Effects of Some Pharmacologic Agents on Gastric Cation Secretion: Ratio of Hydrogen to Potassium Ions[1,2] (35148)

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In earlier experiments from this laboratory concerned with the secretion of sodium (Na) by vagally denervated canine fundic pouches, it was noted that hydrogen (H) and potassium (K) were secreted in a fixed ratio over a wide range of volume rates (1). This experimental observation that K is secreted in a fixed ratio to H suggests they may have a coupled secretory mechanism.

Davenport, Warner, and Code (2), and Berkowitz and Janowitz (3) have demonstrated that there is a normal and relatively slow diffusional exchange of H for Na from lumen to mucosa in the fundus of the resting mammalian stomach. In our cited studies (1) intravenous acetazolamide inhibited the secretory response of the histamine-stimulated canine fundic pouch, with a concomitant and equal reduction in volume, H and K outputs, but a rise in Na output. Werther, Altamarino, and Hollander (4) found that the parenteral acetazolamide led to an increased rate of back-diffusion of H across the surface epithelium of an unstimulated, *in vivo–vitro* canine fundic mucosal preparation. This raised the possibility that the observed decrease in H and K outputs from the acetazolamide-inhibited canine gastric pouch might be the result in part of an increased rate of H and K diffusional exchange for Na across the surface epithelium as well as an effect on the primary secretory process of the parietal cell.

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The present study was undertaken to demonstrate whether inhibition of the histamine-stimulated acid secretion of vagally denervated canine fundic pouches by a variety of other pharmacologic maneuvers would cause a concomitant fall in H and K outputs with little or no change in Na output. A decrease in H and K outputs without correlative changes in Na output would suggest a direct influence on the H–K secretory process.

In dogs, employing histamine, we established steady-state outputs of gastric fluid, H, K, and Na and then altered these outputs by three techniques: (a) the administration of atropine, (b) the administration of cyproheptadine (a compound with antihistaminic, antiserotonin, and anticholinergic activity) (5), and (c) variations in the histamine dosage.

Methods. Vagally denervated fundic pouches were prepared in antrectomized dogs. The animals were allowed to recover from surgery for at least 4 weeks before being studied. All animals were fasted 18 hr prior to each test. For each animal the histamine dose (administered subcutaneously every 10 min) required to produce a half-maximal volume output from the pouch was determined. Four experimental series were performed. (A) The pouch was stimulated to secrete at a half-maximal rate for at least 2.5 hr; then 0.3 mg/kg of atropine sulfate was administered subcutaneously. The administration of histamine at the same dose level was continued until a new steady-state volume output had been established for at least 1 hr. Specimens were collected in graduated centrifuge tubes, volume was measured every 20 min, and aliquots were taken for assay.

Seven such studies were performed in three dogs. Three studies were performed in one dog and the remaining two animals were studied twice. (B) In the same three animals, seven experiments identical to A were performed except that cyproheptadine (3.0 mg/kg) was administered intravenously over a 5-min period, at least 2.5 hr after the onset of steady-state histamine stimulation. Three experiments were performed in one dog and each of the remaining two animals were studied twice. (C) Ten experiments were initiated as in A with histamine being administered subcutaneously to produce a half-maximal volume rate for at least 2.5 hr and then the dose was lowered so as to produce a quarter-maximal response in volume and specimens were collected for another 2.0 hr. Five experiments were performed in one dog, two other dogs had two studies each, and finally, one animal had one study. (D) As a control for the prolonged length of time the animals were stimulated in the above three series, continuous histamine stimulation was maintained for 5 hr at a half-maximal volume rate of secretion. A total of nine such experiments were performed in three animals. Five experiments were done in one animal, three in the second, and one in the third.

In the four sets of experiments, all specimens were assayed for H, Na, K, and Cl. H was measured by titrating to pH 7.0 with standardized 0.1 *N* NaOH. Na and K were determined by flame photometry, and Cl was determined by the method of Cotlove *et al.* (6).

Results. Inhibition by atropine sulfate. The results of a representative experiment are shown in Fig. 1. Na output was high in the initial collection periods and fell to a steady-state level which was unaffected by the inhibitor. In contrast, H, K, Cl, and volume all rose together and were suppressed concomitantly. With atropine suppression, the correlation between the H and K decline was +0.93 and between H and Na it was +0.61 (Table I).

TABLE I. Correlation Coefficients for Change in Outputs.

Experiment[a]	H vs K[b]	H vs Na[b]
Atropine sulfate (7)	+0.93	+0.61
Cyproheptadine (7)	+0.99	+0.66
Change in histamine dose (10)	+0.95	+0.71

[a] Number of studies in parentheses.
[b] p value for all coefficients $<.01$.

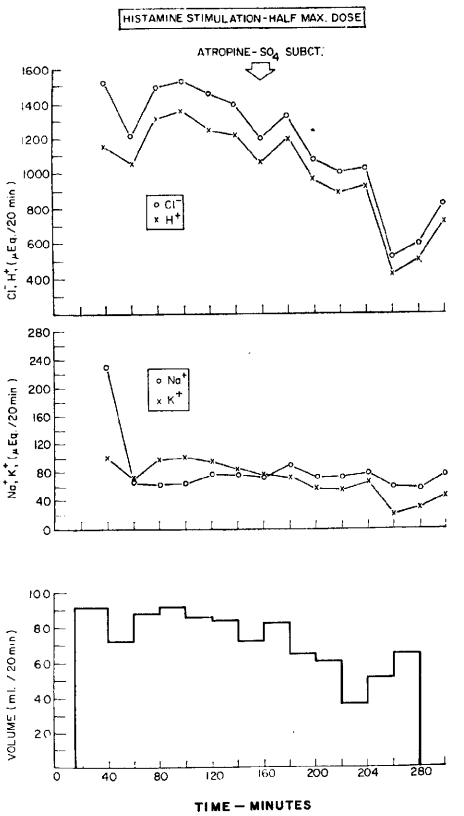

FIG. 1. Typical study showing the effect of atropine sulfate on the steady-state ouput of fluid and electrolytes by half-maximal histamine stimulation.

Inhibition by cyproheptadine. In Fig. 2 (a representative experiment) the pattern of volume and ionic outputs was the same as with atropine, except for the fact that the decline in H, K, Cl, and volume occurred within about 30 min after injection. With atropine, the suppression was frequently not manifest for up to 1 hr later. With cyproheptadine suppression, the correlation between the H and K decline was +0.99 and between the H and Na it was +0.66 (Table I).

Reduction in histamine dosage. Figure 3 sets forth a representative experiment. The pattern of ionic and volume outputs was the

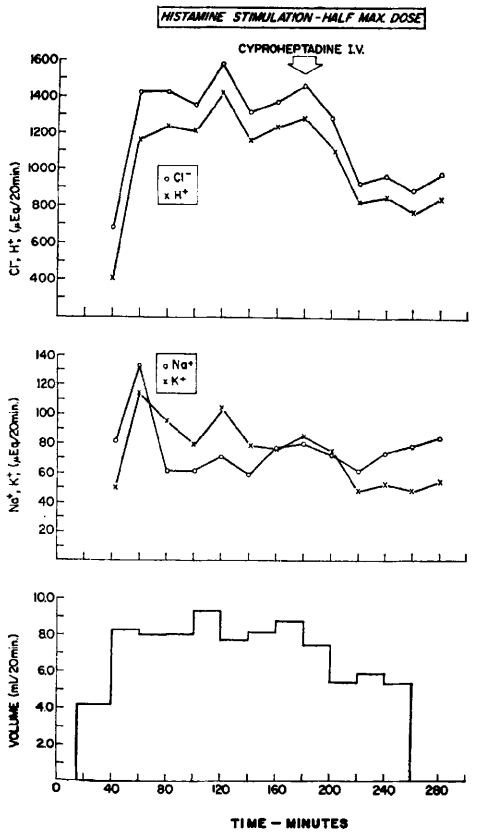

FIG. 2. Typical study showing the effect of cyproheptadine on the steady-state output of fluid and electrolytes by half-maximal histamine stimulation.

same as with the two pharmacologic agents and the fall-off in H, K, Cl, and volume occurred about 30–45 min after the reduction of the dose. The correlation of H with K decline from one steady-state to the other was over +0.95 and in the case of Na it was +0.71 (see Table I).

Histamine steady-state stimulation. Figure 4 is a representative experiment. After the first hour there was steady-state output of H, K, Cl, and volume for 5 hr. During the last hour of three of the nine experiments, there was a fall-off of up to 25% in K output with a concomitant rise in Na output. No change in H output was noted in any experiments. The observed fall-off in K output was not statistically significant ($p > .05$ by the Mann-Whitney U test) (7).

Discussion. The histamine-stimulated, steady-state, half-maximal volume output of a vagally denervated canine fundic pouch was lowered by three pharmacologic maneuvers. A concomitant large fall in H and K output with a much smaller change in Na output was observed. There was a high degree of correlation between the H and K decline but there was poor correlation between the Na and H outputs. The data suggest that Na entry was not dependent on the H:K secretory process and the decline in H and K was not related to an increase in the permeability characteristics of the fundic mucosa. No significant change was observed in H, K, Cl, Na, and volume outputs with 5 hr of steady-state histamine stimulation at a half-maximal volume rate and this served as a control for the studies with the inhibitors and the reduction in histamine dosage. Hirschowitz and colleagues (8–11) recently reported that extended periods of maximal and

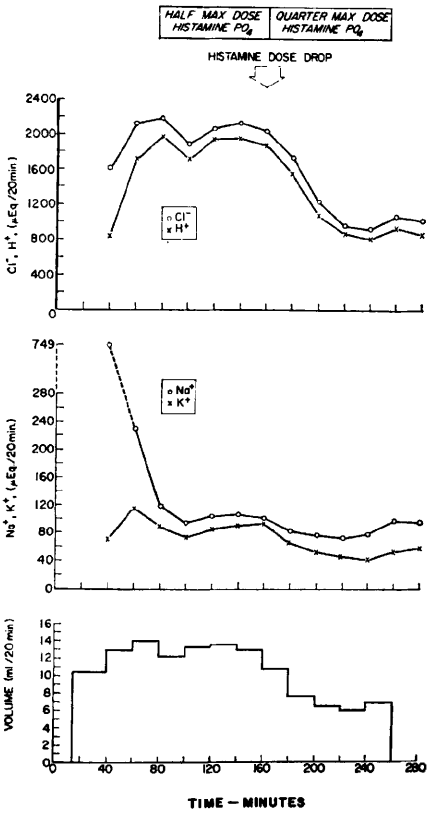

FIG. 3. Typical study showing half-maximal stimulation followed by quarter-maximal histamine stimulation on output of fluid and electrolytes.

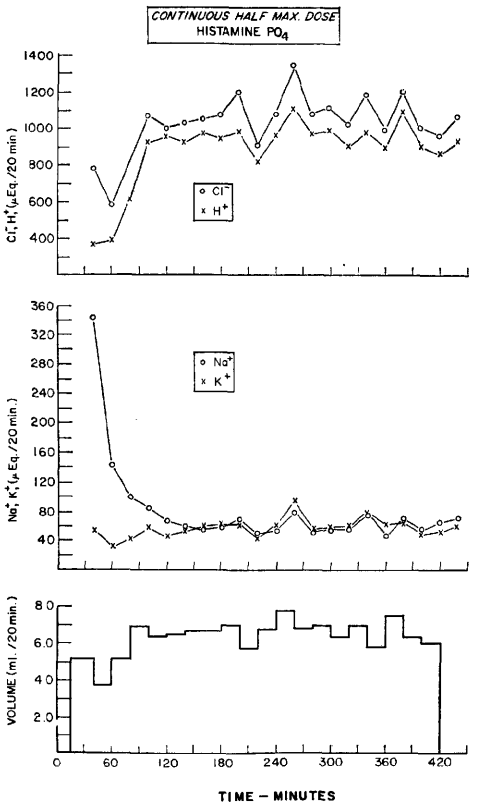

FIG. 4. Typical study showing the effect of steady-state histamine stimulation on the output of water and electrolytes over a 7-hr period.

submaximal stimulation of the entire gastric mucosa results in decline in H and K outputs and that this decline is prevented by the intravenous administration of K. Hirschowitz's experiments were performed in animals with gastric fistula. In Hirschowitz's publications extensive evidence has been presented that in animals in which the entire stomach is stimulated and all the products of secretion are drained externally, there is considerable depletion of K from intra- and extracellular sites. In the preparations employed in our studies, only a small fraction of the entire gastric mucosal secretory output was drained to the outside and we used only half-maximal stimulation.

Our findings of a fixed relationship between H and K over a range of steady-state volume outputs are in keeping with Gray and Bucher's (12) original study comparing H and K secretion by the canine gastric mucosa. Many investigators employing a wide variety of experimental maneuvers, have been able to demonstrate a fixed relationship between the output of H and K by the mammalian gastric mucosa (13–19). It should be pointed out that this relationship does not demonstrate that K enters the gastric lumen only in association with H. Experiments performed in this laboratory, in which isotonic solutions of HCl and mannitol were instilled into antral pouches, have demonstrated that significant amounts of K probably move across the antral mucosa by simple diffusion (20). In contrast, it should be noted that in several studies (1–3, 19) involving the instillation of acid solutions into *in vivo–vitro* fundic mucosal preparations and resting fundic pouches, negligible amounts of K appeared in the instilled solution.

A proposed electrogenic mechanism for the secretion of hydrogen ions would require that H and a major intracellular cation be secreted in a fixed ratio (21). This mechanism is the physicochemical process of membrane polarization, in which there is a field-induced dissociation of water at a fixed negative charge surface in the presence of a dissolved strong electrolyte. The details of the process and its application to gastric secretion have already been described (21). Membrane polarization would require that another cation be secreted at a fixed but lower rate than hydrogen. We and the many others cited above have found that H and K outputs have a fixed ratio. Thull and Rehm (19), Davenport *et al.* (2), and Berkowitz and Janowitz (1) have shown that there is negligible K transport across the resting fundic mucosa into the lumen. This is consistent with the concept that gastric H and K secretion are linked and K crosses the fundic mucosa only with hydrogen ions.

Although Stone and his colleagues (5) have reported that cyproheptadine has one-twentieth the anticholinergic activity of atropine, as well as antihistaminic activity, the effects of cyproheptadine on the gastric secretion of acid have never been reported. It is apparent from our experiments that cyproheptadine in doses 10-fold greater than atropine resulted in suppression of histamine-

induced gastric secretion. The ratio of cyproheptadine to atropine necessary to suppress secretion suggests that the action is probably related to its anticholinergic properties.

Hirschowitz and Sachs have recently studied the effects of the intravenous administration of atropine on insulin, histamine, and pentagastrin-stimulated gastric secretion in gastric fistula dogs (22). In these studies K output fell to a greater extent than the H ion output when atropine suppressed histamine-stimulated gastric secretion. This usually occurred after the second hour in fistula dogs and at the time that depletion of body postassium became significant. The dissociation of the H and K secretory rate in these experiments may be related to K depletion. In our studies, only a small fraction of the entire gastric mucosal output was drained externally with minimal loss of K. The steady-state output of H and K during continuous histamine stimulation for over 5 hr confirms that K loss from pouch draining does not result in suppression of gastric secretory processes.

Atropine and cyproheptadine alter histamine-induced gastric electrolyte secretion differently than acetazolamide. H and K outputs were suppressed to the same degree by these two agents with minimal suppression of Na output, in contradistinction to acetazolamide, which suppressed H and K secretion to the same extent but resulted in a large increase in the output of Na by the gastric mucosa. The difference between the effects of acetazolamide and the two drugs studied are probably the result of an alternation in surface epithelial permeability by acetazolamide, with increased H and K diffusional exchange for Na (4).

Summary. The relationship between H and K output from vagally denervated fundic pouches under steady-state histamine stimulation was determined when secretion was either inhibited by two pharmacologic agents—atropine and cyproheptadine—or the dose of histamine was lowered. The correlation between H and K decline exceeded 0.9 with all three techniques. There was poor correlation between the change in H output and Na output. These findings suggest that gastric H and K secretion are coupled. The relevance of this coupling to a proposed electrogenic mechanism for gastric H secretion is noted. Inhibition of histamine-stimulated gastric secretion with the agents employed altered the pattern of gastric electrolyte secretion in a manner different from that of acetazolamide inhibition.

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