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Pharmacology of JB-9315, A New Selective Histamine H_2-Receptor Antagonist

B. Palacios, M. J. Montero, M. A. Sevilla and L. San Román*

Laboratorio de Farmacognosia y Farmacodinamia, Departamento de Fisiología y Farmacología, Facultad de Farmacia, Universidad de Salamanca, Avenida Campo Charro s/n, 37007 Salamanca, Spain [Tel: 34-23-294530; Fax: 34-23-294515]

ABSTRACT. 1. The histamine H_2-receptor antagonistic activity and antisecretory and antiulcer effects of JB-9315 were studied in comparison with the standard H_2 blocker ranitidine.

2. *In vitro*, JB-9315 is a competitive antagonist of histamine H_2 receptors in the isolated, spontaneously beating guinea-pig right atrium, with a pA_2 value of 7.30 relative to a value of 7.36 for ranitidine. JB-9315 was specific for the histamine H_2 receptor because, at high concentration, it did not affect histamine- or acetylcholine-induced contractions in guinea-pig isolated ileum or rat isolated duodenum, respectively.

3. JB-9315 dose dependently inhibited histamine-, pentagastrin- or carbachol-stimulated acid secretion and basal secretion in the perfused stomach preparation of the anesthetized rat. In the pylorus-ligated rat after intraperitoneal administration, total acid output over 4 h was inhibited by JB-9315 with an ID_{50} of 32.8 mg/kg, confirming its H_2-receptor antagonist properties.

4. JB-9315 showed antiulcer activity against cold stress plus indomethacin-induced lesions with an ID_{50} of 6.8 mg/kg.

5. JB-9315, 50 and 100 mg/kg, inhibited macroscopic gastric hemorrhagic lesions induced by ethanol. In contrast, ranitidine (50 mg/kg) failed to reduce these lesions.

6. These results indicate that JB-9315 is a new antiulcer drug that exerts a cytoprotective effect in addition to its gastric antisecretory activity. GEN PHARMAC 30;2:181–189, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. JB-9315, ranitidine, histamine H_2-receptor antagonist, gastric acid secretion, mucosal protection, cytoprotection

INTRODUCTION

Since Black *et al.* (1972) first defined the H_2 receptor and characterized a number of H_2 antagonists, various types of these compounds have been developed and used clinically. The introduction of cimetidine and ranitidine as H_2-receptor antagonists for the control of peptic ulcer disease (Binder *et al.*, 1978; Debongie, 1992; Grant *et al.*, 1989) has been the catalyst for intense synthetic efforts by medicinal chemists in this therapeutic area to investigate novel histamine H_2-receptor antagonists to find highly efficacious drugs with greater potency.

Structural comparison of these drugs reveals three fundamental units: a basic heteroaromatic ring or aromatic ring substituted by a basic moiety, a four-membered flexible alkyl chain as a spacer group and a urea or amidine equivalent polar group (Katsura *et al.*, 1992; Lumma *et al.*, 1982; Mirossay *et al.*, 1992).

Our efforts to find new H_2 antagonists led to the synthesis of two series of new derivatives. The first series has modifications in the polar group in relation to ranitidine, and the second includes not only the same polar groups as in the first series, but also a piperidinomethylphenoxypropyl moiety as the aromatic part, to obtain lamtidine analogues. The presence of this moiety is reported to confer greater potency and enhanced duration of action (Brittain and Jack, 1983; Katz *et al.*, 1986a, 1996b; Santilli *et al.*, 1988; Torchiana *et al.*, 1983; Van der Goot *et al.*, 1991). After previous studies, we selected JB-9315 in the first series and JB-9322 in the second, which have the same polar group, for further pharmacological characterization. The pharmacological profiles of JB-9322 are described elsewhere (Palacios *et al.*, 1995); the present report relates to the biologic properties of JB-9315, a cyclopropyl derivative of ranitidine, which has been characterized in this laboratory by using both *in vivo* and *in vitro* model systems. Chemically, JB-9315 is *N*-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]-thio]ethyl]-*N'*-ciclopropylmethyl-2-nitro-1, 1-ethenediamine (Fig. 1). Ranitidine was utilized as a reference H_2-receptor antagonist (Bradshaw *et al.*, 1979).

MATERIALS AND METHODS

In vitro *assay techniques*

H_2-RECEPTOR ANTAGONISM IN GUINEA-PIG ISOLATED ATRIA. Male Dunkin-Hartley guinea pigs weighing 350–600 g were killed by cervical dislocation and exsanguination. The hearts were excised from animals and the right spontaneously beating atria were carefully dissected free and suspended in a 10-ml tissue bath with oxygenated Krebs-Henseleit solution at 37°C. After a 30-min stabilization period, three histamine concentration-response curves were established at 20-min intervals by measuring the chronotropic effect of increasing concentrations of histamine given in a cumulative fashion until the maximal response was consistent. Between each curve, the tissues were washed several times and the heart rate allowed to return to the basal level. The second curve served as control, and

*To whom correspondence should be addressed.
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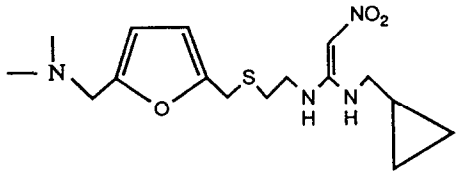

FIGURE 1. Chemical structure of JB-9315.

the third was repeated either 5 or 30 min after the tissue was incubated with various concentrations of JB-9315 or reference H_2-antagonist ranitidine, higher concentrations of histamine being added as needed.

H_1 RECEPTOR ANTAGONISM IN GUINEA-PIG ISOLATED ILEUM. A 2- to 3-cm piece of ileum was removed from the animal and suspended in a 20-ml tissue muscle bath containing atropinized Tyrode's solution at 37°C. After a 30-min stabilization period, cumulative concentration-response curves to histamine were established in 15-min periods until reproducible contractions were obtained. After a 5-min incubation with 10^{-6} mol/l of JB-9315 or ranitidine, the histamine concentration-response curve was repeated.

MUSCARINIC-RECEPTOR ANTAGONISM IN RAT ISOLATED DUODENUM. Male Wistar rats weighing from 250 to 300 g, fed *ad libitum*, were used. Immediately after sacrifice by decapitation, the duodenum was surgically removed. A 2-cm length of duodenum was suspended in a 10-ml tissue bath filled with Tyrode's solution at 35°C and bubbled continuously with air. After a 30-min stabilization period, concentration-response curves to acetylcholine in 10-min periods were carried out in a cumulative form until the maximal response was consistent. When a reproducible contraction was obtained, a new concentration-response curve was repeated after 5-min incubation with 10^{-6} mol/l of JB-9315 or ranitidine.

In vivo *assay techniques*

Wistar rats kept in cages with raised mesh bottoms were deprived of solid diet 24 hr before the onset of the experiment but received a nutritive solution of 8 g/100 ml sucrose in 0.2 g/100 ml NaCl to avoid excessive dehydration.

EFFECTS ON GASTRIC ACID SECRETION. *Measurement of Acid Secretion in the Lumen-Perfused Stomach of the Anesthetized Rat.* The procedure followed for the measurement of gastric acid secretion was first described by Ghosh and Schild (1958) and modified by us (Palacios *et al.*, 1995). Male Wistar rats weighing 200–250 g were anesthetized intraperitoneally (IP) with urethane, and the trachea was intubated. A soft catheter was passed down the esophagus, and a second polyethylene cannula was inserted into the stomach through an incision in the duodenum for the collection of gastric secretion. In studies in which secretion was stimulated, the jugular vein was cannulated for the administration of the secretagogues. The penile vein was cannulated for intravenous (IV) administration of saline or drugs.

When the surgical preparation was completed, the gastric lumen was perfused continuously with warm saline (NaCl 0.9% w/v, 37°C) at a rate of 1 ml/min through the esophageal cannula. After a period of 45 min for stabilization, the perfusate flowing from the stomach was collected at 15-min intervals and H^+ output determined by automatic titration (Crison titrator, micro TT 2050, Barcelona, Spain) with 0.01 N NaOH to pH 7.0. Gastric acid secretion was stimulated by the IV infusion at the rate of 1 ml/hr, or histamine (5 mg/kg/hr) or pentagastrin (10 μg/kg/hr) during a 4.5-hr period or during a 5-hr period for carbachol (10 μg/kg/hr), starting 45 min after determination of three basal values of acid secretion. In the stimulation with histamine and pentagastrin, the acid secretion reached a steady state 60 min after the beginning of infusion of the secretagogues. To reach the steady state in the carbachol-induced gastric acid secretion at 90 min, carbachol (5 μg/kg) was injected as a bolus through the cannulated penile vein when the infusion started.

H_2 antagonists or vehicle (0.9% NaCl solution) were administered IV in a volume of 1 ml/kg 60 min (histamine and pentagastrin stimulation) or 90 min (carbachol stimulation) after commencement of the secretagogue stimulation. Data were expressed as a percentage of the value of acid secretion 60 min (histamine and pentagastrin stimulation) or 90 min (carbachol stimulation) after the initiation of secretagogue infusion. The dose-response relation was established by using the area under the dose-response curve from 60 to 270 min (histamine and pentagastrin) or from 90 to 300 min (carbachol) after the start of secretagogue infusion, determined by the Simfit program (University of Manchester, UK).

In the experiments to determine the effects of JB-9315 and ranitidine on basal acid secretion, drugs or saline were infused continuously through the penile vein at 5 mg/kg/hr, 1 ml/hr, for 150 min. The concentration of H^+ was estimated every 10 min.

Gastric Secretion in Pylorus-Ligated Rat. The pylorus-ligated rat model first described by Shay *et al.* (1945) was used. Male Wistar rats weighing 180–200 g were used. Under light ether anesthesia, a small abdominal incision was made, the pylorus was ligated and test substances or vehicle (0.9% NaCl solution) were administered intraperitoneally in a volume of 1 ml/kg immediately after surgery. The animals were killed 4 hr after ligation of the pylorus, the stomach was excised and opened along the greater curvature and the luminal contents were collected and centrifuged for 15 min at 4500 rpm to remove residual debris. The volume was measure and total acid output, determined by automatic titration, was expressed as microequivalents of H^+ per 4 hours. Pepsin concentration was determined by a colorimetric method (Palacios *et al.*, 1995).

EXPERIMENTALLY INDUCED GASTRIC LESION STUDIES. *Acute Gastric Ulcer: Cold Stress Plus Indomethacin-Induced Lesions.* Female Wistar rats weighing 170–200 g were used. Indomethacin (30 mg/kg, SC) administered rats were immobilized in individual stainless steel cages and left in a cold room (4±1°C) for a period of 5 hr. JB-9315, ranitidine or vehicle (0.9% of NaCl solution) were administered orally (PO) 30 min before the indomethacin treatment. Thereafter, the animals were decapitated, and the stomachs were removed, opened along the greater curvature, gently rinsed under tap water and pinned on a paraffin and plastic polymer plate. The stomachs were then photographed, the photographs were amplified and the area of each macroscopic lesion (mm^2) in the glandular part was measured by computerized planimetry (Ibas Interactive Image Analysis System, Kontron). The total area of the lesions was regarded as the lesion index. The lesion index was expressed as the mean lesion area for each group of rats. The number of lesions in the glandular part of each stomach also was noted.

Cytoprotective Activity in Rats. Gastric protection activity studies were conducted in female Wistar rats weighing 170–200 g by using the method of Robert *et al.* (1979). Rats were pretreated with drugs or vehicle (0.9% of NaCl solution), administered either orally (10 ml/kg) or intraperitoneally (2 ml/kg). Thirty minutes later, the rats were given 1 ml of absolute ethanol orally. Thirty minutes after the ethanol gavage, the animals were sacrificed and the stomachs were

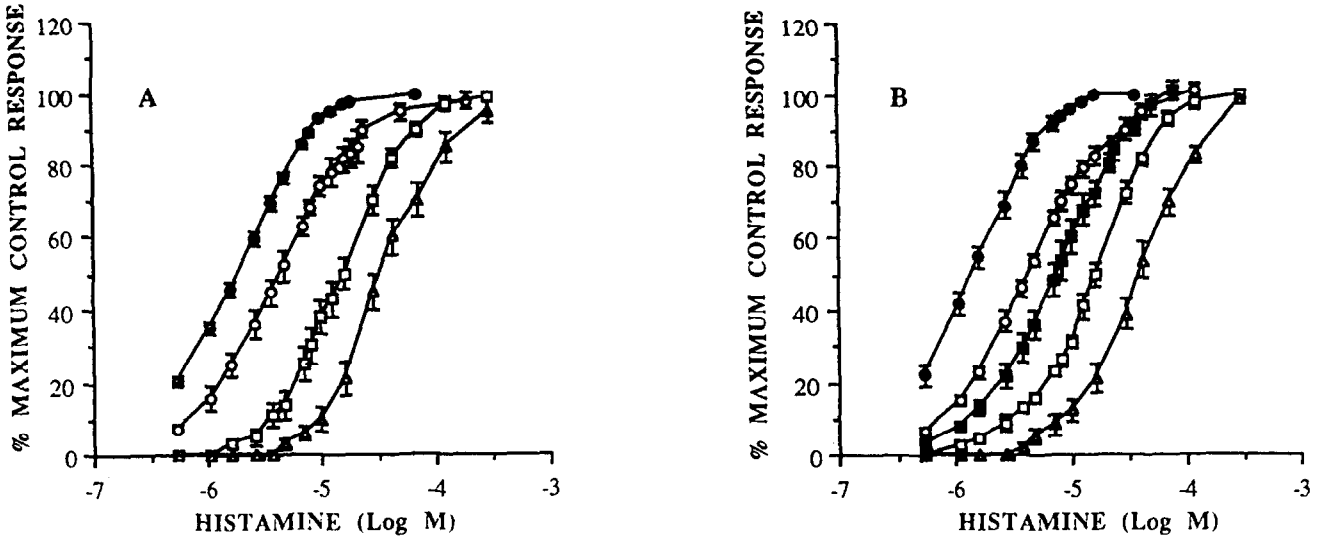

FIGURE 2. Effect of a 5-min incubation with (A) JB-9315 and (B) ranitidine on the positive chronotropic concentration-response curve to histamine in guinea-pig isolated right atrium. Control (solid circles); JB-9315: 3×10^{-6} mol/l (triangles), 10^{-6} mol/l (open squares), 10^{-7} mol/l (open circles); ranitidine: 3×10^{-6} mol/l (triangles), 10^{-6} mol/l (open squares), 3×10^{-7} mol/l (solid squares), 10^{-7} mol/l (open circles). Each curve represents the mean±SEM response obtained in at least five tissues.

excised. The remaining procedure was the same as that described earlier for acute gastric ulcer.

To study the possible mechanism of gastroprotective activity induced by JB-9315, we designed different series of experiments. To determine whether mucosal protection by JB-9315 depends on the synthesis of prostaglandins, rats were given indomethacin (10 mg/kg) subcutaneously 90 min before the oral drug or vehicle pretreatment. This dose of indomethacin has previously been shown to eliminate the production of prostaglandins in gastric mucosa (Ligumsky *et al.*, 1982). Protection was determined as described. The results were expressed as the percentage of the total glandular mucosal area occupied by necrotic lesions.

Estimation of nonprotein sulfhydryl groups (μg of glutation/g of stomach) and gastric mucus (ng of Alcian blue/ml/g of stomach) were done in accord with the methods described by Sedlak and Lindsay (1968) and by Corne *et al.* (1974). JB-9315 or vehicle was administered 1 hr before the animals were sacrificed.

Calculation and statistical analysis

Data are shown as the mean±SEM of at least five experiments. Data from *in vitro* preparations are expressed as a percentage of the maximal agonist response established in the absence of the antagonist for each preparation in the control curve. Concentration-response curves were obtained by plotting the percentage of the maximum control response against log concentration of histamine or acetylcholine. The cumulative concentration-response curves, with and without the antagonists, were analyzed by using the Tallarida and Murray computer program (Tallarida and Murray, 1984) to obtain the EC_{50} values.

In the atrium assay, the pA_2 values and slopes of the Schild regression were calculated according to the method of Arunlakshana and Schild (1959). As in acid secretion studies in cytoprotection assays, the inhibitory ratio (%) was obtained by comparing the values in the treated animals with those of the control group. The dose causing 50% inhibition (ID_{50}) was calculated from the dose-inhibition relations by least-squares regression. Confidence limits of 95% of the ID_{50} values were determined by the Simfit program. The significance of the differences between groups was assessed by using a one-way analysis of variance followed by the Duncan multiple range test, with $P<0.05$ selected as the criterion for statistical significance.

Drugs

The following drugs were used: JB-9315 (synthesized in the Department of Organic Chemistry, Faculty of Chemistry, University of Salamanca), ranitidine hydrochloride, acetylcholine chloride and carbachol (Sigma) and histamine dihydrochloride (Merck). Indomethacin (Inacid, Merck Sharp & Dohme) and pentagastrin (Peptavlon, ICI) were used as the preparations available for clinical use. The compounds were dissolved in isotonic NaCl solution immediately before use. Doses of JB-9315 and ranitidine are expressed as free base.

RESULTS

In vitro *studies*

The histamine H_2-receptor antagonist properties of the test compounds were determined by their inhibition of the chronotropic effects of histamine on isolated guinea-pig atria. In our preliminary experiments, JB-9315 and ranitidine showed the same antagonism of the response to histamine in atria with 5-min and 30-min incubations. Thus, for the assessment of the inhibitory effect of these drugs, 5-min incubation was used. JB-9315 and ranitidine did not affect the resting atrial rate (201.7±4.2 contractions/min) during the period of incubation. JB-9315 (10^{-7}–3×10^{-6} mol/l) and ranitidine (10^{-7} to 3×10^{-6} mol/l) produced a concentration-related parallel shift of the histamine concentration-response curve to the right without affecting the maximal response (297.7±6.9 contractions/min) (Fig. 2). The Schild regressions of log (dose ratio-1) *versus* log molar concentration of these compounds were linear, with slopes not significantly different from unity. JB-9315 and ranitidine showed a competitive antagonistic activity, and their pA_2 values and 95% confidence limits were 7.30 (6.81–7.80) and 7.36 (7.01–7.71), respectively.

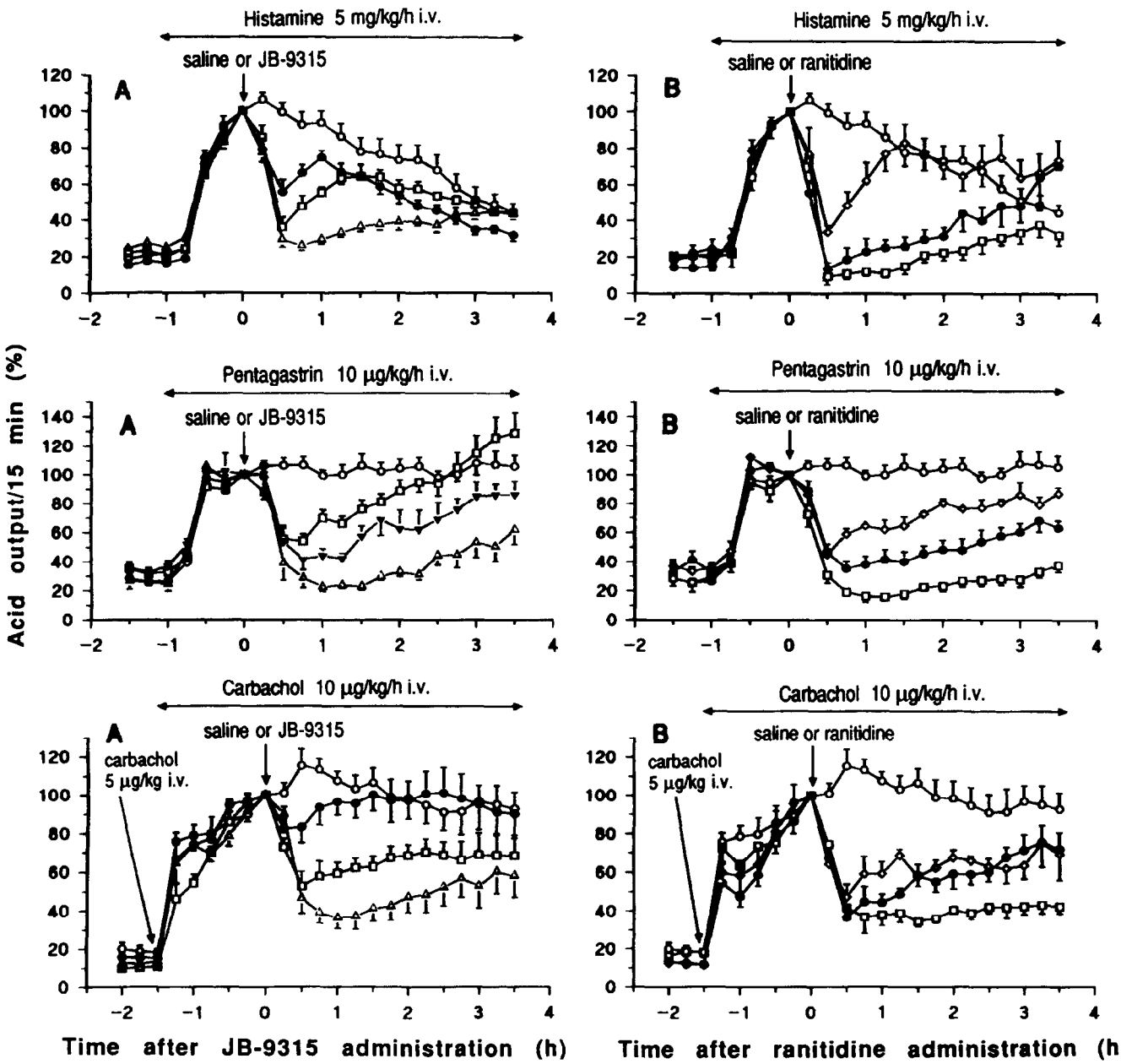

FIGURE 3. Antisecretory effect of (A) JB-9315 and (B) ranitidine on histamine-, pentagastrin- and carbachol-stimulated gastric acid secretion in lumen-perfused rats. Data were expressed as a percentage of value of acid secretion 60 min (histamine and pentagastrin stimulation) or 90 min (carbachol stimulation) after the initiation of secretagogue infusion. Each point indicates the mean±SEM obtained from five to nine rats. Control (open circles). JB-9315 (mg/kg IV): 1 (solid circles), 3 (open squares), 6 (solid triangles), 10 (open triangles). Ranitidine (mg/kg IV): 0.3 (diamonds), 1 (solid circles), 3 (open squares).

JB-9315 and ranitidine 10^{-6} mol/l did not cause any significant displacement of the histamine or acetylcholine concentration-response curve in ileal and duodenal preparations, respectively (data not shown).

In vivo *studies*

EFFECT ON GASTRIC ACID SECRETION. *Effect on Acid Secretion in Gastric Lumen–Perfused Rats.* Basal acid secretion was 3.57±0.20 (µEq H^+/15 min) in the gastric lumen–perfused rats. A stable secretion (24.76±1.25 µEq H^+/15 min) was seen 60 min after the infusion of histamine (submaximal dose: 5 mg/kg/hr). The histamine-induced acid secretion decreased gradually, but significant secretion was observed for at least 4.5 hr after the beginning of the infusion. JB-9315 and ranitidine administered IV inhibited the histamine-stimulated acid secretion with a clear dose-response relation. As shown in Figure 3, the maximum inhibitory effect obtained with JB-9315 and ranitidine was observed 30 min after the IV administration in both cases. However, recovery from the antisecretory activity of JB-9315 was appreciably faster than the recovery from that of

TABLE 1. ID_{50} of JB-9315 and ranitidine for inhibition of histamine-stimulated gastric acid secretion in lumen perfused rats

	ID_{50} (mg/kg)			
	Time after H_2-receptor antagonist administration (hr)			
Treatment (IV)	0–1	1–2	2–3	0.0–2.5
Ranitidine	0.34 (0.12–0.81)	0.75 (0.50–1.51)	2.09 (1.85–2.42)	0.77 (0.44–1.31)
JB-9315	7.05 (6.72–7.24)	5.85 (3.00–14.60)	—	7.90 (4.85–14.20)
Ranitidine/JB-9315	0.048	0.13	—	0.097

Each value represents the ID_{50} with the 95% confidence limits in parentheses. Ranitidine/JB-9315 represents the relative potency of JB-9315 *versus* ranitidine.

ranitidine [JB-9315: $P<0.05$ for 1 (0.0–0.75 hr), 3 (0.0–1.25 hr), 10 mg/kg (0.0–2.5 hr after H_2-receptor antagonist administration); ranitidine: $P<0.05$ for 0.3 (0–1 hr), 1 (0.0–2.5 hr), 3 mg/kg (0.0–2.75 hr)]. The values of ID_{50} at each time interval are listed in Table 1.

JB-9315 and ranitidine also inhibited the pentagastrin- and carbachol-stimulated acid secretion dose dependently, and the maximum inhibitory effect was observed between 30 and 60 min after the IV administration of compounds in both cases. But, as in the case of histamine-stimulation acid secretion, the recovery from the inhibitory activity was faster with JB-9315 [pentagastrin: JB-9315: $P<0.05$ for 3 (0.0–1.75 hr), 6 (0.0–2.75 hr), 10 mg/kg (0.0–3.5 hr); ranitidine: $P<0.05$ for 0.3 (0.0–2.75 hr), 1 (0.0–3.75 hr), 3 mg/kg (0.0–3.5 hr); carbachol: JB-9315: $P<0.05$ for 1 (0.0–0.75 hr), 3 (0–3 h), 10 mg/kg (0.0–3.5 hr); ranitidine: $P<0.05$ for 0.3 (0–3 hr), 1 (0–3 hr), 3 mg/kg (0.0–3.5 hr)] (Fig. 3). The values of ID_{50} for pentagastrin and carbachol at each time interval are listed in Tables 2 and 3, respectively.

JB-9315 (5 mg/kg/hr, IV) decreased the acid concentration of the unstimulated stomach and was as potent as ranitidine at the same dose (Fig. 4).

Gastric Secretion in Pylorus-Ligated Rats. Gastric secretion was evaluated as gastric juice volume, acid output and pepsin output for 4 hr after ligation of the pylorus. These values in control rats given vehicle were 5.13±0.54 ml/4 hr, 383.70±57.51 μEqH^+/4 hr and 26.75±3.86 mg/4 hr, respectively. When administered immediately after ligation, JB-9315 (30, 60 and 100 mg/kg, IP) dose dependently inhibited gastric acid secretion, gastric juice volume and pepsin secretion. Ranitidine (1, 3, 10, 20 and 30 mg/kg, IP) also caused a marked decrease in each secretory parameter. The decrease in pepsin output was due largely to a reduction in gastric volume because the concentration was not greatly changed. However, at higher doses of these compounds, the acid output was inhibited to a greater extent than the volume of secretion (Fig. 5).

The intraperitoneal ID_{50} values and 95% confidence limits for reduction in gastric acid secretion for JB-9315 and ranitidine were 32.85 mg/kg (29.64–37.70) and 3.72 mg/kg (1.23–7.30), respectively.

EXPERIMENTALLY INDUCED GASTRIC LESION STUDIES. *Cold Stress Plus Indomethacin-Induced Lesions.* Severe hemorrhagic lesions were formed in the glandular segment of the stomach in rats by the administration of indomethacin and stress-loading in a cold room for 5 hr. There was evidence of intraluminal bleeding in these animals. Pretreatment with JB-9315 and ranitidine for 30 min prevented the development of these gastric lesions in a dose-related manner, exhibiting an appreciable antiulcer activity (Fig. 6). The ID_{50} values and 95% confidence limits for the lesion index were 6.85 mg/kg (5.24–8.40) and 2.11 mg/kg (0.26–5.70) for JB-9315 and ranitidine, respectively.

Cytoprotective Activity. Oral administration of absolute ethanol produced severe gastric hemorrhagic lesions visible from the outside of the stomach as thick black or red lines. After the stomach was opened, lesions were found in the glandular mucosa and consisted of elongated bands, 2–12 mm long by 2–4 mm wide, usually parallel to the long axis of the stomach. Most were located in the corpus; the antrum was less affected. The control rats with vehicle had gastric lesions of 159.2±29.8 mm^2. Oral administration of JB-9315 at 50 mg/kg did not show a significant effect; however, 100 mg/kg of JB-9315 decreased the formation of these hemorrhagic lesions by 71.6%.

TABLE 2. ID_{50} of JB-9315 and ranitidine for inhibition of pentagastrin-stimulated gastric acid secretion in lumen perfused rats

	ID_{50} (mg/kg)				
	Time after H_2-receptor antagonist administration (hr)				
Treatment (IV)	0–1	1–2	2–3	3.0–3.5	0.0–3.5
Ranitidine	1.24 (0.78–2.02)	0.54 (0.39–0.73)	0.98 (0.86–1.11)	1.24 (1.06–1.49)	0.89 (0.80–0.99)
JB-9315	8.47 (4.64–18.74)	4.35 (3.80–5.00)	6.64 (4.72–10.62)	—	6.20 (5.35–7.41)
Ranitidine/JB-9315	0.15	0.12	0.15	—	0.14

Each value represents the ID_{50} with the 95% confidence limits in parentheses. Ranitidine/JB-9315 represents the relative potency of JB-9315 *versus* ranitidine.

TABLE 3. ID_{50} of JB-9315 and ranitidine for inhibition of carbachol-stimulated gastric acid secretion in lumen perfused rats

	ID_{50} (mg/kg)			
	Time after H_2-receptor antagonist administration (hr)			
Treatment (IV)	0–1	1–2	2–3	0.0–2.5
Ranitidine	2.36 (0.98–5.73)	0.73 (0.59–0.90)	1.73 (0.88–3.80)	1.00 (0.87–1.15)
JB-9315	>10	4.08 (3.54–4.70)	—	6.20 (4.91–8.05)
Ranitidine/JB-9315	—	0.18	—	0.16

Each value represents the ID_{50} with the 95% confidence limits in parentheses. Ranitidine/JB-9315 represents the relative potency of JB-9315 *versus* ranitidine.

In similar experiments, no reduction in ethanol-induced hemorrhagic lesions was seen after the oral administration of ranitidine (50 or 100 mg/kg) (Fig. 7).

To determine whether JB-9315 acted locally or systemically, its cytoprotective activity against ethanol-induced gastric lesions was tested by IP administration. Compared with the control value, ethanol lesions were reduced by JB-9315 (100 mg/kg, IP) to a similar extent as after oral administration. The role of endogenous prostaglandins on the protective effect of JB-9315 was examined in rats that were subcutaneously pretreated with 10 mg/kg of indomethacin. Indomethacin itself significantly increased the amount of lesioning (approximately doubled). However, the protective effect of JB-9315 was not diminished when prostaglandin biosynthesis was inhibited by indomethacin (data not shown).

JB-9315 (100 mg/kg, PO) increased the gastric mucus production from 46±4 (basal value) to 72±6 ng Alcian blue/ml/g of stomach ($P<0.05$); ranitidine at the same dose had no effect (48±3). JB-9315 did not modify the production of nonprotein sulfhydril groups.

DISCUSSION

JB-9315 is a histamine H_2-receptor antagonist possessing mucosal protective properties as well as gastric antisecretory activity.

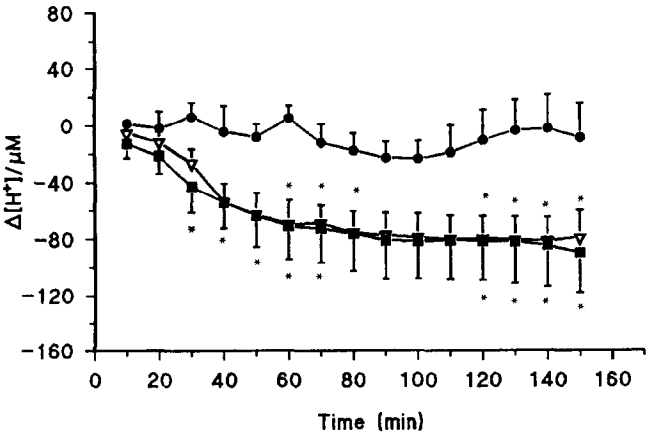

FIGURE 4. Effect of 5 mg/kg/hr of JB-9315 (squares) or ranitidine (triangles) on basal acid secretion in lumen-perfused rats. Control (circles). Data were expressed as the difference between the $[H^+]$ at each 10-min interval during drug infusion and the basal value of $[H^+]$. Each point indicates the mean±SEM obtained from five to seven rats. *Significantly different from the normal saline control group ($P<0.05$).

Chemically, JB-9315 differs from ranitidine in that it possesses a cyclopropylmethyl moiety in the polar group in place of the methyl group of the latter compound. In the guinea-pig atrium assay, ranitidine produced parallel shifts and surmountable antagonism. The observed pA_2 value for ranitidine is in agreement with previously published reports (Daly *et al.*, 1981; Lumma *et al.*, 1982; Katz *et al.*, 1986a, 1986b). JB-9315 also shifted the histamine concentration-response curve in a parallel, surmountable manner. Thus, the nature of the antagonism of the histamine-induced positive chronotropic action in guinea-pig atria was competitive for JB-9315 and ranitidine. A comparison of pA_2 values showed that the potency of ranitidine was slightly greater than that of JB-9315 as an antagonist of the effects of histamine in guinea-pig atria.

This antagonism is selective, because JB-9315 and ranitidine did not inhibit the action of histamine at H_1 receptors or of acetylcholine at muscarinic receptors in guinea-pig isolated ileum and in rat isolated duodenum, respectively.

The antagonism of histamine H_2 receptors by JB-9315 is manifested *in vivo* by gastric antisecretory activity in both the gastric lumen-perfused and the pylorus-ligated rats. In submaximally histamine stimulated rats, the maximum intravenous antisecretory activities of JB-9315 and ranitidine were reached 30 min after administration but, as shown in Figure 3, the recovery from the inhibitory effect was faster with JB-9315 than with ranitidine. On the pentagastrin- and carbachol-stimulated acid secretion, the potency of the inhibitory effect of rantidine also was greater than that of JB-9315 and the duration of the antisecretory action of JB-9315 was also shorter than that of ranitidine. JB-9315 also inhibited the basal gastric secretion in lumen-perfused rats and, in this assay, was as potent as ranitidine. In pylorus-ligated rats, JB-9315, administered immediately after ligation, inhibited gastric acid and pepsin output, as well as gastric juice volume, and was less potent than ranitidine in this test. The antisecretory activity of JB-9315 and ranitidine in these models further indicates that histamine plays an important role in modulating gastric acid secretion.

The inhibitory effect of histamine H_2-receptor antagonist on experimental lesion formation has been previously reported (Isobe *et al.*, 1990; Okabe *et al.*, 1997; Sekiguchi *et al.*, 1993; Shibata *et al.*, 1990; Takeda *et al.*, 1982), showing that these drugs can be very useful for peptic ulcer patients. The antiulcer effects of these H_2-receptor antagonists have been considered to be due mainly to their antisecretory effect (Howard *et al.*, 1985). In the present study, a 30-min pretreatment with JB-9315 and ranitidine inhibited the cold stress plus indomethacin-induced lesions significantly. Arai *et al.* (1987) demonstrated that drugs that are unable to inhibit acid secretion cannot suppress the lesion formation in this model, because

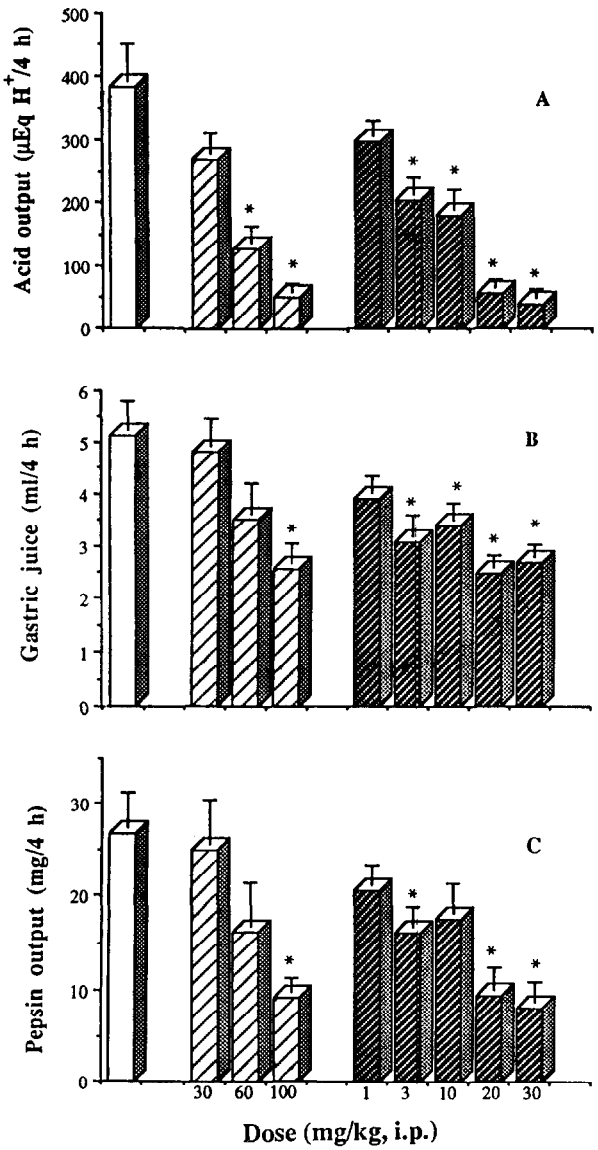

FIGURE 5. Effect of 0.9% NaCl (open), JB-9315 (wide hatch) and ranitidine (narrow hatch) on basal gastric secretion in pylorus-ligated rats: (A) gastric acid secretion; (B) gastric juice volume; (C) pepsin secretion. Each drug was given IP immediately after surgery. Animals were sacrificed 4 hr after pylorus ligation. Each column represents the mean ± SEM of eight animals. *Significantly different from the normal saline control group ($P<0.05$).

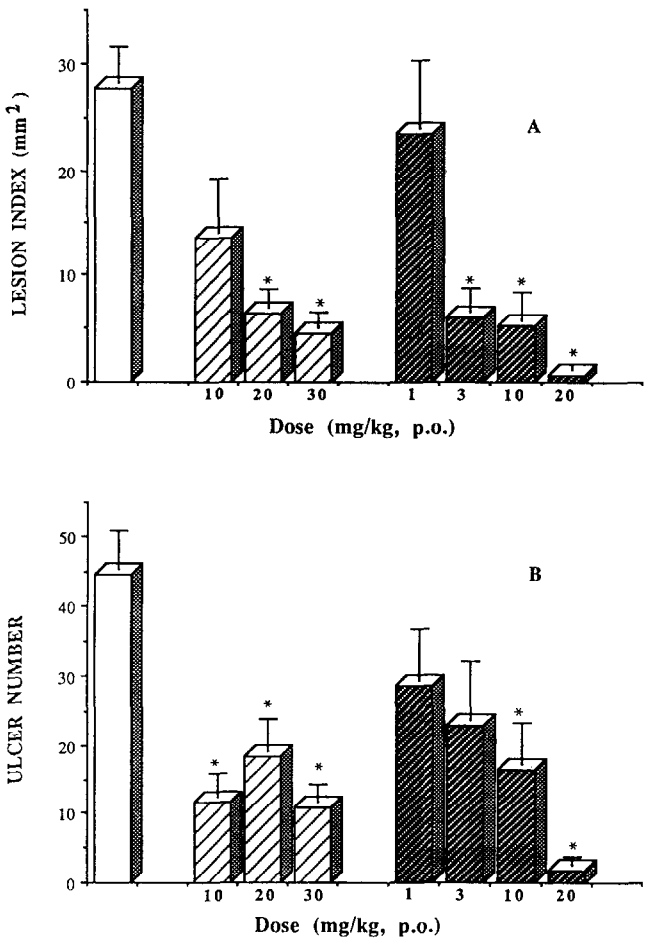

FIGURE 6. Antiulcer effect of 30-min pretreatment with saline (open), JB-9315 (wide hatch) or ranitidine (narrow hatch) on cold stress plus indomethacin-induced gastric lesions: (A) lesion index; (B) ulcer number in the glandular part. Each column represents the mean ± SEM of at least six animals. *Significantly different from the normal saline control group ($P<0.05$).

these lesions are related mainly to the significant increase in acidity of gastric juice and this correlates well with the severity of erosions (Murakami *et al.*, 1985).

Cytoprotection was first demonstrated in rats by Robert *et al.* (1979), who showed that many prostaglandins protect the mucosa of the stomach against the hemorrhagic and erosive effects of intragastric administration of absolute ethanol and other necrotizing agents. This pharmacological property is independent of inhibition of gastric secretion. In the present study, we showed that JB-9315 at 100 mg/kg administered either orally or intraperitoneally prevents the development of gastric lesions provoked by intragastric ethanol, indicating the presence of cytoprotective activity. Ranitidine at 50 or 100 mg/kg orally was inactive, in agreement with the prevailing view that ranitidine is not cytoprotective (Del Soldato *et al.*, 1985; Hakkinen *et al.*, 1991).

It seems likely that JB-9315 exerts such a cytoprotective action mainly through a systemic action because it is present not only when administered orally, but also when administered intraperitoneally; thus its protective effect does not depend on contact of the drug with the gastric mucosa. Moreover, the endogenous prostaglandins may not take part in the mechanism of gastric cytoprotection of JB-9315, because, when the production of prostaglandins in the gastric mucosa was inhibited by indomethacin, the full protective effect afforded by JB-9315 on the gastric mucosa remained. This effect might be explained at least in part by its capacity to stimulate the gastric mucus secretion, thus strengthening of the gastric mucosal barrier (Guth, 1982).

That JB-9315 prevented the stress plus indomethacin-induced ulcers at doses less than the ID_{50} for reduction in the acid output in pylorus-ligated rats might indicate that the gastroprotective effect of this drug does not completely depend on its ability to inhibit gastric secretion, but on its cytoprotective effect as well. In contrast, the effective doses of ranitidine in the inhibition of stress plus indomethacin-induced ulcer are similar to its antisecretory ID_{50} value; thus the

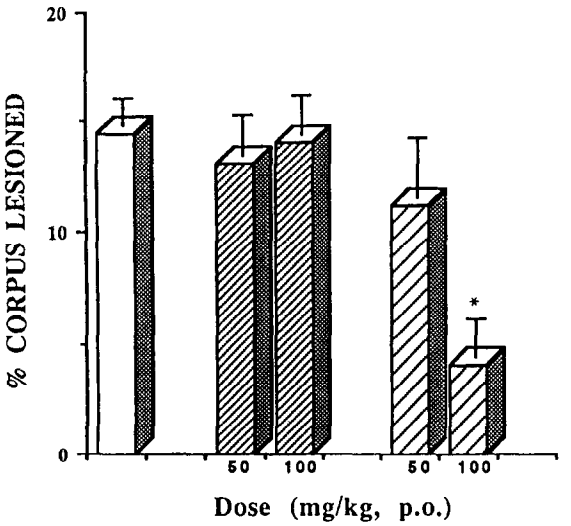

FIGURE 7. Effect of JB-9315 and ranitidine on ethanol-induced gastric hemorrhagic lesions in rats. Rats were orally given saline (open), JB-9315 (wide hatch) or ranitidine (narrow hatch) 30 min before receiving an oral gavage of 1 ml of absolute ethanol. Each column represents the mean ± SEM of at least seven animals. *Significantly different from the normal saline control group ($P<0.05$).

prevention of ulcer formation in this model by ranitidine may be ascribed to the suppression of acid secretion.

In summary, JB-9315 is a specific, competitive histamine H_2-receptor antagonist with antisecretory and antiulcer effects less potent than those of ranitidine, but, unlike ranitidine, it also induces gastroprotective properties.

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