Effect of Secretin and Cholecystokinin on Histidine Decarboxylase Activity in the Rat Stomach* (34068)

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Gastrin and the synthetic gastrin-like peptides have multiple biological effects on the gastrointestinal tract (1). The dominant effect on the stomach is the stimulation of the secretion of acid. Natural gastrin and synthetic pentagastrin, as well as other secretagogues, can activate the histamine (Hm)-forming enzyme histidine (Hd) decarboxylase (EC4.1.1.22) of the rat gastric mucosa from barely measurable activity to high levels (2–6). The physiological significance of this adaptive Hd decarboxylase in the oxyntic gland area of the rat stomach has been speculated upon, but remains unclarified (6–8). Kahlson *et al.* (4) hypothesized that gastrin acts by releasing intramucosal Hm to stimulate acid secretion. Depletion of the Hm stores in turn leads to the activation of the histamine-forming enzyme. Recent evidence indicates that gastrin is an important regulator of Hd decarboxylase in the rat stomach (9). Studies on the effect of compounds which counteract acid secretion elicited by gastrin and/or Hm on the activity levels of Hd decarboxylase should add to a better understanding of the mechanisms of enzyme activation and acid secretion.

The gastrointestinal hormones secretin and cholecystokinin-pancreozymin (CCK-PZ) (10, 11, 12) as well as the commercially available "antigastrin" product SC-15396 [2-phenyl-2-(2-pyridyl)thioacetamide] (13) have been found to reduce gastric acid secretion stimulated by gastrin. It seemed desirable to study the effects of these substances on Hd decarboxylase activity of the rat stomach to see if any correlation exists between Hd decarboxylase activity and inhibition of gastric secretion.

Materials and Methods. Sprague-Dawley rats of both sexes weighing 160–220 g were fasted for 48 hr, but allowed water *ad libitum*. The animals were housed in cages with half-inch mesh raised floors, not more than two rats in a cage. On the day of the experiment, the rats were injected in groups of at least two animals each, 4 hr after an artificial 12-hr dark cycle. The individual groups received the compounds by a single intraperitoneal injection as follows: one group received 250 μg/kg of synthetic pentagastrin (ICI 50,123; Ayerst Laboratories); others received 150 units/kg secretin [Gastrointestinal Hormone (GIH) Research Unit, Karolinska Institute, Stockholm], 150 units/kg CCK-PZ (10% active natural, purified polypeptide hormone, 90% inactive polypeptides, same source), 250 mg/kg SC-15396 (G. D. Searle & Co.) suspended in a 1% solution of gum tragacanth. Other groups received, along with gastrin injections, either secretin, CCK-PZ or SC-15396 in the dosages described above. The control groups received 1 ml of isotonic saline or of the 1% gum tragacanth (S. B. Penick Co.) suspending agent. Ninety minutes after injection,

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the animals were sacrificed by cervical fracture. The stomachs were quickly excised and rinsed at once with ice-cold saline. The oxyntic mucosa was removed by scraping with a sharp-edged microscope slide, and homogenized in 0.02 *M* phosphate buffer, pH 6.5, to a final concentration of 100 mg tissue per ml. The Hd decarboxylase activity in aliquots of 10.0 mg of tissue was assayed by incubation with 1-^{14}C-Hd for 30 min followed by determination of the $^{14}CO_2$ produced. The composition of the incubation mixture (6, 8) and method of collecting $^{14}CO_2$ have been described (14). Corrections were made from heated enzyme blanks. Enzyme activities were expressed as pmoles CO_2 formed per mg tissue under the incubation conditions used. Each sample was determined in quadruplicate. For proper evaluation of the data, the solvents and compounds were tested *in vitro* by adding proper amounts to the incubation mixture and measuring the Hd decarboxylase activity of gastric mucosal tissue from starved and gastrin-injected animals. The data were evaluated by *t* test for unpaired values. Means were considered significantly different if $p<.05$.

Results. The results are summarized in the figure. Pentagastrin increased the Hd decarboxylase activity eightfold above the mean control value ($p<.01$). Secretin injected intraperitoneally along with pentagastrin failed to significantly lessen the stimulatory effect of pentagastrin ($p>.6$), and secretin alone had no apparent effect on Hd decarboxylase activity. Neither CCK-PZ nor SC-15396 effectively antagonized the stimulating effect of pentagastrin on Hd decarboxylase. But both CCK-PZ and SC-15396 significantly stimulated Hd decarboxylase activity when compared to the solvent and saline-injected controls. Activity levels increased approximately fivefold ($p<.01$) and fourfold ($p<.01$) respectively. None of the compounds or the solvents affected the Hd decarboxylase activity when tested *in vitro*.

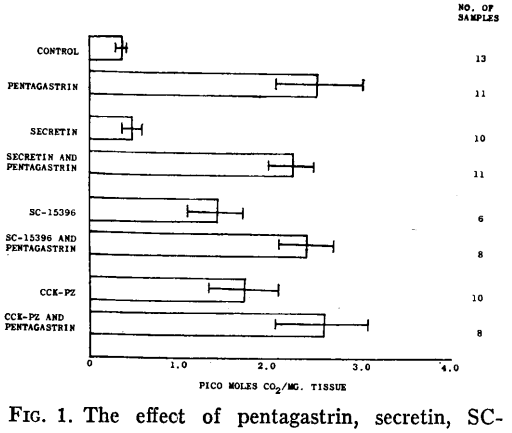

FIG. 1. The effect of pentagastrin, secretin, SC-15396 (2-phenyl-2-[pyridyl]thioacetamide), and cholecystokinin-pancreozymin (CCK-PZ) on the Hd decarboxylase activity (picomoles CO_2/mg tissue) in the rat fundic mucosa. Means and standard errors of the means.

Discussion. Purified preparations of the gastrointestinal hormones, secretin and CCK-PZ, inhibit gastrin-stimulated gastric secretion in the dog (10, 11) and the rat (12). Neither secretin nor CCK-PZ inhibit Hm-stimulated secretion in the dog (11, 15). CCK-PZ was not so strong an inhibitor as secretin in the rat since it produced only an initial 50% inhibition of the gastrin response, and prolonged the secretory response to gastrin (12). SC-15396 has been reported to be a specific inhibitor of pentagastrin-stimulated secretion in the rat (13). In a recent report, however, its specificity has been questioned, apparently extending to Hm-stimulated secretion as well (16). In the current study none of these secretory inhibitors blocked the activation of Hd decarboxylase by pentagastrin.

It has been hypothesized by Kahlson and co-workers (5, 6) that gastrin and parasympathetic drugs act to release Hm from the gastric mucosa. Hm then stimulates gastric secretion presumably acting as the "final common mediator" for all secretory stimuli in the stomach. According to this theory, the release of Hm that stimulates acid secretion depletes Hm stores and thus triggers the synthesis of new Hm as manifested by increased Hd decarboxylase activity (4, 5). In the current study, however, we found that physiological inhibitors of gastric secretion do not decrease Hd decarboxylase activity levels. If the scheme outlined above is correct, this means that secretin and CCK-PZ must in-

terfere with the action of Hm directly, at some point after it is released and before it activates the parietal cells to secrete acid. However, a number of studies show that Hm-stimulated gastric secretion is not inhibited by secretin or CCK-PZ in the dog (17). Although incomplete, preliminary studies confirm the assumption that it is unlikely that secretin inhibits Hm-stimulated acid secretion in the rat. It remains to be shown whether the release of Hm is blocked when the gastrin-stimulated secretion is inhibited. If the model proposed by Kahlson is correct, it would have to be assumed that it is not blocked, because Hd decarboxylase activity increased. But conversely, it can be argued that the release of Hm is inhibited, since the acid secretory response to Hm is not blocked by secretin, and it is not likely that endogenously released Hm would act differently from injected Hm. An alternative hypothesis would be compatible with the latter; namely, that the Hm stores are not involved in the activation of Hd decarboxylase by gastrin. Aures *et al.* (6) proposed that gastric secretagogues, which also stimulate the activation of Hd decarboxylase, exert their action through gastrin as mediator. Basal levels of Hd decarboxylase in antrectomized rats were consistently below the levels of sensitivity of the method of assay (conversion of 0.2 picomoles substrate per mg tissue) (9); and secretagogues such as insulin, urecholine and 2-deoxy-1-glucose did not activate Hd decarboxylase when the source of endogenous gastrin was removed by antrectomy (18). This strongly suggests that gastrin mediates Hd decarboxylase activation. In conjunction with this evidence the results of the current study may be explained by assuming that secretin inhibits the receptors activated by gastrin to stimulate acid secretion, but does not affect the receptors for gastrin related to the activation of Hd decarboxylase. This is compatible with the finding that secretin is a potent inhibitor of gastrin stimulated secretion (12).

CCK-PZ and SC-15396 stimulated Hd decarboxylase activity significantly when given alone, but did not increase the pentagastrin-induced activation of enzyme activity. The dose of pentagastrin used was supramaximal for stimulating acid secretion (12), so Hd decarboxylase activity was increased to the same extent as that produced by gastrin. Activation of Hd decarboxylase in the case of CCK-PZ was no doubt due to its structural similarities to gastrin. Gastrin and CCK-PZ contain the same five C-terminal amino acids (19), and both of these compounds increase Hd decarboxylase activity in the antrectomized rat (18). We have no explanation for the activation of Hd decarboxylase by SC-15396, although our observation confirms that of others (16, 20).

Summary. Secretin and CCK-PZ, hormones which are inhibitors of pentagastrin-stimulated gastric secretion in the dog and the rat, did not block the activation by pentagastrin of Hd decarboxylase in the gastric mucosa of the rat. This supports the concept that pentagastrin acts: (1) directly to activate Hd decarboxylase, and (2) to stimulate acid secretion, and that (1) is not and (2) is inhibited by secretion and CCK-PZ.

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