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Effects of calcium channel blockers on gastric emptying and acid secretion of the rat *in vivo*

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1 Experiments were designed to evaluate the effects of three calcium channel blockers (verapamil, diltiazem and cinnarizine) on gastric emptying and secretion in the rat

2 Pretreatment with the calcium blockers delayed gastric emptying of phenol red in a dose-dependent manner. Verapamil was the most effective of the agents tested.

3 Verapamil and diltiazem inhibited gastric acid secretion in the pylorus-ligated rat without affecting pepsin output. Cinnarizine was ineffective in this model.

4 When the perfused lumen of the anaesthetized rat was used, verapamil was found to inhibit responses to carbachol or histamine more than those to pentagastrin. Further, we found a greater sensitivity to verapamil for basal compared with vagal-stimulated (2-deoxy-D-glucose) acid secretion. Neither diltiazem nor cinnarizine modified gastric acid secretion in this experimental model.

5 These findings are discussed in relation to the role of extracellular calcium in gastric motility and secretion, and the existence of a regional and functional selectivity for calcium blockers is proposed.

Introduction

The pharmacological actions of calcium channel blockers arise from their ability to inhibit directly the influx of calcium ions (Ca^{2+}) across the plasma membrane of excitable cells. Hitherto calcium blockers have been used mainly in the cardiovascular system as inhibitors of muscle contraction. However, since extracellular Ca^{2+} fluxes are not only involved in the stimulus-contraction coupling of this particular system, and contraction is not the only event in which they play an important role, it is likely that calcium blockers could also have effects on a wider range of functions and tissues (Triggle & Swamy, 1983; Katz, 1985).

In the stomach, motility and acid secretion have been shown to be dependent to some extent on Ca^{2+} and are likely to be modified by calcium blockers (Castell, 1985). However, at present there is no experimental evidence to show a major effect of these drugs on gastric motility and reports about their influence on gastric acid secretion are scarce and controversial (Kirkegaard *et al.*, 1982; Levine *et al.*, 1983; Im *et al.*, 1984; Sonnenberg *et al.*, 1984). The present investigation therefore attempts to measure the effect of calcium blockers on both these functions in the rat and to suggest the extent to which Ca^{2+} may be involved. A preliminary account of this work was given at the 9th meeting of the Spanish Society of Pharmacology in Valencia (1985).

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Methods

General

Wistar rats of either sex, weighing 195–225 g, were deprived of food but allowed free access to water 24 h before the onset of the experiments. They were kept in cages provided with a wire net base to avoid coprophagy. Verapamil (Knoll) and diltiazem (Synthelabo) were dissolved in physiological saline solution. Cinnarizine (Lab. Esteve – Division Janssen) was not readily soluble in saline and was dissolved in 0.05% Tween 80 in distilled water before further dilution in saline. All calcium blockers were administered intraperitoneally in a volume of 0.5 ml 200 g^{-1} 30 min before starting the experiments, except in the blood

pressure experiments when they were administered after stabilization. Control rats received, by the same route, a similar volume of saline. During the experiments animals were kept at a temperature of 22–24°C.

Measurement of blood pressure in anaesthetized rats

Rats were anaesthetized with urethane (1 g kg^{-1}, i.p.) and maintained on artificial respiration using a Harvard respiration pump. The systemic blood pressure was continuously recorded from the carotid artery by means of a Hewlett Packard (HP-1280) pressure transducer. Blood pressure was allowed to stabilize for at least 30 min before the responses to calcium blockers were investigated.

Measurement of gastric emptying in conscious rats

The technique was essentially that described by Scarpignato & Calpovilla (1980). The test meal consisted of a solution of 50 mg phenol red in 100 ml of aqueous methylcellulose (1.5%) given by oral intubation as 1.5 ml per rat 30 min after the administration of calcium blockers. All animals were killed by an overdose of anaesthetic (urethane i.p.) 60 min later except those in a control group which were killed immediately after the administration of the test meal. The stomach was exposed by laparotomy, quickly clamped at the pylorus and cardia and then carefully removed. The viscera and its contents were homogenized in 5 ml 0.1 N NaOH and centrifuged (5,000 *g* for 5 min).

The colorimetric assay of phenol red was performed at 560 nm (Unicam S.P. 1800 spectrophotometer) after protein precipitation (20% trichloroacetic acid) and re-alkalinization of the supernatant (borate buffer pH 10).

Results are presented as percentage changes with respect to the average amount of phenol red recovered immediately after the meal from untreated animals, which was therefore taken as a maximum inhibitory response (100%)

Measurement of gastric acid secretion in pylorus-ligated rats

The pylorus-ligated rat model first described by Shay *et al.* (1945) was used. The surgical manipulation was carried out under light ether anaesthesia. Care was taken not to damage the blood supply. The animals were killed 4 h after ligation of the pylorus, the abdomen re-opened and the stomach removed. The gastric content was collected and its volume measured. Aliquots were analyzed for hydrogen ion concentration by electrometric titration to pH 7 with 0.1 N NaOH. The pepsin concentration was estimated by the colorimetric method of Anson (1938) involving haemoglobin digestion (2%, pH 2, 37°C, 15 min), followed by alkaline condensation with Folin-Ciocalteau reagent and spectrophotometric measurement of the absorption at 660 nm.

Measurement of acid secretion in the lumen perfused stomach of the anaesthetized rat

The procedure followed was that described by Ghosh & Schild (1958). In brief, animals were anaesthetized with urethane (1 g kg^{-1} i.p.) and the trachea intubated to facilitate respiration. Two polyethylene cannulae were inserted so that they reached the stomach lumen, one via the oesophagus and the other via the duodenum. The antroduodenal cannula was led outside the abdominal wall. The stomach cavity was then perfused with warm saline (NaCl 0.9% w/v, at 37°C) at a rate of 1 ml min^{-1}, by the use of a peristaltic pump (Watson-Marlow Ltd). After stabilization (30 min), the hydrogen ion activity of the effluent perfusate from the stomach was continuously recorded by means of a glass electrode (Ingold) coupled to a pH-meter (Crison 505) and a potentiometric pen recorder (Phillips). The rate of acid secretion was expressed as mEq H^+ min^{-1}. When the gastric acid output reached a steady state it was recorded for 40 min and considered as basal or unstimulated acid secretion. Thereafter carbachol (10 μg kg^{-1}, Sigma), pentagastrin (20 μg kg^{-1}, ICI), histamine (5 mg kg^{-1}, Merck) or 2-deoxy-D-glucose (2-DDG, 200 mg kg^{-1}, Merck), dissolved in saline in a volume of 0.25 ml, were injected as a bolus through a cannulated jugular vein and the response induced monitored for 2 h.

Statistical analysis

Data are presented as the mean ± s.e.mean obtained from a minimum of 5 animals for each experimental group. When measuring gastric emptying and acid secretion in pylorus-ligated rats, least square regression analysis was used to obtain the line of best fit through the average data points. The ID_{50} (dose causing 50% inhibition) was calculated from the plot. The significance of differences was assessed by the unpaired *t* test and a *P* value of less than 0.05 was considered to be significant.

Results

Effects on blood pressure

Results obtained 90 min after the administration of the calcium blockers are shown in Figure 1. In our experiments blood pressure in the control group remained fairly constant for at least 4 h, and all three

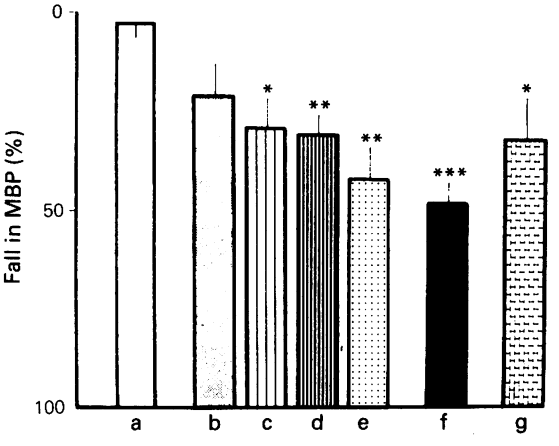

Figure 1 Fall in mean blood pressure (MBP) of the anaesthetized rat 90 min after the administration of saline (a); verapamil 1 mg kg^{-1} (b), 3 mg kg^{-1} (c), 10 mg kg^{-1} (d) and 20 mg kg^{-1} (e); diltiazem 90 mg kg^{-1} (f) and cinnarizine 100 mg kg^{-1} (g). Each column represents the mean of 5 animals and vertical lines show s.e.mean. * $P < 0.05$, ** $P < 0.001$ versus the saline-treated group.

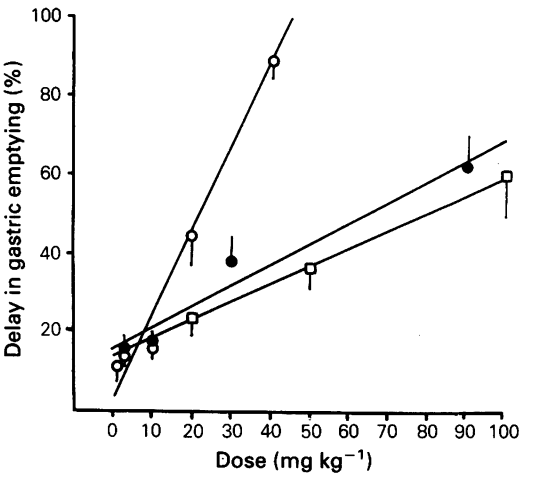

Figure 2 Inhibition of the gastric emptying of phenol red by verapamil (○), diltiazem (●) and cinnarizine (□) in the conscious rat. Each point represents the mean of at least six animals and the vertical lines show s.e.mean. The regression lines were calculated by the method of least squares.

drugs studied induced a hypotension which lasted for at least a similar period of time. Diltiazem (90 mg kg^{-1}) and verapamil (20 mg kg^{-1}) were the most effective agents in lowering blood pressure. Cinnarizine (100 mg kg^{-1}) and intermediate doses of verapamil (3 and 10 mg kg^{-1}) also significantly decreased blood pressure values. Although verapamil 1 mg kg^{-1} exhibited a hypotensive action this effect did not reach statistical significance.

Effects on gastric emptying in conscious rats

All three calcium blockers caused a dose-related delay in the gastric emptying of phenol red (Figure 2) although this was not significant at the lowest doses used. Verapamil was clearly the most potent agent (ID_{50} 21.4 mg kg^{-1}) and its regression line was much steeper than that of diltiazem (ID_{50} 62.5 mg kg^{-1}) or cinnarizine (ID_{50} 76.6 mg kg^{-1}). Under our experimental conditions the stomachs of control animals at the end of the 60 min period contained 13.7 ± 2.4% of the amount of phenol red found in those of control rats killed immediately after the administration of the dye.

Effects on gastric secretion in pylorus-ligated rats

Pretreatment with verapamil and diltiazem reduced, in a dose-dependent manner, both the volume of secretion and the acid output. However, at higher doses, the acid output was inhibited to a greater extent than the volume of secretion (Table 1). Verapamil (ID_{50} acid secretion 12.2 mg kg^{-1}; ID_{50} volume 18.9 mg kg^{-1}) was again more potent than diltiazem (ID_{50} acid secretion 30.2 mg kg^{-1}; ID_{50} volume 45.4 mg kg^{-1}) on both of these parameters. Cinnarizine had no significant effects in the pylorus-ligated rat. Pepsin secretion was not significantly modified in our experiments by any dose of calcium blocker employed.

Effects on acid secretion in the perfused stomach of the anaesthetized rat

Verapamil 1 mg kg^{-1} had no significant effect on basal secretion but pretreatment with 3, 10 and 20 mg kg^{-1} verapamil significantly decreased the acid output of the unstimulated stomach during the 40 min period before the administration of the different secretagogues. The degree of reduction in basal acid secretion was similar using all three doses of this calcium blocker (Figure 3). Verapamil also modified the response elicited by the different stimulants, although the character of this modification varied. Inhibition of responses to carbachol (Figure 4a) or histamine (Figure 4b) was maximal at a dose of 3 mg kg^{-1} of verapamil; increasing the dose to 10 mg kg^{-1} and 20 mg kg^{-1} produced no further increase in inhibition. In contrast, the same dose of verapamil did not decrease secretion stimulated by pentagastrin (Figure 4c), significant inhibition only being obtained at the higher doses. Significant reduction of 2-DDG-stimulated gastric acid secretion was

Table 1 Effects of verapamil, diltiazem and cinnarizine on gastric acid and pepsin production in pylorus-occluded rats (4 h)

	Dose (mg kg^{-1})	n	*Volume* (ml)	*Inhibition* (%)	H^+ (mEq h^{-1})	*Inhibition* (%)	*Pepsin* (mg h^{-1})
Saline		30	4.7 ± 0.5	---	55.3 ± 10	---	1.9 ± 0.6
Verapamil	3	12	3.7 ± 0.3	21.3	43.1 ± 7	22.1	1.6 ± 0.6
	20	12	2.2 ± 0.4*	53.2	12.1 ± 1*	78.1	2.1 ± 0.2
	40	11	0.8 ± 0.2**	83.0	4.7 ± 2*	91.5	2.9 ± 0.6
Diltiazem	10	12	3.9 ± 0.3	17.0	48.0 ± 8	13.2	1.5 ± 0.3
	30	11	2.1 ± 0.3*	55.3	15.6 ± 6*	71.8	2.2 ± 0.4
	100	12	1.1 ± 0.4**	76.6	2.3 ± 3*	95.8	1.9 ± 0.5
Cinnarizine	50	10	3.4 ± 0.4	27.7	38.8 ± 5.6	29.8	2.4 ± 0.3
	100	10	4.1 ± 0.4	12.8	45.2 ± 7.6	18.3	1.8 ± 0.4

Values are expressed as mean ± s.e.mean. * $P<0.01$ ** $P<0.001$, versus the saline pretreated group.

only obtained at the maximum dose of verapamil used, 20 mg kg^{-1} (Figure 4d).

With the same doses previously shown to inhibit gastric emptying (Figure 2) diltiazem and cinnarizine were without significant effects on acid secretion, both basal and stimulated, in this experimental model.

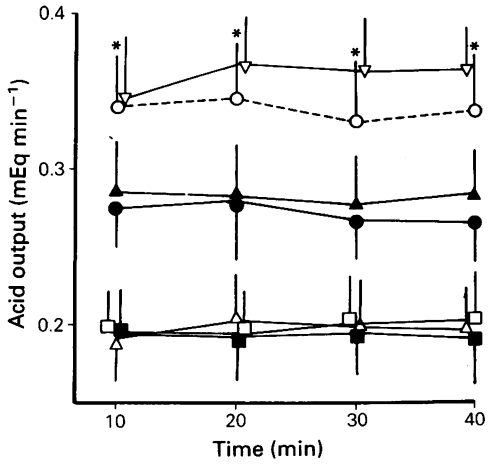

Figure 3 Basal acid output in the perfused stomach of rats. Control (○); pretreated with verapamil 1 mg kg^{-1} (●), 3 mg kg^{-1} (□), 10 mg kg^{-1} (■) and 20 mg kg^{-1} (△); diltiazem 90 mg kg^{-1} (▲) and cinnarizine 100 mg kg^{-1} (▽). Each point represents the mean of at least 24 animals and vertical lines show s.e.mean. * $P<0.05$ versus verapamil 3, 10 and 20 mg kg^{-1}.

Discussion

Calcium blockers relax smooth muscles by inhibiting the influx of Ca^{2+} across the cell membrane. The important inhibitory effects shown by these substances on cardiovascular smooth muscle suggest the possibility of a similar action on the smooth muscle of other regions such as the gastro-intestinal tract. In addition, contraction is not the only physiological function in which fluxes of Ca^{2+} play an important role in cell regulation. Gastric acid secretion has also been shown to be dependent to some degree on Ca^{2+} and, therefore, may be modified by substances which inhibit Ca^{2+} entry.

In the present study gastric emptying of liquids, a parameter which is dependent on the activity of the smooth muscle of the stomach (Kelly, 1981), was shown to be delayed by calcium blockers in a dose-related manner, verapamil being the most effective of the agents used. The order of potency of the drugs as inhibitors of gastric emptying was similar to that of their potency as calcium antagonists *in vitro* (Spedding, 1985). This fact, together with the antagonistic actions on calcium channel exhibited in other tissues of the rat *in vivo* by similar doses of verapamil (Curtis *et al.*, 1984) clearly imply that the delay in gastric emptying is due to calcium antagonist effects. Our results are in keeping with previous studies describing similar inhibitory effects of these drugs, both *in vivo* and *in vitro*, on smooth muscle from other parts of the mammalian gastrointestinal tract such as the oesophagus (Goyal & Rattan, 1980; Hongo *et al.*, 1984; Morales-Olivas *et al.*, 1985) or the colon (Blume *et al.*, 1983; Baumgartner *et al.*, 1985). Although recently Ogle *et al.* (1985) observed that verapamil did not affect the gastric emptying of solids, the low doses used by these authors could account for this difference.

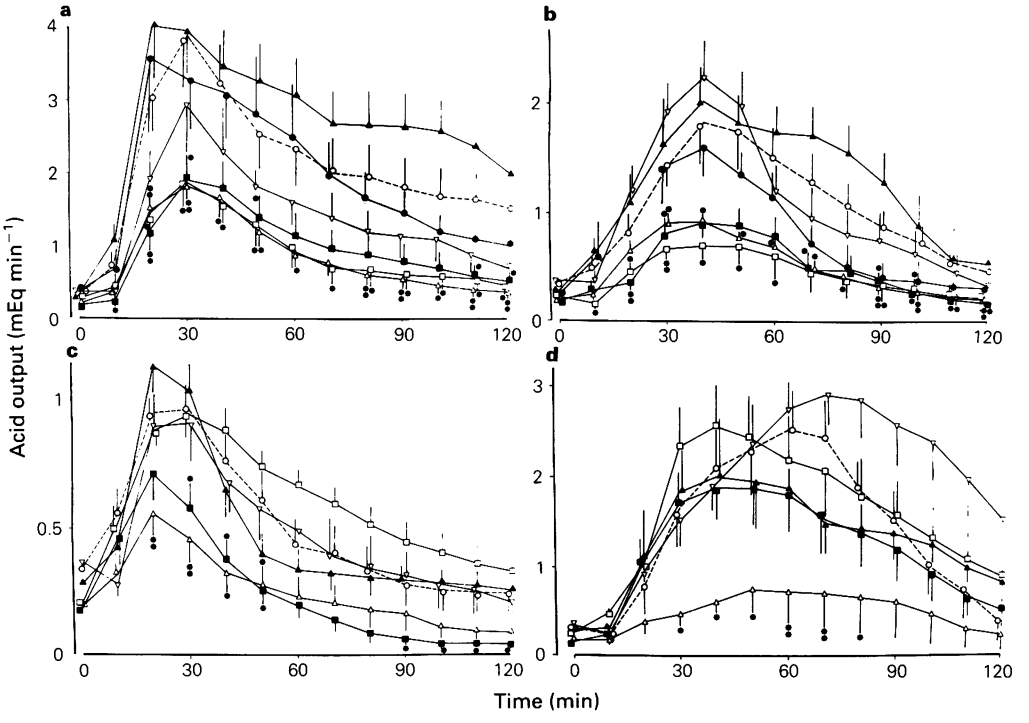

Figure 4 Acid output elicited by a bolus injection of (a) carbachol (10 μg kg^{-1} i.v.), (b) histamine (5 mg kg^{-1} i.v.), (c) pentagastrin (20 mg kg^{-1} i.v.) and (d) 2-deoxy-D-glucose (200 mg kg^{-1} i.v.) in the perfused stomach of rats. Control (O---O); pretreated with verapamil 1 mg kg^{-1} (●), 3 mg kg^{-1} (□), 10 mg kg^{-1} (■) and 20 mg kg^{-1} (△); diltiazem 90 mg kg^{-1} (▲) and cinnarizine 100 mg kg^{-1} (▽). Each point represents the mean of 6 animals and vertical lines show s.e.mean. * $P<0.05$, ** $P<0.01$ versus the control group except in (a) when ** $P<0.001$ versus the control group.

Extracellular Ca^{2+} appears to be an important factor in the control of gastric secretion. Reduction of Ca^{2+} concentration in the nutrient solution modifies the responses to ACh, gastrin and histamine in canine isolated parietal cells (Soll, 1981). Verapamil decreases acid output elicited by histamine in the guinea-pig fundic mucosa (Kirkegaard *et al.*, 1982) and by pentagastrin in the isolated whole stomach of the mouse (Szelenyi 1980). However, it seems unlikely that Ca^{2+} is equally important in the mechanisms of action of the different secretagogues. Although most studies agree on the necessity of this ion for a response to a cholinergic stimulus, studies involving *in vitro* gastrin- or histamine-evoked acid secretion (Main & Pearce, 1978; Bunce *et al.*, 1979; Berglindh *et al.*, 1980) raise some doubts about the importance of the role played by Ca^{2+} in the actions of these two secretagogues. Furthermore, studies in man (Kirkegaard *et al.*, 1982; Levine *et al.*, 1983; Aadland & Berstad, 1983; Sonnenberg *et al.*, 1984) have produced conflicting results on the action of verapamil on acid secretion produced by a wide variety of stimulants. Since low doses of this agent were used in many cases, it is difficult to deduce from these studies whether an intrinsic lack of effect or an insufficient dose is responsible for these differences.

In the present work the effects of different doses of three calcium blockers on two *in vivo* models of gastric secretion are described. In the pylorus-ligated rat both verapamil and diltiazem inhibited the volume of gastric secretion and total acid output in a dose-dependent manner. Although pepsin and acid secretion are usually affected by drugs in the same way (Kontureck, 1980; Esplugues *et al.*, 1982), in this case pepsin secretion remained unaffected by calcium blockers suggesting no significant involvement of Ca^{2+} in the physiological secretion of this enzyme.

The lack of correlation between the hypotension induced by the three calcium blockers and their action on gastric secretion, and on gastric emptying, suggest that the latter effects are not an indirect consequence of the cardiovascular effect of the compounds. It is also probable that the effects on gastric emptying are not a result of an inhibitory effect on secretion as cinnarizine has no significant antisecretory activity.

In the anaesthetized rat verapamil was shown to inhibit gastric secretion. Two patterns of response were found in these experiments. Firstly, basal, carbachol and histamine-stimulated acid secretion were of similar sensitivity to inhibition by verapamil, a maximum effect being observed with 3 mg kg^{-1} verapamil. As this dose of verapamil did not influence gastric emptying it is unlikely that these two actions are related. In contrast, when pentagastrin or 2-DDG was used, their actions showed a greater resistance to this calcium blocker and only higher doses of verapamil were able to modify their effects significantly. Assuming that all Ca^{2+} channels involved in the response to these secretagogues are equally affected by verapamil, these results point to the existence of a different role for extracellular Ca^{2+} depending on the stimulus involved, cholinergic and histaminergic being more dependent on the ion than gastrin-induced ones.

2-DDG does not act directly on the parietal cells but provokes a vagally-mediated stimulus which could be considered to be more physiologically relevant than direct stimulation (Grossman, 1981). It is therefore interesting that gastric acid secretion elicited by 2-DDG administration differs from basal secretion in the continuously perfused lumen, and from that produced in the pylorus-ligated rat, in its sensitivity to Ca^{2+} antagonists. The latter two are affected by verapamil in a similar way to that of responses elicited by carbachol and histamine, and reductions in acid output are achieved by a low dose of this calcium blocker. In contrast, only when 20 mg kg^{-1} of verapamil were given was a significant diminution of responses to 2-DDG seen. Although a possible central effect of verapamil interfering with the action of 2-DDG in triggering the vagal outflow cannot be ruled out, the greater role for gastrin in vagal-stimulated relative to basal secretion, in which acetylcholine and histamine could have a more important contribution to make (Grossman, 1981), may be an explanation for these differences.

From the results obtained in these experiments only hypothetical suggestions can be made to explain the differences between the actions of verapamil and those of diltiazem and cinnarizine on gastric acid secretion. The proposed tissue and functional selectivity of these drugs (Singh *et al.*, 1985; Speeding, 1985) could account for the lack of effect of cinnarizine, but only partially for those of diltiazem since this drug reduces acid secretion in the pylorus-ligated rat but not in the anaesthetized rat. This fact, together with the observation that diltiazem distinguishes between different calcium channels in the antrum smooth muscle cell of the guinea-pig stomach (Ishikawa *et al.*, 1985), raises the question of the possibility of differences in the calcium channels involved in gastric acid secretion in both models.

In conclusion, the data presented here clearly indicate an effect of calcium blockers on gastric emptying and acid secretion and, therefore, an involvement of extracellular Ca^{2+} on these gastric parameters. However, further studies will be necessary to explain the differences between the various calcium blockers, to characterize their effects completely, and to know to what extent they are responsible for the antiulcerogenic activity described for verapamil (Ogle *et al.*, 1985) and other substances characterized as calcium antagonists (Cortijo *et al.*, 1985; Esplugues *et al.*, 1985).

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