a recorder (Elcomatic, UK) and the pressure changes per unit time calculated manually. The difference in motility between the distal and proximal end was estimated, this having been previously shown to be related to the amount of work performed in propelling intraluminal fluid distally.⁷ Thus, the motility index was worked out by integrating the pressure waveform changes over the 3 minutes of maximum response following the infusion of various drugs. The response between intestinal segments was compared by percentage of maximum response. The data was analysed with a computer using the Statistical Package for Social Sciences (SPSSPC+) version 2 software package. The appropriate paired and unpaired paired t-test was then used in the statistical analysis, applying two tailed comparisons for cisapride because the effects may be stimulatory or inhibitory⁴ and 1 tailed comparisons for mebeverine, where the action is known to be only that of inhibition.¹

Results

The baseline spontaneous activity in the ileum consisted of a mean 0.008 (0.003) ml Krebs propelled distally

min⁻¹ [30.1 (5.4)% maximum response] at a frequency of 8.3 (1.6) waves min⁻¹. In the sigmoid colon segments, the spontaneous propulsion waves ejected 0.013 (0.009) ml Krebs min⁻¹ [14.9 (1.8)% maximum response] at a frequency of 3.9 (0.8) waves min⁻¹. Addition of drugs into the 2 L of fluids in the organ bath was too diluted to have any effect. The experiments lasted about 3 h, and there was neither oedema nor any deterioration in the performance of the preparations noted at the end of the procedures. No significant differences were seen between the intestinal segments obtained from abattoir and laboratory animals.

Effects of Cisapride

Cisapride was given initially by the IL route in 4 ileum and 4 sigmoid colon segments, after which cisapride was administered by the IA route, cisapride was given initially by the IA routes in another 4 ileum and 4 sigmoid colon segments, after which cisapride was given by the IL route. The effect of either IL or IA cisapride on the bowel preparations was short lasting (less than 3 min) and the sequence of drug delivery route, whether ini-

tially IL or IA made no difference to the results. In both the ileal and sigmoid colon segments, the response to each given dose of cisapride was higher when given IL then IA (Figs. 2 & 3). The greater response per dose given IL compared to IA was statistically significant ($P < 0.05$) for doses from 3×10^{-11} to 1×10^{-9} M and 1×10^{-7} to 3×10^{-7} M in the ileum and 3×10^{-11} to 3×10^{-9} M in the sigmoid colon experiments (Table I).

Effects of Mebeverine

Doses of IA carbachol were given and then repeated after IA and IL mebeverine administration. Mebeverine was longer acting (up to 3 h). For the purposes of these experiments, a dose of 1×10^{-6} M mebeverine given either IL or IA was found to give optimal suppression of intestinal activity in both the ileal and sigmoid colon preparations. This partially suppressed the intestinal activity to carbachol to a noticeable extent, but not completely abolished any further activity. The latter would make it impossible to compare the difference between administered drugs.

In 4 of the ileal and 4 of the sigmoid colon preparations, 1×10^{-6} M mebeverine was instilled IL, which shifted the carbachol dose response curve downward and to the right (Figs. 4 & 5). The response was further inhibited by an additional similar dose infused IA, to demonstrate the availability of receptors via the alternative route. The inhibition of the carbachol response by IL mebeverine was statistically significant ($P < 0.05$) for the dose range 3×10^{-8} to 3×10^{-6} M carbachol in both the ileum and sigmoid colon. The addition 1×10^{-6} M IA mebeverine further inhibited the response significantly ($P < 0.05$) for the dose range 3×10^{-8} to 3×10^{-6} M carbachol in the ileum, and range 3×10^{-8} to 1×10^{-6} M carbachol in the sigmoid colon.

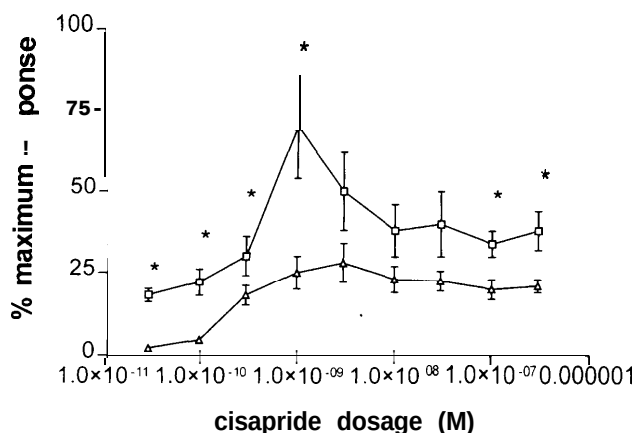


Fig. 2. Response to cisapride instilled intraluminally ($\square$) and intraarterially ($\triangle$) into ileum ($n = 8$). Values given in mean % maximum response of motility index (error bars show SEM). * $P < 0.05$ (paired t-test).

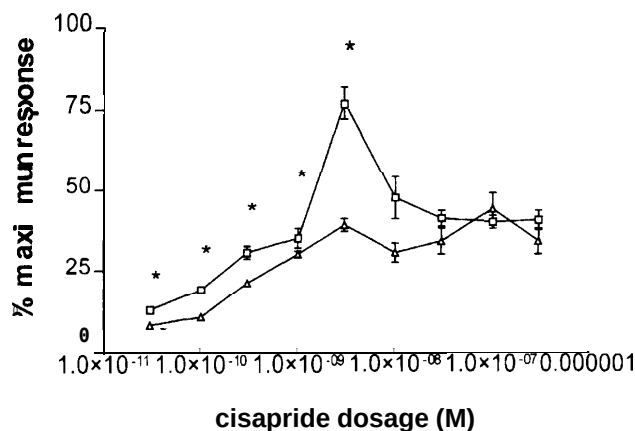


Fig. 3. Response to cisapride instilled intraluminally ($\square$) and intraarterially ($\triangle$) into sigmoid colon ($n = 8$). Values given in mean % maximum response of motility index (error bars show SEM). * $P < 0.05$ (paired t-test).

TABLE I: DIFFERENCES IN RESPONSE TO INTRALUMINAL (IL) AND INTRAARTERIAL (IA) ADMINISTERED CISAPRIDE IN ILEUM AND SIGMOID COLON

Dosage (M)	Ileum		Sigmoid colon	
	(IL - IA) response	P	(IL - IA) response	P
3×10^{-11}	2.6 (1.7)	<0.001	4.9 (1.4)	0.02
1×10^{-10}	18.1 (2.9)	0.003	7.1 (0.8)	<0.001
3×10^{-10}	10 (2.8)	0.002	8.8 (1.5)	0.002
1×10^{-9}	43.6 (11)	0.01	6.2 (1.7)	0.01
3×10^{-9}	27.7 (15.1)	0.14	37.1 (8.1)	0.004
1×10^{-8}	13.2 (5.1)	0.06	15.2 (12.5)	0.3
3×10^{-8}	16.8 (9.6)	0.14	7.4 (5.5)	0.2
1×10^{-7}	13.4 (2.3)	0.004	-5.1 (10.6)	0.6
3×10^{-7}	15.6 (4)	0.01	5.4 (9.1)	0.6

(IL - IA) response was calculated by subtracting the response obtained with IA from the response obtained with IA cisapride. This response was measured as the % maximum response of the motility index obtainable from each of the isolated perfused preparations. Values are given as mean (SEM). Two tailed paired t-test used for statistical analysis.

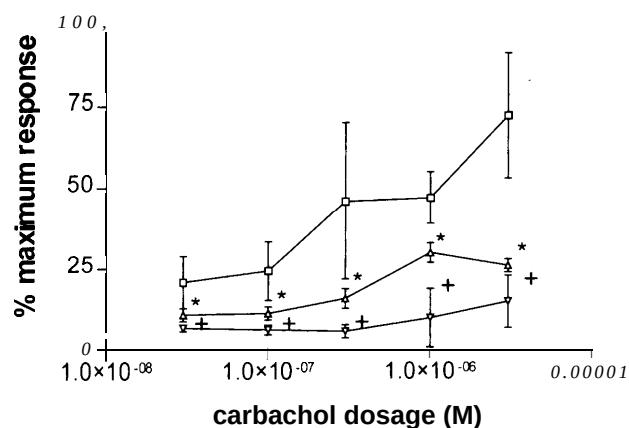


Fig. 4. Carbachol response in ileum ($n=4$) before ($\square$) and after intraluminal instilled mebeverine (A; 1×10^{-6} M). * $P < 0.05$ (paired t-test). Further inhibition to carbachol response with intra-arterial mebeverine (∇ ; 1×10^{-6} M). + $P < 0.05$ (paired t-test). Values given in mean % maximum response of motility index (error bars show SEM).

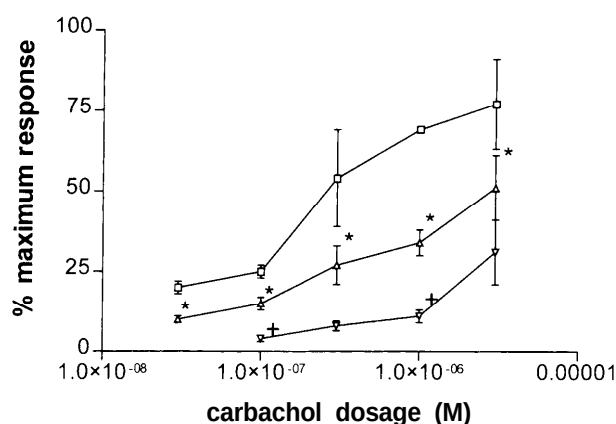


Fig. 5. Carbachol response in sigmoid colon ($n=4$) before ($\square$) and after intraluminal instilled mebeverine (Δ ; 1×10^{-6} M). * $P < 0.05$ (paired t-test). Further inhibition to carbachol response with intra-arterial mebeverine (∇ ; 1×10^{-6} M). + $P < 0.05$ (paired t-test). Values given in mean % of maximum response of motility index (error bars show SEM).

In the remaining 4 ileum and 4 sigmoid colon segments, 1×10^{-6} M mebeverine was delivered IA which resulted in inhibition of the carbachol dose response curve (Figs. 6 & 7). The suppression of the carbachol response was statistically significant ($P < 0.05$) for the dose of 3×10^{-6} M in the ileum, and for the dosage range 1×10^{-7} to 1×10^{-6} M carbachol in the sigmoid colon. A further dose of 1×10^{-6} M IA mebeverine gave additional retardation of the carbachol response, significant ($P < 0.05$) at the dosage range of 1×10^{-7} to 1×10^{-6} M carbachol in both the ileum and in the sigmoid colon segments.

The inhibitory effects of IL and IA mebeverine were compared (Table II). IL administration usually produced a greater degree of suppression of the carbachol response, which was statistically significant at a dose of 3×10^{-8} M carbachol in the ileal segments and 1×10^{-7} M carbachol in the sigmoid segment preparations.

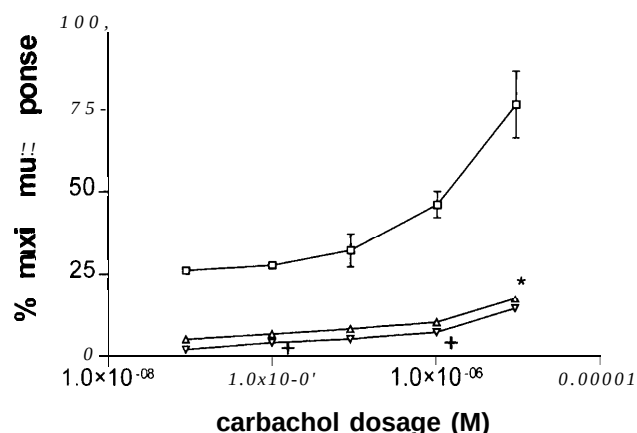


Fig. 6. Carbachol response in ileum ($n=4$) before ($\square$) and after intraarterial instilled mebeverine (Δ ; 1×10^{-6} M). * $P < 0.05$ (paired t-test). Further inhibition to carbachol response with intraluminal mebeverine (∇ ; 1×10^{-6} M). + $P < 0.05$ (paired t-test). Values given in mean % of maximum response of motility response (error bars show SEM).

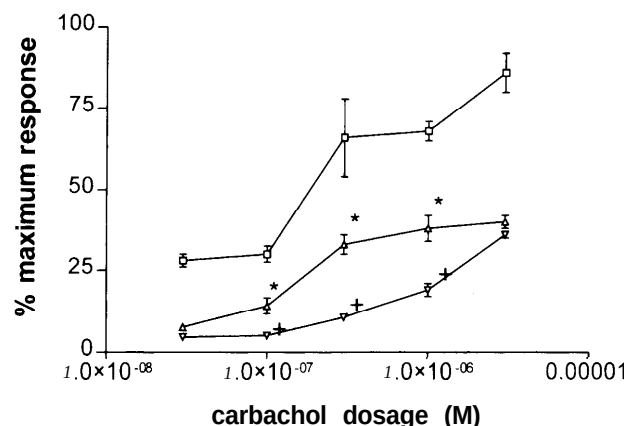


Fig. 7. Carbachol response in sigmoid colon ($n=4$) before ($\square$) and after intraarterial instilled mebeverine (Δ ; 1×10^{-6} M). * $P < 0.05$ (paired t-test). Further inhibition to carbachol response with intraluminal mebeverine (∇ ; 1×10^{-6} M). + $P < 0.05$ (paired t-test). Values given in mean % of maximum response of motility index (error bars show SEM).

Discussion

An isolated perfused model for studying motility in cat intestine^{1,12} was adapted to work for pig intestine, which is anatomically and physiologically more similar to humans. We found that with this experimental set-up, the pig intestine preparation remained viable for at least 3 h. We were able to observe spontaneous propulsive waves at 8.3 min^{-1} in the ileum and 3.9 min^{-1} in the sigmoid colon, which were faster than that in cats.^{1,12} Assuming that the motility dynamics were likely to be similar to the cat model, where the propulsive activity may be influenced by the intraluminal pressure^{12,13} the intestinal segments were loaded with fluid to an equal column of pressure. Furthermore, care was taken in instilling the IL drugs so that extra fluid was not introduced into the system and that mechanical distension of the intestinal segments was kept minimal.

We showed previously that cisapride and mebeverine

TABLE II: COMPARISON OF INTRALUMINAL (IL) WITH INTRAARTERIAL (IA) MEBEVERINE INHIBITION OF CARBACHOL RESPONSE IN ILEUM AND SIGMOID COLON

Dose (M)	Ileum			Sigmoid colon		
	IL inhibition	IA inhibition	P	IL inhibition	IA inhibition	P
3×10^{-8}	21.1 (0.9)	1.8 (1.8)	0.02	20.1 (1.5)	9.2 (3)	0.06
1×10^{-7}	20.8 (3.3)	13.9 (7.7)	0.5	20.7 (3.2)	9.1 (3.5)	0.05
3×10^{-7}	26.7 (5)	29.5 (19.7)	0.9	32.3 (9.7)	42.9 (10)	0.7
1×10^{-6}	34.2 (2.1)	14 (8.1)	0.09	28.8 (0.5)	32.5 (3.4)	0.4
3×10^{-6}	53.8 (9.2)	46 (14.6)	0.7	45.4 (7)	33.1 (1.8)	0.4

IL inhibition and IA inhibition were the amount of change in the carbachol dose response, after administration of 1×10^{-6} M of mebeverine via the IL or IA route. These were measured as the % maximum response of the motility index obtainable from each of the isolated perfused preparations. Values are given as mean (SEM). Two tailed unpaired t-test used for statistical analysis.

have a localised effect upon the colon when instilled IL, which would be expected from their typical pharmacological properties, i.e. stimulatory and inhibitory respectively, in the anaesthetised pig.¹ The response to IL cisapride was dose dependent and these properties would not be expected if it acted by non-specific chemical irritation of the colon. The maximum effect achieved with IL administration was compared with that attained with intravenous drug delivery. A greater response observed with IL cisapride than intravenous cisapride and the lack of drug action in the ascending colon when instilled into the sigmoid colon suggested a local mode of action rather than a systemic action following absorption in the colon. In our present study, the *in vitro* isolated perfused pig intestine was an ideal model for comparing the effects of IL instilled drug with equivalent doses of IA drug, which could be delivered in high concentrations without dilution into the body fluid volume. Adding the drugs to the 2 L organ bath was too diluted to be effective and, therefore, the action of IA administered drugs was systemic. Another unproven assumption of this model was that when motility returned to baseline levels, the drugs instilled had no further effect upon the tissue receptors or had been completely inactivated by metabolism. However, despite the higher systemic concentrations delivered regionally, IL instillation still resulted in a higher response than IA infused drugs. Furthermore, the response achieved in these extrinsically denervated *in vitro* preparations showed that central nervous system reflexes, such as the stimulation of pancreatic secretion by intraluminal amino acids and fatty acids via vagal reflexes^{5,6} was not essential. The response to IL drugs in the isolated perfused model was also free of endocrine influence.

Cisapride is- a substitute of piperidinyl benzamide with prokinetic properties, lacking the dopaminergic receptor antagonistic central nervous system side-effects.¹⁴ Its action on the ileum involves facilitation of myenteric plexus cholinergic transmission¹⁵ but, in the colon, may involve a direct action upon the smooth

muscle.^{2,4} Despite this difference, it was found that intraluminal drugs were effective and the pattern of response similar, in both the ileum and colon. We also found that even *in vitro*, both IA and IL cisapride had a biphasic response with peak effects in the intermediate doses (the effects of higher doses being more variable), and this was consistent with the experience of *in vivo* systemic administration in ponies.¹⁶ Mebeverine is a B-phenylethylamine derivative with mainly musculotrophic spasmolytic properties.¹⁷ Its action in the guinea pig, involves atropine-like cholinergic inhibition, decreasing muscle cell sodium permeability and limiting the availability of calcium mobilised by stimulated colonic smooth muscle.¹⁸ Cisapride and mebeverine have a promising role in the clinical management of functional bowel disorders.¹⁹⁻²² Further work is required to clarify if IL administration by slow release preparations and enemas may provide a more efficacious and specifically localised pharmacological management for these disorders.

Conclusions

Our experiments have shown that the isolated perfused intestinal segment model for studying fluid propulsion under various pharmacological influences was effectively applicable to pigs. Pig colon is physiologically more similar to that of man than in other laboratory animals studied, and the results therefore more likely to be applicable clinically. We have confirmed that the *in vitro* IL instillation of cisapride and mebeverine affected the colonic motility, in a manner expected of their typical pharmacological properties, in both the ileum and the sigmoid colon. A higher response was obtained with IL cisapride than that achievable with equivalent doses administered IA, where high drug concentrations undiluted by body fluid distribution were attainable. Extrinsic denervation and endocrine deprivation did not qualitatively affect the outcome of IL administration of these drugs.

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