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Effect of Electrical Current on the Secretory Status and Tissue Potassium of Amphibian Fundic Mucosa (40542)[1]

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Previous studies (1, 2) have shown that secretion of H^+ by the gastric fundic mucosa could be inhibited by electric current (EC) passed across the mucosa from secretory (S) to nutrient (N) surfaces. This effect was attributed to altered electrical properties (circuit parameters) of the mucosa brought about by increased transport of H^+ into the surface cells (SC) and acceleration of the Cl^- pump (3). More recent studies on the amphibian fundic mucosa have shown that the effect of EC is more likely a true inhibition of the H^+-secretory pump than an accelerated electrochemical back-diffusion of H^+ (4, 5). Electron microscopy has confirmed that, when EC is passed from S → N, the oxyntic cell (OC) returns to its resting state, with involution of the secretory membrane and reconstitution of the intracellular tubulovesicular system (5).

It has been shown that washing out K^+ from S and N solutions (6–9) inhibits H^+ secretion, and although Davis *et al.* (8) were unable to show any changes in tissue K^+ concentration ($[K^+]_t$) as a result of this maneuver, others have been able to do so (6, 9). Furthermore, Sedar and Wiebelhaus (7) have demonstrated that the inhibition of secretion resulting from the replacement of the bathing media with K^+-free solutions is accompanied by conversion of the electron microscopic appearance of the OC to the resting state.

Raising the K^+ concentration of the S solution *ab initio* prevents the inhibition of H^+ secretion by EC and the OC retains its secretory configuration by electron microscopy (5). If K^+ is added to the S solution after secretion has been inhibited by EC the secretory rate is restored to within the normal range (5). A forced exchange of K^+ for H^+ at the secretory membrane is unlikely (10). A more tenable explanation would appear to be replenishment of intracellular K^+ carried by the current across the secretory membrane.

This study was designed to investigate the effect of EC on the frog gastric fundic mucosa $[K^+]_t$ and the relationship of $[K^+]_t$ to H^+ secretion. As Ba^{2+} is known to compete for K^+ conductance sites at the nutrient membrane (11), the effects of Ba^{2+} on $[K^+]_t$ and H^+ secretion were also examined.

Materials and methods. Bullfrogs (*Rana catesbeiana*) which had been kept in 120 m*M* NaCl at 4° were used in all experiments. The frogs were pithed and the stomachs removed immediately. Using sharp and blunt dissection, the mucosa was stripped off the muscularis and the antral portion discarded. Paired isolated sheets of fundic mucosa from the same frog (surface area 1.96 cm^2) were mounted between two halves of lucite chambers and connected to Ussing chambers. A bicarbonate-buffered Ringers solution (containing in m*M*: Na^+ 105, K^+ 5, Mg^{2+} 2, Ca^{2+} 2, Cl^- 93, HCO_3^- 18, PO_4^{3-} 1, and glucose 10) at pH 7.2 bathed N of all tissues. Ba^{2+} 1 m*M* (as BaCl) was added to N during the course of some experiments. Either 120 m*M* NaCl or 100 m*M* KCl/20 m*M* NaCl bathed S in all the studies (Table I).

Both S and N solutions in all groups were maintained at room temperature and circulated with a gas lift of 95% O_2 and 5% CO_2, and S in all groups was fixed at pH 4.8 with a pH-stat (Radiometer, Copenhagen) using 50 m*M* NaOH as a titrant.

In groups 4 and 5 the electrical potential difference (PD) across the mucosa was measured between two salt–agar bridges connected to a high input impedance voltmeter (Keithley) through calomel reference electrodes. The resistance (R) of the tissue was calculated from the change in PD 0.5 sec after passing 100 μA direct current across the mucosa and correcting for the R of the bathing solutions. The EC was derived from two

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TABLE I. DETAILS OF THE SECRETORY SOLUTION, PASSAGE OF EC, AND PRESENCE OR ABSENCE OF Ba^{2+}.

Group 1 (n = 5):
(a) 120 m*M* NaCl on S, no EC
(b) 120 m*M* NaCl on S, plus EC
Group 2 (n = 5):
(a) 120 m*M* NaCl on S, plus EC
(b) 100 m*M* KCl/20 m*M* NaCl on S, plus EC
Group 3 (n = 3):
(a) 100 m*M* KCl/20 m*M* NaCl on S, no EC
(b) 100 m*M* KCl/20 m*M* NaCl on S, plus EC
Group 4 (n = 5):
(a) 120 m*M* NaCl on S, no Ba^{2+} on N, plus EC
(b) 120 m*M* NaCl on S, 1 m*M* Ba^{2+} on N, plus EC
Group 5 (n = 5):
(a) 100 m*M* KCl/20 m*M* NaCl on S, no Ba^{2+} on N, plus EC
(b) 100 m*M* KCl/20 m*M* NaCl on S, 1 m*M* Ba^{2+} on N, plus EC

45-V batteries connected in series, controlled with a microammeter, and passed across the tissue via two other salt–agar bridges in contact with Ag–AgCl electrodes. When continuous EC was applied to the mucosa to inhibit secretion it was always 500 $\mu A/cm^2$, passed from S → N.

Once spontaneous secretion had stabilized, the H^+ secretory rate was recorded at 15-min intervals throughout the remainder of the experiment. After 45 min, in groups 1, 2, and 3, EC was passed across the appropriate mucosa for 1 hr. In groups 4 and 5, in which the electrical parameters were also measured, Ba^{2+} was added to N after 45 min. After an additional 45 min EC was passed across the appropriate mucosa for 1 hr. Electrical parameters were not measured during this period.

At the completion of each experiment the disks of mucosa were rapidly removed from the chambers and approximately 2 mm of the circumference was trimmed away, to avoid including areas of edge damage. The tissue was gently blotted with damp filter paper to remove any mucus and extraneous fluid, and then dried in an oven for 48 hr. The desiccated tissue was weighed and then digested with concentrated sulfuric acid at 180°, which was subsequently discharged with hydrogen peroxide (6). The resultant solution was made up to a standard constant volume with deionized water and the K^+ concentration measured on an atomic absorption spectrophotometer. A minimum of three readings on separate days was made, and only the average of two or more readings within 10% of each other was accepted. The concentration of K^+ was expressed in terms of microequivalents of K^+ per gram of dry weight tissue—or $[K^+]_t$. The use of dry-weight values has been recommended by Davis *et al.* (10). In each experiment a sample of resting mucosa from the same stomach was treated in identical fashion to act as a control.

The $[K^+]_t$ was corrected to allow for K^+ contributed by residual S solution on the tissues which had been bathed by a high $[K^+]$ on S. The differences in percentage weight (water) loss, resulting from desiccation, between these tissues and their individual controls was determined (mean 6.9%, range 0–13%). This difference was regarded as the S solution fraction of the wet weight of the tissue. The volume and K^+ (in μEq/gram dry wt) contributed by the S solution was calculated for each tissue and the latter subtracted from the gross value to give a true $[K^+]_t$.

Statistical analysis was by Student's t test for paired results.

Results. 120 mM NaCl on S with or without passage of EC. The passage of EC across the mucosa significantly reduced the rate of acid secretion while the mucosa not exposed to EC continued to secrete at rates no different from the control period (Fig. 1). The passage

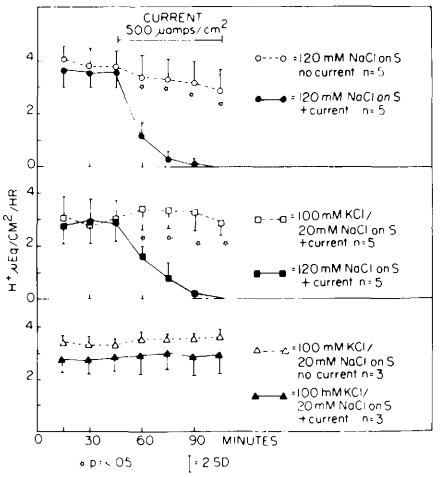

FIG. 1. H^+ secretory rates with or without passage of electrical current and with or without high K^+ (100 m*M*) in secretory solution.

of EC did not change the $[K^+]_t$ of the tissues (Table II).

K^+ free or high K^+ on S, both with passage of EC. EC inhibited acid secretion by the mucosa bathed with saline only but had no significant effect on those bathed with the K^+-containing solution (Fig. 1). The mean $[K^+]_t$ of the inhibited mucosae was no different from that of the control tissues, but the mean $[K^+]_t$ of the mucosae which continued to secrete was significantly increased above values for the controls and inhibited tissues (Table II).

High K^+ on S with or without passage of EC. The mean secretory rates of both sets of tissues bathed with 100 m*M* KCl/20 m*M* NaCl remained unchanged whether or not they were exposed to EC (Fig. 1). The mean $[K^+]_t$ of the tissues exposed to EC, however, was significantly greater than that of either control tissues or the tissues not exposed to EC. The latter showed no significant increase above values for controls (Table II).

K^+ free on S with or without Ba^{2+} on N and both with passage of EC. The addition of Ba^{2+} 1 m*M* to N of mucosae bathed with 120 m*M* NaCl on S did not affect the H^+ secretory rate (Fig. 2), but did result in a significant and progressive decrease in PD (21.9 ± 2.1 to 13.2 ± 2.6 mV/cm^2, $n = 5$, $P = < 0.05$) and rise in R (157.0 ± 47.1 to 264.2 ± 73.6 Ωcm^2, $n = 5$, $P = < 0.05$). Passage of EC produced the expected decrease to zero in H^+ secretion of those tissues not pretreated with Ba^{2+}, while those treated with Ba^{2+} continued to secrete at a low rate. There was no significant change in $[K^+]_t$ of either set of tissues, whether compared with each other or with controls (Table III).

High K^+ on S with or without Ba^{2+} on N and both with passage of EC. The addition of Ba^{2+} to N of mucosae bathed with 100 m*M* KCl/20 m*M* NaCl did not affect the H^+ secretory rate (Fig. 2), but did result in a significant and progressive decrease in PD (10.1 ± 2.7 to 6.0 ± 2.3 mV/cm^2, $n = 5$, $P = < 0.05$) and rise in R (90.0 ± 14.0 to 132.0 ± 12.0 Ωcm^2, $n = 5$, $P = < 0.05$). Passage of EC produced a small inhibition of H^+ secretion in both circumstances, but there was no significant difference between the two. The

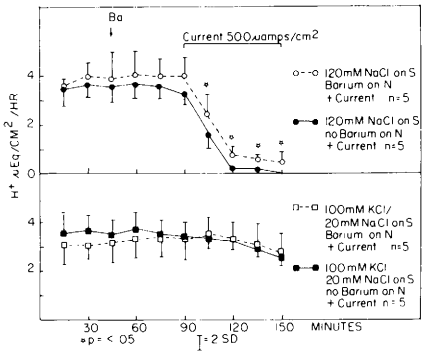

FIG. 2. H^+ secretory rates before and after passage of electrical current with or without high K^+ in secretory solution and with or without Ba^{2+} in nutrient solution.

TABLE II. TISSUE K^+ CONCENTRATIONS (μEq/g dry wt) OF FUNDIC MUCOSAE WITH OR WITHOUT PASSAGE OF EC AND WITH OR WITHOUT HIGH $[K^+]$ IN THE SECRETORY SOLUTION.

		Secretory solution			
		120 m*M* NaCl		100 m*M* KCl/20 m*M* NaCl	
n	Control (resting mucosa)	Without EC	With EC	Without EC	With EC
5	199.5 ±35.7[a]	189.9 ±20.1	189.3 ±29.4		
5	183.6 ±13.7		186.2 ±37.2		277.4* ±46.1
3	223.3 ±35.7			286.8 ±71.8	321.9* ±69.5
					**

[a] Mean ± SD.
* $P < 0.05$, compared with controls.
** $P < 0.005$, between groups with and without EC.

TABLE III. TISSUE K^+ CONCENTRATION (μEq/g dry wt) OF FUNDIC MUCOSAE SUBJECTED TO ELECTRIC CURRENT WITH OR WITHOUT HIGH $[K^+]$ IN THE SECRETORY SOLUTION AND WITH OR WITHOUT Ba^{2+} 1 m*M* IN THE NUTRIENT SOLUTION.

		Secretory solution and EC			
		120 m*M* NaCl + EC		100 m*M* KCl/ 20 m*M* NaCl + EC	
n	Control (resting mucosa)	No Ba on N	With Ba on N	No Ba on N	With Ba on N
5	137.9 ±33.6[a]	116.7 ±50.1	119.2 ±54.9		
5	173.2 ±33.5			210.5* ±10.9	186.55 ±43.7

[a] Mean ± SD.
* $P < 0.05$, compared with controls.

mean $[K^+]_t$ of tissues not treated with Ba^{2+} was significantly increased above that of controls, whereas tissues treated with Ba^{2+} showed no increase in $[K^+]_t$ (Table III).

Discussion. Although K^+ has been recognized as essential to H^+ secretion by the gastric mucosa (10, 12), its role has not been exactly defined, and it is likely that it has multiple functions. An important K^+-stimulated ATPase system has been identified (13) and some form of K^+–H^+ exchange at the secretory membrane has been shown by Sachs *et al.* (14). The latter investigator postulated that there is probably a K^+–H^+ exchange pump with a passive KCl limb, i.e., K^+ is needed on S for H^+ to be released from the secretory membrane. This principle would appear to be supported by the demonstration of a histamine-sensitive K^+ flux from N → S which accompanies histamine-stimulated H^+ secretion *in vitro* (15). The increased flux of K^+ from N → S in response to histamine suggests that this flux is not entirely passive and that movement of K^+ into the S solution may be a prerequisite to the subsequent release of H^+ from the S membrane.

In their studies on the distribution of K^+ within the fundic mucosa Davenport and Alzamora (9) concluded that, in terms of cations (Na^+ and K^+), there existed two distinct populations of cells. They suggested that the SC were predominantly K^+ containing, with little Na^+, and that the OC contain plentiful Na^+ and no K^+, although being readily permeable to K^+. It is difficult to conceive that any cell could be totally deficient in K^+ and yet still maintain essential metabolism. If their theory is, at least in part, correct and there is biased distribution of K^+ in the cells of the fundic mucosa, one could postulate that the SC (or perhaps a specialized and yet unidentified cell in the epithelium) provides the K^+ on S for the K^+–H^+ exchange at the S membrane of the OC, with little change in $[K^+]_t$. If EC is carried by K^+ (as the present series of experiments infers), K^+ would be driven back into the SC, thus limiting the K^+–H^+ exchange with consequent inhibition of H^+ secretion. Large quantities of exogenous K^+ in the S solution for the K^+–H^+ exchange would rescue H^+ secretion with resultant increase in $[K^+]_t$. There is no evidence to support this concept.

Inhibition of H^+ secretion by simple depletion of the K^+ from the OC by EC, as suggested by Forte (16), is not borne out by these studies on $[K^+]_t$. Whether the mucosa is in a basal secretory state or inhibited by EC, the $[K^+]_t$ remains unchanged. Although we measured "tissue K^+" which would include the extracellular fluid (ECF) and its K^+, K^+ is predominantly an intracellular cation and the ECF is presumed to be in equilibrium with the N solution (K^+ = 5 m*M*). For this reason it is unlikely that ECF-K^+ contributes much to $[K^+]_t$ in our measurements. K^+ driven from the cells to the ECF compartment would rapidly equilibrate in the relatively large volume (15 ml) of the N solution. The significant increment in $[K^+]_t$ when EC is passed through an S solution with a high K^+ concentration cannot be attributed to a gain in ECF-K^+.

Takeguchi *et al.* (6) defined two K^+ compartments ("regions") within the OC—a "bound K^+" compartment and a "free K^+" compartment. These investigators concluded that the latter was concerned with the secretory membrane and that, by reducing the K^+ concentration on N, K^+ was lost only from this compartment, resulting in decreased H^+ secretion. By constructing the model in Fig. 3, where the H^+ secretory compartment contains the "free K^+," we can explain inhibition of H^+ secretion by EC without alteration in $[K^+]_t$. The current drives K^+ from one compartment to the other, depriving the H^+-secretory mechanism of K^+ necessary for *de novo* proton formation. Inhibition of the K^+-dependent ATPase is the most plausible explanation. Replenishment of this compartment by providing K^+ on S to be carried into the cell by EC would rescue H^+ secretion and be accompanied by an overall gain in $[K^+]_t$.

Studies on the effect of Ba^{2+} on the nutrient membrane (11) and on the secretory mem-

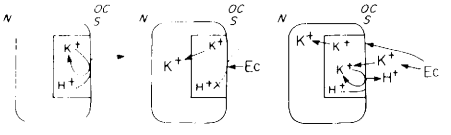

FIG. 3. Proposed model depicting a K^+–H^+ exchange at the S membrane (left), the effect of EC passed through a K^+-free S solution producing an intracellular K^+ shift and inhibition of secretion (center), and the rescue of secretion by replenishment of the secretory compartment with K^+ when EC is passed through a K^+-containing S solution (right).

brane (17) have revealed a Ba^{2+}:K^+ antagonism, with alterations in the conductive properties of these membranes. The present studies have shown that passage of K^+ across the nutrient membrane, and therefore, out of the OC, is not a factor in the inhibitory mechanism of EC. Consequently, the addition of Ba^{2+} to N would be expected to have little effect on the inhibition of secretion as the K^+ shift caused by EC is intracellular. With 120 m*M* NaCl on S and 1 m*M* Ba^{2+} added to N, however, passage of EC does not result in total inhibition of H^+ secretion, the implications being that sufficient K^+ remains within the secretory compartment to sustain secretion, albeit reduced, and $[K^+]_t$ remains constant. Adding Ba^{2+} to the N solution when there is a high K^+ concentration in the S solution does not significantly influence the response to EC in terms of secretory pattern but does prevent the expected increment in $[K^+]_t$. This strongly suggests that Ba^{2+} not only competes for conductance sites at the nutrient membrane but may alter intracellular conductance pathways governing movement of K^+ within the cell, as well as influencing conductance across the secretory membrane.

We conclude that the concept of two K^+ compartments in the OC, one of which is directly concerned with *de novo* proton formation and H^+ secretion, is probably valid. The evidence suggests that the inhibitory action of EC is effected by driving K^+ from this compartment to the other without altering the K^+ content of the cell, rather than depleting the cell as a whole of K^+. When EC is passed through an S solution with a high K^+ concentration, K^+ is driven into the cell, thus replenishing the H^+ secretory compartment and providing the K^+ necessary to sustain secretion. The movement of K^+ within the cells probably takes place along specific conductance pathways, the character of which appears to be influenced by the presence of Ba^{2+} on N.

Summary. The effect of direct electric current (EC) on the tissue K^+ concentration ($[K^+]_t$) of inhibited and secreting frog gastric mucosa was studied in order to investigate further our previous observation that a high K^+ concentration in the secretory solution prevents the inhibitory effect on H^+ secretion of EC passed from secretory (S) to nutrient (N) side. No differences in the $[K^+]_t$ of resting, secreting or inhibited mucosae bathed with K^+-free S solutions (120 m*M* NaCl) was observed. All N solutions contained 5 m*M* K^+. A significant increment in $[K^+]_t$ of secreting mucosae bathed on S with 100 m*M* KCl/20 m*M* NaCl was observed when EC was passed from S → N through this solution. Addition of Ba^{2+} (1 m*M*) to the N solution had no effect on $[K^+]_t$, but did prevent complete inhibition of H^+ secretion by EC passed through a K^+-free S solution. A mechanism whereby EC exerts its inhibitory action and the "rescue" effect of K^+ on S is postulated. Interference with intracellular conductance pathways by Ba^{2+} on N is suggested.

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