Aldosterone Induction of Electrogenic Sodium Transport in the Apical Membrane Vesicles of Rat Distal Colon*

Vazhaikkurichi M. Rajendran, Michael Kashgarian, and Henry J. Binder

From the Departments of Internal Medicine and Pathology, Yale University, New Haven, Connecticut 06510

Na-H exchange is present in apical membrane vesicles (AMV) isolated from distal colon of normal rats. Because in intact tissue aldosterone both induces amiloride-sensitive sodium transport and inhibits electroneutral sodium absorption, these studies with AMV were designed to establish the effect of aldosterone on sodium transport. An outward-directed proton gradient stimulated $^{22}\text{Na}$ uptake in AMV isolated from distal colon of normal and dietary sodium depleted (with elevated aldosterone levels) experimental rats. Unlike normal AMV, proton gradient-dependent $^{22}\text{Na}$ uptake in experimental AMV was inhibited when uptake was measured under voltage-clamped conditions. 10 $\mu\text{M}$ amiloride inhibited the initial rate of proton gradient-dependent $^{22}\text{Na}$ uptake in AMV of normal and experimental rats by 30 and 75%, respectively. In contrast, 1 $\text{mM}$ amiloride produced comparable inhibition (90 and 80%) of $^{22}\text{Na}$ uptake in normal and experimental AMV. Intravesicular-negative potential stimulated $^{22}\text{Na}$ uptake in experimental but not in normal AMV. This increase was inhibited by 90% by 10 $\mu\text{M}$ amiloride. An analogue of amiloride, 5-(N-ethylisopropyl)amiloride (1 $\mu\text{M}$), a potent inhibitor of electroneutral Na-H exchange in AMV of normal rat distal colon, did not alter potassium diffusion potential-dependent $^{22}\text{Na}$ uptake. Increasing sodium concentration saturated proton gradient-dependent $^{22}\text{Na}$ uptake in normal AMV. However, in experimental AMV, $^{22}\text{Na}$ uptake stimulated by both proton gradient and potassium diffusion potential did not saturate as a function of increasing sodium concentration. We conclude from these results that an electrically sensitive conductive channel, not electroneutral Na-H exchange, mediates $^{22}\text{Na}$ uptake in AMV isolated from the distal colon of aldosterone rats.

Active sodium absorption is present in the distal colon of both rat and rabbit (1, 2). Studies in intact tissue reveal that there are both qualitative and quantitative differences in the characteristics of sodium transport in these two epithelia. In the rat colon, sodium absorption is predominantly electroneutral, chloride-dependent, and inhibited by cyclic AMP. It is likely that overall electroneutral NaCl absorption is the result of the coupling of Na-H and Cl-HCO$_3$ exchanges. In contrast, sodium absorption in the rabbit distal colon is electrogenic, chloride-independent, and not inhibited by cyclic nucleotides, and it is generally accepted that an amiloride-sensitive sodium conductive channel is present in apical membranes of the rabbit distal colon.

Both electroneutral and electrogenic sodium absorptive processes are regulated by aldosterone, a mineralocorticoid hormone (3). In vitro adduction of aldosterone enhanced the membrane conductance and existing electrogenic sodium absorption in rabbit distal colon (4). In contrast, in the rat distal colon, elevated aldosterone levels produced by either the continuous infusion of aldosterone or dietary sodium depletion converted chloride-dependent sodium absorption into chloride-independent, amiloride-sensitive electrogenic processes (5).

The mechanism by which amiloride regulates sodium transport is not known. To understand the regulatory mechanism(s), a study at the membrane level is necessary. Isolation of apical and basolateral membrane vesicles from epithelial cells have greatly expanded knowledge of the regulation of epithelial ion transport by permitting better identification of specific transport processes and the driving forces responsible for ion movement (6, 7). Due to lack of an enzyme marker, there have been relatively few studies (8-12) of ion transport with colonic apical membrane vesicles (AMV) compared to the numerous studies with vesicles prepared from small intestine and renal tubules. Brasitus and Keresztes (13) isolated AMV from rat colon utilizing cysteine-sensitive alkaline phosphatase as an enzyme marker, and with this preparation, Foster et al. (9), using acridine orange fluorescence, reported that an outward-directed sodium gradient induced intravesicular acidification. This marker has been questioned by Stieger et al. (14), who developed a novel procedure independent of enzyme markers, to isolate colonic apical membrane. Binder et al. (8) in studies with AMV, prepared from rat distal colon by the method of Stieger et al. (14), identified Na-H exchange in that an outward-directed proton gradient stimulated $^{22}\text{Na}$ uptake (8). However, their vesicles did not demonstrate transient accumulation (overshoot), and at equilibrium there was a significant difference in $^{22}\text{Na}$ uptake in presence and absence of proton gradient (8).

The aim of the present study was to establish and validate an AMV preparation from rat distal colon to study colonic sodium transport and to contrast aldosterone-induced alterations of sodium transport with that found in normal rat colon. In this present report, we have employed a monoclonal antibody, developed by Gorr et al. (15) directed against colonic apical membranes, to establish that these vesicles are derived...
Electrogenic Sodium Transport

from apical membranes; we have modified the uptake conditions so that the equilibrium values are similar in both the presence and absence of a proton gradient and that transient accumulation is observed in the presence of a proton gradient. This study demonstrates that aldosterone induces amiloride-sensitive potassium diffusion potential-dependent and proton gradient-linked sodium conductive channel in AMV of rat distal colon.

MATERIALS AND METHODS

Sprague-Dawley rats weighing 250–300 g were divided into normal and experimental groups. The normal group was fed a standard Purina rat chow containing 25 meq of sodium per 100 g of food. The experimental or chronic secondary hyperaldosterone group was fed a sodium-free diet, which was prepared in our laboratory, for 7–9 days, as described previously (16).

Although in this present study the effects of aldosterone were studied in sodium-depleted animals with secondary aldosteronism, previous studies in this laboratory have established that the changes in sodium transport induced by sodium depletion are identical to those observed in animals that have received continuous infusion of aldosterone for 7–10 days (5).

Preparation of Apical Membrane Vesicles—AMV of normal and experimental rat distal colon were isolated by the method of Stieger et al. (14), as described by Binder et al. (8), with additional modification in the final centrifugation. In this step the Polytron (Brinkmann Instruments) homogenate was centrifuged at 20,000 × g for 15 min; this modification of the original procedure, which represented a significant reduction of the centrifugal force and time compared to that originally described, was adapted because the pellets obtained at higher centrifugal force were found to be too hard to resuspend with a fine needle (26 G). By reducing the centrifugal force, only 6–8% of the total protein was lost.

The final pellet was resuspended in an appropriate media at a concentration of 5–6 mg of protein/ml and stored in aliquots at −70 °C. The frozen vesicles were thawed and diluted into an appropriate protein concentration with respective medium and resuspended with a fine needle. The vesicles were incubated for 90–60 min and kept at room temperature throughout the experimental period. The results of uptake determined with fresh and frozen vesicles were both qualitatively and quantitatively similar. The isolated membranes were assessed by both electron microscopic examination and immunofluorescent microscopy using a monoclonal antibody provided by Hans-Peter Hauri, Biozentrum, University of Basel, Basel, Switzerland (15).

Membrane Identification Studies—Immunofluorescence and immunoblotting studies were carried with monoclonal antibodies CP1/126 and CD1/62 directed against apical membranes of proximal and distal rat colon, respectively, as described by Gorr et al. (15).

Transport Studies—Transport of 22Na (Radiochemical Center, Amersham Corp.) into AMV was determined by rapid filtration techniques as reported previously (8). Protein was assayed by the method of Lowry et al. (17) using bovine serum albumin as standard.

Representative experiments that are illustrated are mean ± S. E. of triplicate assays. Standard errors of the mean less than 5% are not shown. Kinetic constants were calculated using Enzfitter program in an IBM P.C. All experiments were repeated at least three times with different membrane preparations.

RESULTS

Assessment of Membranes—Electron microscopic examination of the fraction isolated by Percoll gradient centrifugation revealed intact brush border caps with minimal attached lateral membranes (Fig. 1A). The final pellet obtained from the brush border caps contained microsomal vesicles of varying sizes (Fig. 1B). Tissue sections of distal colon demonstrated that the monocular antibodies CP1/126 and CD1/62 (not shown), directed against isolated apical membranes of rat proximal and distal colon, respectively, labeled only the apical membranes of epithelium (Fig. 2, A and B). Immunofluorescent examination of the AMV fraction demonstrated that nearly all of the microsomal fragments were positive for the apical membrane protein identified by these monoclonal antibodies (Fig. 2, C and D). Evidence of enrichment of the brush border-specific antigen identified by CD1/62 was also documented by immunoblotting in both the normal and experimental groups. Protein samples were subjected to electrophoresis on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting with CD1/62. Examination of these gels (Fig. 3) reveals that there was a substantially greater amount of antigen in AMV preparation (lanes 2 and 4) as compared to homogenate (lanes 1 and 3) in both normal and experimental groups and that enrichment was quantitatively greater in the normal than in the experimental AMV.

Uptake Studies—In our previous experiments, an outward-directed proton gradient stimulated 22Na uptake in AMV from normal rat distal colon (8). However, uptake at equilibrium was substantially greater in the proton gradient AMV than in the absence of proton gradient, and transient accumulation of 22Na over equilibrium was not present (8). Preliminary studies demonstrated that the presence of intravesicular cations (e.g. Mg2+, K+) resulted in equal uptake of 22Na at equilibrium in proton gradient and non-proton gradient conditions and transient accumulation of 22Na uptake in the presence of outward-directed proton gradient (18). Fig. 4 presents the effect of proton gradient on 22Na uptake in normal and experimental AMV in the presence of potassium. In these experiments, proton gradient stimulated 22Na uptake in both groups. In normal AMV, 22Na uptake was not altered.

Fig. 1. Electron micrograph of brush border cap (A) and apical membrane vesicles (B) isolated from normal rat distal colon. Thin sections of samples fixed in glutaraldehyde and post-fixed in osmium tetroxide were stained with uranyl acetate/lead citrate. Magnification: ×25,000.
by voltage clamping \( (K_i = K_o + \text{valinomycin}) \). However, proton gradient-dependent \(^{22}\text{Na}\) uptake in experimental AMV was significantly inhibited when uptake was measured under similar voltage-clamped conditions. These results suggest that proton gradient-dependent \(^{22}\text{Na}\) uptake in experimental AMV occurs via a different mechanism than in the normal group: an electrically sensitive coupled diffusion (i.e. electrodif\- fusional coupling) in the former but an electroneutral process in the latter.

To test whether proton gradient-dependent \(^{22}\text{Na}\) uptake is the result of electrodif\- fusional coupling or electroneutral Na-H exchange, the effect of \(10 \mu M\) and \(1 \text{mM}\) amiloride, concentrations shown to inhibit electrogenic and electroneutral sodium absorption, respectively, in other epithelia, was examined (19). As the results show in Fig. 5, \(10 \mu M\) amiloride inhibited proton gradient-dependent \(^{22}\text{Na}\) uptake by 75 and 29% in experimental and normal AMV, respectively. \(1 \text{mM}\) amiloride did not produce any further inhibition of proton gradient-dependent \(^{22}\text{Na}\) uptake in experimental AMV; however, in normal AMV, inhibition was increased to 90%.

Because these results suggest that proton gradient-dependent \(^{22}\text{Na}\) uptake in experimental AMV occurs primarily as a result of electrodif\- fusional coupling, the effect of intravesicular-negative membrane potential on \(^{22}\text{Na}\) uptake in AMV of experimental and normal was examined, and the results are shown in Fig. 6. Imposing an intravesicular-negative potential by valinomycin added to vesicles with an outward-directed potassium gradient significantly stimulated \(^{22}\text{Na}\) uptake, and transient accumulation over that seen at equilibrium (90 min) in experimental AMV was observed; in normal AMV \(^{22}\text{Na}\) uptake was not stimulated by a potassium diffusion potential. In both normal and experimental AMV an outward directed potassium gradient alone, in absence of valinomycin, did not stimulate \(^{22}\text{Na}\) uptake more than that seen in the absence of an outward potassium gradient.

A time course of the initial rate of \(^{22}\text{Na}\) uptake was determined in normal and experimental AMV. Fig. 7 demonstrates that the initial rate of proton gradient-stimulated \(^{22}\text{Na}\) uptake in AMV from normal animals and both proton gradient- and potassium diffusion potential-stimulated \(^{22}\text{Na}\) uptake in experimental AMV were linear up to 10 s. As a result, all further studies were performed with incubation period of 6 s.

The effect of low dose (10 \(\mu M\)) amiloride, a concentration that inhibits electrogenic sodium absorption (but not Na-H exchange) (19) and 5-(N-ethylisopropyl) amiloride (EIPA), an analogue of amiloride, a potent inhibitor of electroneutral Na-H exchange (20), was tested on potassium diffusion potential-dependent uptake of \(^{22}\text{Na}\). Fig. 8 shows that \(^{22}\text{Na}\)
Electrogenic Sodium Transport

FIG. 5. Effect of amiloride on initial rate of proton gradient-dependent \(^{22}\)Na uptake. Apical membrane vesicles isolated from normal (A) and experimental (B) rat distal colon were preloaded with 50 mM MES-Tris (pH 5.5), 300 mM mannitol, and 10 mM MgSO\(_4\). \(^{22}\)Na uptake was determined by incubating the vesicles for 6 s in medium that contained either 50 mM MES-Tris (pH 5.5) (pH, 5.5/pH, 5.5; ○) or 50 mM Hepes-Tris (pH 7.5) without amiloride (pH, 7.5/pH, 5.5; □) or 50 mM Hepes-Tris (pH 7.5) with 10 \(\mu\)M amiloride (□) or 50 mM Hepes-Tris with 1 mM amiloride (■). All medium also contained 300 mM mannitol, 10 mM MgSO\(_4\), and 0.1 mM \(^{22}\)Na. Uptake obtained with (pH, 7.5/pH, 5.5) was considered as 100%.

FIG. 4. Time course of proton gradient-dependent \(^{22}\)Na uptake. Apical membrane vesicles isolated from normal (A) and experimental (B) rat distal colon were preloaded with 50 mM MES-Tris (pH 5.5), 150 mM K-gluconate, and 10 mM MgSO\(_4\). \(^{22}\)Na uptake was determined by incubating the vesicles in medium that contained either 50 mM MES-Tris (pH 5.5) (open circles) or 50 mM Hepes-Tris (pH 7.5) with 25 \(\mu\)M valinomycin (filled squares), or 50 mM Hepes-Tris without valinomycin (filled circles). All medium also contained 150 mM K-glucanate, 10 mM MgSO\(_4\), 0.1 mM \(^{22}\)Na, and 0.8% ethanol. Valinomycin was dissolved in ethanol.

FIG. 3. Immunoblotting. Protein (50 \(\mu\)g) samples of homogenates (lanes 1 and 3) and isolated AMV (lanes 2 and 4) of normal (lanes 1 and 2) and experimental (lanes 3 and 4) rat distal colon were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5% acrylamide), electrophoretically transferred to nitrocellulose filters, and probed with CD1/62 antibody. Labeled bands were detected with an alkaline phosphatase-coupled secondary antibody. A broad band of approximately 116 kDa is detected in homogenate and is enriched in AMV. The molecular mass of this 116-kDa species is same as that identified by Gorr et al. (15) in rat colonocytes.

Uptake was stimulated significantly (163%) by intravesicular negative potential (induced by a potassium gradient and valinomycin) in experimental AMV, which was almost completely (90%) inhibited by 10 \(\mu\)M amiloride. 1 mM amiloride did not produce any further inhibition (results not shown), and 1 \(\mu\)M EIPA did not inhibit \(^{22}\)Na uptake stimulated by potassium diffusion potential. In contrast, \(^{22}\)Na uptake in the presence of a potassium diffusion potential in normal AMV was not altered by 10 \(\mu\)M amiloride.

The effect of EIPA on proton gradient-dependent \(^{22}\)Na uptake was examined in normal and experimental AMV. The results presented in Fig. 9 show that \(^{22}\)Na uptake stimulated by proton gradient was not significantly altered by 1 \(\mu\)M EIPA in experimental AMV. However, EIPA inhibited proton gradient-dependent \(^{22}\)Na uptake by 75% in normal AMV.

Because these results suggest that \(^{22}\)Na uptake in experimental AMV occurs via a conductive pathway and the satu-
Electrogenic Sodium Transport

FIG. 7. Initial time course of proton gradient and potassium diffusion potential-dependent 22Na uptake. Apical membrane vesicles isolated from normal (A) and experimental (B) rat distal colon were preloaded with 50 mM MES-Tris (pH 5.5), 300 mM mannitol, 10 mM MgSO₄. 22Na uptake was considered as 100%.

FIG. 8. Effect of 10 μM amiloride and EIPA on potassium diffusion potential-dependent 22Na uptake. Apical membrane vesicles isolated from normal (A) and experimental (B) rat distal colon were preloaded with 50 mM MES-Tris (pH 5.5), 150 mM K-glucuronate, and 10 mM MgSO₄. 22Na uptake was determined by incubating the vesicles in medium that contained 50 mM MES-Tris (pH 5.5), 150 mM TMA-glucuronate, 10 mM MgSO₄, 0.1 mM 22Na, and 25 μM valinomycin dissolved in ethanol (0.8%).

FIG. 9. Effect of EIPA on proton gradient-dependent 22Na uptake. Apical membrane vesicles isolated from normal (A) and experimental (B) rat distal colon were preloaded with 50 mM MES-Tris (pH 5.5), 300 mM mannitol, and 10 mM MgSO₄. 22Na uptake was determined by incubating the vesicles for 6 s in medium that contained 50 mM Hepes-Tris (pH 7.5), 300 mM mannitol, 10 mM MgSO₄, 0.1 mM 22Na, and either 0.5% dimethyl sulfoxide alone or 1 μM EIPA dissolved in dimethyl sulfoxide. Uptake obtained in the absence of EIPA was considered as 100%. □ pH 7.5/pH 5.5; ■ plus 1 μM EIPA.

Alcohol and EIPA on potassium diffusion potential-dependent 22Na uptake. Apical membrane vesicles isolated from normal (A) and experimental (B) rat distal colon were preloaded with 50 mM MES-Tris (pH 5.5), 300 mM mannitol, and 10 mM MgSO₄. 22Na uptake was determined by incubating the vesicles for 6 s in medium that contained 50 mM Hepes-Tris (pH 7.5), 300 mM mannitol, 10 mM MgSO₄, 0.1 mM 22Na, and either 0.5% dimethyl sulfoxide alone or 1 μM EIPA dissolved in dimethyl sulfoxide. Uptake obtained in the absence of EIPA was considered as 100%. □ pH 7.5/pH 5.5; ■ plus 1 μM EIPA.

Discussion

Aldosterone stimulates active sodium absorption in several epithelia including the mammalian colon (3, 24). Studies over the past several years have established that the overall increase in sodium absorption is associated with an increase in basolateral Na,K-ATPase activity and the number of apical membrane amiloride-sensitive sodium channels. Controversy has existed whether the primary effect of aldosterone is at the apical membrane (and thus the increase on Na,K-ATPase activity is secondary to enhanced sodium entry into the epithelial cell) or whether aldosterone directly affects both apical and basolateral membranes (25).

Although the mammalian colon is a known aldosterone-responsive epithelia, the rat distal colon is a unique epithelium to study the effects of aldosterone action on sodium (and potassium) transport. Although most aldosterone-responsive epithelia have amiloride-sensitive sodium channels in the basal state, no evidence of amiloride sensitivity is present in the distal colon of normal rat (5). Aldosterone, therefore, induces rather than amplifies aldosterone-induced processes in this epithelium.

In aldosterone-treated rat distal colon, amiloride-sensitive sodium transport is the sole mechanism for sodium absorption when studied under voltage-clamped conditions across isolated mucosa (16). In contrast, electroneutral NaCl absorption (most likely the result of Na-H and Cl-HCO₃ exchanges) is the predominant transport process identified in normal rat...
 Electrogenic Sodium Transport

Fig. 10. Effect of sodium concentration on $^{22}\text{Na}$ uptake. Apical membrane vesicles isolated from normal (A) and experimental (B) rat distal colon were preloaded with 50 mM MES-Tris (pH 5.5), 300 mM mannitol, and 10 mM MgSO$_4$. $^{22}\text{Na}$ uptake was determined by incubating the vesicles for 6 s in medium that contained 50 mM Hepes-Tris (pH 7.5), 10 mM MgSO$_4$, and varying concentrations of Na-gluconate (0.1–50 mM) and mannitol to maintain an isosmotic medium. C, apical membrane vesicles isolated from experimental rat distal colon were preloaded with 50 mM MES-Tris (pH 5.5), 150 mM K-gluconate, and 10 mM MgSO$_4$. $^{22}\text{Na}$ uptake was determined by incubating the vesicles for 6 s in medium that contained 50 mM MES-Tris (pH 5.5), 10 mM MgSO$_4$, 25 $\mu$M valinomycin, and varying concentrations of Na-gluconate (0.1–50 mM) and TM$_2$-gluconate to maintain an isosmotic medium. A and B are proton gradient-dependent $^{22}\text{Na}$ uptake (uptake obtained in presence of proton gradient (pH$_7.5$/pH$_{5.5}$) minus uptake in absence of proton gradient (pH$_{5.5}$/pH$_{5.5}$)). Values shown in C are potassium diffusion potential-sensitive $^{22}\text{Na}$ uptake (uptake in presence of an outward-directed potassium gradient ($K_t > K_o$) with 25 $\mu$M valinomycin minus uptake in the absence of potassium gradient ($K_t = K_o$) plus valinomycin). The best fit curves were drawn using Michaelis-Menten equation.

distal colon (5). These present experiments confirm that $^{22}\text{Na}$ uptake in AMV isolated from normal rat distal colon is an electroneutral Na-H exchange (Fig. 4A). $^{22}\text{Na}$ uptake enhanced by an outward-directed proton gradient was not affected by voltage clamping but was inhibited by 1 mM amiloride and 1 $\mu$M EIPA. No evidence of a sodium conductive channel was found in these membrane vesicles in that proton gradient-dependent uptake was inhibited by only 30% by 10 $\mu$M amiloride and potassium diffusion potential did not enhance $^{22}\text{Na}$ uptake.

The present studies demonstrate amiloride-sensitive sodium uptake was enhanced by both a proton gradient and a potassium diffusion potential in experimental AMV (Figs. 5B and 8B). The characteristics of proton gradient-dependent $^{22}\text{Na}$ uptake, which was significantly inhibited by voltage clamping in experimental AMV, was qualitatively different from those of electroneutral $^{22}\text{Na}$ uptake stimulated by proton gradient in normal AMV. However, the characteristics of $^{22}\text{Na}$ uptake stimulated by both proton gradient and potassium diffusion potential in experimental AMV were qualitatively similar: both were inhibited by 10 $\mu$M amiloride, were insensitive to 1 $\mu$M EIPA, and did not manifest saturation kinetics up to 50 mM sodium. Thus, $^{22}\text{Na}$ uptake stimulated by either proton gradient or potassium diffusion potential probably represents uptake via the same pathway.

It appears that in experimental AMV an outward-directed proton gradient creates an intravesicular negative potential, and as a result, proton gradient-stimulated $^{22}\text{Na}$ uptake represents electrodiffusional coupling, not a tightly coupled electroneutral Na-H exchange. Such electrodiffusional coupling requires the presence of a H$^+$ conductance and a sodium conductive channel. However, not all epithelia with cortical-active sodium channels also have an H$^+$ conductance. Bridges et al. (10) in membranes isolated from dexamethasone-treated rats have identified an amiloride-sensitive sodium uptake that was stimulated by a potassium diffusion potential but not by a pH gradient. Although these investigators concluded that these membranes were of apical origin, they did not substantiate that premise with specific markers. Nonetheless, aldosterone appears to induce a channel(s) with affinity for both sodium and H$^+$, whereas dexamethasone may induce a channel with affinity for sodium but not H$^+$. Palmer (26) has identified a sodium channel in the toad bladder with an even higher affinity for H$^+$ than for sodium. To establish whether aldosterone-induced H$^+$ movement is via a specific H$^+$ conductive channel or via a sodium channel with high affinity for H$^+$, an extensive study with proton and potential-sensitive dyes will be necessary.

As noted above, stimulation of $^{22}\text{Na}$ uptake by potassium diffusion potential created in presence of its ionophore valinomycin is also a result of uptake via a sodium conductive channel. Aldosterone induction of active potassium secretion in the rat distal colon is associated with luminal membrane potassium channel (27). It is somewhat surprising that there was minimal enhancement of $^{22}\text{Na}$ uptake by an outward-directed potassium gradient in the absence of valinomycin, which suggests that these apical membranes are relatively impermeant to potassium ions. There are at least three potential explanations to account for the absence of potassium channels in the isolated AMV: 1) the potassium channel demonstrated in intact tissue may be present only in crypt cells but not in surface cells, as these AMV are primarily derived from the brush border caps isolated from the surface cells; 2) the existing potassium channel in AMV may not be in an active (open state) form that may require an energy source or second messenger for activation; and 3) the potassium channel is inactivated during the membrane preparation procedure.

In normal AMV, increasing the sodium concentration saturated proton gradient-dependent $^{22}\text{Na}$ uptake and resulted in an apparent affinity constant ($K_a$) of 10.6 mM. In contrast, in experimental AMV, increasing the sodium concentration did not saturate either proton gradient- or potassium diffusion potential-dependent $^{22}\text{Na}$ uptake (Fig. 10, B and C). These results suggest that $^{22}\text{Na}$ uptake stimulated by either proton gradient or potassium diffusion potential occurs via similar pathways in experimental AMV but differs from that of proton gradient-dependent $^{22}\text{Na}$ uptake of normal AMV. Our results with experimental AMV are comparable to observa-
tions of the sodium channel in frog skin with current fluctuation analysis reported by Van Driessche and Lindemann (21) who also did not find saturation up to 60 mM sodium. However, our observations contrast with recent studies with A6 cell and rat cortical tubules (22, 23). Olans et al. (22) in reconstituted amiloride-sensitive sodium channel isolated from A6 cell line demonstrated saturation with a half-maximal concentration ($K_a$) of 18 mM, and Palmer and Frindt (23) using patch clamp techniques have shown saturation of sodium channel in rat collecting tubules with a $K_a$ of 75 mM. The significant variation in apparent affinity constants may represent difference between amphibian and mammalian sodium channels.

We conclude from these results that in AMV of experimental rat distal colon proton gradient-dependent and potassium diffusion potential-dependent $^{22}$Na uptake occurs via an electrically silent process in AMV differ markedly from those in normal AMV in which a tightly coupled electroneutral Na-H exchange is present. We did not find any evidence of this electrically silent process in experimental AMV.

Acknowledgments—We are indebted to Dr. Hans-Peter Hauri of Biozentrum, University of Basel, who provided the monoclonal antibody used in these studies. We also acknowledge Professor Heini Murer for suggestions and thoughtful discussions, Andrea Mann for technical assistance, and Irene Pollard for secretarial assistance.

REFERENCES
1. Binder, H. J., and Sandle, G. I. (1987) in Physiology of the Gastrointestinal Tract (Johnson, L. R., ed) pp. 1389–1418, Raven Press, New York
2. Schultz, S. G. (1984) Annu. Rev. Physiol. 46, 435–451
3. Sandle, G. I., and Binder, H. J. (1987) Gastroenterology 93, 188–196
4. Frizzell, R. A., and Schultz, S. G. (1978) J. Membr. Biol. 39, 1–26
5. Haley, J. J., Budinger, M. E., Hayslett, J. P., and Binder, H. J. (1986) Gastroenterology 91, 1227–1233
6. Murer, H., and Kinne, R. (1980) J. Membr. Biol. 55, 81–95
7. Sachs, G., Jackson, R. J., and Rabon, E. C. (1980) Am. J. Physiol. 238, G151–G164
8. Binder, H. J., Stange, G., Murer H., Stieger, B., and Hauri, H. P. (1986) Am. J. Physiol. 251, G382–G390
9. Foster, E. S., Dudeja, P. K., and Brasitus, T. A. (1986) Am. J. Physiol. 250, G781–G787
10. Bridges, R. J., Garty, H., Benos, D. J., and Rummel, W. (1986) Am. J. Physiol. 254, C484–C490
11. Jackson, R. J., Stewart, H. B., and Sachs, G. (1977) Cancer (Phila.) 40, 2847–2896
12. Kaunitz, J. D., and Sachs, G. (1996) J. Biol. Chem. 261, 14005–14010
13. Brasitus, T. A., and Kereztes, R. S. (1984) Biochim. Biophys. Acta 773, 290–300
14. Stieger, B., Marzer, A., and Hauri, H. P. (1986) J. Membr. Biol. 91, 19–31
15. Gorr, S. U., Stieger, B., Fransen, J. A., Kedinger, M., Marzer, A., and Hauri, H. P. (1986) J. Cell Biol. 106, 1937–1946
16. Foster, E. S., Zimmerman, T. W., Hayslett, J. H., and Binder, H. J. (1986) Am. J. Physiol. 254, G668–G675
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
18. Rajendran, V. M., and Binder, H. J. (1988) Gastroenterology 94, A365
19. Benos, D. J. (1982) Am. J. Physiol. 242, C131–C145
20. Haggerty, J. G., Gregoe, E. J., Jr., Slayman, C. W., and Adelberg, E. A. (1985) Biochem. Biophys. Res. Commun. 127, 759–767
21. Van Driessche, W., and Lindemann, B. (1979) Nature 282, 519–520
22. Olans, L., Sariban-Sohraby, S., and Benos, D. J. (1984) Biophys. J. 46, 831–835
23. Palmer, L. G. (1984) Biophys. J. 46, 831–835
24. Garty, H. (1986) J. Membr. Biol. 90, 193–205
25. Edelman, I. S., and Fanestil, D. D. (1970) in Biochemical Action of Hormones (Litwack, G., ed) pp. 324–364, Academic Press, New York
26. Palmer, L. G. (1984) J. Membr. Biol. 80, 153–165
27. Sweiry, J. H., and Binder, H. J. (1989) J. Clin. Invest. 83, 844–851