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VB20B7, a Novel 5-HT-ergic Agent with Gastrokinetic Activity. II. Evaluation of the Gastroprokinetic Activity in Rats and Dogs*

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Abstract

The gastrokinetic activity of 2[1-(4-piperonyl)piperazinyl]benzothiazole (VB20B7), a new compound with 5-HT_4 receptor agonist and weak 5-HT_3 receptor antagonist properties has been studied in rats and dogs.

The effects of VB20B7 were investigated in physiological conditions and in a model of gastroparesis induced by the α_2-adrenergic agonist UK-14304 and compared with cisapride.

In rats, both VB20B7 and cisapride enhanced gastric emptying of indigestible solids (steel spheroids) and liquids (phenol red) at doses of 5–10 mg kg^{-1} by mouth. Gastric emptying of solid radiopaque markers in fasted beagle dogs was enhanced significantly by VB20B7 (0·25–1 mg kg^{-1} p.o.) whereas the effect of cisapride (0·5–2 mg kg^{-1} p.o.) did not reach statistical significance. Similar results were found when the radiopaque markers were given to the dogs following a standard solid meal The delayed gastric emptying of indigestible solids and radiopaque markers by UK-14304 was reversed by oral administration of VB20B7 in both rats and dogs. Cisapride, however, was only effective in rats. In addition, gastric emptying of a digestible solid/liquid meal was assessed by quantitating the rate of appearance of the radioactive markers in the duodenum of dogs. VB20B7 (0·2–1 mg kg^{-1}, i.v.) enhanced gastric emptying of both solid and liquid phases while cisapride only enhanced emptying of the solid phase.

The present study indicates that acute oral and intravenous administration of VB20B7 accelerates gastric emptying of both solids and liquids in different animal models.

Prokinetic drugs stimulate gastric emptying and small intestine motility in man and in various animal species (King & Sanger 1988; Kilpatrick et al 1990). Prokinetic benzamides with 5-HT_4 receptor agonist activity, such as metoclopramide and cisapride are widely used in the treatment of gastrointestinal tract disorders (Harrington et al 1983). It has been reported that cisapride may be useful in patients with idiopathic gastric stasis (Jian et al 1985), intestinal pseudo-obstruction (Camilleri et al 1985) or chronic constipation with laxative abuse as well as in diabetics with constipation (Lederer et al 1985). Among benzamide derivatives, cisapride (Schuurkes et al 1985) and renzapride (Sanger 1987) stimulate gastrointestinal motor activity through an enhancement in cholinergic transmission, like in experiments with isolated gastrointestinal preparations of the guinea-pig (Buchheit et al 1985; Schuurkes et al 1985). This effect may be due to an activation of 5-HT_4 receptors, which in turn produces a stimulation of the enteric cholinergic neurons (Craig & Clarke, 1990; Ramírez et al 1994). Several 5-HT_3 receptor antagonists are also prokinetic agents in rats and other animal species (Costall et al 1987; Buchheit et al 1989) but not in dogs (Schiavone et al 1990; Eglen et al 1993). Moreover, it is known that some potent 5-HT_3 receptor antagonists, such as LY277359, lack any prokinetic effect (Cohen et al 1990).

2[1-(4-Piperonyl)piperazinyl]benzothiazole (VB20B7; Monge et al 1994; Ramírez et al 1996) is a 5-HT-ergic agent whose chemical structure markedly differs from that of known prokinetic drugs (Monge et al 1994). In-vitro studies have shown that VB20B7 is a 5-HT_4 receptor agonist and a weak 5-HT_3 receptor antagonist (Ramírez et al 1996). The present study was aimed at characterizing comparatively the effects of VB20B7 and cisapride on gastric emptying in rats and in dogs, first in physiological conditions using both indigestible and digestible meals and also in a model of gastroparesis induced by an α_2-adrenergic agonist. In regard to a possible antiemetic activity in ferrets, VB20B7 was compared with ondansetron, the typical 5-HT_3 receptor antagonist. A preliminary study of the effects of VB20B7 on spontaneous motor activity and rectal temperature in mice was finally carried out. It is known that typical prokinetic benzamides such as cisapride and metoclopramide show some affinity at central D_2 receptors and extrapyramidal side effects have been occasionally reported with the latter drug (Pinder et al 1976). The results of the present study indicate that VB20B7 is a new drug with gastrokinetic properties in rats and dogs, particularly in the latter species.

*A preliminary report of this study was presented at the 7th European Symposium on Gastrointestinal Motility (Roca et al 1994).

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Materials and Methods

Gastroprokinetic activity in rats

Gastric emptying of steel spheroids. Male Wistar rats weighing 160 ± 10 g were fasted overnight with free access to water. Vehicle or drugs were administered orally. After 60 min, rats received 40 steel spheroids (1 mm diameter) in 2 mL of a 3% solution of carboxymethylcellulose by gavage as described (Jacoby & Brodie 1967). The animals were killed by CO_2 inhalation 60 min later. The spheroids remaining inside the stomach were counted and the number of spheroids emptied were calculated. Gastric emptying induced by drugs was

expressed as percentage of the number of spheroids emptied by control rats according to the formula:

$$(\text{no. spheroids test} - \text{no. spheroids control}) \times 100/40 \quad (1)$$

Gastric emptying of phenol red. Male Wistar rats (220–260 g) fasted overnight with water ad libitum were used. The animals were treated with test drugs suspended in 3% carboxymethylcellulose. After 60 min, each rat received by gavage 0·4 mL of a phenol red suspension (3·75 mg mL^{-1} in distilled water at 40°C) as described (Megens et al 1991). The rats were killed by cervical dislocation 30 min later and the stomach was extracted with 100 mL of 0·1 N NaOH, shaken and filtered. The phenol red content of this extract in the control and treated animals was assayed colorimetrically at 558 nm in a Unicam 8625 UV/Vis spectrophotometer and expressed in extinction units. Gastric emptying was determined as follows:

$$\text{gastric emptying } (\%) = (1 - A/B) \times 100 \quad (2)$$

where A and B were the average values for treated and control animals, respectively.

Antagonism of gastroparesis induced by the α_2-adrenergic agonist UK-14304. Male Wistar rats (230–250 g) were fasted overnight with water ad libitum. Animals were divided into 4 groups: vehicle, vechicle + UK-14304, cisapride + UK-14304 and VB20B7 + UK-14304. Sixty minutes after administration of the drugs, 40 steel spheroids in 2 mL of 3% carboxymethylcellulose were given by gavage. Immediately after, UK-14304 (0·1 mg kg^{-1} s.c.) suspended in Tween 80 and saline was administered. The animals were killed at different times (1, 2, 3 and 4 h). Percentages of gastric emptying were calculated as above.

Gastroprokinetic activity in dogs

Gastric emptying of solid radiopaque markers. Beagle dogs (10–15 kg) of both sexes were used. Animals were treated several times, with a minimum 7-day resting period between treatments. Assays were performed with animals fasted overnight. Test compounds were administered orally. After 1 h, the animals received 30 barium sulphate spheroids (4 mm diameter) following or not a standard solid meal (200 g of canned food, JAKAN-M). Measurement of gastric emptying was achieved by means of X-ray location (75 kv, 40 mA, 0·37 s). The passage of the markers was monitored for 2 h. Markers located between the second and the third intercostal spaces were considered to be inside the stomach. In fasted dogs, the results were expressed as percentage of animals emptying all the radiopaque markers. In dogs receiving the markers with a meal gastric emptying was calculated from the formula:

$$(\text{no. spheroids test} - \text{no. spheroids control}) + 100/30$$

Antagonism of gastroparesis induced by the α_2-adrenergic agonist UK-14304. Beagle dogs (10–15 kg) of both sexes were used. Gastroparesis was induced using the α_2-adrenergic model of gastroparesis (Gullikson et al 1991b). The highly potent and selective α_2-agonist UK-14304 (Cambridge 1981) was used. Animals were fasted overnight with free access to water. They received test drugs (VB20B7 and cisapride) by mouth 1 h before the oral administration of 30 barium sulphate spheroids. Immediately after, UK-14304, 0·03 mg kg^{-1}, was injected subcutaneously. The animals were observed for 5 hours. Gastric emptying was calculated from the formula:

$$(\text{no. spheroids test} - \text{no. spheroids control}) \times 100/30$$

Gastric emptying of a radioactive solid/liquid test meal. Beagle dogs weighing 13–15 kg were used. Under halothane anaesthesia, a Thomas cannula was placed on the greater curvature of the gastric body at about 10 cm from the pylorus and brought through the left abdominal wall at 5 cm from the last rib and 10 cm from the midline. The solid meal was prepared with liver from a sheep injected intravenously with 0·4 μCi kg^{-1} of [^{57}Co]cyanocobalamin (Hinder & Kelly 1977). The liquid phase consisted of 100 mL of tap water containing 50 mg of polyethyleneglycol 4000 (PEG), a non-absorbable water marker with 0·5 μCi of [^{14}C]PEG. VB20B7 and cisapride were given intravenously 10 min before the meal. One hour after meal ingestion, the stomach contents were collected by gravity, weighed, the volume measured and then carefully homogenized. Then 2 series of samples (4–5 mL) were rapidly taken. Each sample of the first series was accurately weighed and introduced into the gamma counter for [^{57}Co] determination. The second series was used for [^{14}C] determination. The samples were centrifuged (12 000 rev min^{-1}, 20 min) and 0·5 mL of supernatant used for liquid scintillation counting. Gastric emptying of solids was calculated from the formula:

$$\text{gastric emptying } (\%) = [(C_o \cdot W - C_t \cdot W_t) \times 100]/C_o \cdot W \quad (3)$$

where C_o = counts min^{-1} (g of marked liver introduced into the meal)$^{-1}$; W = weight of liver introduced into the meal; C_t = counts min^{-1} (g of homogenized gastric contents)$^{-1}$; W_t = weight of contents present in the stomach at time t. Gastric emptying of liquids was determined using the formula:

$$\text{gastric emptying } (\%) = ([^{14}\text{C}]\text{PEG}_i\, V_i - [^{14}\text{C}]\text{PEG}_t\, V_t) \; 100/[^{14}\text{C}]\text{PEG}_i\, V_i \quad (4)$$

where $[^{14}\text{C}]\text{PEG}_i$ = initial concentration of the liquid phase of the meal; V_i = initial volume of water added; $[^{14}\text{C}]\text{PEG}_t$ = concentration of homogenized meal at time t; V_t = volume of the homogenized meal.

Antiemetic activity in ferrets

Male fitch ferrets weighing 1–1·5 kg were used. They were purchased from Marshall Farm, North Rose, N.Y., USA. The animals were given free access to cat chow before being used in the study. Then they were fasted for 24 h with free access to water and a solid meal was provided 2 h before the beginning of the assay. VB20B7 and ondansetron were administered orally 30 min before the intraperitoneal administration of cisplatinum (15 mg kg^{-1}). The animals were housed in individual cages and observed for 5 h. Emetic episodes were defined as a series of retches or vomits, separated by at least a 5-min interval.

Central nervous system (CNS) activity

Spontaneous locomotor activity in mice. Male Swiss mice (20–25 g) were used. After the administration of drugs, mice were

placed in a black wooden open-topped box (65 × 65 × 45 cm height), divided into 4 equal compartments (walls of 30 cm), being thus possible to work with 4 animals simultaneously. Distance travelled in cm (locomotor activity) was measured by using a digital VIDEOMEX-V system (Columbus Instruments, USA) working with the appropriate computer program. After a 75-min period, divided into 5 sessions of 15 min, distance travelled in cm was recorded for each animal.

Rectal temperature in mice. Male Swiss mice (20–25 g) were used. Rectal temperature for each animal was determined using a digital thermometer (Panlab, Spain) before administration of test drugs or vehicle. After 40 min, the rectal temperature was determined again.

Drugs

VB20B7 (2[1-(4-piperonyl)piperazinyl]benzothiazole) was synthesized at the Department of Organic Chemistry, CIFA, University of Navarra. Other drugs and chemicals were obtained from the sources indicated: cisapride (Vita Laboratories, Spain), metoclopramide (Delagrange, Spain), cis-platinum (Wasserman, Spain), UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]quinoxaline bitartrate) (Pfizer, USA), phenol red (Aldrich, USA), carboxymethylcellulose (Panreac, Spain), [^{57}Co]cyanocobalamine (Amersham) and [^{14}C]PEG (New England Nuclear, USA).

Statistical analysis

Unpaired *t*-tests were used for comparing gastric emptying in treated and control rats. In studies with fasted beagle dogs, the number of animals responding with total emptying was analysed using a 2 × 2 Fisher test, while Student's *t*-test for paired values was used to compare control and treatment observations for each dog canine gastric emptying (dogs with a standard meal or emptying of a radioactive solid/liquid meal). After gastroparesis induced by UK-14304, analysis of variance followed by Scheffé test was used to determine statistical significance between drugs treatment and controls. Spontaneous motor and changes in rectal temperature in mice were analysed with a Student's *t*-test for paired values.

Results

Gastroprokinetic activity in rats

Gastric emptying of steel spheroids. VB20B7 (5–10 mg kg^{-1}, p.o.) significantly enhanced gastric emptying of steel spheroids in a dose-dependent manner with a potency practically similar to cisapride (Table 1). A higher activity for cisapride was only observed at the intermediate dose tested (7·5 mg kg^{-1}).

Gastric emptying of a phenol red suspension. Animals treated either with cisapride or VB20B7 had a content of phenol red in the stomach lower than controls, indicating an increase in

Table 1. Effect of VB20B7 and cisapride on gastric emptying of steel spheroids in rats.

Treatment	Dose (mg kg^{-1} p.o.)	n	Gastric emptying (%)	ED50 (mg kg^{-1} p.o.)
Control	—	16	30·0 ± 7·7	—
Cisapride	2·5	10	49·5 ± 5·8	3·60 (1·89–6·87)
	5·0	10	77·3 ± 8·7*	
	7·5	10	89·5 ± 4·1**	
	10·0	10	97·0 ± 1·5**	
VB20B7	2·5	8	42·2 ± 7·1	4·16 (2·49–6·95)
	5·0	8	75·0 ± 2·6*	
	7·5	9	73·3 ± 1·4*	
	10·0	8	92·8 ± 4·5**	

Rats given by gavage, 40 one-mm diameter steel spheroids 60 min after drugs. *$P < 0.05$; **$P < 0.01$ vs. controls (unpaired *t*-test). Gastric emptying, means ± s.e.m.; ED50, 95% confidence limits.

Table 2. Effect of VB20B7 and cisapride on gastric emptying of a phenol red suspension in rats.

Treatment	Dose (mg kg^{-1} p.o.)	n	Extinction units	Gastric emptying (%)
Control	—	6	0·82 ± 0·06	—
Cisapride	5	4	0·37 ± 0·09**	56
VB20B7	5	4	0·62 ± 0·10	24
Control	—	5	1·16 ± 0·22	—
Cisapride	10	4	0·54 ± 0·13*	53
VB20B7	10	5	0·61 ± 0·09*	48
Control	—	5	1·04 ± 0·16	—
Cisapride	20	4	0·61 ± 0·22*	41
VB20B7	20	5	0·63 ± 0·22*	39

Drugs given 60 min before phenol red. Animals killed 60 min later. *$P < 0.05$; **$P < 0.01$ vs the corresponding controls (unpaired *t*-test). Extinction units, mean ± s.e.m.

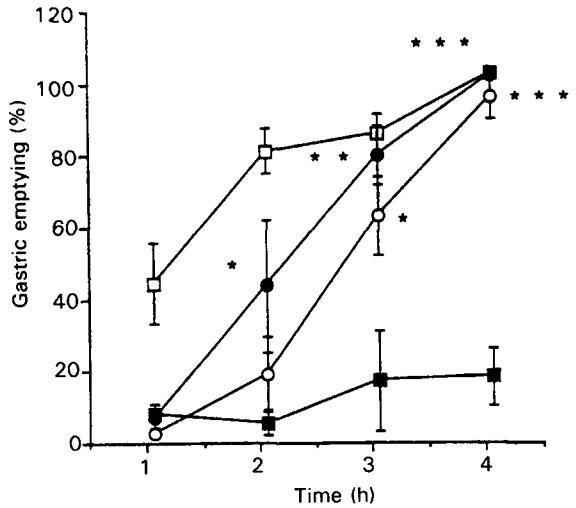

FIG. 1. Effect of VB20B7 (○) and cisapride (●) on the gastroparesis induced by the α_2-adrenergic agonist UK-14303 (■, 0·1 mg kg^{-1} s.c.) in rats. Vehicle only (□). Data are means ± s.e.m. of 4–9 animals. Prokinetic drugs given by mouth (10 mg kg^{-1}) 60 min before the administration of 40 steel spheroids (1 mm diameter); UK-14304 was injected immediately after. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs controls (unpaired *t*-test).

gastric emptying. Cisapride produced approximately the same percentage increase at the 3 doses tested (from 5 to 10 mg kg^{-1}, p.o.). The effect of VB20B7 at 5 mg kg^{-1} did not reach statistical significance. With higher doses (10–20 mg kg^{-1}, p.o.) the effect of VB20B7 did not substantially differ from that of cisapride (Table 2).

Antagonism of gastroparesis induced by the α_2-adrenergic agonist UK-14304. UK-14304 (0·03–0·1 mg kg^{-1}, s.c.) caused a dose-related inhibition in gastric emptying of steel spheroids. After the higher dose, gastric emptying in the first 2 h was practically absent, and averaged less than 20% of controls 3–4 h after administration. This dose was selected for further experiments. Cisapride and VB20B7, administered 1 h before the α_2-agonist, counteracted the delay in gastric emptying induced by UK-14304. Four hours after UK-14304 administration, the effect of the α_2 agonist was fully antagonized both by cisapride and VB20B7 (Fig. 1).

Gastroprokinetic activity in dogs

Gastric emptying of radiopaque markers. In fasted animals, there was a tendency to an all-or-none effect in the emptying of barium sulphate spheroids. Results were consequently expressed as percentage of animals emptying all the markers. None of the control dogs was able to empty all the markers over the 2 h of the experiment. In the cisapride group, the effect was practically identical 1 or 2 h after drug administration. The effect of VB20B7 was not dose-related. The results depicted in Table 3 show that VB20B7 produced a statistically significant effect in the second hour.

In control dogs receiving the radiopaque markers after a standard solid meal, there was only a moderate emptying of radiopaque markers over the observation period, averaging approximately 30%. At variance with the results in dogs not receiving a digestible meal there was a graded drug effect. Both drugs increased gastric emptying but there was a high dispersion of results and only VB20B7 produced a statistically significant effect (Table 4).

Antagonism of gastroparesis induced by the α_2-adrenergic agonist UK-14304. Pilot experiments showed that dogs receiving UK-14304, 0·1 mg kg^{-1} subcutaneously, exhibited a profound sedation along with emetic episodes. After a lower dose, 0·03 mg kg^{-1}, the sedation was only moderate and all of the radiopaque markers remained in the stomach for at least 5 h. In 5 out of the 7 animals treated with VB20B7, there was already some emptying of the spheroids in the second hour. Total gastric emptying occurred in 2 of these animals in the fourth hour. With cisapride, the gastroparesis induced by UK-14304 was only moderately attenuated in one of the animals, no effect being apparent in the other dogs. The results obtained are depicted in Fig. 2.

Gastric emptying of a radioactive solid/liquid test meal. VB20B7, 0·2 mg kg^{-1} intravenously, significantly increased, compared with controls, gastric emptying of solid and liquid phases. With a higher dose, 1 mg kg^{-1}, the acceleration of emptying of the solid phase was no longer apparent while that of the liquid phase was augmented. Cisapride only tended to increase significantly the evacuation of solids, but not of liquids with the lower dose tested, 0·2 mg kg^{-1} (Table 5).

Antiemetic activity in ferrets

Cis-platinum, 15 mg kg^{-1} intraperitoneally, induced in the ferret a potent emetic response characterized by retches and vomiting. The first emetic episodes in control animals started approximately 90 min after cis-platinum. Most retches were seen within the 120–180-min period and vomiting within the 90–120-min period after cis-platinum administration. The

Table 3. Effect of VB20B7 and cisapride on gastric emptying of radiopaque markers in fasted beagle dogs.

Treatment	Dose (mg kg^{-1} p.o.)	n	Animals with total emptying (%) 1 h	2 h
Control	—	16	0	0
Cisapride	0·5	7	14·3	14·3
	1·0	7	28·6	28·6
	2·0	6	16·7	16·7
VB20B7	0·25	7	42·8	57·1*
	0·5	7	57·1*	57·1*
	1	7	28·6	57·1*

Drugs were given 1 h before the administration by gavage of 30 barium sulphate spheroids. $*P < 0.05$ vs control (Fisher 2 × 2 test).

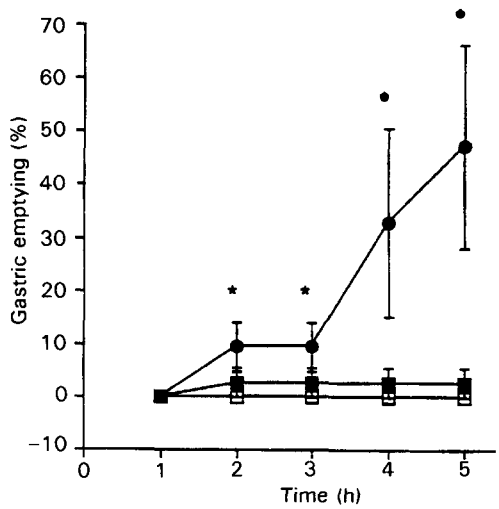

FIG. 2. Effect of VB20B7 (●, 0·5 mg kg^{-1}) and cisapride (■, 1 mg kg^{-1}) on the gastroparesis induced by the α_2-adrenergic agonist UK-14304 (□) (0·03 mg kg^{-1} s.c.) in fasted beagle dogs. Data are means ± s.e.m. of 6–7 animals. Prokinetic drugs given by mouth 60 min before administration of radiopaque spheroids; UK-14304 was injected immediately after. *$P < 0·05$ vs controls (one-factor analysis of variance followed by Scheffé test).

maximum inhibitory effect of VB20B7 (10 mg kg^{-1} p.o.) was reached within the 120–150-min period (60·6% and 92·9% for retches and vomiting, respectively) and was statistically significant. Ondansetron (0·5 mg kg^{-1}, p.o.) fully protected all of the animals, no retches or vomiting being observed in any treated ferret.

CNS activity

Spontaneous motor activity in mice. High doses of VB20B7, 20 mg kg^{-1} intraperitoneally or 200 mg kg^{-1} orally, did not modify spontaneous motor activity in mice while identical doses of cisapride produced a highly significant reduction (Table 6).

A highly significant decrease in spontaneous motor activity was also found when cisapride was injected intracerebroventricularly (5–10 μg per mouse) and the activity recorded between 5 and 20 min after the intracerebroventricular injection. Identical doses of VB20B7 dit not produce any significant reduction in motor activity (Table 6).

Rectal temperature in mice. Cisapride and metoclopramide, especially the latter drug, produced a profound decrease in rectal temperature when given at the high dose of 100 mg kg^{-1} intraperitoneally. An identical dose of VB20B7 did not change the rectal temperature of mice (Table 7).

Discussion

The results of the present work show that VB20B7 stimulates gastric emptying of solids and liquids in rats, in physiological conditions and in a situation of drug-induced gastroparesis. Lower doses of this new drug also accelerate gastric emptying in dogs.

VB20B7 facilitated gastric emptying in rats, both of solids (steel spheroids) and liquids (phenol red suspension). Both VB20B7 and cisapride showed a dose-related effect on gastric emptying of solids but not of liquids. Differences in the effect of compounds on gastric emptying of solids and liquids are probably due to different emptying patterns since liquid emptying is regulated by tonic contractions of the proximal part of the stomach, whereas the peristaltic activity of the distal part (antrum) is responsible for solid emptying (Minami & McCallum 1984; McCallum et al 1988; Gullikson et al 1991a).

VB20B7 also enhanced gastric emptying of radiopaque markers in fasted beagle dogs whereas cisapride only produced a weak effect that did not reach statistical significance. In these experiments, drug treatment usually produced a non dose-related all-or-none pattern of gastric emptying. Moreover, drug effect was not linear with time, particularly in the case of cisapride which only produced some gastric emptying in the first hour of the observation period. Like in healthy subjects and in patients with gastroparesis (Feldman & Smith 1987), emptying of indigestible solids in fasted dogs does not allow simple statistical analysis. The experiments were repeated in the same beagle dogs following a standard solid meal in an attempt to mimic normal physiological conditions. In this new situation, animals showed a gradual gastric emptying over the

Table 4. Effect of VB20B7 and cisapride on gastric emptying of radiopaque markers in beagle dogs with a standard meal.

Treatment	Dose (mg kg^{-1} p.o.)	n	Gastric emptying (%)	
			1 h	2 h
Control	—	11	11·2 ± 3·6	29·1 ± 6·6
Cisapride	0·5	3	7·8 ± 4·5	24·5 ± 7·8
	1·0	4	25·8 ± 10·4	47·5 ± 14·7
	2·0	4	49·2 ± 28·4	51·7 ± 27·0
	4·0	5	27·3 ± 15·2	50·7 ± 20·7
VB20B7	0·1	3	16·7 ± 15·0	32·2 ± 19·3
	0·25	6	21·7 ± 16·0	67·2 ± 20·7*
	0·5	4	55·0 ± 7·3*	67·5 ± 5·5*
	1·0	4	40·0 ± 3·6*	55·0 ± 4·0

Drugs given 1 h before the administration by gavage of 30 barium sulphate spheroids. Gastric emptying was calculated individually as % change (mean ± s.e.m.) in emptying over own control. *$P < 0·05$ vs controls (paired t-test).

Table 5. Effect of vehicle, VB20B7 and cisapride on gastric emptying of a radioactive solid or liquid meal.

	Dose (mg kg^{-1} i.v.)	Gastric emptying (%)	
		Solid meal	Liquid meal
Vehicle	—	32 ± 5	27 ± 5
VB20B7	0·2	39 ± 4*	33 ± 6*
Cisapride	0·2	38 ± 4	27 ± 2
Vehicle	—	32 ± 5	27 ± 5
VB20B7	1·0	35 ± 7	37 ± 5*
Cisapride	1·0	34 ± 7	28 ± 4

VB20B7 and cisapride were administered intravenously, 10 min before the meal and determinations were carried out 60 min later. *$P < 0.05$ vs controls (Student's t test for paired values). Data are means ± s.e.m., n = 8.

Table 6. Effect of VB20B7 and cisapride on spontaneous locomotor activity in mice.

Treatment	Dose[a]	n	Motor activity (cm travelled)
Control	—	8	5817 ± 1042
Cisapride	20 (i.p.)	6	1498 ± 90***
VB20B7	20 (i.p.)	8	5225 ± 877
Control	—	7	5219 ± 335
Cisapride	200 (p.o.)	8	222 ± 69***
VB20B7	200 (p.o.)	8	4616 ± 719
Control	—	5	4580 ± 235
Cisapride	5 (i.c.v.)	5	1145 ± 93***
	10 (i.c.v.)	5	870 ± 45***
VB20B7	5 (i.c.v.)	5	4213 ± 390
	10 (i.c.v.)	5	4992 ± 410

[a]Doses given in mg kg^{-1} (i.p. and p.o.) or in μg (mouse)$^{-1}$ (i.c.v.). Motor activity recorded for 75 min. Data are mean ± s.e.m. ***$P < 0.001$ vs the corresponding controls (paired t-test).

2 hours of the observation period. Both drugs enhanced gastric emptying of fed dogs. VB20B7 showed a more marked effect than cisapride, that produced again an increase in gastric emptying which did not reach statistical significance. It has been reported that under physiological conditions the control mechanisms of gastric emptying in dogs may not be markedly accelerated by cisapride (Wulschke et al 1986) and it seems that prokinetic drugs only accelerate emptying in the dog when gastroparesis is present (Gullikson et al 1991a). Since the dose-response curve for the effect of cisapride on gastric emptying in this model was bell-shaped, it should not be expected in principle that higher doses would promote a more marked emptying. Stimulation of antral motility may elicit inhibitory reflexes reducing gastric motility and this may result in a failure to accelerate gastric emptying (Wulschke et al 1986). Interestingly, VB20B7 was also able to increase gastric emptying in the dog in physiological conditions. These results may be of particular relevance since dogs are probably a better model for man with regard to gastrokinesis (e.g. review by Ford & Clarke 1993). Even though emptying of radiopaque markers has not been frequently used, to our knowledge, for the study of prokinetic drugs in dogs, this method has been

Table 7. Effect of VB20B7, metoclopramide and cisapride on rectal temperature in mice.

Treatment	Dose (mg kg^{-1} i.p.)	Temperature (°C)	
		Baseline	Treatment
Vehicle	—	38·24 ± 0·16	37·92 ± 0·14
Metoclopramide	100	38·74 ± 0·05	33·56 ± 0·58**
Cisapride	100	38·86 ± 0·08	35·86 ± 0·44**
VB20B7	100	38·93 ± 0·03	38·08 ± 0·32

Data are means ± s.e.m. of 5 mice. Temperature recorded before treatment and 40 min after treatment. **$P < 0.01$ vs the corresponding controls (paired t-test).

extensively used in studies in humans since it is a simple and highly sensitive procedure for evaluating gastric motor dysfunction and the effect of prokinetic agents (Bertrand et al 1980; Feldman & Smith 1987; Kawagishi et al 1993).

Prokinetic drugs like cisapride clearly stimulate gastric and intestinal contractions in dogs and normal healthy subjects, but gastric emptying may not be enhanced (Wulschke et al 1986; Edwards et al 1987). It was therefore of interest to induce gastroparesis pharmacologically in rats and in dogs (Muller-Lissner et al 1986; Gullikson et al 1991b) in order to demonstrate clearly the effects of prokinetic benzamides on gastric emptying. Use of the α_2-adrenergic canine model of gastroparesis has permitted the demonstration of the gastrokinetic activity of benzamides, such as renzapride (Gullikson et al 1991b). The highly potent and selective α_2-adrenoceptor agonist UK-14304 (Cambridge 1981) was able to induce a situation of gastroparesis in rats and in dogs with a complete inhibition of gastric emptying. VB20B7 reversed gastroparesis in both animal species while cisapride was more effective in rats but lacked any apparent effect on gastric emptying in dogs. It could be supposed that the reversion of gastroparesis induced by VB20B7 was due to an α_2-receptor antagonism. However, in other experiments (not shown) performed in the electrically stimulated longitudinal muscle myenteric plexus, VB20B7 failed to antagonise the inhibitory effect of the α_2-adrenoceptor agonist clonidine. The results obtained with cisapride in dogs with gastroparesis are not in keeping with those reported by Gullikson et al (1991a), although this discrepancy may come simply from the use of a non-nutrient solid (i.e. barium sulphate beads) in the present study. Control mechanisms for emptying of indigestible solids are obviously very different from those operating in the emptying of solid food (Hinder & Kelly 1977; Feldman et al 1984; Kawagishi et al 1993).

In most studies with prokinetic drugs, non-nutrient solids and liquids that do not initiate a true digestive state have been used to measure gastric emptying, which is known to be affected by both the amount and the composition of meals (Mizuta et al 1990; Gullikson et al 1991a). Consequently, the enhancement of gastric emptying of solid and liquid radioactive meals was also studied. VB20B7 accelerated gastric emptying of both solid and liquid meals whereas cisapride only tended to modify significantly gastric emptying of the solid meal. Like in the previous study with radiopaque spheroids, a loss of effect was found by increasing the dose of both VB20B7 and cisapride. This appears to be a general finding for several prokinetic compounds (e.g. Rizzi et al 1994) and is

most likely due to a strong effect on the amplitude of antral contractions, preventing antral filling from the gastric body (Schuurkes 1990). However, it should also be noted that in dogs with Thomas cannulas the physiological control of emptying by the intestine has been eliminated.

The enhancement of contractile activity in the upper gastrointestinal tract by prokinetic benzamide drugs has been demonstrated to be cholinergically-mediated (Wulschke et al 1986; Bermúdez et al 1990). Thus, an increase in acetylcholine release in the myenteric plexus of the gut has been described for metoclopramide (Kilbinger et al 1982), cisapride (Schuurkes et al 1985), BRL 24924 (Sanger 1987) and AS-4370 (Yoshida et al 1989). VB20B7 appears to be a 5-HT_4 receptor agonist in the periphery (Ramírez et al 1996) and a stimulation of this receptor leads to an activation of the enteric cholinergic neurons (Ramírez et al 1994). This mechanism of action has previously been suggested by Gullikson et al (1993). However, the enhancement of gastric motility may be via separate pathways, not involving 5-$HT_{1,2,3,4}$ receptors, at least in the canine antrum (De Ridder & Schuurkes 1993).

As indicated in the Introduction, VB20B7 was a weak 5-HT_3 receptor antagonist. Consistent with this modest activity, VB20B7 was scarcely effective as an antagonist of cis-platinum- induced emesis. Even though VB20B7 was not directly compared with cisapride, it seems that the latter drug is a more potent antiemetic (Gullikson et al 1991a). Obviously, the slight antagonist activity of VB20B7 at 5-HT_3 sites does not appear to account at all for the prokinetic effect of this drug. Admittedly, 5-HT_3 receptor antagonists are able to promote gastric emptying in rats but not in dogs or in humans (Nielson et al 1990; Schiavone et al 1990; Eglen et al 1993). Cisapride was more potent than VB20B7 as an antagonist at 5-HT_3 receptors of the guinea-pig ileum (Ramírez et al 1996). 5-HT_3 receptor antagonists such as tropisetron or granisetron not only do not stimulate but inhibit antral contractile activity in dogs (Gullikson et al 1991a). It is then possible to speculate that 5-HT_3 receptor antagonism may determine the comparatively lower effect of cisapride in the studies of gastric emptying in dogs.

Dopamine is known to suppress gastric motility by inhibiting the release of acetylcholine from the nerve terminals via stimulation of dopamine D_2 receptors (Kusunoki et al 1985). In binding assays, VB20B7 even at a 10^{-4} M concentration had no affinity at the [^{3}H]spiperone-labelled D_2 receptors of rat cortical membranes whereas cisapride had a relative high affinity at this site, with an IC50 of 0·68 μM (Monge et al 1994), a result in agreement with previous data (Yoshida et al 1991). The decrease in spontaneous locomotor activity of mice induced by high doses of cisapride and not by the same doses of VB20B7 may be a consequence of the differential activity of both drugs at central dopamine D_2 receptors. Since qualitatively identical results were obtained after intracerebroventricular administration of the drugs, these data suggest that the lack of effect VB20B7 is not a consequence of a poor penetration of the blood-brain barrier. Another effect associated with D_2 receptor antagonism is the decrease of rectal temperature. Cisapride, and more markedly metoclopramide, produced a significant decrease whereas VB20B7 did not produce any effect when administered at the same high dose.

In conclusion, VB20B7 is a prokinetic agent whose effects are possibly mediated through an agonist activity at peripheral 5-HT_4 receptors. Its pharmacological profile is partially different from that of cisapride as it lacks any affinity at dopamine D_2 receptors. From the clinical point of view, VB20B7 may be a gastrokinetic agent similar to cisapride.

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