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Protection by the Calcium Antagonist Wy-47,037 Against Stress Ulceration in the Rat

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NIELSEN, S. T. AND T. S. SULKOWSKI. *Protection by the calcium antagonist Wy-47,037 against stress ulceration in the rat.* PHARMACOL BIOCHEM BEHAV **29**(1) 129–132, 1988.—Wy-47,037, a novel compound with both intracellular and extracellular calcium-blocking properties, was evaluated for its effects on cold/restraint stress induced ulceration in the rat. Wy-47,037 dose-dependently inhibited ulcerogenesis; with an ED_{50} of 8 mg/kg, PO, it was approximately twice as potent as nitrendipine (ED_{50}=15 mg/kg). Wy-47,037 also reduced basal gastric acid secretion (ED_{50}=7 mg/kg) and gastrointestinal motility (ED_{50}=16 mg/kg). It is thus possible that Wy-47,037's alteration of basal gastric acid secretion and/or of gastrointestinal motility may contribute to its therapeutic efficacy against stress induced ulcer formation.

Calcium antagonist Stress induced ulceration Wy-47,037 Nitrendipine Verapamil Nifedipine Gastric emptying Acid secretion

STRESS induced by cold exposure to lightly restrained rats causes gastric mucosal ulcer formation [9]. The pathological basis for this lesion development has been postulated to be multifactorial in nature and, in fact, can be blocked by a variety of pharmacologic agents [2]. Included in this multifactorial cast may be phenomena such as increased platelet aggregation and secondarily the compromise of mucosal blood flow and perfusion [20]. In addition Ogle and coworkers have recently invoked the triad of decreased gastric mast cell amine release, stomach wall relaxation and reduced gastric acid secretion as key elements militating against mucosal damage [15]. Since these physiological processes are calcium-dependent, their modulation may influence stress induced ulcer formation. Indeed, drugs such as verapamil which owe their pharmacologic activity to calcium blocking properties, inhibit cold/restraint stress induced gastric ulceration [15]. Given these findings, we determined the effect on cold/restraint stress induced ulcerogenesis of Wy-47,037, a novel compound with calcium blocking properties which is being developed as a therapeutic agent in the treatment of hypertension [19].

METHOD

Materials

Wy-47,037 was prepared by Dr. T. S. Sulkowski, Wyeth Laboratories. Nifedipine was synthesized in-house. Nitrendipine was obtained from Miles Labs, Elkhart, IN. Verapamil was obtained from Knoll Pharmaceutical Co., Whippany, NJ. All other materials were reagent grade or better, and were obtained from standard laboratory suppliers.

Cold/Restraint Stress Induced Gastric Ulcers

Ulcerogenesis in the rat was induced using the method of Hanson and Brodie [9]. Male Charles River rats weighing between 120–160 g were deprived of food for 18 hour with water ad lib. The rats were divided into groups of ten and dosed by the oral route with drug or vehicle control, 0.25% methyl-cellulose, in a volume of 5 ml/kg. Immediately after dosing the animals were inserted into aluminum restraining tubes measuring 4.1 cm in diameter by 20.3 cm and placed in the cold (6±1°C) for 3 hours. At the end of the test period the animals were killed by CO_2 asphyxiation, the duodenum and esophagus ligated, and the stomach removed. The stomachs were inflated with water, opened along the lesser curvature, spread over the index finger, and the mucosa wiped to remove superficial adherent blood or mucus. The number of ulcers was counted by visual observation and recorded. The incidence of ulcerogenesis was recorded, the mean ulcer number ±S.E.M. was calculated for each drug treated group and this was compared to that calculated for the control group.

Gastrointestinal Motility

Effects of drugs on gastrointestinal motility were assessed by quantitating gastrointestinal transit of a charcoal meal, as described by Macht and Barba-Gose [14]. Male 100–150 g, Charles River SD/CD strain rats were fasted for 24 hours with access to tap water ad lib until the test. Groups of 10 rats each were assigned to either control or drug treatment. The control vehicle or drug in vehicle was administered as a single, oral 1 ml/kg bolus. Thirty min later a 5% suspension

TABLE 1

EFFECT OF CALCIUM BLOCKING AGENTS ON COLD/RESTRAINT STRESS-INDUCED ULCEROGENESIS IN THE RAT

Drug	Dose (mg/kg)	Incidence of Rats with Ulcers (%)	Percent Inhibition of Ulcerogenesis Incidence	No. of Ulcers per Rat (mean ± S.E.M.)*	Percent Reduction in Mean No. of Ulcers
Wy-47,037	control	100	—	13.0 ± 2.41	—
	5	90	10	12.9 ± 3.00	0.8
	10	40	60	2.3 ± 1.38†	82
	20	50	50	1.7 ± 0.65†	87
Nitrendipine	control	100	—	11.1 ± 3.57	—
	1	100	0	8.5 ± 1.38	23
	8	100	0	7.7 ± 1.51	31
	32	30	70	1.7 ± 1.29†	85

*Number of animals per treatment group=10.
†Significantly different from control, $p<0.05$.

of finely ground charcoal in 0.25% aqueous methylcellulose (10 ml/kg) was given orally. After an additional 30 min interval, the rats were sacrificed by CO_2 asphyxiation. The small intestine from the gastroduodenal to the ileocecal junction was removed and the total length measured and recorded. The distance that the charcoal meal advanced through the gut lumen (charcoal front) was also measured. The ratio of these two parameters—charcoal front/total intestinal length—was calculated.

Pylorus-Ligated Rat

Pylorus ligation was carried out as described previously [17]. Male 190–260 g Charles River rats (strain SD/CD) were fasted for 24 hours with access to tap water ad lib until the test. Groups of 10 rats each were assigned to either control or drug treatment. Under methohexital anesthesia (40 mg/kg), a midline laparotomy was performed and a ligature tightly secured around the pylorus. Either control vehicle (0.25% aqueous methylcellulose) or drug in control vehicle was administered intraduodenally 1 ml/kg, immediately after ligating the pylorus. The abdominal incision was closed, the rats were allowed to recover from anesthesia, and then were sacrificed by CO_2 asphyxiation four hours after ligation. The stomachs were removed and the contents emptied into a graduated centrifuge tube.

The volume of gastric juice was recorded and the acid concentration of 1.0 ml sample aliquots was measured by electrometric titration to pH 7.0 using 0.1 N sodium hydroxide. The product of the gastric volume and acid concentration was used to calculate the total acid output. Total acid output after drug administration was compared with that obtained in control animals and results expressed as percent inhibition. Any samples having coprophagic contamination were excluded.

RESULTS

The effects of Wy-47,037 and nitrendipine on cold/restraint stress induced ulceration in the rat are shown in Table 1. With respective ED_{50}'s of 7 and 15 mg/kg, Wy-47,037 was approximately twice as potent as nitrendipine at reducing the mean number of ulcers per stomach. Both agents also decreased the incidence of ulcerogenesis (see Table 1).

When evaluated for the ability to decrease basal acid secretion in the pylorus-ligated rat, each of the four agents tested was dose-dependently active, as shown in Fig. 1, with the following order of potency: Wy-47,037 (ED_{50}=7 mg/kg) > nitrendipine (ED_{50}=15 mg/kg) > nifedipine (ED_{50}=12 mg/kg) > verapamil (ED_{50}=24 mg/kg). The decreases in acid secretion elicited by these calcium blocking agents were achieved through decreases in both parameters used to calculate total acid output (TAO), i.e., hydrogen ion concentration and secretory volumes (data not shown).

The effects of Wy-47,037 and nitrendipine on gastrointestinal motility were also investigated. This parameter was assessed by quantitating transit of a charcoal meal administered orally as a bolus. This assay, which is influenced by effects on gastric emptying and intestinal motility, indicated that Wy-47,037 was inhibitory at doses of 10 and 20 mg/kg (ED_{50}=16 mg/kg). In contrast nitrendipine elicited changes which, while significantly different from control at both the 8 and 32 mg/kg doses, were relatively small in magnitude (see Table 2).

DISCUSSION

Factors suggested as being causally related to stress induced gastric lesion formation, such as gastric acid secretion and smooth muscle contraction, are known to be calcium-dependent. Wy-47,037 is a novel, orally active antihypertensive agent with both intracellular and extracellular calcium blocking properties [19]. It was therefore of interest to determine whether Wy-47,037's therapeutic efficacy might extend as well to the alleviation of cold/restraint stress induced gastric ulcerogenesis in the rat. The selected doses of Wy-47,037 were based on their relevance to antihypertensive studies. Thus, for example, Wy-47,037 at 5 mg/kg, PO, caused a modest but significant reduction in blood pressure in the spontaneously hypertensive rat, while a maximal effect was observed with a dose of 20 mg/kg (R. Wendt, personal communication). Wy-47,037 showed no effect at 5 mg/kg, PO but at doses of 10 and 20 mg/kg, PO, it significantly prevented the development of cold restraint stress induced ulcers in the rat. It appeared more potent than ni-

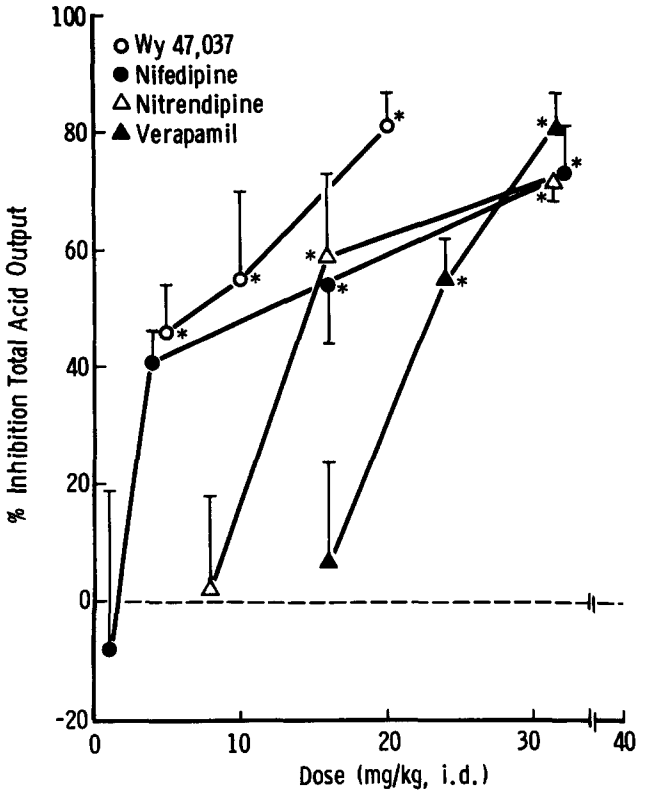

FIG. 1. Inhibition of acid secretion in the four-hour pylorus-ligated rat by calcium antagonists. Calcium antagonists were suspended in 0.25% methylcellulose and administered intraduodenally (ID) at the time of pylorus ligation. Four hours later the animals were sacrificed, the stomachs removed, and the acid content of the stomach (total acid output) determined as described in the Method section. Each group contained 10 animals, however any sample having coprophagic contamination was discarded. Acid secretion in drug-treated groups was compared to that in vehicle-treated groups and the results expressed as percent inhibition relative to the untreated control. Data points shown represent the mean±S.E.M., and asterisks indicate those values significantly different from control ($p<0.05$) by analysis of variance. Control (i.e., uninhibited) basal acid secretory values (mean±S.E.M.), in milliequivalents for each of the individual experiments were as follows: verapamil, 0.477±0.072; nifedipine, 0.538±0.098; nitrendipine, 0.527±0.081; and Wy-47,037, 0.591±0.062.

trendipine in this regard (see Table 1). These observations are in agreement with studies previously reported in which verapamil, which acts primarily by blocking extracellular calcium influx, was shown to attenuate both the incidence and the severity of stress induced gastric ulceration [15,20].

As was found with the ulceration studies, the 5 mg/kg dose of Wy-47,037 elicited no effect on gastrointestinal motility, but higher doses (10 and 20 mg/kg) significantly retarded the transit of a charcoal meal bolus administered intragastrically (see Table 2). Nitrendipine also decreased gastrointestinal motility, but its effects appeared less marked than Wy-47,037's in this regard (see Table 2).

Gastrointestinal smooth muscle function is variably subject to alteration by calcium antagonists, depending on the specific tissue and contractile stimulus involved. Contractile responses of rat duodenum were shown to be more strongly affected by verapamil and nifedipine than those of proximal colon [13]. Gastric emptying of a liquid has been reported to

TABLE 2

EFFECTS OF WY-47,037 AND NITRENDIPINE ON GASTROINTESTINAL MOTILITY IN THE RAT AS INDICATED BY TRANSIT OF A CHARCOAL MEAL

Drug	Dose (mg/kg)	Ratio* (mean ± SEM)	Percent Inhibition
Wy-47,037	control	0.62 ± 0.07	—
	5	0.62 ± 0.07	0
	10	0.41 ± 0.13	34†
	20	0.22 ± 0.11	64†
Nitrendipine	control	0.67 ± 0.01	—
	1	0.63 ± 0.02	6
	8	0.59 ± 0.02	12†
	32	0.55 ± 0.02	18†

*Mean ratio of charcoal meal front to total small intestinal length; all values are mean of ten animals per treatment group.
†Significantly different from control $p<0.05$, Students t-test.

be delayed by calcium antagonists [4]. Also, gastric emptying is known to be delayed by the cold/restraint procedure, and the calcium blocker verapamil has been reported to exacerbate this delay [11]. We might therefore postulate that the diminished gastrointestinal motility observed with nitrendipine and Wy-47,037, of which gastric emptying is a component, would be more pronounced under conditions of cold/restraint.

Since the acid secretory state of the animal may influence stress induced ulceration, the effect of calcium blocking agents on acid secretion was evaluated in the pylorus-ligated rat. Of the four agents examined, Wy-47,037 was the most potent at inhibiting basal acid secretion in the rat. Of the three agents having only extracellular calcium-blocking properties, nifedipine most closely approached Wy-47,037's potency, nitrendipine's potency was roughly half that of Wy-47,037, and verapamil was the least potent. Correlation between experimental observations in animal models and clinical observations regarding effects on gastric acid secretion are somewhat problematical. Thus, for example, verapamil reduces gastric acid secretion in the dog and the rat experimentally, but human studies on this point have been conflicting [1, 3, 8, 10, 12, 16, 18]. It is also difficult to define a relationship between (a) the present findings on the inhibition of basal acid secretion in the rat in vivo and (b) in vitro studies conducted with secretagogue-stimulated rabbit gastric glands. In that latter system nifedipine had no effect and other calcium antagonists, including verapamil, evinced only non-specific effects [5]. This illustrates the complexities which may be involved in the regulation of acid secretion under differing circumstances, and factors such as dose, secretagogue and the particular drug under study may all influence the experimental outcome [4,5].

Various attempts have been made to explain the etiology of stress induced ulcer formation. For example, decreased gastric mucosal blood flow secondary to vasoconstriction and platelet aggregation has been discussed in this context [20]. Indeed, this might be expected to be a contributing factor to the therapeutic effect of Wy-47,037, which has vasodilator properties. Likewise, the present data are consistent with the hypothesis that it is a combination of effects (e.g., increased mucosal blood flow, altered contractile function

and decreased acid secretion) which is responsible for Wy-47,037's inhibition of cold/restraint stress induced ulcerogenesis. In the rat, verapamil effectively antagonized bethanechol-induced gastric acid secretion as well as decreasing gastric glandular ulceration, mast cell degranulation and the increased stomach wall contractions elicited by cold/restraint stress [15]. Based on these findings the decrease in ulceration is postulated to be secondary to decreased mast cell amine release, to decreased acid secretion and to stomach wall relaxation. Moreover, in recent studies, Garrick *et al.* found that anesthetized rats which developed gastric lesions also had extended duration, high amplitude, gastric smooth muscle contractions [6,7]. Wy-47,037 inhibited charcoal meal passage, and this may reflect either a decrease in gastric emptying, a decrease in the contractile propulsive movements of the upper bowel, or some combination of the two parameters. Thus, while one may speculate that Wy-47,037 would decrease the sustained, high amplitude contractions which occur under conditions of cold/restraint, this remains to be unequivocally demonstrated. However, factors such as this, together with decreased basal acid secretion and possible vasodilation effects could conceivably contribute to Wy-47,037's therapeutic impact in terms of gastric mucosal protection in certain clinical settings.

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