Initiation of motility in canine ileum by short chain fatty acids and inhibition by pharmacological agents

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SUMMARY We have previously shown that short chain fatty acids (SCFA) stimulate motility in the canine ileum. Concentrations of SCFA in the ileum are normally low but would be expected to increase after coloileal reflux; thus, this phenomenon could have pathophysiological relevance. The present studies were designed to seek pharmacological means by which this response could be blocked. Four dogs were prepared with isolated, ileocolonic fistulae into which physiological concentrations of SCFA could be instilled so as to stimulate ileal motility. Pretreatment of the ileum with topical lidocaine abolished the response to luminal SCFA but general anaesthesia did not. Indomethacin stimulated ileal motility and prostacyclin abolished the ileal response to SCFA. Naloxone and a calcium channel blocker also negated the response to SCFA; blockage of muscarinic, adrenergic and 5H-T receptors did not. We conclude that the motor response to SCFA is probably a local neural reflex which is sensitive to local anaesthetics, opiates and the prostanoids.

The terminal ileum, ileocolonic junction and proximal colon act in concert to regulate distal transit of chyme.¹ In addition to the motility patterns characteristic of the interdigestive cycle and the postprandial period, the ileum of dog and man shows two distinctive motor phenomena.¹⁻⁴ Spontaneous bursts of phasic waves (discrete clustered contractions (DCC)), usually lasting longer than one minute, propagate regularly through the ileum.

Although variable in prevalence among individual animals, their mean frequency in the dog is three to 10 per hour. Prolonged propagated contractions (PPC) are single, high pressure waves which span several slow waves;¹⁻⁵ they occur spontaneously once every several hours in the canine ileum.^{2,4} Patterns similar to DCCs and PPCs are evoked by distension of the ileum.² Instillation of short chain fatty acids (SCFA) into the lumen stimulates PPCs and bursts which are of higher amplitude and longer duration than spontaneous DCCs.³ Short chain fatty acids are produced in the colon by anaerobic bacterial metabolism of carbohydrates and, as such, can be considered as 'markers' of colonic contents. We have

proposed previously³ that, when SCFA reflux into the ileum, they provide a chemical stimulus for motility by which the ileum is, thereby, cleared of coloileal reflux.

Patients with irritable bowel syndrome exhibit more PPCs in the ileum than do control subjects⁶ and these motor patterns are often accompanied by abdominal symptoms. In addition, patients with ileoanal pouches after proctocolectomy generate in the 'neorectum' contractions which have features similar to PPCs.⁷ Thus, pharmacological blockade of the motor patterns stimulated by SCFA is of potential clinical interest.

We used a convenient canine model in which ileal motility could be stimulated by SCFA³ and we report here experiments which were aimed at the pharmacological blockade of ileal motility. Spontaneous propulsive motor patterns in the ileum require integration by the enteric nervous system (ENS), mechanisms which involve both cholinergic and serotonergic synapses.⁸ There is also evidence that prostaglandins are local regulatory agents in canine ileal circular muscle.⁹ Thus, the pharmacological agents we used were designed to characterise the neural circuits involved in the muscular responses to SCFA.

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Methods

DOGS

In four female dogs, ileocolonic loops were constructed under pentobarbital sodium anaesthesia. The loops comprised 30 cm distal ileum, the ileocolonic sphincter (ICS), and 5 cm proximal colon.^{1,2} Neuromuscular continuity was maintained by a sero-muscular bridge; the loop, however, was isolated from the intestinal stream and ended as a colostomy. Intestinal continuity was restored by ileocolonic anastomosis. A plastic infusion catheter (od 2 mm) was inserted into the proximal end of the loop. Six perfused manometry catheters were placed in the loop, with pressure ports at the ICS, and 5, 10, 15, 20, and 25 cm proximal. The catheters were exteriorised through a metal cannula. Animals were allowed two weeks to recover from the effects of surgery, during which time they were trained to stand quietly while restrained gently in a Pavlov sling. The effects of ileal distension, and the instillation of nutrients, bile acids, and SCFA on ileal motility were documented; these results have been reported separately.³ The responsiveness of the loops to SCFA did not decrease over a four month period. At the time of death, mucosal histology of the loops was normal and there was no evidence of degenerative changes in the nervous plexuses or muscularis layers. This protocol was approved by the Animal Care Committee of Mayo Clinic in August 1985.

RECORDING SYSTEMS

Manometric catheters were perfused at 0.1 ml/min with a low compliance, hydraulic capillary infusion system driven by a head of nitrogen. Pressures were recorded using Statham Gould P23 pressure transducers (Statham Instruments Inc, Halto Rey, Puerto Rico) and the output displayed on a Honeywell 1600 (Honeywell Technical Instruments Inc, Denver, CO) multichannel pen recorder with a chart speed of 25 mm/min.

TEST SOLUTIONS

The SCFA mixture was in physiological proportions³ consisting of 66% acetic acid (Baker, Phillipsburg, NJ), 24% propionic acid (Mallinckrodt, Paris, KY), and 10% butyric acid (Fisher, Springfield, NJ). Total concentration of SCFA was 145 mM, the pH was adjusted to 6.5 with NaOH and the osmolality to 295 mOsmkg⁻¹. The concentration of the SCFA mixture is within the range found in the dog stool.³ Concentrations of SCFA in the test solutions were confirmed by gas liquid chromatography.¹⁰

CONDUCT OF EXPERIMENTS

The animals were studied after fasting for 18 hours. The SCFA solution was instilled into the loop as 15 ml boluses at 10 minute intervals, for a total of six instillations each day; intraluminal pressures in the loop were recorded continuously. We have shown earlier that repeated 15 ml boluses of saline do not evoke ileal contractions³ thereby precluding any effects related to volume distension alone in the present study. Pretreatment drugs were given intravenously, using saline as a control; lidocaine was placed directly into the loop. There was an interval of at least one week between each set of studies. Before and after each day's experiment, loops were flushed with saline until the effluent was clear.

EFFECT OF ANAESTHESIA

General anaesthesia

One set of experiments was performed after dogs were anaesthetised with pentobarbital (30 mg/kg; pentobarbital sodium, Fort Dodge, Iowa).

Local anaesthesia

In conscious dogs, loops were pretreated with 15 ml of 2% lidocaine (xylocaine topical solution, Astra Pharmaceutical Products, Inc, Worcester, MA). After five minutes, a series of three boluses of SCFA

Fig. 1 Ileal motility in the dog after indomethacin (5 mg/kg, iv). There was a striking increase in number of pressure waves, which precluded any further testing of the stimulatory effects of SCFA.

Ileal motility in the dog

solution was instilled; five minutes after the third instillation, 15 ml 2% lidocaine was again placed in the loop and three more boluses were instilled.

EFFECT OF POTENTIAL INHIBITORY AGENTS

Drugs were tested on conscious dogs in two separate blocks. In the first set, the effects of adrenergic, cholinergic, and opiate blockade were studied.

Adrenergic blockade

Propranolol (Inderal; Ayerst Laboratories, New York, NY) was given as an intravenous bolus (300 µg/kg), followed by an infusion of 300 µg/kg/h. Phentolamine (Regitine; Ciba, Summit, NJ), as a bolus of 300 µg/kg followed by an infusion of 1.5 mg/kg/h, was given simultaneously but in a different vein.

Cholinergic blockade

Atropine (Abbott Laboratories; North Chicago, IL) was given as a bolus dose of 100 µg/kg, followed by an infusion of 100 µg/kg/h. The heart rate increased by a mean of 75% after the drug.

Opiate blockade

Naloxone (Narcan; Du Pont Pharmaceuticals, Manati, Puerto Rico) was administered as a bolus, 70 µg/kg followed by an infusion of 70 µg/kg/h.

Control experiments featured intravenous normal saline alone

In all studies, an intravenous infusion of normal saline was started 30 minutes before the first instillation of SCFA and was continued until the end of the study. The bolus injections were given 30 minutes

before the first instillation of SCFA. Adrenergic, cholinergic, and opiate blockade were tested in all four dogs; on a given day, only one manipulation was tested in each dog. The agents were given in random sequence.¹¹ These doses of adrenergic and cholinergic blocking agents predictably cause cardiovascular effects.¹² The dose of naloxone used blocks morphine induced gastric relaxation in dogs.¹³

In the second set of experiments, the effects of prostanoids, a calcium channel blocker, non-selective 5HT and selective 5HT₂ blockade were tested.

The effects of indomethacin (Merck, Sharp and Dohme Research Laboratories; Rahway, NJ) and prostaglandin E₂ (Upjohn Diagnostics; Kalamazoo, MI) were addressed in preliminary studies. Indomethacin (5 mg/kg iv) induced almost constant motor activity in all dogs, precluding the opportunity to evaluate any further stimulating effects of SCFA (Fig. 1). The motor activity stimulated by indomethacin was similar to phase 3 activity. Prostaglandin E₂ was studied at two doses, 25 µg/kg given iv before each instillation of SCFA into the loop, and as a constant infusion of 40 µg/kg/h. Both doses caused side effects, including salivation, vomiting, and defecation. Despite these, however, features of overdosage, SCFA were still able to stimulate motility (Fig. 2). Indomethacin and prostaglandin E₂ were not studied further and inhibitory effects were sought systematically with: (i) Prostacyclin (Upjohn Diagnostics; Kalamazoo, MI), which was made as a fresh solution of 5 µg/ml, in 0.05 M tris buffer at pH 9.0 and infused at 5 µg/kg/h. (ii) A calcium channel blocker, verapamil (Calan; Searle Pharmaceuticals, Chicago, IL), which was given as an infusion of 8.3

Fig. 2 Ileal motility in a dog after PGE₂ (40 µg/kg/h, iv). Although side effects were prominent (note artifacts due to retching), short chain fatty acids (SCFA) stimulated bursts of propagated phasic activity.

µg/kg/min. (iii) Methysergide maleate (gift from Sandoz Research Institute; East Haven, NJ) was used as a non-selective 5-HT blocker at 1 mg/kg. (iv) Ketanserin (gift from Janssen Life Sciences Products; Beerse, Belgium), a selective 5HT₂ blocker, was used at 0.16 µg/kg. Both methysergide and ketanserin were given intravenously over three minutes, 10 minutes before the first instillation of SCFA. A second bolus of ketanserin was given five minutes after the third instillation of SCFA.

ANALYSIS OF DATA

Bursts of rhythmic motility stimulated by SCFA were discrete clusters of phasic pressure waves with an amplitude greater than 40 mmHg. To be so defined, they had to last for more than 60 seconds and migrate over at least two channels; they were readily distinguished from phase 3 of the MMC.³ Prolonged propagated contractions (PPC) were defined as high pressure waves (>60 mmHg), of duration greater than 12 seconds, which migrated rapidly over at least two channels. A positive response to SCFA was judged to be present if either a burst or a PPC was recorded within 60 seconds of instillation of the SCFA solution.

Records were evaluated in a blinded fashion by two observers and the data in each of the two sets of blocking studies were compared using analysis of variance within a 4×4 Graeco-Latin square design¹¹ with the four variables being dogs, days, study number, and drug.

Results

QUALITATIVE FEATURES

Bursts and PPCs were recorded only with SCFA stimulation; during these experiments, they did not occur spontaneously. When pretreatment was with saline alone, boluses of SCFA regularly induced PPCs or bursts (Table 1, Fig. 3), confirming earlier observations.³ Indeed, bursts and PPCs may be related phenomena or of a common origin. Motor events which started as a burst could be propagated

Fig. 3 Ileal motility in a dog after a 15 ml bolus of short chain fatty acids (SCFA). Bursts of clustered contractions are recorded in the proximal leads but as the motor phenomenon progresses distally the pattern changes to a single, broad high pressure wave (PPC). A second PPC was stimulated by this bolus (right of tracing).

as a PPC (Fig. 3). Both PPCs and bursts are thought to be propulsive in the ileum² and have, therefore, been analysed together. Ileal motor responses evoked by SCFA were independent of the interdigestive cycle. A phase 3 'front' could be interrupted by PPCs or bursts of phasic waves which were evoked by SCFA or, occasionally, by a spontaneous event (Figs 4 and 5). It is obvious from Figures 4 and 5 that PPC's and bursts could be stimulated during all phases of the interdigestive cycle. As the prevalence of spontaneous PPCs has also been noted before² not to be influenced by phases of the interdigestive cycle, boluses of SCFA were not timed to coincide with a particular phase of the interdigestive cycle. We confirmed that SCFA stimulated ileal motility just as often when phase III activity was passing through the loop as when there was no such activity (p=0.3).

QUANTITATIVE INHIBITION OF MOTILITY EVOKED BY SCFA

Effect of anaesthesia

Short chain fatty acids still stimulated ileal contractions when dogs were under general anaesthesia. Local anaesthesia completely blocked all propulsive activity; however, no PPCs or phasic bursts were stimulated by boluses of SCFA after treatment with lidocaine. In one dog, four hours after lidocaine,

Table 1 Ileal motor responses to instillates of SCFA*

		Pretreatment			
Instillates		Saline	Phentolamine-propранolol	Atropine	Naloxone†
Dog (n)					
1	6	6	6	2	2
2	6	5	4	3	0
3	6	5	4	5	0
4	6	4	6	3	3

*Number of positive motor responses to instillation of boluses of short chain fatty acids (SCFA); †p<0.05 v saline.

boluses of SCFA were again instilled into the loop and ileal motility was stimulated promptly.

Effect of pharmacological blockade

The results of the first block of tests are summarised in Table 1. Contractions were stimulated by SCFA after 20 of 24 boluses when animals were pretreated with control saline. Adrenergic blockade with phentolamine and propranolol did not reduce the stimulatory effects of SCFA. Motor responses were reduced, though not significantly so ($p>0.05$), after muscarinic blockade with atropine. The opiate blocker, naloxone, significantly decreased the potential SCFA to stimulate ileal motility ($p<0.05$). Spontaneous motor activity was not affected by any of the drug treatments.

The second set of results is shown in Table 2. Contractions were stimulated 15 of 24 times in the methysergide treated dogs, and 22/24 after ketanserin. Verapamil decreased the sensitivity to SCFA ($p<0.05$), while prostacyclin blocked all contractile responses to SCFA. During prostacyclin infusions, no side effects were noted.

Discussion

Our assay for chemosensitive stimuli of ileal motility was based on earlier observations³ which showed that physiological concentrations of SCFA stimulated ileal motility; equal volumes of saline instilled into

Table 2 Ileal motor responses instillates of SCFA*

Dog (n)	Instillates	Pretreatment			
		Methysergide	Ketanserin	Verapamil†	Prostacyclin†
1	6	5	5	3	0
2	6	4	5	0	0
3	6	2	6	2	0
4	6	4	6	2	0

*Number of positive motor responses to instillation of boluses of short chain fatty acids (SCFA); † $p<0.05$ v saline.

the loop did not. In the present studies we used 145 mM mixed solutions of SCFA to stimulate motility maximally,³ so that the effects of potential blockers could be assessed. Clustered bursts of contractions (DCCs) are a feature of normal ileal motility in the dog,^{1,3} and they occur on average each 10–20 minutes. We suspect that the ileal bursts seen in response to SCFA represent a different phenomenon. To qualify for our definition of a motor sequence evoked by SCFA required a pressure sequence with an amplitude, duration, and propagation that occurs rarely, if ever, spontaneously. Together with the temporal relationship to the SCFA bolus, this meant that evoked patterns could be recognised easily. Prolonged propagated contractions also occur spontaneously in the canine ileum;^{2,3} but they are infrequent, occurring only each three to

Fig. 4 Motility recording in a fasting dog, showing migration of a phase III 'front' (MMC) through the ileum. Bolus instillations of short chain fatty acid (SCFA) at 10 minutely intervals stimulated single, high pressure phasic contractions (PPC) which propagated through the ileum and interrupted the MMC (channel 3, middle of recording).

Fig. 5 Motility in a fasting dog, showing migration of a phase III front (MMC) through the ileum. Spontaneous, high pressure waves interrupt passage of the MMC through the ileum.

four hours. Thus, we had no difficulty in identifying those evoked by SCFA.

The phenomenon whereby SCFA evoke a pattern of motility which probably empties the distal small bowel,² is of functional significance. As proposed previously,^{2,3} it represents a potentially protective mechanism against colo-ileal reflux, but the phenomena may also be relevant clinically. Powerful ileal contractions have been recorded proximal to Brooke ileostomies¹⁴ and in ileoanal pouches created after proctocolectomy.⁷ In both circumstances, the motility patterns were propulsive and contents were expelled from the small intestine. In another context, Kellow⁶ has described increased numbers of PPCs in the ileum of patients with irritable bowel syndrome; these motor events were often accompanied by episodes of abdominal pain. Further, Mathias^{15,16} described a propulsive pattern in the rabbit ileum (MAPC) which was stimulated by a known laxative (ricinoleic acid, castor oil) and also by a diarrhoeogenic bacterial toxin.

Ileal motility was stimulated predictably by SCFA; our control observations, which featured pretreatment with saline only, confirmed earlier findings.³ Thus, we had a model in which the possible inhibitory effects of pretreatment could be evaluated. We assume that lidocaine, which stabilises neuronal membranes so as to inhibit nerve impulses, functioned as a local anaesthetic. Lidocaine had no effect

on basal motility. Inhibition of the contractile response to SCFA after lidocaine, which was used twice because of its brief duration of action, suggests that the action of SCFA is, in part, neurally mediated.

Naloxone blocks opiate receptors and opiates increase phasic and tonic activity of the circular muscle in man,^{17,18} dogs,¹⁹ and cats.²⁰ The mechanisms by which opiates stimulate smooth muscle, however, are not clear. Projections of nerve fibres with immunoreactivity for enkephalin have been found in the myenteric and submucosa ganglia, and in longitudinal and circular muscle layers in the canine ileum.²¹ Enkephalins decrease the release of acetylcholine from myenteric plexus neurones,²² but they also contract smooth muscle in preparations devoid of neural opiate receptors.²³ Contractions in the feline ileum²⁰ are mediated through delta receptors, thought to be located on muscle. It is clear that naloxane could have several possible sites of action in our study.

Verapamil, a calcium channel blocker, which inhibits the entry of calcium into cells and its mobilisation from intracellular stores was also effective in our system. Inhibition of contractions by verapamil presumably indicates that the effects of SCFA can be blocked at the level of the smooth muscle cell. Calcium channel blockers have been shown to inhibit pendular movements of the rabbit

isolated ileum²⁴ and contractions in strips of human colon muscle.²⁵ Nifedipine, another calcium channel antagonist, has been used in the treatment of irritable bowel syndrome; it decreased colonic motility indices and numbers of contractions.²⁶

Prostaglandins are synthesised by all layers of the bowel wall,²⁷ and indomethacin, which inhibits prostaglandin biosynthesis, alters ileal motility in dogs,⁹ guinea pigs,²⁸ and rabbits.²⁹ Indomethacin also increases spike activity, an effect which can be reversed by PGE₂ and prostacyclin. Indeed, inhibitory prostaglandins may be normally present in the ileum in quantities sufficient to control spontaneous contractions, as pretreatment with indomethacin induced constant motor activity in our canine loops, as it does in the rabbit.²⁹ The dose of PGE₂ used in our studies produced side effects, but did not block the SCFA effect. Prostacyclin (PGI₂), which is not inactivated in the lung, however, and which may behave as a circulating 'hormone',³⁰ could be given in doses that were well tolerated, and which blocked completely the motility evoked by SCFA.

Adrenergic and cholinergic blockade were studied to characterise the effect on the sympathetic and parasympathetic nervous systems. Although muscarinic blockade decreased the number of contractions, the effect was not significant and our data suggest that these systems are not involved when SCFA stimulate ileal contractions; nor is 5HT. Serotonin (5HT) is believed to be released by opiate action in the ileum.³¹ Several lines of evidence suggest that 5HT is the neurotransmitter for the slow EPSP in myenteric ganglia.³² Excitatory postsynaptic potentials influence the duration of excitability of effector muscle and thus ensure coordinated responses over lengths of bowel. 5HT, however, did not seem to be involved in SCFA induced ileal contraction as the 5HT blocker methysergide and the 5HT₂ receptor blocker, ketanserin were inactive.

Confirmation that SCFA induced ileal contractions might be a local reflex comes from the observation that motility could be stimulated even under general anaesthesia, suggesting that higher control was not essential. The local reflex over-rides the interdigestive myoelectric complex (IDMEC) and can interrupt temporarily the propagation of the MMC (Figs 4 and 5). Spontaneous PPCs have also been observed to interrupt the MMC.³ Our results suggest that SCFA act by a local pathway which is sensitive to local anaesthetics, and which involves opiates and the prostanoids.

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