Biochemical Pharmacology, Vol. 29, pp. 2755–2758.

0006-2952/80/1015-2755 $02.00/0

SECRETAGOGUE-INDUCED TRANSPORT OF H^+ AND K^+ BY *IN VITRO* AMPHIBIAN GASTRIC MUCOSA*

TUSHAR K. RAY† and LARRY L. TAGUE

Department of Surgery, SUNY, Upstate Medical Center, Syracuse, NY 13210, and University of Texas Medical School at Houston, Houston, TX, U.S.A.

(*Received* 17 *December* 1979; *accepted* 11 *April* 1980)

Abstract—The effects of histamine, pentagastrin (PG), urecholine (UC) amd dibutyryl cyclic AMP (cAMP) on the chambered fundic mucosa from *Rana catesbeiana* were studied to determine the H^+ and K^+ transport characteristics of the *in vitro* preparation. The three secretagogues (histamine, PG and UC) elicited very similar responses—a sustained steady-state secretion of H^+ within 15–30 min and a transitory efflux of K^+ that returned to control level within 60 min. Inhibition of H^+ transport of the secretagogue-stimulated mucosa by 2.5×10^{-4} M *p*-chloromercuribenzene sulfonic acid (pCMBS) caused an increased efflux of K^+. Replacement of pCMBS by β-mercaptoethanol (2×10^{-3} M) rapidly restored the H^+ transport, with a concomitant decrease of K^+ efflux, to the pre-stimulated control level. The effects of cAMP were qualitatively similar to those of other secretagogues except that there was a delayed peak response for both H^+ and K^+. Our data are compatible with a K^+ conductive pathway and a membrane-recycyling mechanism for K^+ in exchange for H^+ at the secretory membrane of the gastric cells. Thiocyanate inhibited H^+ transport, and the effect appeared to be due to inhibition of the transport and coupling of K^+ to the K^+/H^+ exchange mechanism.

Potassium ions are known to play a prime role in gastric hydrochloric acid secretion. The absolute dependence of H^+ transport on K^+ has been demonstrated recently [1–3] in isolated bullfrog gastric mucosa, where elimination of K^+ from the nutrient solution reversibly abolished the H^+ transport. K^+ is a normal component of gastric juice, and the concentration of K^+ varies with the pH and the volume of gastric juice [4, 5]. Experiments with histamine-stimulated denervated canine fundic pouch have shown that a good correlation exists between H^+ and K^+ output, suggesting that gastric H^+ and K^+ secretion are coupled [6]; but the precise relationship existing between H^+ and K^+ transport in gastric mucosa is not known.

We recently reported [7] that resting bullfrog gastric mucosa secretes K^+ at a steady rate; histamine causes sustained increase in H^+ transport and a transient increase in K^+ efflux, that promptly returns to the resting level within 60 min. Our data led us to suggest [7] the presence of a mechanism for recycling of luminal K^+ back into the cell, possibly mediated by the K^+–ATPase system at the apical membrane of the parietal cells.

The purpose of the present investigation has been to study the relation between the H^+ and the K^+ transport characteristics of bullfrog gastric mucosa in response to a variety of secretagogues, exploring further the K^+-recycling mechanism proposed previously [7]. We report that the well-known gastric secretagogues histamine, pentagastrin, and urecholine elicit strikingly similar patterns of H^+ and K^+ transport by bullfrog gastric mucosa. The effects of dibutyryl cAMP, which are somewhat similar to those of other secretagogues on the transport parameters of H^+ and K^+ are discussed. The modes of action of *p*-chloromercuribenzene sulfonic acid (pCMBS) and thiocyanate on gastric H^+ and K^+ transport are discussed in terms of the mechanism of gastric acid secretion.

MATERIALS AND METHODS

Experiments were done with *in vitro* preparations of gastric mucosa of *Rana catesbeiana*, isolated as described previously [7]. The fundic mucosa was carefully separated from the submucosa and mounted over one end of a plastic tube (13 × 100 mm) with the mucosal surface facing out. The area of each mounted mucosa was 1.5 cm^2. The mounted tissue was placed vertically inside a 25-ml container. The compositions of the bathing solutions [2] were as follows. The nutrient solution contained (mM): Na^+, 102; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 82.6; HCO_3^-, 25; PO_4^{2-}, 1; and glucose, 11; the luminal side was bathed in an unbuffered 104 mM NaCl solution. The volumes of nutrient and mucosal solutions were 2.5 and 12.5 ml, respectively. Both solutions were bubbled with 95% O_2–5% CO_2. The mucosal solution was stirred continuously with a magnetic stirrer. Secretagogues were added to the nutrient solutions so the final concentrations were 1×10^{-4} M histamine, 1×10^{-6} M pentagastrin, and 1×10^{-4} M urecholine; dibutyryl cyclic AMP was added to a final concentration of 1×10^{-4} M. For the dibutyryl cAMP experiment the mucosa was incubated overnight according to the method of Kasbekar [8] to bring it to resting (zero H^+ secretion) level. The mucosal solution was collected at 15-min intervals and placed in thoroughly washed plastic vials. The K^+ content of the secretory medium was determined with either a flame photometer (Brinkman) or an atomic absorption spectrophotometer

* Part of the work was presented at the AGA, 1978.

† Author to whom all correspondence should be addressed.

(360 Perkin Elmer). The H^+ secretion was quantified by titration with 1 mM NaOH to pH 6.5 while gassing with 100% N_2 using an automatic buret and pH stat (Radiometer).

Student's unpaired *t*-test was used to compare secretory data with initial steady-state values, and the differences were regarded as statistically significant when $P < 0.05$. The variability of the samples is expressed as the mean ± S.E.

RESULTS AND DISCUSSION

We previously reported [7] that in bullfrog gastric mucosa, using histamine as a secretagogue, there is a pathway for the transport of K^+ from an intracellular compartment into the lumen and a mechanism for recycling of K^+ from the luminal environment back into the cell in exchange for H^+. The present data with three secretagogues (histamine, pentagastrin and urecholine) confirm and extend our previous observations [7], demonstrating the generality of the phenomenon. Gastric K^+-stimulated ATPase, located at the apical and tubulovesicular membranes, is widely believed to be the vehicle for transport of H^+ by the parietal cells [9] and has been implicated in the recycling of K^+ in intact mucosa [7] as well as in isolated gastric microsomal vesicles [10, 11].

The data in Figs. 1–3 showing the patterns of H^+ and K^+ transport in response to the three secretagogues under various conditions are strikingly similar. The secretagogue-induced stimulation of K^+ transport appears much earlier than at 15 min, the earliest time reported in this paper (Figs. 1–3). For example, histamine enhanced K^+ tranport as early

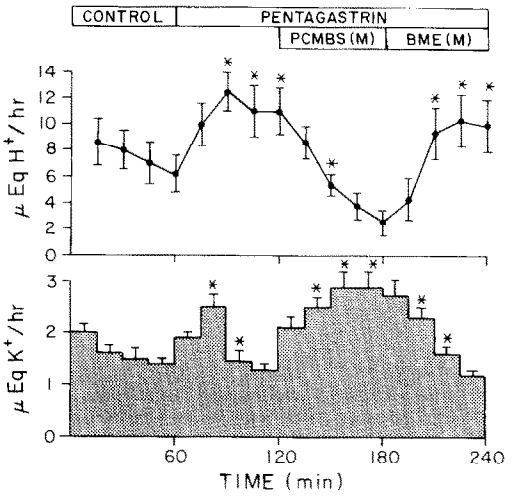

Fig. 2. Effects of pentagastrin (10^{-6} M) on the transport of H^+ and K^+ by the spontaneously secreting bullfrog gastric mucosa *in vitro*. Experimental conditions were identical to those given in the legend of Fig. 1. Note that the reduced transport of H^+ and the enhanced transport of K^+ caused by 2.5×10^{-4} M pCMBS were completely reversed by the substitution of pCMBS with 2×10^{-3} M BME in the mucosal solution. N = 8. An asterisk (*) indicates a statistically significant difference from the prior steady-state value ($P < 0.05$).

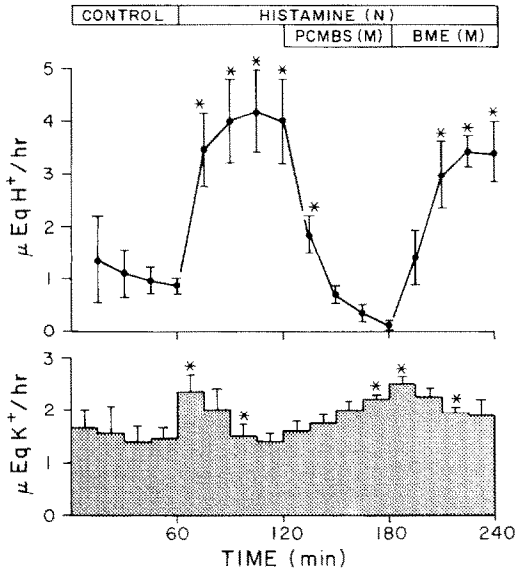

Fig. 1. Effects of histamine (10^{-4} M) on the transport of H^+ and K^+ by bullfrog gastric mucosa *in vitro*. The spontaneously secreting mucosa was washed thoroughly with several changes of regular frog Ringer solution and mounted in a chamber. The mucosal solution was collected every 15 min and assayed for H^+ and K^+. The standard errors for K^+ are shown in the middle of each of the 15-min periods, whereas those for H^+ are shown at the end of each 15-min period. The concentrations of pCMBS and BME were 2.5×10^{-4} and 2×10^{-3} M, respectively. Details are given in Materials and Methods. N = 6. An asterisk (*) indicates a statistically significant difference from the prior steady-state value ($P < 0.05$).

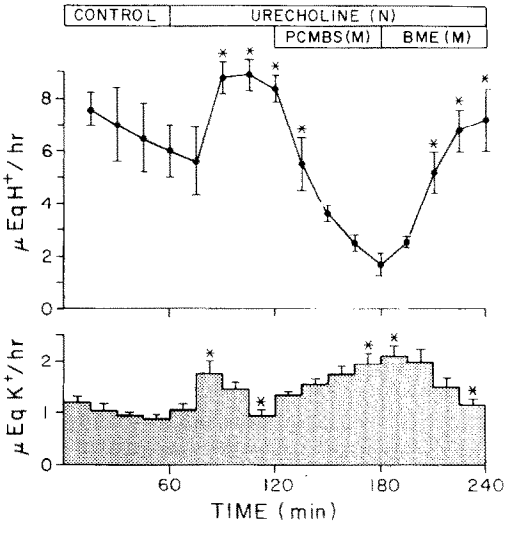

Fig. 3. Effects of urecholine (10^{-4} M) on the transport of H^+ and K^+ by the spontaneously secreting bullfrog gastric mucosa *in vitro*. The conditions of the experiments were the same as described in the legends of Figs. 1 and 2. N = 5. An asterisk (*) indicates a statistically significant difference from the prior steady state value ($P < 0.05$).

as 2 min, at which time H^+ secretion was still at the basal level (unpublished data). The rapidity of the effects of pCMBS on H^+ and K^+ transport and its ready reversibility by β-mercaptoethanol (BME) suggest that this sulfhydryl agent acts at or near the membrance surface [12]. Since pCMBS has been shown [7] not to affect K^+ transport in the resting (zero acid secretion) mucosa, the effects of pCMBS appear to be specifically on the H^+ transport mechanism at the apical membranes of the oxyntic cells. The decrease in K^+ efflux following the transient increase after stimulation by the secretagogues (Figs. 1–3) and the similar decrease after replacement of pCMBS by BME (Figs. 1–3) are very likely due to recycling of the luminal K^+ back into the cells. The increase in the transport of K^+ after inhibition of H^+ secretion by pCMBS is consistent with such a recycling mechanism (Figs. 1–3). Two alternative explanations for the K^+ transients—they are a simple washout phenomenon, or they result from a secretagogue-induced transient change in the mucosal permeability to K^+—were previously excluded by appropriate control experiments [7].

Our data demonstrate that secretagogues with widely differing physicochemical natures elicit similar H^+ and K^+ transport responses, suggesting a common intracellular mediator for these two separate transport events. Cyclic AMP has been strongly suggested to be the intracellular mediatior for H^+ transport by the bullfrog gastric mucosa [13, 14]. In the present study, dibutyryl cyclic AMP (Fig. 4) exhibited a response, with respect to H^+ and K^+ transport, qualitatively similar to that of other secretagogues (Figs. 1–3). There was a delayed peak response for both H^+ and K^+ however, and the effect of dibutyryl cAMP on K^+ transport occurred much earlier than its effect on H^+. The delayed response to H^+ was not due to overnight preparation of the mucosae since such tissues showed peak response to H^+ transport within 30 min after histamine stimulation (unpublished data), as demonstrated by Kasbekar [8]. The effect of cAMP on K^+ transport was observed within 15 min (Fig. 4), suggesting that permeability to dibutyryl cAMP was not the limiting factor in the delayed response of H^+. A lag in H^+ is to be expected if the rate of intracellular conversion of dibutyryl cAMP into physiologically active forms, such as monobutyryl and unsubstituted cAMP, is very slow. The stimulation of K^+ transport by cAMP prior to the onset of H^+ (Fig. 4) is in accord with a K^+-recycling mechanism. According to such a scheme, the transport of H^+ by gastric mucosa would be dependent on the availability of K^+ at the K^+-recycling site for exchange with H^+.

Resting mucosa, maintained in a K^+-free medium, can be made to secrete acid by introducing K^+ into the secretory solution (Fig. 5). This observation is consistent with other reports [1–3] and suggests that, even though the permeability of the mucosal membrane to K^+ is considerably lower than that of the nutrient membrane [2], the amount of K^+, at or near the apical membrane that is necessary for H^+ transport can be procured solely from the mucosal side. While maintaining mucosal K^+ (10 mM), addition of SCN^- to the same medium inhibited the H^+ transport which was completely reversed by increasing

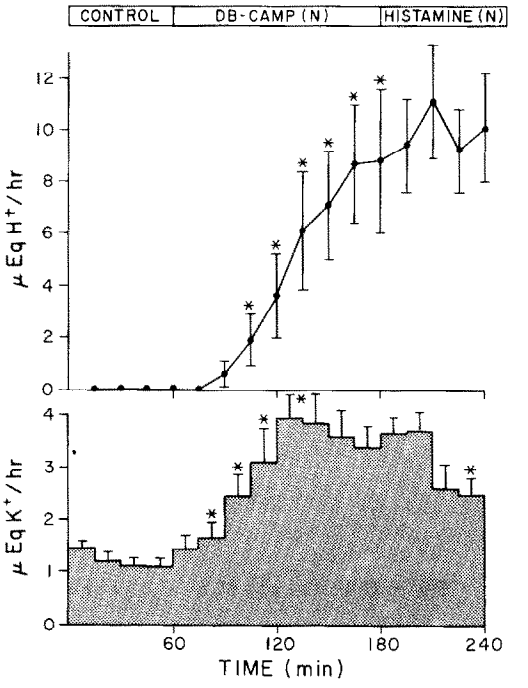

Fig. 4. Effects of dibutyryl cyclic AMP (10^{-4} M) on the transport of H^+ and K^+ by resting bullfrog gastric mucosa *in vitro*. The mucosae were brought to the zero acid secreting state by overnight incubation following the method of Kasbekar [8]. No change in H^+ transport was observed by stimulation of the mucosa with 10^{-4} M histamine following DB-cAMP stimulation. N = 6. An asterisk (*) indicates a statistically significant difference from the prior steady-state value ($P < 0.05$).

the mucosal K^+ (Fig. 5). Thus, the data in Fig. 5 demonstrate a competitive antagonism between K^+ and SCN^- in the reaction sequence leading to H^+ transport. The data suggest some kind of coupling of K^+ to the H^+ transport mechanism at the apical membrane [9] and SCN^- appears to interfere with that control mechanism. Evidently detailed study is needed to understand the precise mechanisms by which SCN^- exerts its inhibitory effect on the K^+/H^+ exchange system.

The proposed K^+/H^+ exchange mechanism is consistent with the recent findings of Kivilaakso *et al.* [15] who demonstrated, using chambered bullfrog gastric mucosa, that the inhibition of H^+ transport induced by an opposing electric current can be completely reversed by high concentrations of K^+ in the secretory solution of the gastric mucosa. The present data, however, do not provide us with any information regarding the stoichiometry of the K^+/H^+ exchange system and the electrical characteristics [16] of the secretory membrane, known to be associated with gastric ion transport. It should be pointed out that, in a recent report, Sachs *et al.* [17] demonstrated a neutral exchange of H^+ and K^+ mediated by the gastric K^+-stimulated ATPase in the isolated gastric microsomal vesicles.

It is evident that the details on the K^+/H^+ mechanism in intact gastric mucosa need to be worked

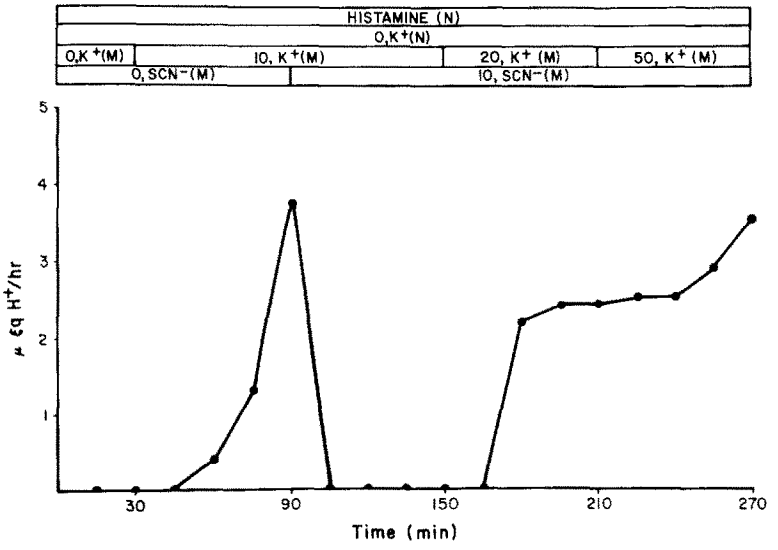

Fig. 5. Effects of 10 mM SCN^- and various concentrations of K^+ in the mucosal solution (M) on H^+ transport by the chambered bullfrog gastric mucosa bathed in a K^+-free (O, K^+) nutrient solution (N). The O,K^+ nutrient medium was regular frog Ringer without any K^+. Details are given in Materials and Methods. Data are from a representative experiment from three separate studies.

out. Future exploration of the precise mechanisms involved in the coupling of K^+ to the K^+/H^+ exchange system and its relation to the Cl^- transport mechanism may eventually enable us to understand the basic mechanisms underlying the electrongenicity of the apical membrane.

Acknowledgements—We gratefully acknowledge the help of Dr Parimal C. Sen in the thiocyanate experiment and Miss Susan Golden for typing the manuscript. This work was supported by a grant from U.S. Public Health Service (AM 25544). T. K. Ray is the recipient of Research Career Development Award AM 00623.

REFERENCES

1. J. B. Harris, F. Heddy and I. S. Edelman, *Am. J. Physiol.* **195**, 499 (1958).
2. S. S. Sanders, D. H. Noyer, S. G. Spangler and W. S. Rehm, *Am. J. Physiol.* **224**, 1254 (1973).
3. T. Noriake, I. Horikoshi and M. Hattari, *Am. J. Physiol.* **232**, E294 (1977).
4. S. Linde and K. J. Obrink, *Acta physiol. scand.* **21**, 54 (1950).
5. F. Hollander, *Gastroenterology* **40**, 477 (1961).
6. J. M. Berkowitz, R. P. Warner, H. D. Janowitz and I. F. Miller, *Proc. Soc. exp. Biol. Med.* **135**, 804 (1970).
7. T. K. Ray and L. L. Tague, *Acta physiol. scand.* (Special Suppl.) 283 (1978).
8. D. K. Kasbekar, *Proc. Soc. exp. Biol. Med.* **125**, 267 (1967).
9. G. Sachs, J. G. Spenney and W. S. Rehm, in *International Review of Physiology* (Ed. R. K. Crane), University Park Press, Baltimore (1977) Vol. 12, p. 127.
10. J. G. Forte and H. C. Lee, *Gastroenterology* **73**, 921 (1977).
11. G. Sachs, H. Chang, E. Rabon, R. Shackman, H. M. Sarau and G. Saccomani **73**, 931 (1977).
12. L. A. Solberg and J. G. Forte, *Am. J. Physiol.* **220**, 1404 (1971).
13. D. K. Kasbekar, in *Gastric H⁺ Transport* (Eds. G. Sachs, E. Heinz and K. I. Ulrich), pp. 203–224. Academic Press, New York (1976).
14. T. K. Ray and J. G. Forte, in *Hormonal Receptors in Digestive Tract Physiology* (Ed. S. Bonfils), p. 335. Elsvier/North Holland (1976).
15. E. Kivilaakso, R. Crass, J. G. Allison, S. Ito, D. Fromm and W. Silen, *Gastroenterology*, **76**, 795 (1979).
16. J. G. Forte, in *Membrane and Ion Transport* (Ed. E. Bittar) Vol. 3. pp. 111–165. John Wiley, London (1971).
17. G. Sachs, H. H. Chang, E. Rabon, R. Schackmann, M. Lewin and G. Saccomani, *J. biol. Chem.* **251**, 7690 (1976).