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ACTIVATION OF HISTIDINE DECARBOXYLASE BY H_2-RECEPTOR BLOCKADE: MECHANISM OF ACTION

R. HÅKANSON
Department of Pharmacology, University of Lund, Lund, Sweden

J. HEDENBRO & G. LIEDBERG
Department of Surgery, University of Lund, Lund, Sweden

J.F. REHFELD
Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark

F. STADIL
Department of Gastroenterology C, Rigshospitalet, Copenhagen, Denmark

1 Treatment with histamine H_2-receptor antagonists, which inhibit basal acid secretion, was found to activate rat stomach histidine decarboxylase. At the same time the serum gastrin concentration was greatly increased.

2 In antrectomized rats neither the enzyme activity nor the serum gastrin concentration was affected by the treatment.

3 In analogy with previous observations on other inhibitors of acid secretion we suggest that the H_2-receptor antagonists stimulate gastrin release through their effect on acid secretion and that the raised serum gastrin level is responsible for the enzyme activation.

Introduction

A histamine-forming enzyme, histidine decarboxylase, is found in the rat stomach. Recently, Maudsley, Kobayashi, Williamson & Bovaird (1973) reported that treatment with H_2-receptor antagonists, burimamide or metiamide, activates this enzyme.

In previous studies evidence was obtained that the activity of rat stomach histidine decarboxylase varies with the serum gastrin level (Håkanson, Kroesen, Liedberg, Oscarson, Rehfeld & Stadil, 1974). Furthermore, the enzyme-activating effect of various surgical and pharmacological treatments was abolished by antrectomy whereas that of gastrin or gastrin analogues was not (Johnson, Jones, Aures & Håkanson, 1969; Aures, Johnson & Way, 1970; Håkanson & Liedberg, 1970, 1971a, b; 1972; Håkanson, Liedberg & Sjölund, 1972; Håkanson, Liedberg & Oscarson, 1973a; Håkanson, Liedberg, Oscarson, Rehfeld & Stadil, 1973b). This study is concerned with the role of endogenous gastrin in the enzyme activation after H_2-receptor blockade.

Methods

Adult male albino rats (Wistar strain, 150-200 g body weight) were used. Antrectomy was performed on eight rats by resection of the distal half of the glandular stomach (the pyloric gland area together with the adjacent portion of the oxyntic gland area) and the duodenal bulb. Gastrointestinal continuity was re-established by gastroduodenostomy end-to-end (for details see Håkanson & Liedberg, 1970; 1972). Operated rats were allowed to recover for at least three weeks before they were used in experiments.

Burimamide or metiamide (Smith, Kline and French) were dissolved in 0.1 M sodium acetate-acetic acid buffer, pH 5.0, at 70-80°C to a concentration of 10 mg/ml. After cooling at room temperature the drugs were injected intraperitoneally in doses of 100 mg/kg three times at 2 h intervals. Controls received acetate buffer. The rats were fasted for 48 h (free access to water) before experiments, and they were killed 2 h after the last injection. Blood was drawn (until exsanguination) from the aorta under diethyl ether anaesthesia.

The serum was analysed for gastrin by radioimmunoassay (Stadil & Rehfeld, 1971, 1973). The antiserum (No. 2604; Rehfeld, Stadil & Rubin, 1972) employed in the present study was used in a final dilution of 1 : 200 000. It binds four gastrin components of different molecular size (Rehfeld, Stadil & Vikelsøe, 1974). Monoiodinated [^{125}I]-synthetic human gastrin I was employed as tracer (Stadil & Rehfeld, 1972). The only known naturally occurring peptide which cross-reacts in the assay is cholecystokinin; the ratio in molar terms between ID_{50} for synthetic human gastrin I and pure porcine cholecystokinin is 0.002. The accuracy of the assay for the determination of rat serum gastrin has been evaluated previously (Håkanson *et al.*, 1974). The gastrin concentration is expressed as pg equivalent of synthetic human gastrin I per ml.

The stomachs were taken out, cut open along the major curvature and washed with ice-cold 0.9% w/v NaCl solution (saline). The mucosa was scraped off the oxyntic gland area and homogenized in 0.1 M phosphate buffer, pH 7.0 (final tissue concentration 100 mg wet weight per ml). Enzyme activity was measured as $^{14}CO_2$ produced from [1-^{14}C]-L-histidine. The reaction mixture (0.5 ml) contained 0.4 ml homogenate, 10^{-5} M pyridoxal-5-phosphate, 5×10^{-4} M glutathione and 4×10^{-4} M [1-^{14}C]-L-histidine (1.3 mCi/mM) added in this order. The mixture was gassed with nitrogen for 5 min at 0°C. After 1 h at 37°C the reaction was terminated by the addition of 0.5 ml 10% trichloroacetic acid, and the evolved $^{14}CO_2$ was trapped during 30 min at 37°C on a filter paper strip, previously immersed in Protosol and placed in a centre well in the reaction vessel. The radioactivity of the filter paper strip was then determined in a liquid scintillation spectrometer. The results were corrected for non-enzymatic decarboxylation by incubating identical samples with [1-^{14}C]-D-histidine instead of [1-^{14}C]-L-histidine. Blanks never exceeded 190 ct/minute. Duplicate assays were run in all experiments. The reproducibility of the assay was such that the standard deviation of 15 identical samples (aliquots of a pooled extract of gastric mucosa from freely fed rats) was 6.6%. Enzyme activities are expressed as pmol CO_2 produced per mg tissue (wet weight) and hour. For further details of the procedure see Håkanson (1970) and Håkanson *et al.* (1974).

Gastric secretion was studied in 300-350 g rats fitted with chronic gastric cannulae, by a slight modification of the technique described by Bel, Levrat, Nesmos & Girard (1966). Before experiments the fistula rats were deprived of food but not of water for 24 hours. The fistula rats were immobilized without anaesthesia in Bollman type cages, the cannulae opened and the stomachs rinsed with warm saline. Saline (10 ml) was given subcutaneously to replace fluid loss during the experiment. The cannulae were allowed to drain

Table 1 Effect of H_2-receptor blockade on serum gastrin concentration and gastric histidine decarboxylase activity

	Treatment	Serum gastrin (pg SHG Eq/ml)	Histidine decarboxylase activity (pmol CO_2 mg^{-1} h^{-1})
		Mean ± s.e. mean (n)	
Unoperated rats	Controls	46 ± 4 (30)	3.7 ± 0.3 (23)
	Burimamide (100 mg/kg)	118** ± 21 (15)	15.8*** ± 3.0 (10)
	Metiamide (100 mg/kg)	223*** ± 48 (14)	33.0*** ± 4.0 (14)
Antrectomized rats	Controls	35 ± 7 (4)	3.1 ± 0.4 (4)
	Metiamide (100 mg/kg)	41 ± 3 (4)	3.5 ± 1.0 (0.4)

Determinations of serum gastrin (in terms of synthetic human gastrin I (SHG)) and histidine carboxylase activity were usually made on the same animal. All rats received three intraperitoneal injections of burimamide or metiamide 2 h apart and were killed 2 h after the last injection.
Significant differences between controls and drug-treated animals are marked by ** for $0.001 < P < 0.01$ and *** for $P < 0.001$ (Student's t test).

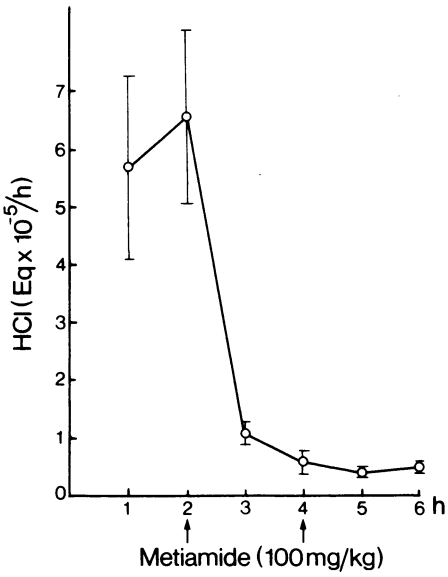

Figure 1 Inhibitory effect of metiamide on basal acid secretion in seven gastric fistula rats. Mean values are given, vertical bars indicate s.e. mean.

freely for 1 h, and two 1 h samples of basal secretion were collected. Metiamide (100 mg/kg) was given intraperitoneally after which two 1 h samples were collected, the injections were repeated and two more 1 h samples were collected. The volumes were measured and the hourly acid output was determined by titration with 0.02 N NaOH, using phenolphthalein as indicator. Acid output is expressed as mEq HCl per hour.

Results

In confirmation of previous reports we found that H_2-receptor antagonists inhibited basal acid secretion (Figure 1) (cf. Black, Duncan, Durant, Ganellin & Parsons, 1972; Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973) and activated gastric histidine decarboxylase (Table 1) (cf. Maudsley *et al.*, 1973). We also found that the gastrin concentration in serum was greatly increased (Table 1). In antrectomized rats, the H_2-receptor antagonists failed to activate the enzyme and the serum gastrin concentrations were not raised above the basal level (Table 1).

Discussion

The present results show that H_2-receptor antagonists raise the serum gastrin level as well as

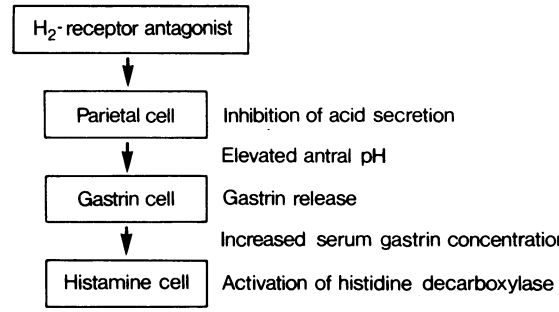

Figure 2 Proposed chain of events leading to activation of histidine decarboxylase in the rat stomach after H_2-receptor blockade. The H_2-receptor antagonists act on the parietal cells to inhibit acid secretion. As a consequence, pH in the antral lumen is raised. This stimulates the gastrin cells to release gastrin. The increased serum gastrin concentration causes activation of gastric histidine decarboxylase.

the histidine decarboxylase activity. This is in agreement with the hypothesis that the activity of this enzyme is regulated by the serum gastrin concentration. The great majority of gastrin cells in the digestive tract of the rat are found in the antrum and in the regions immediately adjacent to the antrum (Larsson, Håkanson, Rehfeld, Stadil & Sundler, 1974). However, the serum gastrin concentration was only lowered to a minor extent by antrectomy, possibly because there exists an as yet unrecognized source of gastrin immunoreactivity outside the digestive tract. The H_2-receptor antagonist metiamide failed to raise the serum gastrin level in antrectomized rats. The histidine decarboxylase activity of these animals was also unaffected by metiamide. This observation lends further support to the view that gastrin is a mediator of the enzyme activation.

The inhibition of basal acid secretion produced by injection of metiamide may possibly explain why H_2-receptor antagonists increase the serum gastrin level (Figure 2). Conceivably, the elevation of antral pH that results from the inhibition of acid secretion facilitates gastrin release (Becker, Reeder & Thompson, 1973). The H_2-receptor antagonists may thus be added to the list of agents that non-specifically activate rat stomach histidine decarboxylase through an inhibitory effect on acid secretion. This list includes 'anti-gastrin' (SC-15396) (Håkanson & Liedberg, 1971b), prostaglandin E_1 (Håkanson, *et al.*, 1973a), deglycyrrhizinized liquorice (Håkanson *et al.*, 1973b), atropine and hexamethonium (Håkanson *et al.*, 1974).

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