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GASTRIC ACID INHIBITORY PROFILE OF EBROTIDINE, A NOVEL H_2-RECEPTOR ANTAGONIST IN HUMANS

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This study was designed to assess the gastric secretory effects of ebrotidine, a novel H_2 receptor antagonist, in humans. Three groups (A, B and C) of male subjects with normal gastric mucosa were used. Group A (6 subjects) was used to determine the dose-dependency of gastric inhibitory effect of ebrotidine on basal and pentagastrin-induced maximal acid output. Group B (8 subjects) was employed to examine the duration of the inhibitory effect of ebrotidine on basal and pentagastrin-induced acid secretion. In group C (6 subjects), the 24 h pH-metry was assessed using intraluminal pH-electrode placed in the gastric corpus and connected to a portable recording unit. Single oral dose of ebrotidine (200, 400 or 800 mg) caused a dose-dependent reduction in basal and pentagastrin-induced acid secretion that at a dose of 800 mg amounted to about 89% and 93%, respectively. This inhibition was still observed after 6 h and averaged 72% and 50%, respectively. After 12 and 24 h upon the drug intake, both basal and pentagastrin-induced acid secretion returned to the control values. Single oral dose of ebrotidine (800 mg) caused a significant reduction in circadian acidity and resulted in a marked and significant reduction of intragastric acidity for about 6 h upon the administration. This inhibition was accompanied by a transient increase in basal and postprandial gastrin levels. We conclude that ebrotidine is highly effective inhibitor of basal, pentagastrin-induced and circadian gastric acid secretion in humans.

Key words; *stomach, gastric acid, histamine H_2-receptor, gastrin*

INTRODUCTION

Ebrotidine is a novel competitive antagonist of H_2-receptors characterized by the presence of N-sulphonyl formamidine substructure with antisecretory activity comparable to that of ranitidine (1). Studies on experimental animals demonstrated that ebrotidine exhibits gastric inhibitory effects comparable to that of ranitidine and shows also a remarkable gastroprotective effect (2) but little information is available whether this agent inhibits also gastric acid secretion in humans.

This study was designed to assess the effects of ebrotidine on gastric acid secretion under basal conditions and in response to maximal pentagastrin stimulation as well as on circadian gastric acidity and gastrin release in humans.

MATERIAL AND METHODS

Subjects

Three groups (A, B and C) of male subjects, 24 to 29 years of age (mean, 26 years), weighing 67 to 81 kg body weight (mean weight, 75 kg) were used in this study. These investigations were approved by the Institutional Human Research Review Committee.

All subjects had a history of duodenal ulcer disease, documented by duodenoscopy 2—3 years before, but they were asymptomatic and without ulcer at a time of the study. Subjects with a history of drug or ethanol abuse were eliminated from the study. All subjects abstained from drug or alcohol intake for 14 days and underwent routine physical examinations, laboratory tests and upper gastrointestinal endoscopy.

Six subjects of group A underwent 4 tests at least 4—5 days apart in a placebo controlled, randomized double-blind cross-over study. After 12 h of fasting period, each subject was given orally either placebo or ebrotidine at a dose of 200, 400 or 800 mg. One hour after the ingestion of placebo or ebrotidine tablet, the residual gastric content was aspirated and rejected. Continuous gastric aspiration was started and maintained for 30 min to determine basal acid outputs and then for 90 min when pentagastrin was infused i. v. (2 μg/kg-h) to assess maximal acid output in these subjects. Ebrotidine and placebo tablets were supplied by (Dr. J. Torres from Centro de Investigacion, Gruppo Ferrer, Barcelona, Spain). The ebrotidine tablet contained 200, 400 or 800 mg of the active agent and the placebo tablets contained cornstarch and microcrystalline cellulose.

The study on the time course of gastric inhibition by ebrotidine was performed on 8 subjects of group B. The 30 min basal and 90 min pentagastrin (μg/kg-h) infusion test was first performed on each subject. In a separate test on another test day, a single oral dose of 800 mg of ebrotidine was administered and then 6, 12 and and 24 h later, basal and pentagastrin infusion test was repeated in each of 8 subjects tested.

The volume of each sample of gastric juice aspirated during tests on subjects of group A and B was recorded and titratable acidity was determined with an automatic titration assembly (Radiometer, Copenhagen, Denmark) using 100 mM NaOH (endpoint pH 7.0). Acid outputs were expressed in millimoles (mmol) per 30 min.

Group C subjects (6 subjects) underwent two 24 h recording tests, at least 7 days apart, in a placebo-controlled, randomized, double-blind, cross-over study. Each patient was given either placebo or ebrotidine (800 mg) on two separate occasions with a wash-out intervals of at least one week. Drugs were orally administered as single dose at 11.30 h.

Gastric acidity was assessed during 24 h period by means of an intraluminal system, including an antimony electrode (Monocrystal model 91—0215, Synectics AB, Sweden) connected to the portable unit which permitted the pH recording to be sampled every 4 s (Digitrapper MKII, 6200, Synectis AB, Sweden). The antimony electrode used an external reference on thorax with contact jelly (Hellige 217, 177 01/02, Fritz Hellige and Co., FRG). At the beginning of each examination the antimony electrodes were accurately calibrated at 21°C using a standard buffers with pH 7.01 and pH 1.07 (5001 and 5002, Synectics, AB, Sweden) and a temperature correction for intragastric reading (37°C) was performed. The pH electrodes were passed through an anesthetized nostril and

were positioned in the gastric corpus under fluoroscopic control approximately 10 cm below the lower esophageal sphincter. The connecting wires were fixed to the nares with adhesive tape. The data recorder of intraluminal mini-electrodes was carried in a small bag so that it did not interfere with normal daily life. The pH recordings started at 11.30 and lasted for 24 h. A further fluoroscopic check was performed at the end of each test. Meal timing for breakfast (8.00—8.15), lunch (13.00—13.30) and supper (18.00—18.15) and meal composition were standardized as proposed previously (3, 4). Patients were instructed not to lie down during the day or to take any additional meals or alcoholic drinks and carbonated beverages during the examination period.

Calculations and statistics

The results obtained from subjects of group A and B are expressed as means plus minus standard error of the mean. The differences in gastric acid outputs between the control tests and those with ebrotidine were determined by Student's t test as used in the text, significance indicates $P < 0.05$.

Data obtained in subjects of group C from studies with continuous intragastric pH monitoring with antimony electrodes were transferred to IBM compatible computer (PC AT12) programmed with Gastrogram version 5.50, serial No E1024 (Gastrosoft Inc., Sweden) for calculating median pH values from each 24 h test. Data from all 6 patients treated with placebo or ebrotidine were analyzed with the use of program STATpHAC II/PHARM, version 2.16 D3 (Gastrosoft Inc., Sweden). Gastric acidity was expressed as pH and values from each patient have been transformed into 10 min median values. The circadian median pH values were calculated and compared using Wilcoxon and Spearman signed rang test. Statistics of pH profile was calculated and compared in medians with significance level of 0.05. The statistical evaluation of the median values of pH was performed in the following time intervals; 11:30—11:29 (24 h) and 11:30—03:00. The P values were corrected for multiple testing. The frequency distribution of pH readings scaled with 0.1 pH incremental steps were also calculated for each treatment periods. Sperman's rank test was used to compare the percentages of the time spent at the various pH units by each of two medications in the same period.

Blood samples were withdrawn 30 min before and 30 min after breakfast, lunch and supper for determination of serum gastrin levels. Blood was taken from peripheral vein and allow to clot. The serum was collected and frozen for the radioimmunoassay of gastrin using gastrin antiserum 4652 (gift of Professor J. F. Rehfeldt, Aarhus, Denmark as described previously (5). The intraassay and interassay precisions were about 10% and 12%, respectively.

RESULTS

Effects of ebrotidine on basal and pentagastrin-induced gastric acid secretion

The effects of gradually increasing doses of ebrotidine on basal and pentagastrin-induced peak gastric acid output (calculated from the sum of the two highest consecutive 15 min outputs) in subjects of group A are shown on *Fig. 1.* Ebrotidine at a dose of 200 mg did not affect basal acid secretion but reduced significantly the pentagastrin-induced acid output by about 36%. At a dose of 400 mg, ebrotidine inhibited basal acid output by about 92% and

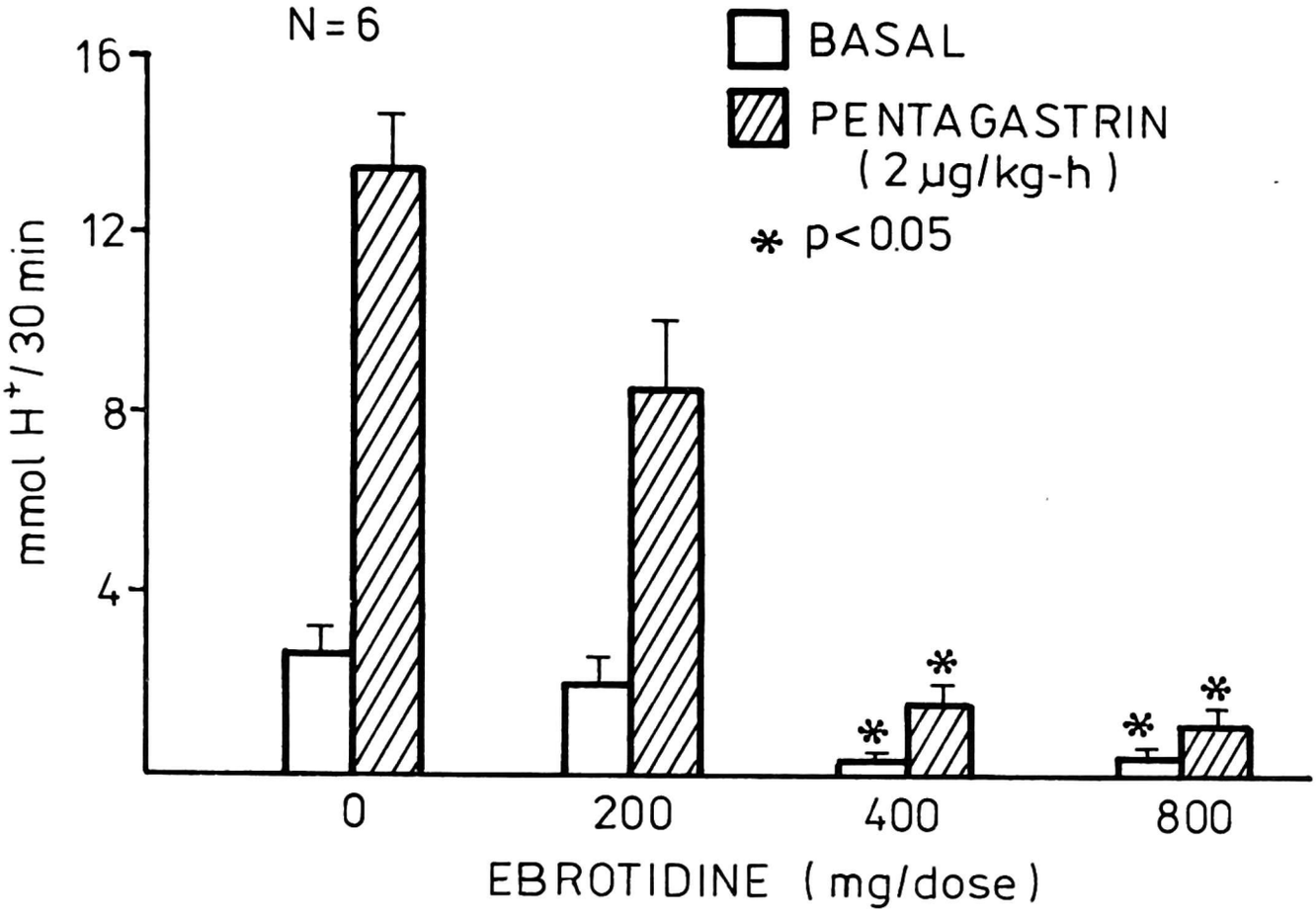

Fig. 1. Effect of oral administration of ebrotidine in various doses (200, 400 or 800 mg) or placebo (0) on basal and peak acid outputs in response to pentagastrin (2 µg/kg-h) i. v. infusion. Means ± SEM of 6 tests on six subjects. Asterisk indicates significant reduction below the control value obtained in placebo-treated subjects.

pentagastrin-induced secretion by about 88%. With doubling the dose of ebrotidine to 800 mg, the inhibition of basal and pentagastrin-induced secretion averaged about 89%, respectively, and this inhibition was not significantly different from that obtained with a dose of 400 mg.

The results showing the time-course of the inhibitory action of ebrotidine given orally in a single dose of 800 mg are presented in *Fig. 2.* Basal and peak pentagastrin-induced acid secretion was suppressed significantly after 6 h following the intake of ebrotidine and the degree of inhibition amounted to about 72% for basal secretion and 50% for pentagastrin-induced secretion. After 12 and 24 h, ebrotidine did not affect significantly basal or pentagastrin-stimulated gastric acid secretion.

Effects of ebrotidine on luminal acidity and plasma gastrin levels before and after meals

The 24 h pH profiles of the placebo and ebrotidine treatments in subjects of group C are reported in *Fig. 3.* Ebrotidine caused significant suppression of gastric acidity for the whole 24 h examination time. In tests with ebrotidine,

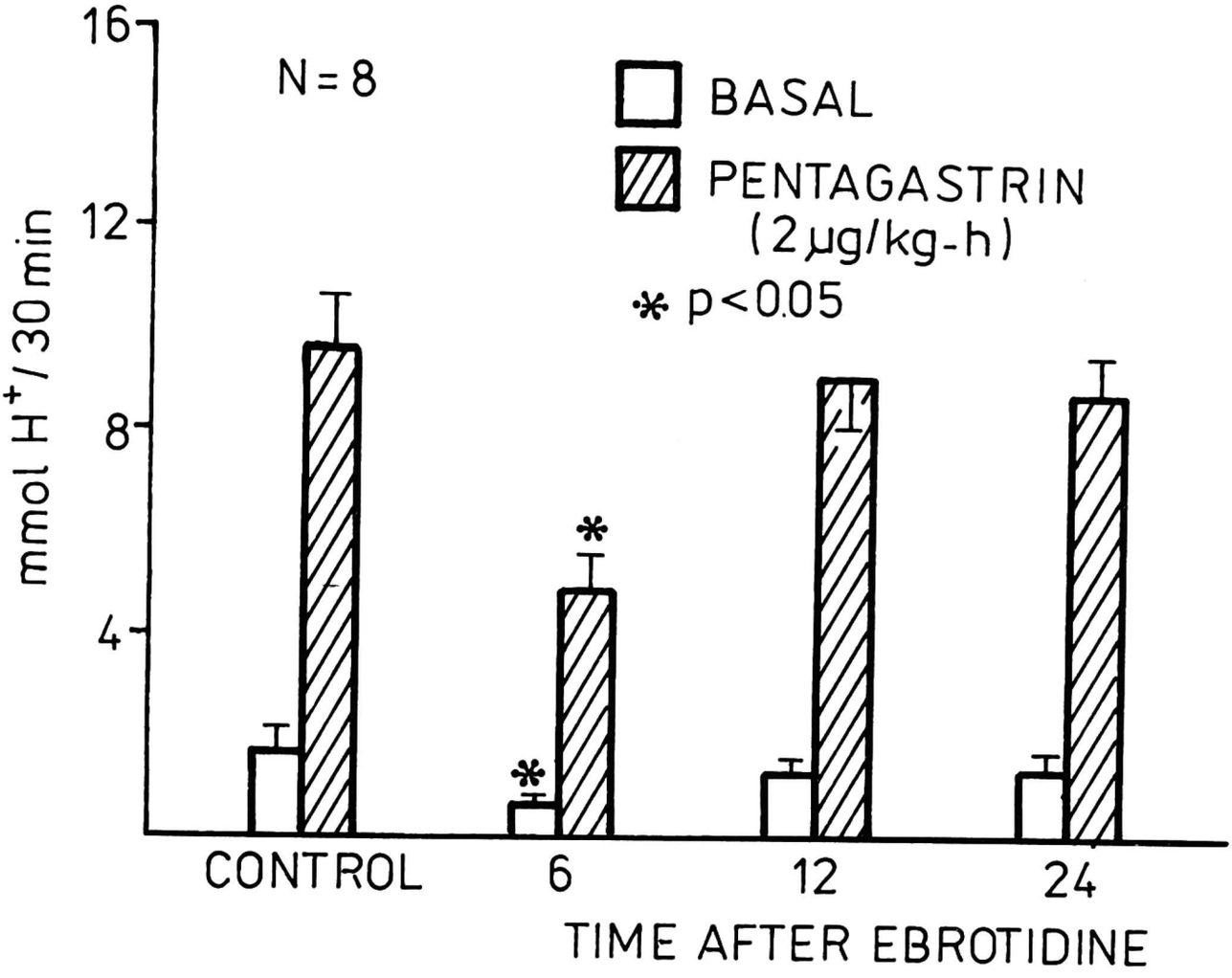

Fig. 2. Basal and peak pentagastrin-induced peak acid output before and 6, 12 or 24 h after oral administration of a single dose of ebrotidine (800 mg). Means ± SEM of 8 tests on 8 subjects. Asterisk indicates significant reduction below the value obtained in placebo-treated subjects.

middle median gastric acidity [3.40 (74.2—0.01 mM/1)] was significantly lower than that obtained in control tests with placebo [49.84 (200.1—0.02)]. The 24 h acidity in tests with ebrotidine and placebo control is presented on *Fig. 4*. The frequency distribution curves of pH readings for 24 h period confirms the pronounced alkaline shift in subjects treated with ebrotidine as compared to placebo. The percentages of time spent above pH 4.0 are significantly higher for ebrotidine (27%) than for placebo (9%) *(Fig. 5)*.

Effects of ebrotidine on luminal acidity and serum gastrin levels before and after meals.

Table 1 shows intragastric acidity (as determined by intragastric pH-electrode) for 60 min before and after each meal and serum gastrin concentrations determined 30 min before and after each meal in placebo- and ebrotidine-treated subjects. After each meal, plasma gastrin level in placebo-treated

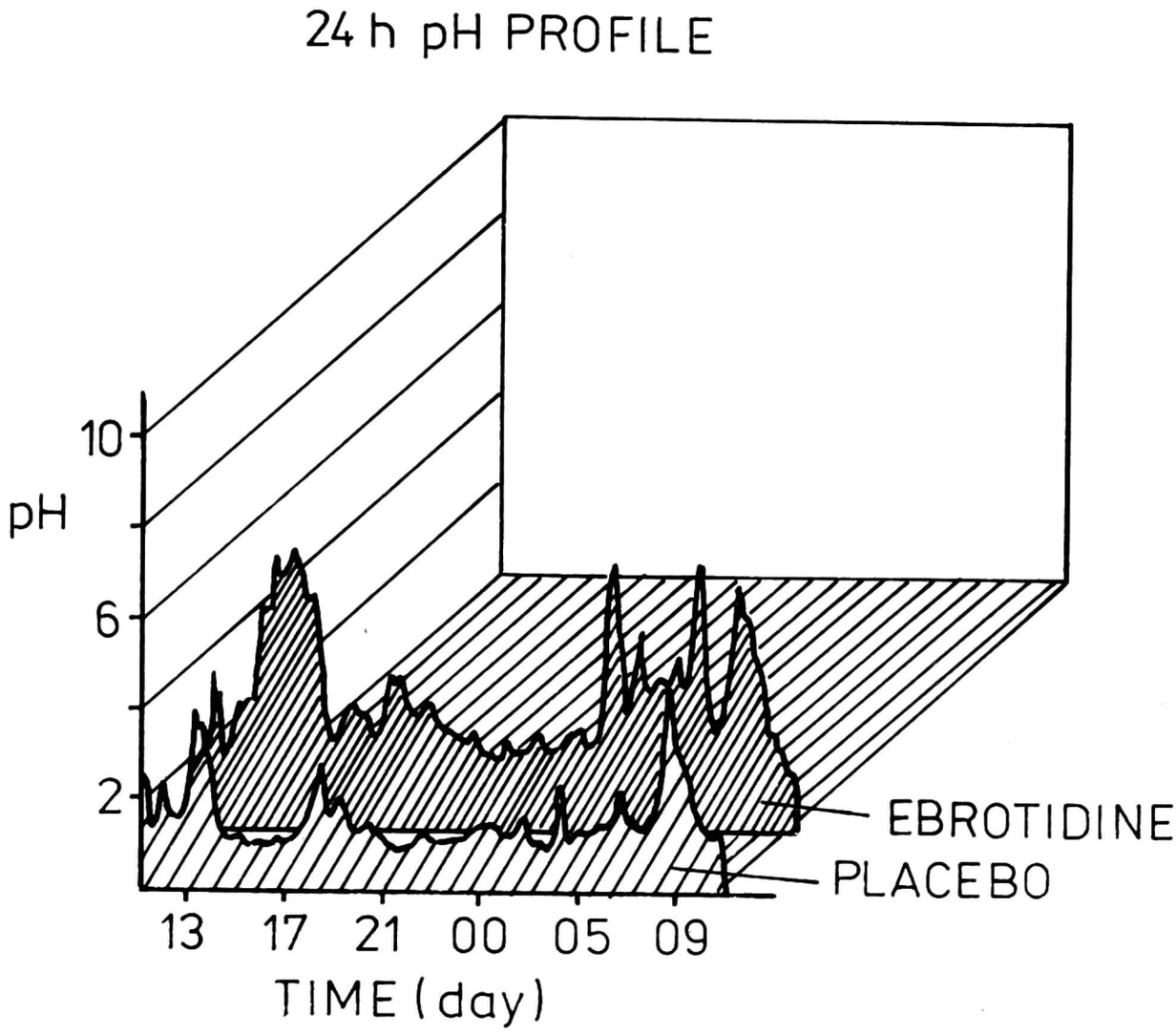

Fig. 3. Graph showing medians for the groups of subjects treated with single dose ebrotidine or placebo.

Table 1. Serum gastrin concentrations 30 min before and 30 min after meal (breakfast, lunch, supper) and gastric acidity as recorded for 60 min before and 60 min after meal during 24 h pH-metry in placebo- and ebrotidine-treated subjects.

		PLACEBO		EBROTIDINE	
		Acidity (mM/l)	Gastrin (pM/l)	Acidity (pM/l)	Gastrin (pM/l)
LUNCH	Before	27+6	22+4	8+2^{+}	51+7^{+}
	After	10+4*	65+12*	2+1^{+}	80+14*$^{+}$
SUPER	Before	61+12	19+4	4+2^{+}	45+6^{+}
	After	14+3*	38+5*	2+1^{+}	96+15*$^{+}$
BREAKFAST	Before	25+4	21+3	3+1^{+}	24+6
	After	4+2*	47+6*	4+2^{+}	51+8*

* — Significant ($P < 0.05$) increase as compared to the value before meal

$^{+}$ — Significant ($P < 0.05$) change as compared to the value obtained in placebo-treated subjects.

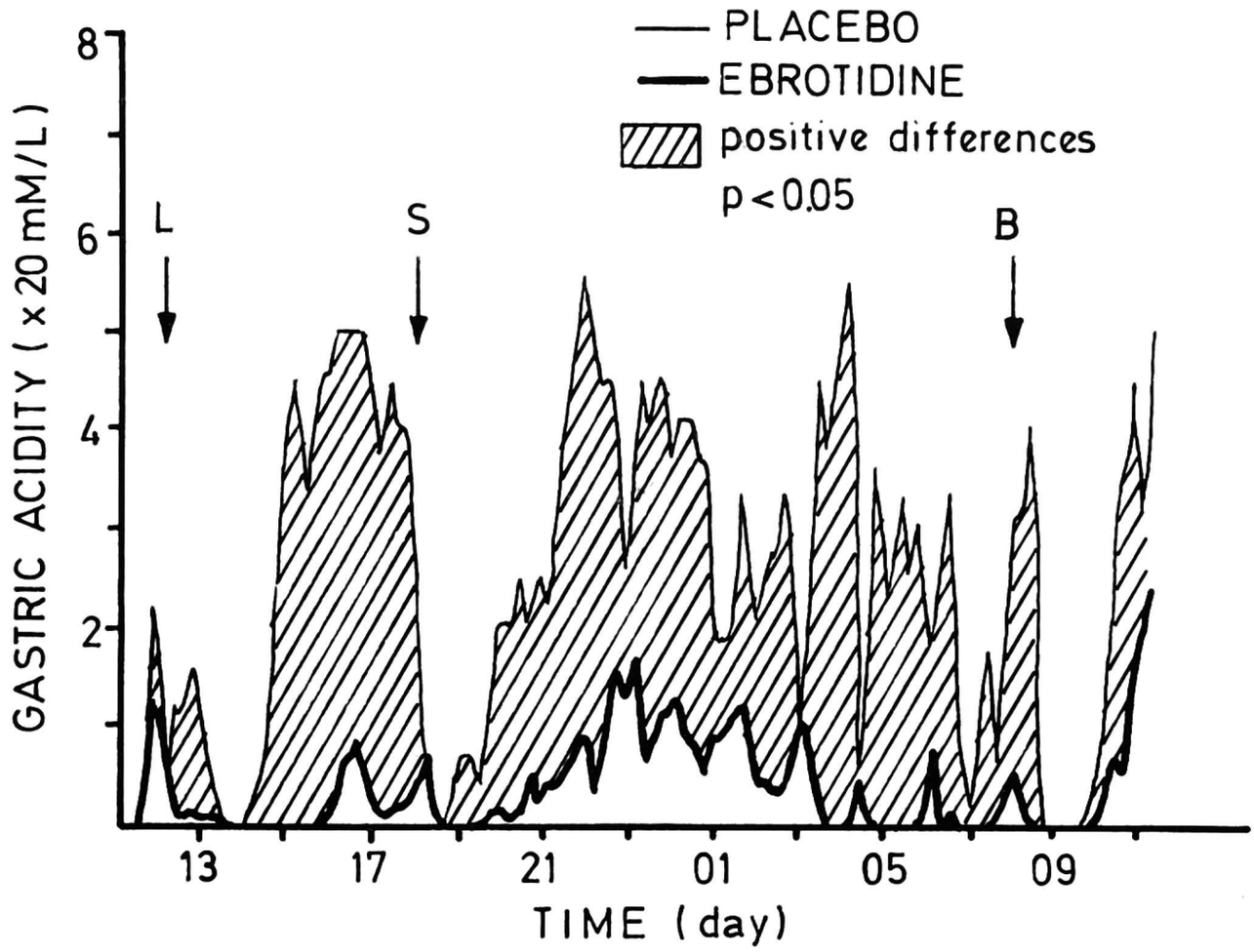

Fig. 4. Graph showing 24-h acidity in a 10 min steps medians in subjects treated with ebrotidine or placebo. Cross-hatched area indicates significant ($P < 0.05$) difference between medians.

subjects was almost doubled. In ebrotidine-treated subjects, serum gastrin levels recorded before lunch and supper were roughly twice higher than in placebo-treated control tests and following these meals, serum gastrin levels rose 2—3 times above the pre-meal values. About 20 h after the intake of ebrotidine (next day morning), the values of plasma gastrin before and after breakfast were similar to those in placebo-treated controls. Gastric acidity recorded before and after meals in ebrotidine-treated subjects was significantly lower than in placebo-controls (Table 1).

DISCUSSION

Previous studies showed that ebrotidine has unique ability to protect gastric mucosa against the damage by various irritants and ulcerogens (1, 2). This protective properties of ebrotidine has also been demonstrated in humans and it was suggested to be independent from its gastric acid inhibitory effect (6).

Although the gastroprotective activity of ebrotidine may be an important factor in the prevention of acute gastric lesions such as caused by ethanol or non-steroidal antiinflammatory drugs (7), there is a little doubt that the enhancement of healing of chronic gastroduodenal ulcerations by H_2-receptor

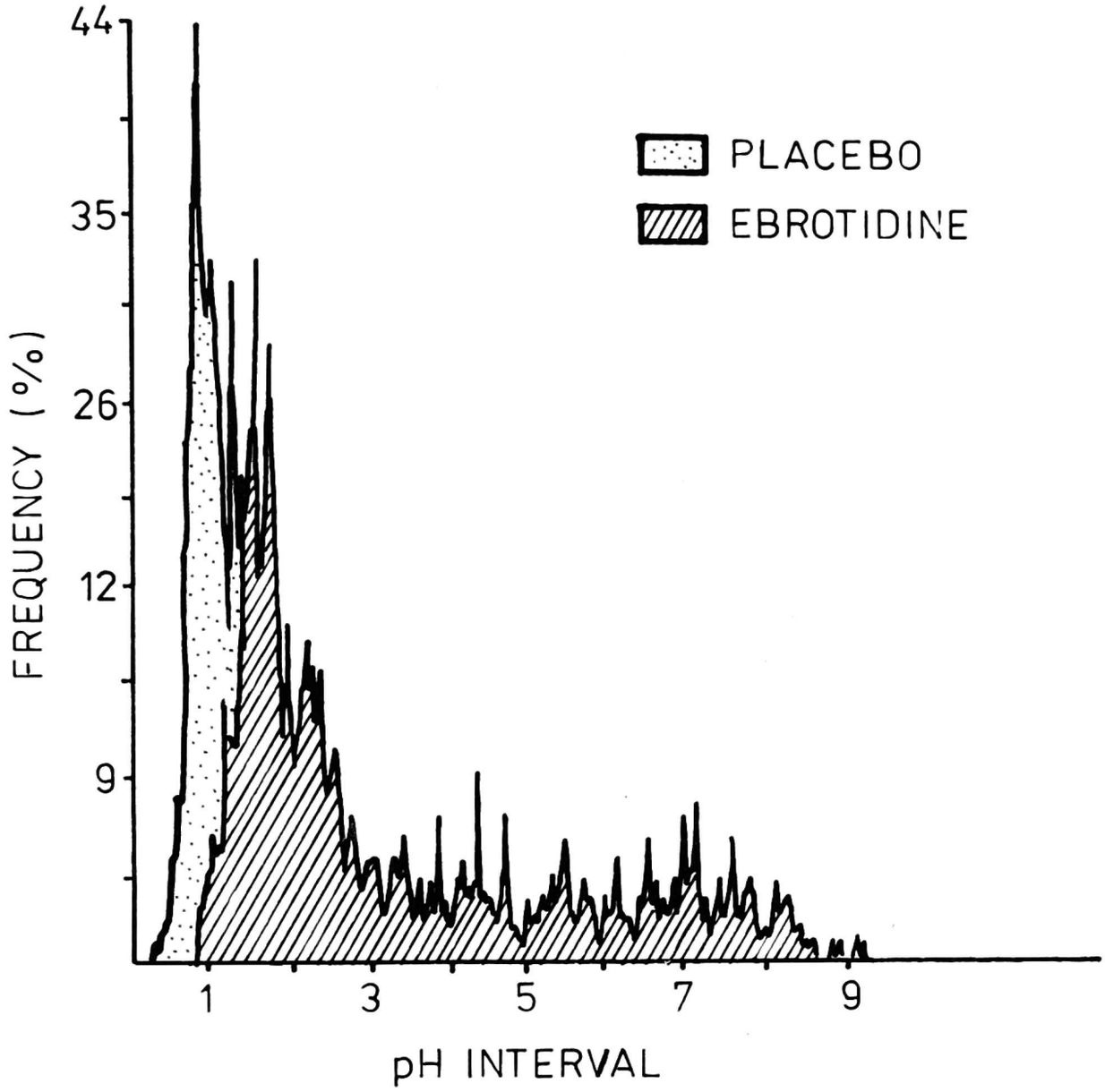

Fig. 5. The distribution of percent time frequency of pH during 24 pH-metry in subjects treated with ebrotidine and control.

antagonists requires an inhibition of gastric acid secretion. Patients with duodenal ulcer tend to have increased basal acid secretion throughout the day and night (8, 9) and also show a prolonged secretory response to food (10, 11) possibly due to defective autoregulation in that acid output is only partially reduced at a lower intragastric pH (pH below 2.5).

Initially, the therapy of duodenal ulcer with H_2-receptor antagonist was intended to reduce gastric acid for prolonged periods but more recently attention has been shifted to the suppression of nocturnal acid secretion selectively (12). Using meta analyses of clinical trials of various ulcer-healing drugs, the ulcer healing was found to be most significantly correlated with the suppression of nocturnal gastric acidity; no further ulcer healing was associated with the suppression of daytime acidity (13).

This study provides an evidence that single oral dose of ebrotidine (800 mg) is an effective inhibitor of basal and maximal pentagastrin-stimulated

gastric acid secretion. This inhibition is dose-dependent and at a dose of 400—800 mg is almost complete with respect to both basal and maximally stimulated (by pentagastrin) acid secretion. The time course of the gastric inhibitory effects of ebrotidine shows that this inhibition lasts for over 6 h after single oral dose ingestion and thus is comparable to that of ranitidine (14). The inhibitory effects of ebrotidine disappear 12—24 h after the administration of the drug. This antisecretory profile suggests that the suppression of nocturnal gastric acidity by ebrotidine can be achieved after single dose of the drug applied at the bed time.

A new technique for continuous measurement of intragastric pH (or acidity) using intragastric electrode has been shown to detect consistent changes in luminal pH greater that 0.1 unit over 24 h period. This provides the most physiological assessment of gastric acidity and allows for an accurate determination of the onset and the duration of the antisecretory drugs (16, 17, 18). Duodenal ulcer patients were found to have lower postprandial pH elevation and smaller decline in intragastric acidity during early morning hours when compared to normals (19, 20). This technique was used in this study to assess the inhibitory action of ebrotidine on gastric acidity and to quantify the effect of the intake of this drug on median 24 h intragastric pH and plasma gastrin profile. Our studies with placebo-treated controls showed that intragastric pH fluctuates to reach the highest levels after meals and the fall to the lowest values at night. The typical frequency pH shows the unimodal distribution with a heavy tail at lower pHs that is characteristic for the secretory pattern recorded in duodenal ulcer patients (16—19) and this is in keeping with the fact that our subjects had a history of duodenal ulcer disease but have been in the remission during the study period. Following the administration of ebrotidine in a single dose (800 mg), the significant inhibition of acid secretion started about 2 h and lasted for about 6 h, indicating again, that the drug should be taken at the bed-time to maintain a high intragastric pH during the night.

The analysis of the percent time spent above pH 4.0 shows that it was significantly higher for ebrotidine than for placebo in the same period. The reduction in gastric acidity probably explains the significant increase of plasma gastrin level observed before and after meals in ebrotidine-treated subjects. Thus, ebrotidine appears to cause significant inhibition of gastric acid secretion and, like other gastric inhibitors (8, 9), results in the transient rise in plasma gastrin level, likely, due to the increase in luminal pH.

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