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Effects of the enterokinetic prucalopride (R093877) on colonic motility in fasted dogs

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Abstract *The novel enterokinetic drug prucalopride was tested at various intravenous and oral doses in fasted dogs to assess: (i) the effects on colonic contractile motility patterns; and (ii) the mediation of these effects by 5-hydroxytryptamine (5-HT$_4$) receptors. Colonic motility patterns were assessed in conscious dogs with four chronically implanted strain-gauge force transducers that were sutured on the serosal side of the colon. Prucalopride altered colonic contractile motility patterns in a dose-dependent fashion by stimulating high-amplitude clustered contractions in the proximal colon and by inhibiting contractile activity in the distal colon. Prucalopride was equipotent after oral and intravenous administration, as reflected by the values for the effective dose that induced 50% of maximum effect (95% confidence limits): 0.04 mg kg^{-1} p.o. (0.01–0.1 mg kg^{-1}) and 0.01 mg kg^{-1} i.v. (0.006–0.04 mg kg^{-1}). Prucalopride also caused a dose-dependent decrease in the time to the first giant migrating contraction (GMC); at higher doses of prucalopride, the first GMC generally occurred within the first half-hour after treatment. Subcutaneous pretreatment with the 5-HT$_4$ receptor antagonist GR125487 (40 μg kg^{-1} bodyweight) completely prevented the effects of orally administered prucalopride (0.31 mg kg^{-1} bodyweight). Prucalopride, given orally or intravenously, alters colonic motility in the fasted conscious dog in a dose-dependent fashion. It induces GMCs and causes proximal colon stimulation and distal colon inhibition of contractile motility patterns by stimulating 5-HT$_4$ receptors.*

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INTRODUCTION

Prucalopride (R093877), a novel enterokinetic compound, induces defecation and accelerates total gut and colonic transit in healthy subjects.[1–4] The compound is currently being evaluated in phase III clinical trials for efficacy in relieving chronic constipation. *In vitro* studies on isolated strips have shown that prucalopride has no effect on serotonin (5-hydroxytryptamine; 5-HT) 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_3$ receptors and muscarinic cholinoceptors, and does not inhibit cholinesterases.[5] Instead, prucalopride has been shown to be a potent, highly specific and selective 5-HT$_4$ receptor agonist in isolated gut tissues from various species (pEC$_{50}$: guinea-pig, 7.4; rat, 7.5).[5] Radioligand-binding studies have confirmed the high specificity and selectivity of prucalopride ($pK_i \approx 8.0$ to human 5-HT$_4$ binding sites) and showed that, using up to 3 μM, there is no binding to other 5-HT receptor subtypes or various monoamine and peptide receptors.[6] These data suggest that prucalopride is one of the most specific and selective 5-HT$_4$ receptor ligands available today.

Colonic motor patterns have been studied extensively in dogs.[7–10] The current study was designed to assess the effects of various intravenous and oral doses of prucalopride on fasted colonic motor patterns in freely moving, healthy dogs equipped with four chronically implanted strain-gauge force transducers on the serosal side of the colon. Using different dogs, a second set of experiments was done to determine if the effects of prucalopride on colonic motility are blocked after pretreatment with GR125487, a selective, potent, and metabolically stable, 5-HT$_4$ receptor antagonist.[11]

MATERIALS AND METHODS

Surgery and experimental set-up

Female beagles weighing between 9 and 13 kg were used. They were housed according to the EEC Council Directive 24 November 1986. The dogs had free access to tap water and were fed a standard dog feed daily (approximately 350 g) between 0800 and 0900 hours. The cages had light from 0600 to 1900 hours.

Strain-gauge force transducers were constructed (9×16 mm)[12] and calibrated[13] before implantation. Dogs were implanted with the strain gauges under general anaesthesia (N_2O/O_2 + halothane) and aseptic precautions. After a median laparatomy, four transducers were sutured in the circular direction on the serosal side of the colon at 8, 16, 24 and 32 cm distally to the ileocaecal valve. The wires of the strain gauges were led through a subcutaneous tunnel on the left costal flank through a stab wound between the scapulae. The connector was soldered to the lead wires and protected by a canvas jacket. After surgery, each dog received a single ampoule of Dipidolor® analgesic (containing 15 mg piritramide per ampoule, Janssen-Cilag, Neuss, Germany) and were given 0.1 mg kg^{-1} once daily of Duplocilline® antibiotic (containing 150 000 U mL^{-1} each of benzylpenicillin procaine and benzylpenicillin benzathinine, Mycofarm, Mechelen, Belgium) for 1 week to prevent infection. This regimen did not affect stool frequency or consistency. Dogs were allowed a recovery period of at least 2 weeks.

The dogs were permanently housed in a special room in cages with glass pervious to light in one direction. The dogs could not see out of their cages, but the investigators could observe the dogs for behavioural changes and defecation. A telemetric system was used to capture the transducer signals. The system allows simultaneous recording of motility signals from a maximum of eight dogs with four force transducers each. The transducer signals were transmitted in digitized form (sample frequency 5 Hz) to a central computer by a small transmitter box (Pharmatel 88, Glonner Electronic GmbH, Planegg, Germany) that was placed in the canvas jacket. Data were stored on a VAX 3500 computer for overnight analysis.

Experiments were started after the dogs had fasted for approximately 20 h, during which time water was available. During the experiments, dogs were free to move in their cages. Prucalopride and GR125487 (1-2-(methyl sulphonyl, amino]ethyl)-4-piperidinyl-methyl 5-fluoro-2-methoxy-1*H*-indole-3-carboxylate) were synthesized in-house (Janssen Research Foundation, Beerse, Belgium). Compounds were dissolved in saline directly prior to intravenous administration.

Experimental protocol 1: intravenous and oral effects of prucalopride

One set of dogs ($n = 5$) was used for these experiments. After a basal period of at least 4 h of recording (starting between 0600 to 0800 hours), at $t = 0$, the dogs received tap water (solvent for oral treatment), or saline (solvent for intravenous treatment) or a dose of prucalopride as an aqueous solution (0.3 mL kg^{-1}) in randomized order. Motility recordings were made for a minimum of 10 h ($t = 10$). The dogs' behaviour and defecation were observed until 4 h after treatment. The same dogs were used for testing the various oral ($n = 5$) and intravenous ($n = 4$) doses of prucalopride, with at least 1 day between experiments.

Experimental protocol 2: involvement of 5-HT_4 receptors

Another set of dogs ($n = 6$) was used in four treatment schemes. After a basal period of at least 3.5 h of recording (starting between 0600 and 0800 hours), at $t = -0.5$, either solvent alone (saline) or GR125487 (40 μg base equivalent kg^{-1} bodyweight) was administered subcutaneously. Thirty minutes later, at $t = 0$, the dogs received either solvent (water) or prucalopride (0.31 mg base equivalent kg^{-1} body weight) orally. Thus, the four treatments administered in randomized order were solvent/solvent, solvent/prucalopride, GR125487/solvent, and GR125487/prucalopride. Motility recordings were made for a minimum of 10 h ($t = 10$). The dogs' behaviour and defecation were observed for 4 h after treatment. Each dog was used for each treatment, with at least 1 day between the different experiments.

Data analysis

The recorder tracings of colonic motility were visually analysed: the motility patterns in the 4-h period before treatment ($t = -4$ to 0) were compared with those in the 4-h period after treatment ($t = 0$–4). Changes in colonic contractile patterns were scored after blinding the traces, irrespective of the nature of the changes, by three persons who were not involved in conducting the experiments. The scoring was scaled from 0 (no change in colonic motor activity) to 4 (definite change in colonic motor activity). To quantify motility, the computer generated a motility index that was defined as the integrated area between baseline and

contractions per hour. The mean motility index during the 1- to 4-h post-treatment period was divided by the mean motility index during the –4 to 0-h pretreatment period (0 h being the time of drug treatment), yielding the motility index ratio. The first hour was not included in the motility index calculation because prucalopride induced giant migrating contractions (GMCs) in the first hour after treatment. The exact time to the first GMC after treatment was recorded for all tracings, with a GMC defined as a single high-amplitude propagated contraction (HAPC) propagated toward the anus, which was not related to a cluster of contractile activity. However, the contractile events that occurred simultaneously with defecation also were considered a single GMC.

Statistical methods

Statistical analyses were performed using StatXact-3 (statistical software for exact nonparametric inference; Cytel Software Corp., Cambridge, USA). For experimental protocol 1 and determining the effects of prucalopride, the Jonckheere –Terpstra test was used to test for a dose-related effect for summed scores, motility indices and time to first GMC. Individual differences between treatments were compared to solvent (placebo) and analysed using the Mann–Whitney–Wilcoxon *U*-test. For experimental protocol 2, with two-way layout experiments (six dogs receiving four treatments each), the Friedman test was used to explore for differences among the treatments for summed scores and motility indices. Individual differences between solvent (placebo) and each treatment were further analysed with the Friedman test. For all tests used, $P < 0.05$ was considered statistically significant. If scores were analysed, the results from the three scorers were summed for each treatment per dog. The values for the effective dose that induced 50% of maximum effect (ED_{50} values) and 95% confidence limits were estimated using an iterative sigmoidal curve-fitting procedure (GraphPad Prism, San Diego, CA, USA), using all data points for a single fit.

RESULTS

Dose-related effects of intravenous and oral prucalopride

To illustrate the effect of prucalopride on colonic contractile motility, an example of the colonic contractile motility patterns of a dog receiving an oral dose of prucalopride is shown (Fig. 1a). Figure 1(b) zooms in on the massive contractile event (a GMC) approximately 30 min after prucalopride administration. In the proximal and midcolon ≤ 24 cm from the ileocolonic junction (Fig. 1, tracings labelled COLON-IC8CM, -IC16CM, and -IC24CM), the amplitude and frequency of clustered contractions were enhanced by prucalopride, but were variable in onset, duration, and quality. The motility index did not adequately reflect drug-induced changes in motility induced by prucalopride. However, in the distal colon 32 cm from the ileocolonic junction (Fig. 1, tracings labelled COLON-IC32C), prucalopride inhibited motility by reducing the intensity of contractile clusters. The consistency of prucalopride-induced distal inhibition enabled the distal force transducer to generate data for motility index calculations. Therefore, prucalopride dose responses were examined in the distal colon. A dose-dependent response in the distal colon was reflected by a decrease in the motility index ratio with increasing prucalopride doses ($P < 0.0001$, oral administration; $P = 0.0035$, intravenous administration; Jonckheere–Terpstra test; Fig. 2). The dose-dependent change in contractile motility patterns due to prucalopride was also determined by visual analyses and scoring of blinded traces ($P < 0.0001$, oral administration; $P = 0.0001$, intravenous administration: Jonckheere–Terpstra test; Fig. 3). After both oral and intravenous administration, the dose–response curve had a sigmoidal shape and allowed estimation of ED_{50} values (95% confidence intervals): for intravenous administration, 0.01 mg base equivalent kg^{-1} bodyweight (0.006–0.04 mg kg^{-1}); for oral administration, 0.04 mg base equivalent kg^{-1} bodyweight (0.01–0.1 mg kg^{-1}).

Prucalopride facilitated the occurrence of GMCs after treatment (Fig. 1a,b) and also decreased the time to the first GMC, in a dose-dependent fashion. The dose dependency was more obvious after intravenous ($P = 0.0009$: Jonckheere–Terpstra test; Fig. 4a) than after oral ($P = 0.0001$: Jonckheere–Terpstra test; Fig. 4b) administration. A prucalopride-induced GMC occurred in the first hour after treatment, especially at higher doses (Fig. 4). After this time, a GMC was equally likely to occur in a dog given solvent only or prucalopride (results not shown). Prucalopride, at any of the doses tested, did not increase the number of stools produced by dogs in the 4 h after treatment (results not shown).

In about 6% of the defecations, no concomitant high-amplitude single contraction could be detected on any of the strain-gauge recordings. Prucalopride did not affect stool consistency. None of the doses given to the dogs caused changes in their behaviour or visible adverse effects.

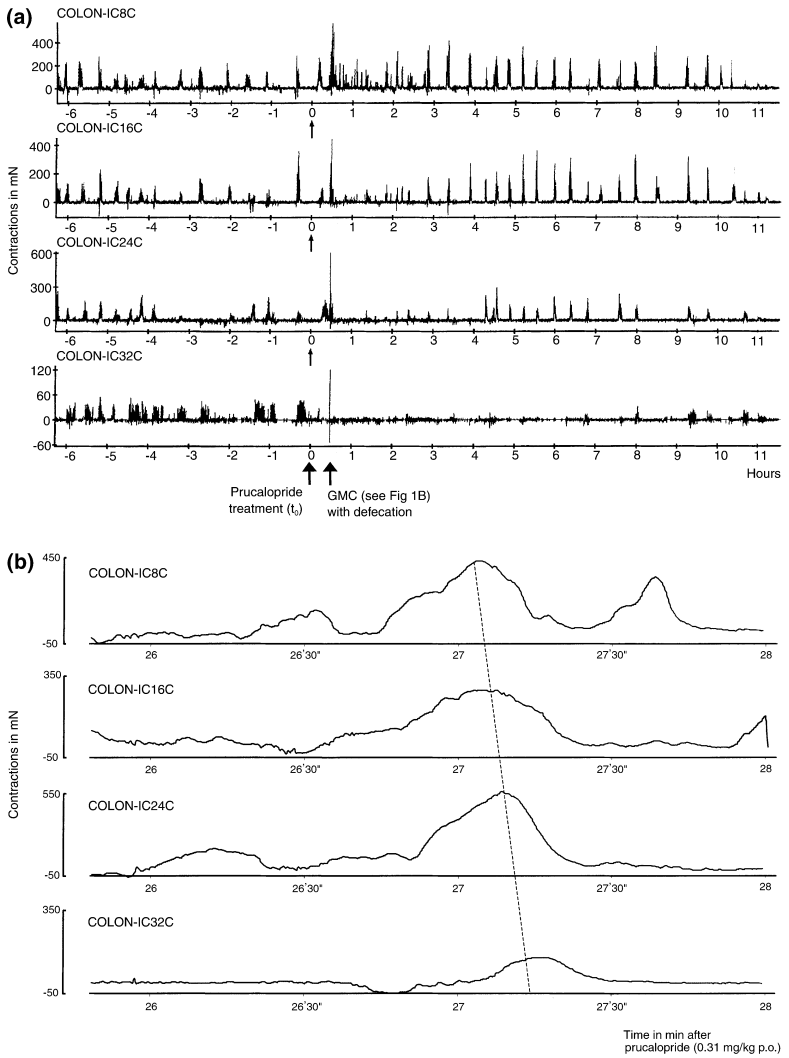

Figure 1 Effects of orally administered prucalopride (0.31 mg kg^{-1}) on colonic motility patterns in conscious dogs. (a) Motility recordings from four serosal force transducers implanted on the colon at 8, 16, 24, and 32 cm from the ileocolonic (IC) junction, over a period exceeding 17 h. (b) A zoom-in on the same motility recordings (thus in the same dog) over a period of 2 min (26–28 min after prucalopride addition), clearly displaying a giant contraction migrating from the proximal to the distal recording site. In this case, the GMC was accompanied by production of a solid stool.

5-HT_4 receptor antagonism and prucalopride-induced effects

A different set of dogs ($n = 6$) was used to examine the effects of the 5-HT_4 antagonist GR125487 on prucalopride-induced activity. When prucalopride alone was orally administered (0.31 mg kg^{-1} bodyweight), it significantly ($P = 0.0313$; Friedman test) induced alterations in the colonic motor pattern (median score 12; Fig. 5) compared with solvent-only treatment (median score 1) and also caused distal inhibition of contractile activity (Fig. 6). Treatment with GR125487 prevented prucalopride-induced motility effects (Figs 5 and 6). When GR125487 alone was administered, it caused some alteration of colonic motility (median score 3; Figs 5 and 6), but this was not statistically different when compared with solvent treatment ($P = 0.1250$ based on scores, $P = 0.2188$ based on motility index; Friedman test).

The effects of prucalopride and GR125487 on GMCs were also analysed. In the first hour after treatment with prucalopride, four of six dogs experienced a GMC (which in two dogs led to defecation), whereas none of the six dogs experienced a GMC after treatment with GR125487 and prucalopride. None of the six dogs given solvent alone had a GMC; one dog had a GMC with concomitant defecation after GR125487 was given without prucalopride.

DISCUSSION

Prucalopride clearly alters canine colonic motility in a dose-dependent fashion, irrespective of the route of administration. These effects are characterized not only by proximal stimulation and distal inhibition of clustered contractile activity but also by induction of GMCs. The effects were reproducible, as indicated by

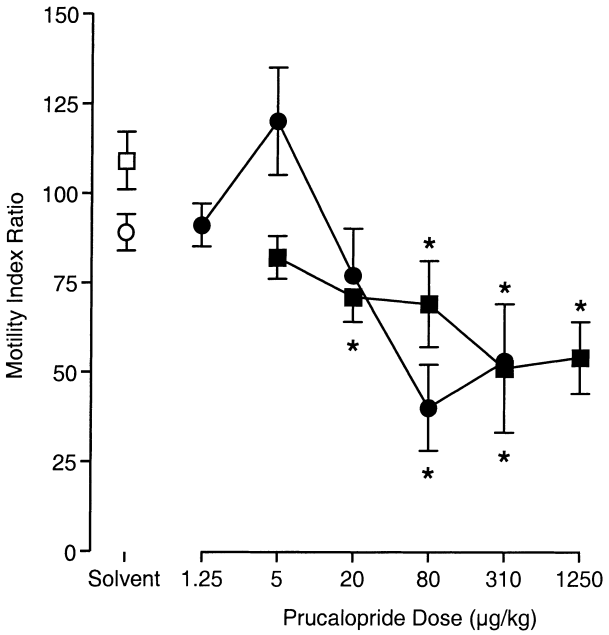

Figure 2 Motility in the distal colon (motility index ratio) relative to prucalopride dose. At each of the doses administered either orally (squares; $n = 5$ at each dose) or intravenously (circles; $n = 4$ at each dose), the mean motility index ratios (± SEM) are shown. $^*P < 0.05$; Mann–Whitney–Wilcoxon *U*-test.

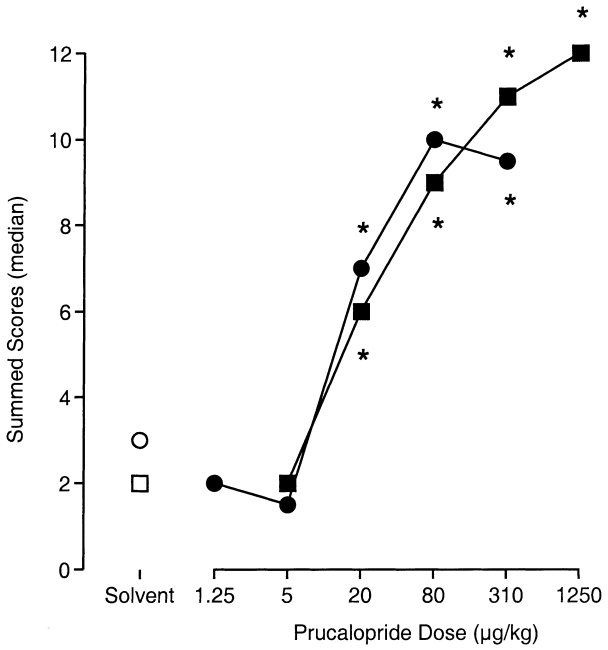

Figure 3 Colonic motility patterns (summed scores) relative to prucalopride dose. Three persons scored blinded traces, assigning scores from 0 (no change in motility patterns) to 4 (definite change in motility patterns), comparing the 4-h period after treatment with the 4-h period before treatment. Summed scores are shown for each of the doses administered either orally (*squares*; $n = 5$ at each dose) or intravenously (*circles*; $n = 4$ at each dose). Shown are median values; $^*P < 0.05$; Mann–Whitney–Wilcoxon *U*-test.

the median activity scores of 11 and 12 in the two groups of dogs after they received an oral dose of prucalopride (0.31 mg kg^{-1} bodyweight).

GMCs are the main motor event underlying fast propulsion of intestinal (colonic) contents over longer distances.[7,8] Bassotti and coworkers[14,15] have shown that in chronic constipation, the number, duration and intensity of HAPCs (the human equivalent of canine GMCs) are reduced compared with those in healthy volunteers. Thus, inducing HAPCs in constipated patients could be a way to normalize bowel habits and treat chronic constipation. It is not known what triggers GMCs/HAPCs and how they are controlled chemically and electrically. In dogs, many GMCs originate in the distal ileum.[9] Segmental contractile activity in the colon may block these GMCs from propagating distally over longer distances. By causing distal inhibition in the colon, it may well enhance the likelihood that these GMCs will travel distally over a longer distance because no disturbing contractile activity is present to block their propagation.

Interestingly, at higher doses, prucalopride induced GMCs without concomitant defecation. Although it is not known what parameters trigger a GMC, it is likely that the degree of filling, and thus the wall distension that stimulates stretch receptors of the colon, is one of the main stimuli. Apparently, high doses of prucalopride can evoke a GMC in these fasted dogs without the wall distension stimulus (i.e. without sufficient material in the colon to produce a stool).

Detection of GMCs is a much more sensitive parameter than counting stools to evaluate the effects of enterokinetic drugs such as prucalopride, for two reasons. Firstly, when a GMC propels faecal material into the rectum, the distension of the rectal wall induces an urge to defecate. Whether defecation actually occurs is subject to the dog's will, which may bias the interpretation of stool count. Secondly, the dogs in this study fasted for at least 20 h, which rendered their colons relatively empty. This was done to ensure the occurrence of interdigestive motility patterns, which are more constant over time (period of measurement >8 h) than postprandial motility.[10] A disadvantage, however, of using fasted dogs is the greater difficulty in inducing defecation. As stated, prucalopride did not stimulate defecation in the current studies. In a study with 12 conscious healthy cats having access to food at will, oral prucalopride (0.64 mg kg^{-1} bodyweight) induced defecation in five of 12 cats in the first hour

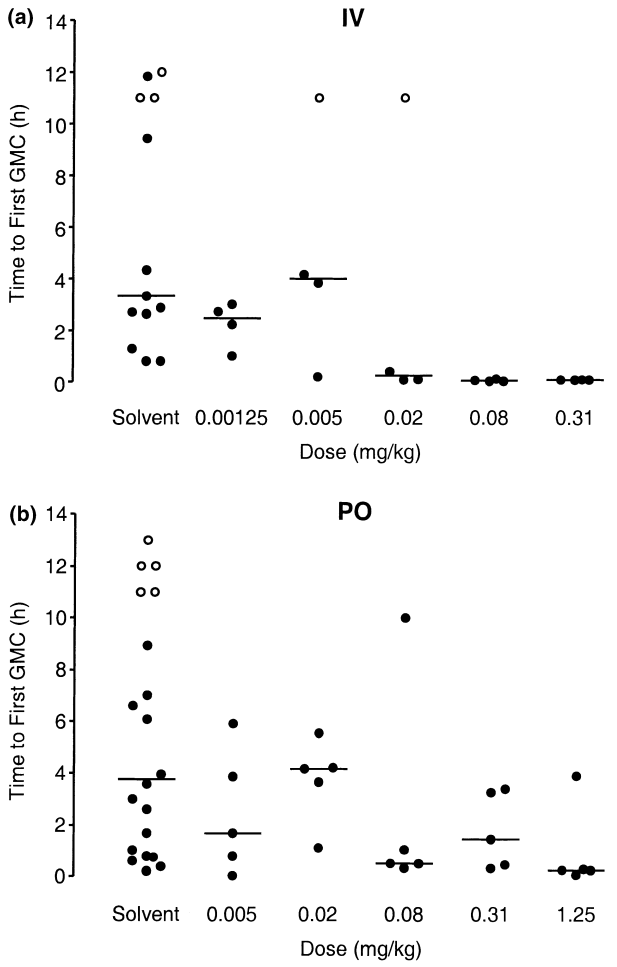

Figure 4 Time to the first giant migration contraction (GMC) relative to prucalopride dose. •, time to the first GMC; ◦, no GMC observed (total time that was measured after treatment, 11–13 h). IV, intravenous administration (a); PO, oral administration (b). The horizontal bars represent median values.

after treatment (solvent alone caused defecation in one of the 12 cats).[16] In other studies, bowel movements were stimulated in human healthy volunteers given prucalopride.[1,3] It is not known why the GMCs in dogs who received prucalopride seemed to occur only in the first hour after treatment, whereas the other motility effects (proximal stimulation and distal inhibition) persisted for at least 8 h. These long-lasting effects are in agreement with the relatively long half-life of prucalopride in dogs ($t_{1/2} = 7.0$ h after 1.25 mg kg^{-1} intravenously; Janssen Research Foundation, data on file). The discrepancy between the two prucalopride-based events, GMC induction vs. proximal stimulation/distal inhibition, suggests that they might be separate phenomena. Hypothetically, GMCs should only occur during the peak plasma levels of prucalopride, directly after the drug is administered, while proximal

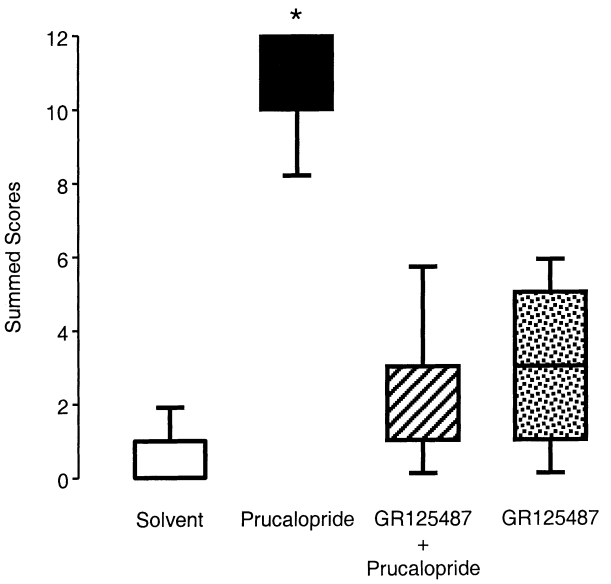

Figure 5 Colonic motility (summed scores) in dogs administered four treatments. Prucalopride (0.31 mg kg^{-1} bodyweight) was administered orally. The 5-HT$_4$ receptor antagonist GR125487 (40 μg kg^{-1} bodyweight) was subcutaneously administered 30 min before prucalopride or its solvent. Shown in the box plot are the median, 10th (lower whisker), 25th, 75th (lower and upper part of box), and 90th (upper whisker) percentiles; $n = 6$; $^*P < 0.05$; Friedman test.

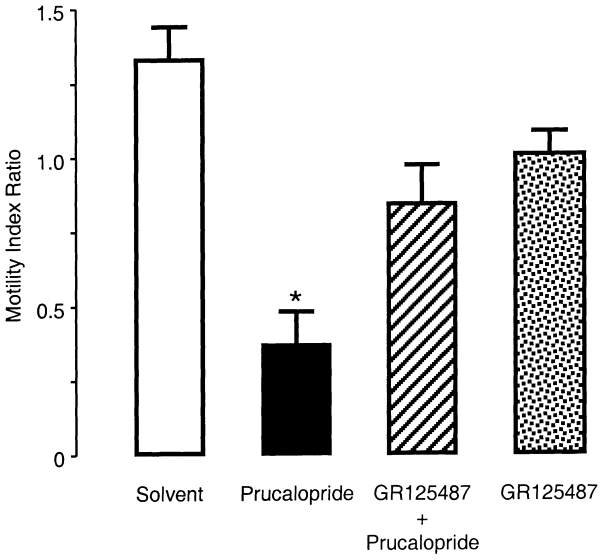

Figure 6 Motility in the distal colon (motility index ratio) in dogs administered four treatments compared to the solvent control. Prucalopride (0.31 mg kg^{-1} bodyweight) was administered orally. The 5-HT$_4$ receptor antagonist GR125487 (40 μg kg^{-1} bodyweight) was subcutaneously administered 30 min before prucalopride or its solvent. Shown are mean values ± SEM; $n = 6$; $^*P < 0.05$; Friedman test.

stimulation/distal inhibition should occur as long as sufficient prucalopride was present. If this assumption is true, it would explain why the dose–effect relationship of prucalopride, with respect to the time to first GMC, is more pronounced after intravenous than after oral treatment, when peak plasma concentrations are expected to be lower compared with levels after intravenous administration.

The dose–response curves and the ED_{50} values were analogous for intravenously and orally administered prucalopride. The similarity suggests a high bioavailability of prucalopride after oral administration in dogs. Indeed, a pharmacokinetics study in dogs showed that bioavailability of prucalopride averaged 77% after oral administration of 1.25 mg kg^{-1} bodyweight (Janssen Research Foundation, data on file). In addition, *in vitro* prucalopride is a highly selective and specific 5-HT_4 receptor agonist in gastrointestinal tissue from rat and guinea-pig.[5] We have demonstrated that smooth muscle 5-HT_4 receptors on the circular muscle of the dog rectum mediate relaxation.[17] In the human colon, 5-HT_4 receptors have been found on the circular but not on the longitudinal smooth muscle, mediating inhibition of spontaneous contractile activity and relaxation[18] through elevation of intracellular cyclic adenosine monophosphate.[19,20] Thus, these observations in isolated muscle strips from canine and human colon parallel the observation of inhibition of distal colonic motility patterns seen in the current study. This suggests that distal colonic inhibition is mediated by smooth muscle 5-HT_4 receptors.

Nagakura and coworkers[21] found that systemic treatment with the nonselective 5-HT_4 receptor agonist renzapride stimulated proximal, mid-, and distal colonic motility in conscious fasted dogs; this effect was blocked by the 5-HT_4 receptor antagonist SDZ 205 557 and by the nicotinic cholinoceptor antagonist hexamethonium. Thus, the excitatory component seen after 5-HT_4 receptor stimulation may be neurally mediated. Conversely, Graf and Sarna[22] reported that close intra-arterial infusion of 5-HT to the proximal colon in conscious dogs stimulated phasic contractions, and that this effect was insensitive to pretreatment with the 5-HT_4 receptor antagonist GDS 20352R, suggesting that the effects of 5-HT observed in this study were mediated via receptors other than 5-HT_4 receptors. Very recently, we have shown that excitatory 5-HT_4 receptors located on cholinergic neurones in the circular and longitudinal muscle layers of dog proximal colon.[23] The facilitation of cholinergic neurotransmission that results from activation of these 5-HT_4 receptors may explain the proximal motility stimulation observed in this study.

Our data on 5-HT_4 receptor-mediated stimulation of colonic motility with prucalopride are in good agreement with published data on the indole carboxamidamide, SDZ HTF 919, a potent partial 5-HT_4 agonist. This compound was shown to reduce colonic transit time in dogs (0.03–0.3 mg kg^{-1} s.c.)[24] as well as in humans (25–100 mg, twice daily).[25] In the study on dogs, manometry was used to determine the effects of SDZ HTF 919 on colonic motility, while the dogs were placed in a Pavlov sling. This may explain the lack of significant effects of the compound (except at 0.03 mg kg^{-1} on colonic contractions >30 s) on colonic motility in this study. Our telemetry set-up allows us to measure motility in dogs in their normal environment.

In conclusion, prucalopride administered orally and intravenously alters colonic motility in the fasted conscious dog in a dose-dependent manner. It induces GMCs and causes proximal stimulation and distal inhibition of contractile motility patterns by stimulating 5-HT_4 receptors.

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