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Sodium Transport in *Rana pipiens* Gastric Mucosa (41839)

L. L. SHANBOUR, Y.-J. KUO, AND M. J. JACKSON

Department of Physiology and Cell Biology, University of Texas Medical School at Houston, Houston, Texas 77025, and Department of Physiology, George Washington University School of Medicine, Washington, D.C. 20037

Abstract. The transport properties of frog gastric mucosa *in vitro* have been reexamined in conditions analogous to those used in studies on mammalian systems in which net movements of sodium were observed. Net transport of sodium across frog gastric mucosa was not observed to occur when the mucosal surface was bathed with a well-buffered solution of near neutral pH, indicating that failure to demonstrate sodium transport across frog stomach in previous work could not be ascribed to the low pH value of the solution usually used as the mucosal fluid. Addition of 5×10^{-4} *M* amphotericin B to the mucosal solution elicited net transport of sodium and an increase in short-circuit current. These findings indicate that sodium transport may contribute to the electrolyte physiology of frog gastric mucosa in some experimental conditions, and may limit the utility of the three-variable model proposed by Hogben.

Studies on *in vitro* preparations of gastric mucosa from several mammalian species (1–5) have suggested that the mammalian stomach differs in an important respect from the extensively studied frog gastric mucosa (6–8). The mammalian tissues have generally been found to maintain net transport of sodium from the secretory, to nutrient surface in the absence of transmural gradients of electrochemical potentials, but sodium transport across frog gastric mucosa has previously been reported to occur only under hypoxic conditions (9, 10).

A survey of the literature suggested that this discrepancy may reflect a difference in the conditions used to study amphibian and mammalian tissues. In general, an unbuffered solution of pH less than 5 has been used as the mucosal medium in studies on frog stomach, but a well-buffered solution of pH greater than 7 has usually been employed in studies on mammalian systems. Because net sodium transport across rabbit gastric mucosa was inhibited when the pH of the secretory medium was decreased (11), and sodium transport was observed in a study on an amphibian species (*Necturus*) in which the pH of the secretory medium was maintained greater than 7 (12), the transport properties of the frog gastric mucosa were reexamined using conditions analogous to those employed in experiments on mammalian tissue.

Methods. The preparation of frog (*Rana pipiens*) gastric mucosa used in the present experiments was essentially similar to that described in several previous studies (7, 8, 13). The tissue was taken from the fundic region of the stomach, separated from the muscle coats by dissection, and mounted between lucite chambers with an exposed surface area of 1 cm^2.

For studies on the transport of sodium and chloride, both surfaces of the tissue were bathed by 10 ml of a well-buffered incubation solution with the following composition (m*M*): NaCl, 70; NaHepes, 20; $NaHCO_3$, 19; KCl, 3; KH_2PO_4, 2; $MgSO_4$, 1; glucose, 10. The solutions were equilibrated with 95% O_2/5% CO_2 before, and during the experiment, and maintained the pH value of both nutrient and secretory solutions at 7.2 throughout the study. The experiments were conducted at room temperature. Transmucosal potential difference (PD), resistance (*R*), and short-circuit current (I_{sc}) were evaluated using the automatic voltage clamp system described previously (14). For flux estimation, 3 μCi of ^{22}Na or ^{36}Cl were added to the solution on one side of the tissue, a 1-hr period allowed for equilibration, and the flux estimated from the appearance of tracer in the initially unlabeled solution during 20-min periods.

For studies on acid secretion, the solution bathing the mucosal surface of the tissue was replaced by an isoosmotic solution of NaCl. The pH of this solution was maintained con-

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stant by titration with 5 mN NaOH by means of a Radiometer pH stat.

Amphotericin B was obtained from the Sigma Chemical Company, and was applied as a concentrated stock solution in dimethylsulfoxide (DMSO). Results were expressed as means ± SM. Statistical significance was estimated by the t test for paired data, and the differences with a P value less than 0.05 were considered to be significant.

Results. Preliminary studies showed that, in control conditions, the *in vitro* preparation of frog gastric mucosa used in our experiments was stable for at least 3 hr of incubation. Figure 1 shows that addition of DMSO to the mucosal solution produced no change in the electrical variables (PD, R, I_{sc}), but addition of a DMSO solution of amphotericin B (to give a final concentration of 5×10^{-4} M in the bath) elicited a prompt increase in I_{sc} and a decrease in R. The changes in I_{sc} and R were of equivalent magnitudes with the result that PD did not change significantly.

Figure 2 shows that the opposed unidirectional fluxes of sodium did not differ significantly from each other in the control condition, indicating that a net movement of sodium did not occur in this situation. Addition of DMSO to the mucosal solution did not change the sodium fluxes, but exposure to 5×10^{-4} M amphotericin B increased both of the sodium fluxes. The increase in the mucosal-to-serosal flux was larger than that of the serosal-to-mucosal flux, indicating that a net movement of sodium in the mucosal-to-serosal direction was maintained in the presence of amphotericin B.

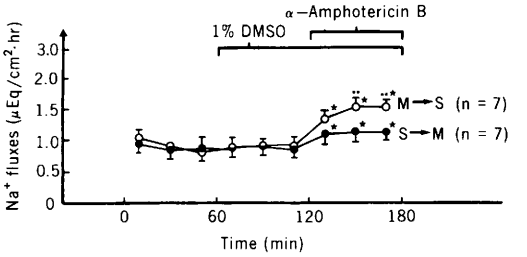

FIG. 2. Unidirectional fluxes of sodium during DMSO (1%) and α-amphotericin B conditions (5×10^{-4} M). N = 7. Single asterisk denotes significant difference at $P < 0.05$ from value at 120 min. Double asterisks denote significant difference at $P < 0.05$ between unidirectional fluxes.

The mean value of the net flux of sodium observed in the presence of amphotericin B was 0.35 ± 0.04 μeq cm^{-2} hr^{-1}, and the increase in I_{sc} associated with exposure to amphotericin B was 0.48 ± 0.04 μeq cm^{-2} hr^{-1}, suggesting that the transport of sodium was equivalent to more than 70% of the change in I_{sc}.

Figure 3 shows that the preparation maintained a net secretory movement of chloride

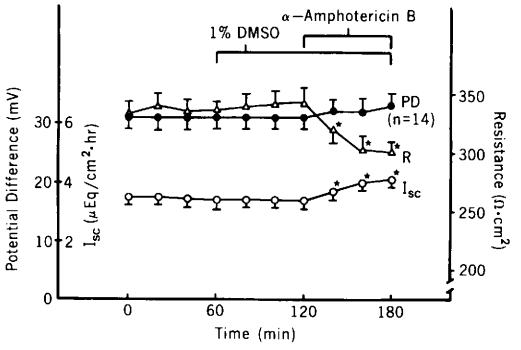

FIG. 1. Effects of DMSO (1%) and α-amphotericin B (5×10^{-4} M) on potential difference, short-circuit current and electrical resistance in isolated frog gastric mucosa. N = 14. Asterisk denotes significant difference at $P < 0.05$ from value at 120 min.

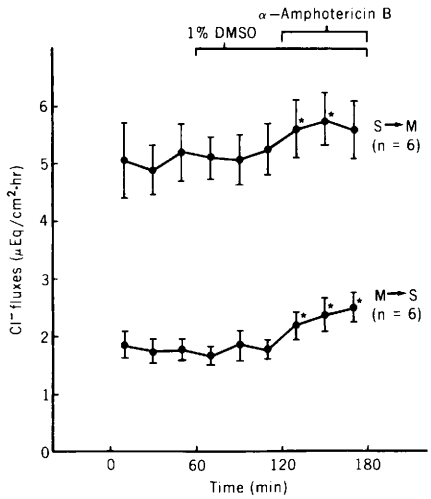

FIG. 3. Unidirectional chloride fluxes using DMSO (1%) and α-amphotericin B conditions (5×10^{-4} M). N = 6. Asterisk denotes significant difference at $P < 0.05$ from value at 120 min.

in the control condition, and after addition of DMSO to the mucosal fluid. Exposure to 5×10^{-4} *M* amphotericin B produced significant increases in both chloride fluxes, but the changes in the two fluxes were equivalent and the net movement of chloride did not change after addition of amphotericin B. Thus, in the control condition, the net movement of chloride was -3.3 ± 0.08 μeq cm^{-2} hr^{-1}, and after addition of amphotericin B this quantity was -3.2 ± 0.16 μeq cm^{-2} hr^{-1}.

Figure 4 shows that the basal secretion of acid remained constant during the control period of incubation, and after addition of DMSO to the mucosal fluid. Exposure to 5×10^{-4} *M* amphotericin B resulted in a prompt decrease in acid secretion, but the effect was transient and lasted for only one sampling period. Subsequently the basal secretion of acid returned to the level observed in the control period, and was maintained at this value during the remainder of the experiment.

Discussion. The primary objective of these experiments was to examine the transport of sodium across frog gastric mucosa *in vitro* when the mucosal surface of the tissue was bathed with a well-buffered solution of near neutral pH. The experiments showed that the tissue did not maintain a net movement of sodium in this condition. Several observations indicated that the failure to demonstrate net sodium transport in these studies could not be ascribed to a nonspecific depression of tissue function in the conditions used. Thus the rates of chloride transport and basal acid secretion, and the electrical variables were all of comparable magnitudes to values reported in previous studies. In addition, our experiments showed that net transport of sodium was maintained in tissue treated with amphotericin B, indicating that a sodium transport process was demonstrable when the properties of the tissue were modified. Accordingly, it was concluded that the frog gastric mucosa did not normally effect net transport of sodium when the mucosal surface was bathed with a well-buffered solution of pH greater than 7, and that the lack of sodium transport demonstrated in previous studies on the tissue could not be ascribed to the low pH of the solution used as the mucosal medium in most of the earlier work.

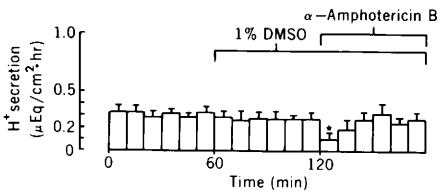

FIG. 4. Basal acid secretion during DMSO (1%) and α-amphotericin B conditions (5×10^{-4} *M*). $N = 6$. Asterisk denotes significant difference at $P < 0.05$ from value at 120 min.

The mechanism of action of amphotericin B in eliciting the sodium transport process in frog gastric mucosa *in vitro* was not investigated. In other systems it has been shown that the action of amphotericin B on sodium transport was associated with the introduction of cation selection channels into the apical membrane of epithelial cells (15, 16), and it may be that a similar mechanism contributed to the effects of amphotericin B observed in the present experiments. This suggestion carries the implication that the principal difference between the frog gastric mucosa and the mammalian systems, with respect to sodium transport, lies in the presence of sodium entry channels in the sodium transporting cells of mammalian stomach, and the absence of such channels in the frog tissue. However, the effects of amphotericin B on frog gastric mucosa were not restricted to sodium movements. The agent also increased permeability to chloride, and transiently modified the basal rate of acid secretion, and the possibility that the action of amphotericin B was associated with modification of cellular regulatory mechanisms of more general significance cannot be discounted at present. The latter proposal suggests that the frog gastric mucosa includes mechanisms controlling the sodium transport process that are not present, or depressed in the corresponding mammalian systems.

Previously, Flemström and Öbrink (9) and Flemström (10) have shown that net transport of sodium across frog gastric mucosa *in vitro* may be induced by decreasing the partial pressure of oxygen to which the tissue is exposed. In contrast to our findings, the hypoxia induced stimulation of sodium transport was not associated with a variation in I_{sc}, suggesting that the actions of amphotericin B and hypoxia may be mediated through the differ-

ent mechanisms. Thus the present studies, together with those of Flemström and Öbrink (9) and Flemström (10), raise a question concerning the general validity of the simple three-component model proposed by Hogben (6) as a description of the transport properties of frog gastric mucosa *in vitro*. This model is summarized by the expression:

$$I_{sc} = J^{Cl}_{net} - J^{H^+}$$

in which J^{Cl}_{net} represents the rate of chloride transport, and J^{H^+} represents the rate of acid secretion. The earlier studies (9, 10) and those described here demonstrate that the assumption that only chloride and acid require consideration as transported species is not generally valid, and that net movements of sodium may be stimulated in some experimental conditions. In addition, the present work has suggested that in at least some situations the sodium transport process may contribute to the electrical characteristics of the tissue.

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