Sodium-Lithium Exchange and Sodium-Proton Exchange Are Mediated by the Same Transport System in Sarcolemmal Vesicles From Bovine Superior Mesenteric Artery

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Several laboratories have reported that Na^+-Li^+ countertransport activities are increased in red blood cells from patients with essential hypertension. It has been proposed that the activity of this red blood cell transport system might reflect the activity of a similar system in vascular smooth muscle. We previously demonstrated Na^+-Li^+ exchange in sarcolemmal vesicles from canine artery and proposed that this transport function might be mediated by the Na^+-H^+ exchanger. In the present studies, however, we were unable to demonstrate Na^+-Li^+ countertransport in canine red blood cells. Since bovine red blood cells have a vigorous Na^+-Li^+ exchanger and we previously demonstrated Na^+-H^+ exchange in sarcolemmal vesicles from bovine artery, we wished to determine whether bovine sarcolemmal vesicles mediate Na^+-Li^+ exchange and whether this transport function is mediated via the Na^+-H^+ exchanger. We found that an outwardly directed proton or Li^+ gradient stimulated $^{22}Na^+$ uptake in sarcolemmal vesicles from bovine superior mesenteric artery. Li^+ gradient–stimulated Na^+ uptake was not due to electrical coupling between the two ions, was not affected by a change in membrane potential, and could not be explained by the parallel operation of Li^+-H^+ and Na^+-H^+ exchange. External Li^+ inhibited proton gradient–stimulated Na^+ uptake, and external protons inhibited Li^+ gradient–stimulated Na^+ uptake. Na^+ efflux from vesicles was stimulated by inwardly directed gradients for Li^+ or protons, and these effects were not additive. Proton efflux from vesicles was stimulated by inwardly directed gradients for Na^+ or Li^+, and these effects were not additive. Finally, Na^+-H^+ exchange and Na^+-Li^+ exchange in sarcolemmal vesicles were inhibited by 5-(*N*-ethyl-*N*-isopropyl)amiloride in an identical dose-dependent manner. In conclusion, Na^+-Li^+ countertransport could not be demonstrated in canine red blood cells, but as is the case with bovine red blood cells, sarcolemmal vesicles from bovine artery mediate Na^+-Li^+ countertransport. This transport function and sarcolemmal Na^+-H^+ exchange are mediated via a single 5-(*N*-ethyl-*N*-isopropyl)amiloride–sensitive cation exchanger with affinity for Na^+, Li^+, and protons. The cow, as opposed to the dog, may be a good animal model to test whether the activity of red blood cell Na^+-Li^+ countertransport is predictive of the activity of Na^+-Li^+ (and Na^+-H^+) exchange in vascular smooth muscle. (*Circulation Research* 1989;65:818–828)

Many laboratories have looked for Na^+ transport abnormalities in red blood cells from patients with essential hypertension in the hope of finding a defect that, if present in other tissues such as kidney or vascular smooth muscle, could account for increased blood pressure.[1-6] Of the several red blood cell Na^+ transport defects that have been reported in hypertensive patients, increased Na^+-Li^+ countertransport activity has been one of the most consistently reported observations.[2,5,6] This transport system, in the presence of the very low physiological concentrations of Li^+, would be expected to mediate Na^+-Na^+ exchange,[7] which would not affect net Na^+ transport in any

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tissue. Thus, the relation between red blood cell Na^+-Li^+ countertransport and the hypertensive process is obscure.

Several authors have suggested that red blood cell Na^+-Li^+ countertransport may be an operative mode of a Na^+-H^+ exchanger[8,9] and that increased activity of the former in patients with essential hypertension may be a marker for increased Na^+-H^+ exchange activity in the kidney or vascular smooth muscle.[9,10] This attractive hypothesis has gained support by data demonstrating reduced renal Li^+ clearance, which is an indication of increased proximal tubular Na^+-H^+ exchange activity, in a group of patients with essential hypertension.[11] Other studies have shown that Na^+-H^+ exchange activity in red blood cells from hypertensive patients[12,13] and lymphocytes[14] and neutrophils[15] from hypertensive rats is increased. Nevertheless, there are data that detract from this hypothesis. Whereas Na^+-Li^+ countertransport is easily demonstrable in human red blood cells under control conditions,[2,5-7] Na^+-H^+ exchange can only be elicited under special experimental conditions.[16,17] In addition, Na^+-Li^+ countertransport in human red blood cells is insensitive to amiloride,[18] whereas this drug readily inhibits Na^+-H^+ exchange in these cells.[17]

To assess whether red blood cell Na^+-Li^+ countertransport activity might be predictive of the activity of a similar transport system in vascular smooth muscle, we previously performed studies that demonstrated the presence of a Na^+-Li^+ exchange transport process in sarcolemmal vesicles from the superior mesenteric artery of the dog.[19] This exchanger shared several properties with the human red blood cell Na^+-Li^+ countertransport system, and the data suggested that canine sarcolemmal Na^+-Li^+ exchange may be mediated by the Na^+-H^+ exchanger in that tissue.[19] In the present study, we wished to see if the canine sarcolemmal Na^+-Li^+ exchanger was homologous with a putative Na^+-Li^+ countertransport system in the canine red blood cell. As opposed to red blood cells from humans or rabbits, however, we were not able to demonstrate the presence of Na^+-Li^+ countertransport in canine red blood cells.

If the activity of a particular transport system in the red blood cell is to be considered a marker for transport activity in vascular smooth muscle, it is obviously important that both tissues contain the same transport system in question. Unlike the canine red blood cell, the bovine red blood cell has a very vigorous Na^+-Li^+ countertransport system,[7,9,20,21] and we have previously demonstrated that bovine sarcolemmal vesicles from the superior mesenteric artery contain a Na^+-H^+ exchanger.[22] Therefore, we wished to document the presence of Na^+-Li^+ exchange in bovine sarcolemmal vesicles. Furthermore, due to the confusion over whether Na^+-Li^+ countertransport in any tissue is truly an operative mode of a Na^+-H^+ exchanger, we wished to determine whether Na^+-Li^+ exchange and Na^+-H^+ exchange in bovine sarcolemmal vesicles are mediated by the same transport system.

Materials and Methods

Red Blood Cell Na^+-Li^+ Countertransport

Fresh blood was drawn into a heparinized syringe from the antecubital vein of a human volunteer, from the central ear artery of an adult male New Zealand White rabbit, or from a foreleg vein of a male mongrel dog. The whole blood was centrifuged at 5,000*g* for 5 minutes at 4° C, and the plasma and buffy coat were removed. The efflux of Li^+ from red blood cells was measured by methods modified from Canessa et al[2] and are described as follows. One volume of packed red blood cells was incubated in a shaking water bath for 3 hours at 37° C with five volumes of (mM) LiCl 150, glucose 10, ouabain 0.1, and Tris–3-(*N*-morpholino)propanesulfonic acid (MOPS) 10, pH 7.4. The cells were centrifuged at 5,000*g* for 5 minutes at 4° C, and the packed cells were resuspended in ice-cold washing solution that contained (mM) $MgCl_2$ 75, sucrose 85, glucose 10, and Tris-MOPS 10, pH 7.4. This suspension was centrifuged at 5,000*g* for 5 minutes at 4° C, and five successive identical washing and centrifuging steps were performed to remove extracellular Li^+. The hematocrit of the washed suspension was measured. The efflux of Li^+ from the preloaded washed red blood cells was initiated by incubating cells at 37° C with different external media. At time zero and at specified intervals thereafter, three 1-ml aliquots from each suspension were pipetted into individual ice-cold plastic tubes and centrifuged at 5,000*g* for 3 minutes at 4° C, and the supernatants were removed and stored in plastic tubes. The Li^+ concentrations of the supernatants were measured with a flame photometer (model 450, Corning, Medfield, Massachusetts). The mean concentration of Li^+ in the initial supernatants was subtracted from the mean concentrations in the subsequent supernatants, and the efflux of Li^+ into the different external media was calculated and expressed as millimoles per liter of red blood cells. Each red blood cell transport experiment was performed on at least three separate occasions.

Membrane Vesicles

Fresh bovine superior mesenteric arteries were obtained from a local slaughterhouse. Adhering connective tissue, fat, veins, and nervous tissue were removed in the cold. A sarcolemmal-enriched vesicle preparation was obtained by previously described methods,[22] which are outlined as follows. The arteries were thoroughly minced with scissors, suspended in 10 ml/g wet wt in (mM) mannitol 200, Tris 10, and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) 16, pH 7.5 at 4° C, and homogenized with a Polytron homogenizer. Magnesium sulfate was added to the homogenate to a final concentration of 10 mM, and the homogenate

was incubated on ice for 30 minutes. The suspension was centrifuged at 1,035*g* for 4 minutes, and the resultant supernatant was filtered through four layers of gauze and centrifuged at 48,000*g* for 30 minutes. The resultant pellet was resuspended in homogenizing medium with 10 mM $MgSO_4$ and incubated on ice for an additional 30 minutes. This suspension was centrifuged at 1,035*g* for 4 minutes. The resultant supernatant was centrifuged at 48,000*g* for 30 minutes to yield the final pellet, which was resuspended and recentrifuged twice in the original homogenizing medium without $MgSO_4$ and resuspended to a protein concentration of about 10 mg/ml. Protein was measured by the method of Lowry using bovine serum albumen as a standard. These methods yield a membrane vesicle preparation that is enriched in the sarcolemmal marker, ouabain-sensitive K^+ phosphatase, 20-fold and fourfold relative to arterial homogenate and a microsomal fraction, respectively.[22] The final membranes were not enriched relative to the microsomal fraction in the sarcoplasmic reticular marker, NADPH cytochrome *c* reductase, or the mitochondrial membrane marker, cytochrome *c* oxidase.[22]

Brush border membrane vesicles were prepared from the renal cortexes of New Zealand White rabbits by a Mg^{2+} aggregation and differential centrifugation technique, as previously described.[23] These vesicles were enriched more than 10 times relative to the cortical homogenate in the brush border membrane enzyme, alkaline phosphatase, but not enriched in the basolateral membrane marker enzyme, Na^+,K^+-ATPase.

Sodium Transport

The transport of Na^+ by the vesicle preparation was studied using $^{22}Na^+$ and a rapid Millipore filtration technique (Millipore, Bedford, Massachusetts). In general, the transport of $^{22}Na^+$ was measured under conditions in which the external and intravesicular ion concentrations had been preset to certain values according to the goals of each particular experiment. Aliquots of membranes (about 100 μg protein) that had been preincubated in the desired solutions at 22° C for 90 minutes were incubated with 1 mM $^{22}Na^+$ at 22° C as described in the figure legends. Uptake was terminated at the desired time points by rapidly diluting the incubating membranes with 3.5 ml cold (0–4° C) "stop solution" that contained (mM) $MgSO_4$ 112, Tris 1, and HEPES 1.6, pH 7.5. The membranes were separated from external media by Millipore filtration (0.65 μm, DAWP filter) and washed three times with 3.5-ml aliquots of cold stop solution. The filters were immersed in scintillation cocktail and counted. Sodium uptake by the membranes was determined by subtracting a filter blank, which was obtained in the absence of membranes. Sodium efflux studies were performed by preincubating vesicles with 1 mM $^{22}Na^+$ for 90 minutes at 22° C and diluting them 50-fold in sodium-free media at 22° C. At 0- and 30-second time points, the sodium content of the vesicles was determined by the same cold stop, Millipore filtration, and washing technique just described. Sodium efflux was calculated as the difference in the sodium content between the 0- and 30-second time points. In all experiments, each determination was performed in triplicate.

Proton Transport

The transport of protons by vesicles was measured by monitoring the fluorescence quenching of acridine orange as previously described.[24] Acridine orange is a fluorescent weak base that rapidly enters the intravesicular space and is trapped in its protonated form if the intravesicular pH (pH_{in}) is lower than external pH (pH_{out}). This results in quenching of acridine orange fluorescence.

To see whether an outwardly directed Li^+ gradient resulted in acidification of sarcolemmal vesicles, 15 μl vesicle suspension (about 150 μg protein), which had been preincubated with buffer containing 25 mM LiCl or choline chloride, pH 7.5, was rapidly mixed with 1 ml external media containing 6 μM acridine orange plus 5 mM LiCl, pH 7.5. Fluorescence was recorded over time by activating at 493 nm and recording the emission at 530 nm with a fluorescent spectrophotometer (model 650-10S, Perkin-Elmer, Norwalk, Connecticut) attached to a chart recorder. The addition of the vesicles to the cuvette resulted in immediate quenching of fluorescence, even though no initial pH gradient was present across the membranes. This finding is presumably due to increased turbidity of the solution caused by the membranes per se. It was determined whether Li^+-preloaded vesicles, as compared with choline-preloaded vesicles, would result in further fluorescence quenching and whether the subsequent imposition of an inwardly directed Na^+ gradient would reverse the fluorescence quenching. Such a result would indicate that Li^+-H^+ exchange acidified the intravesicular space and that Na^+-H^+ exchange realkalinized it. A similar experiment was performed with rabbit renal brush border vesicles, which were preloaded with 100 mM sodium gluconate, pH 7.5, and mixed with external media containing 6 μM acridine orange plus 100 mM gluconate salt of *N*-methyl-D-glucamine (NMG^+, a control cation), pH 7.5.

To see the effects of inwardly directed Na^+ and/or Li^+ gradients on the dissipation of an outwardly directed proton gradient, 10 μl sarcolemmal vesicle suspension (about 100 μg protein), which had been preincubated with buffer at pH 5.0, was rapidly mixed with 1 ml external media containing 6 μM acridine orange plus the desired salts at pH 7.5 as previously described.[24] Fluorescence was measured over time as described above. The fluorescence signal was immediately quenched to low values due to the sequestration of acridine orange in the relatively acidic intravesicular space. As the pH gradient dissipated, acridine orange left the vesicles,

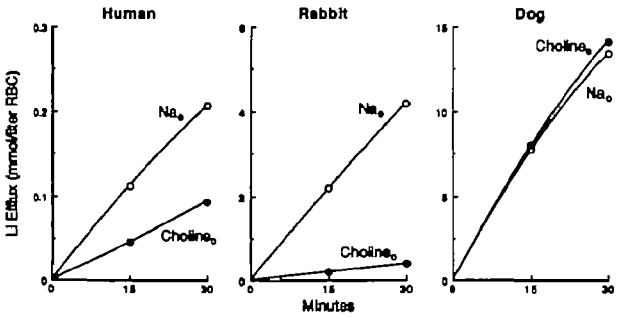

FIGURE 1. *Graphs showing effects of external Na^+ on Li^+ efflux from red blood cells (RBCs). Human, rabbit, or dog RBCs were preloaded with Li^+, and the efflux of Li^+ at 37° C was assayed by incubating the cells in media containing (mM) $MgCl_2$ 4, sucrose 4, glucose 10, ouabain 0.1, and Tris-MOPS 10, pH 7.4, plus NaCl (Na_o) or choline chloride ($Choline_o$) 150. Data presented are the results of representative experiments.*

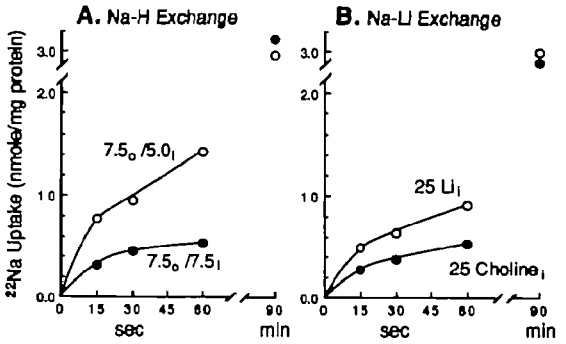

FIGURE 2. *Graphs showing effects of outwardly directed proton (panel A) or Li^+ (panel B) gradients on Na^+ uptake in bovine sarcolemmal vesicles.* Panel A: *Vesicles were preincubated with (mM) mannitol 181, Tris 17, HEPES 28, pH 7.5 (●; $7.5_o/7.5_i$, external and internal pH, respectively), or mannitol 181, MES 40, Tris 2, and HEPES 3.2, pH 5.0 (○; $7.5_o/5.0_i$ external and internal pH, respectively) for 90 minutes at 22° C, and the uptake of 1 mM $^{22}Na^+$ was assayed at 22° C in the presence of (mM) Cl^- 1; mannitol 104, MES 8, Tris 47, and HEPES 65, pH 7.5.* Panel B: *Vesicles were preincubated with (mM) mannitol 150, choline chloride (●; $choline_i$) or LiCl (○; Li) 25, plus Tris 10, and HEPES 16, pH 7.5, for 90 minutes at 22° C, and the uptake of 1 mM $^{22}Na^+$ was assayed at 22° C in the presence of (mM) $choline^+$ 5, Li^+ 5, Cl^- 11, mannitol 178, Tris 10, and HEPES 16, pH 7.5. Data presented are the results of representative experiments.*

thereby reducing fluorescence quenching. The fluorescence signal increased with time until a steady-state level was achieved, at which point protons were in electrochemical equilibrium across the membrane. The fluorescence data were fitted by computer analysis to the general equation: $F=c\times e^{-kt}$, where F is the difference between final fluorescence (at 20 minutes) and fluorescence at time, t, after mixing (3–20 seconds), and c and k are constants. In each experiment, the data fitted the above equation with a regression coefficient of 0.99 or greater. The value for k was taken to represent the first-order rate constant for proton gradient dissipation.

$^{22}Na^+$ (200 Ci/g) was obtained from Amersham (Arlington Heights, Illinois), and acridine orange and valinomycin were obtained from Sigma (St. Louis, Missouri). 5-(*N*-Ethyl-*N*-isopropyl)amiloride (ethylisopropylamiloride) was synthesized as previously described.[25] Statistical analysis was performed with paired data using Student's *t* test.

Results

Li^+ Transport in Red Blood Cells

To determine whether canine red blood cells mediate Na^+-Li^+ countertransport, red blood cells were preloaded with Li^+, and the efflux of Li^+ was measured into external media containing 150 mM NaCl or choline chloride. For comparison, the same experiment was performed with human and rabbit red blood cells. As shown in Figure 1, the efflux of Li^+ from human and rabbit red blood cells was faster into Na^+ media than $choline^+$ media. The increment in Li^+ efflux due to external Na^+ (Na^+_{out}) can be taken as the activity of Na^+-Li^+ countertransport. However, the efflux of Li^+ from canine red blood cells into Na^+ and choline media was the same. Thus, Na^+-Li^+ countertransport in canine red blood cells is either absent or is quiescent under conditions where the transport system is demonstrable in red blood cells from humans and rabbits. It would seem, then, that the dog is an unsuitable animal model to test whether the activity of red blood cell Na^+-Li^+ countertransport is predictive of the activity of Na^+-Li^+ (or Na^+-H^+) exchange in vascular smooth muscle. Bovine red blood cells, on the other hand, do mediate Na^+-Li^+ countertransport.[7,9,20,21] Thus, the presence of Na^+-Li^+ exchange was assessed in vascular smooth muscle sarcolemmal vesicles from this species.

Na^+-H^+ and Na^+-Li^+ Exchange in Sarcolemmal Vesicles

The presence of Na^+-H^+ exchange in bovine sarcolemmal vesicles was confirmed by demonstrating that an outwardly directed proton gradient stimulated the uptake of Na^+. As shown in Figure 2A, the presence of an inside acidic pH gradient (pH_{in}, 5.0; pH_{out}, 7.5) increased the uptake of Na^+ at early time points relative to the absence of a pH gradient (pH_{in} and pH_{out}, 7.5). The presence of Na^+-Li^+ exchange in the vesicles was assessed by demonstrating that an outwardly directed Li^+ gradient (25 mM intravesicular Li^+ [Li^+_{in}], 5 mM Li^+_{out}) stimulated the uptake of Na^+ at early time points relative to the absence of such a gradient (0 Li^+_{in}, 5 mM Li^+_{out}). These data are shown in Figure 2B. Since an initial outwardly directed proton or Li^+ gradient did not affect Na^+ uptake at the 90-minute equilibrium time points, the effects of proton or Li^+ gradients on Na^+ uptake could not be explained by increased

Na^+ binding to the membranes or by increased intravesicular space. Thus, the data in Figures 2A and 2B indicate that the vesicles can mediate both Na^+-H^+ exchange and Na^+-Li^+ exchange.

We have previously demonstrated the presence of Na^+-H^+ exchange in sarcolemmal vesicles from bovine superior mesenteric artery by showing that gradients for Na^+ or protons stimulated the transport of the counter ion in the opposite direction.[22] In that study, we showed that the link between Na^+ and proton transport was not merely due to electrical coupling between the two ions via separate conductive pathways. This was demonstrated by showing that an inwardly directed Na^+ gradient stimulated proton efflux in vesicles where membrane voltage was clamped to zero by setting equal the internal and external K^+ concentration (K^+_{in} and K^+_{out}, respectively) in the presence of the K^+ ionophore, valinomycin. In the present study, we wished to show that the stimulation of Na^+ uptake by an outwardly directed Li^+ gradient in bovine sarcolemmal vesicles was not merely due to stimulation of conductive Na^+ influx by a relatively inside negative Li^+ diffusion potential. We also wished to assess the effect of a change in membrane potential on Li^+ gradient–stimulated Na^+ uptake in these vesicles.

The 30-second uptake of 1 mM $^{22}Na^+$ was measured in vesicles preloaded with 25 mM choline$^+$ or Li^+, in the presence of valinomycin and equal K^+_{in} and K^+_{out} (55 mM), or in an outwardly directed K^+ gradient (K^+_{in}, 55 mM; K^+_{out}, 11 mM). The former condition should clamp membrane potential to zero, whereas the latter condition should clamp potential to an inside electronegative value. As shown in Figure 3, either in the presence of zero membrane potential ($K^+_{in}=K^+_{out}$) or in an inside negative membrane potential ($K^+_{in}>K^+_{out}$), Li^+_{in} stimulated Na^+ uptake relative to intravesicular choline. Thus, the stimulation of Na^+ uptake by an outwardly directed Li^+ gradient cannot be explained by a relatively inside negative Li^+ diffusion potential since transport was assessed under voltage-clamped conditions. Although Na^+ uptake by Li^+- or choline$^+$-preloaded vesicles was stimulated when the intravesicular space was rendered electronegative compared with electroneutral vesicles (Figure 3), it is noteworthy that the Li^+ gradient–stimulated component of Na^+ uptake was the same under both voltage conditions (0.27 and 0.28 nmol Na^+/mg protein · 30 sec for electroneutral and electronegative vesicles, respectively). These data suggest that Na^+-Li^+ exchange in bovine sarcolemmal vesicles is an electroneutral process.

Prior reports have shown that the Na^+-H^+ exchanger in several different tissues has affinity for Li^+.[26–29] It was possible that the stimulation of Na^+ uptake in sarcolemmal vesicles via an outwardly directed Li^+ gradient was actually the result of intravesicular acidification via Li^+-H^+ exchange followed by proton gradient–stimulated Na^+ uptake

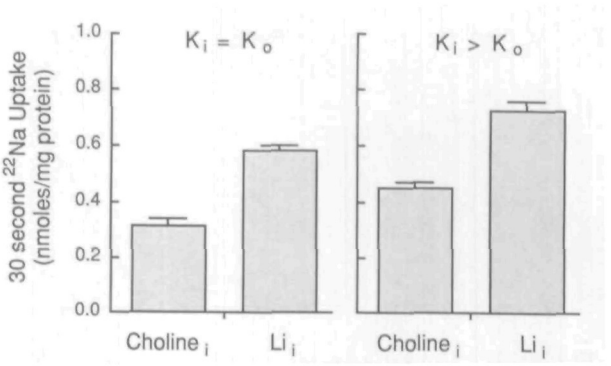

FIGURE 3. *Bar charts showing effect of membrane voltage on Na^+-Li^+ exchange in bovine sarcolemmal vesicles. Vesicles were preincubated with (mM) LiCl (Li_i) or choline chloride (Choline$_i$) 25, KCl 55, mannitol 40, and HEPES-Tris 16, pH 7.5, plus 50 μg/ml valinomycin for 90 minutes at 22° C.* Left panel: *30-second uptake of 1 mM $^{22}Na^+$ was assayed at 22° C in the presence of (mM) Li^+ 5, choline$^+$ 5, K^+ 55, Cl^- 66; mannitol 68, and HEPES-Tris 16, pH 7.5.* Right panel: *30-second uptake of 1 mM $^{22}Na^+$ was assayed at 22° C in the presence of (mM) Li^+ 5, choline$^+$ 49, K^+ 11, Cl^- 66, mannitol 68, and HEPES-Tris 16, pH 7.5. Values are the mean±SEM of a representative experiment done in quadruplicate. K_i, internal K^+ concentration; K_o, external K^+ concentration.*

via Na^+-H^+ exchange. In other words, it was possible that a direct Na^+-Li^+ exchange process was not present in the vesicles but that the parallel operation of Li^+-H^+ and Na^+-H^+ exchange gave rise to Li^+ gradient–stimulated Na^+ uptake. This scenario would be ruled out if it could be shown that an outwardly directed Li^+ gradient, under conditions where it stimulated Na^+ uptake, did not in fact acidify the intravesicular space.

Sarcolemmal vesicles were preloaded with 25 mM LiCl or choline chloride, pH 7.5, mixed with media containing 5 mM LiCl, pH 7.5, and pH_{in} monitored by measuring the fluorescence quenching of acridine orange. The intravesicular and extravesicular ion and buffer concentrations were the same as those used in the experiment shown in Figure 2B. As shown in Figure 4, after addition of Li^+-preloaded vesicles to external media, the fluorescence tracing was relatively flat and was not different from the tracing obtained after adding choline$^+$-preloaded vesicles to external media. Furthermore, as also shown on these tracings, the addition of 25 mM Na^+_{out} was without effect on the fluorescence tracings. If an outwardly directed Li^+ gradient had resulted in intravesicular acidification, imposition of an inwardly directed Na^+ gradient should have collapsed the pH gradient via Na^+-H^+ exchange, resulting in a rise in fluorescence. As a positive control, a similar experiment was performed with rabbit renal brush border vesicles, which have very vigorous Na^+-H^+ exchange activity relative to bovine sarcolemmal vesicles.[22,30] As shown on the left side of Figure 4, after renal brush border vesicles, which had been preloaded with 100

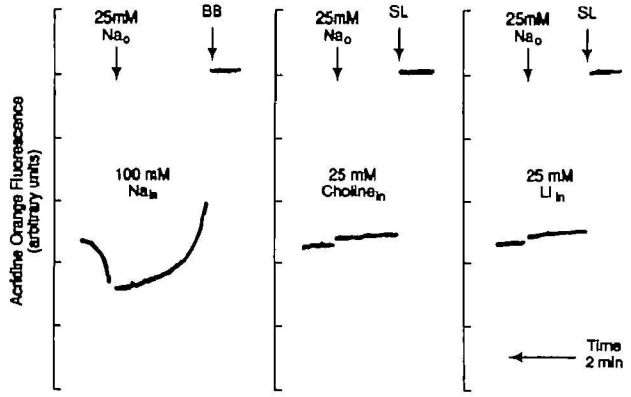

FIGURE 4. *Tracings showing effect of an outwardly directed cation gradient on intravesicular pH. Sarcolemmal vesicles (SL) were preincubated with (mM) mannitol 150, LiCl 25 (Li_{in},* right tracing*) or choline chloride ($Choline_{in}$,* middle tracing*), plus Tris 10, and HEPES 16, pH 7.5, for 90 minutes at 22° C. Fifteen microliters of vesicles (about 150 μg protein) were rapidly mixed (at SL arrow) with 1 ml buffer containing (mM) acridine orange 0.006, choline$^+$ 5, Li^+ 5, Cl^- 10, mannitol 170, Tris 10, and HEPES 16, pH 7.5. Fluorescence was measured by activating at 493 nm and recording the emission at 530 nm with a chart recorder (right to left). Sodium gluconate (Na_o) (25 mM) was added to the cuvette where indicated from a 1 M stock. Rabbit renal brush border vesicles (BB)* (left tracing) *were incubated with (mM) sodium gluconate (Na_{in}) 100, Tris 10, and HEPES 16, pH 7.5, for 90 minutes at 22° C. Ten microliters of vesicles (about 100 μg protein) were rapidly mixed (at BB arrow) with 1 ml buffer containing (mM) acridine orange 0.006, NMG gluconate 100, Tris 10, and HEPES 16, pH 7.5, and fluorescence was recorded as described above. Na_o (25 mM) was added to the cuvette where indicated. Tracings are representative experiments that were performed at least five times with two separate sarcolemmal and brush border preparations.*

mM sodium gluconate, pH 7.5, were suspended in external media containing 6 μM acridine orange plus 100 mM NMG gluconate, pH 7.5, there was rapid acridine orange fluorescence quenching that was reversed by adding 25 mM external sodium gluconate. Thus, the methods used in Figure 4 can detect acidification of a membrane vesicle preparation due to an outwardly directed cation gradient.

These data indicate that under the conditions used, an outwardly directed Li^+ gradient did not measurably acidify the inside of sarcolemmal vesicles. Therefore, the stimulation of Na^+ uptake by an outwardly directed Li^+ gradient under identical conditions (Figure 2B) cannot be explained by parallel operation of Li^+-H^+ exchange and Na^+-H^+ exchange but was probably due to Na^+-Li^+ exchange per se.

Relation Between Na^+-H^+ and Na^+-Li^+ Exchange in Sarcolemmal Vesicles

If Na^+-H^+ exchange and Na^+-Li^+ exchange in these vesicles are mediated by the same transport

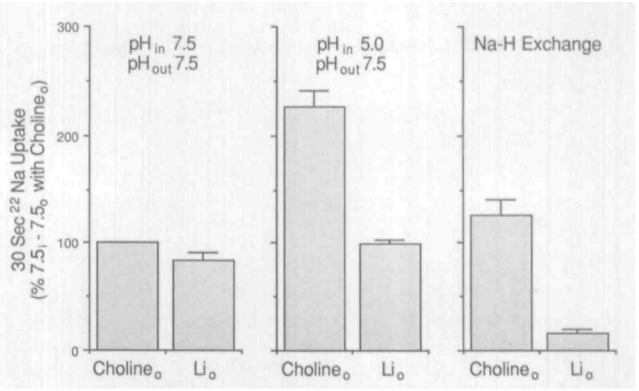

FIGURE 5. *Bar charts showing effect of external Li^+ on Na^+-H^+ exchange in bovine sarcolemmal vesicles. Vesicles were preincubated with buffer at pH 7.5 or 5.0 as described in the legend of Figure 2A. pH_{in}, internal pH; pH_{out}, external pH. The 30-second uptake of 1 mM $^{22}Na^+$ was assayed at 22° C in the presence of (mM) mannitol 54, Cl^- 26, choline$^+$ or Li^+ 25, plus MES 8, Tris 47, and HEPES 65, pH 7.5. In each experiment, data are expressed as a percent of uptake by vesicles preincubated at pH 7.5 and incubated with $^{22}Na^+$ in the presence of choline$^+$. In the presence of external choline$^+$ ($Choline_o$) or Li^+ (Li_o), Na^+-H^+ exchange activity is calculated as the uptake of Na^+ by vesicles preincubated at pH 5.0 minus uptake by vesicles preincubated at pH 7.5. Data represent the mean±SEM of four separate experiments.*

system, it would be expected that Li^+_{out} would inhibit proton gradient–stimulated Na^+ uptake and external acidity would inhibit Li^+ gradient–stimulated Na^+ uptake. These predictions were tested in the following two experiments. As shown in Figure 5, vesicles were preloaded to pH_{in} 7.5 (left panel) or 5.0 (middle panel), and the 30-second uptake of 1 mM Na^+ was assayed in external media at pH 7.5. These measurements were made in the presence of 25 mM Li^+_{out} or external choline$^+$. As expected, in the presence of external choline$^+$, preloading vesicles at pH_{in} 5.0 stimulated Na^+ uptake by over 100%. However, in the presence of Li^+_{out}, the stimulation of Na^+ uptake by internal acidity was markedly attenuated. As shown in the right panel of Figure 5, Na^+-H^+ exchange activity, which is taken as the proton gradient–stimulated component of Na^+ uptake, was inhibited 87% by 25 mM Li^+_{out}. These data are in keeping with the possibility that Li^+ competes with Na^+ for the Na^+ binding site of the Na^+-H^+ exchanger in these vesicles.

As shown in Figure 6, vesicles were preloaded at pH_{in} 7.5 with 25 mM choline$^+$ (left panel) or Li^+ (middle panel), and the 30-second uptake of 1 mM Na^+ was assayed. These measurements were made at pH_{out} 7.5 or 6.0. Li^+_{in} stimulated the uptake of Na^+ by about 100% at pH_{out} 7.5, but this stimulation was attenuated at pH_{out} 6.0. Na^+-Li^+ exchange activity, which is taken as the Li^+ gradient–stimulated component of Na^+ uptake (right panel), was inhibited 61% by pH_{out} 6.0. These data are consistent with the idea that protons compete with

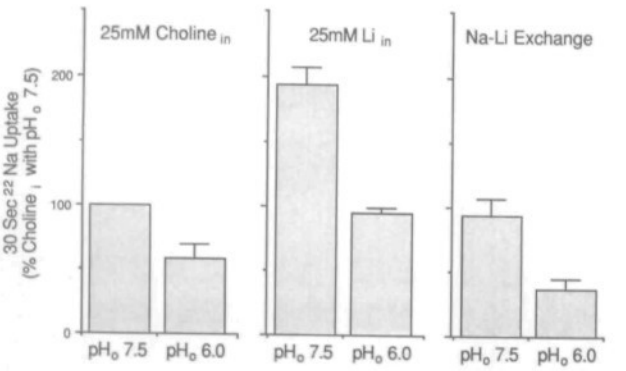

FIGURE 6. *Bar charts showing effect of external acidity on Na^+-Li^+ exchange in bovine sarcolemmal vesicles. Vesicles were preincubated with Li^+ (Li_{in}) or choline$^+$ ($Choline_{in}$) as described in the legend of Figure 2B, and the 30-second uptake of 1 mM $^{22}Na^+$ was assayed at 22° C in the presence of (mM) choline$^+$ 5, Li^+ 5, Cl^- 11, mannitol 178, plus Tris 10, and HEPES 16, pH 7.5, or Tris 6, HEPES 3.2, and MES 16.8, pH 6.0. In each experiment, data are expressed as a percent of uptake by choline$^+$-preloaded vesicles, external pH (pH_o) 7.5. At pH_o 7.5 or 6.0, Na^+-Li^+ exchange activity is calculated as the uptake of Na^+ by Li^+-preloaded vesicles minus uptake by choline$^+$-preloaded vesicles. Data represent the mean±SEM of three separate experiments.*

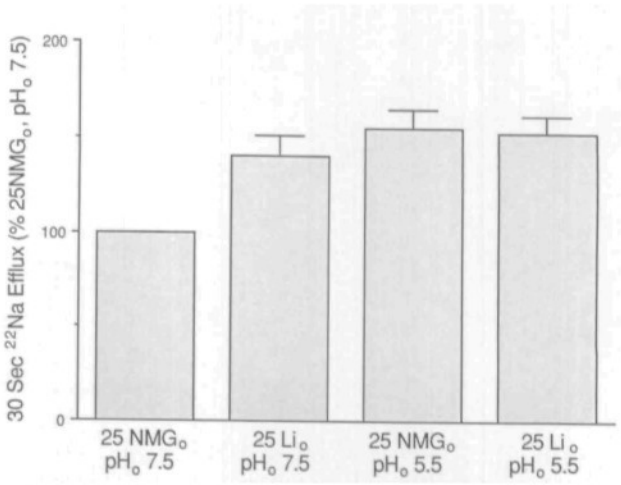

FIGURE 7. *Bar charts showing effects of external Li^+ and protons on Na^+ efflux from bovine sarcolemmal vesicles. Vesicles were preincubated for 90 minutes at 22° C with (mM) $^{22}NaCl$ 1, NMG_2SO_4 12.5, mannitol 160.5, Tris 10, and HEPES 16, pH 7.5. The 30-second efflux of $^{22}Na^+$ was assayed at 22° C by incubating a 10-μl vesicle suspension preloaded with $^{22}Na^+$ with a 0.5-ml solution containing (mM) NMG_2SO_4 (NMG_o) or Li_2SO_4 (Li^+_o) 12.5, mannitol 114.5, plus Tris 28.5, and HEPES 45.5, pH 7.5, or Tris 10 and MES 64, pH 5.5. In each experiment, data are expressed as a percent of efflux into media containing NMG^+, pH 7.5, which was significantly less ($p<0.05$) than efflux into the other three media. Data represent the mean±SEM of four separate experiments.*

Na^+ for the Na^+ binding site of the Na^+-Li^+ exchanger.

Another prediction that would be expected if Na^+-H^+ and Na^+-Li^+ exchange were mediated by the same transport pathway is that the stimulation of Na^+ efflux induced by high concentrations of Li^+_{out} or external protons would not be additive. This was tested in the following experiment. The 30-second efflux of Na^+ at pH_{in} 7.5 was measured in external media at pH 7.5 containing 25 mM NMG or 25 mM Li^+. Na^+ efflux was also measured in external media at pH 5.5 containing 25 mM NMG^+ or 25 mM Li^+. Compared with control conditions where the external media contained NMG^+ at pH 7.5, the presence of Li^+_{out}, pH 7.5, stimulated the efflux of Na^+. These data, which are shown in Figure 7, are in keeping with the presence of Na^+-Li^+ exchange in the vesicles. As also shown, the presence of external acidity (pH_{out} 5.5) also stimulated Na^+ efflux compared with control conditions, consistent with the presence of Na^+-H^+ exchange. However, when both Li^+_{out} and external acidity were present, Na^+ efflux was not faster than when either Li^+_{out} or external acidity alone was present. These data suggest that Na^+ efflux via Na^+-Li^+ exchange was saturated at 25 mM Li^+_{out}, that Na^+ efflux via Na^+-H^+ exchange was saturated at pH_{out} 5.5, and that Li^+ and protons occupy the same external transport site when exchanging for internal Na^+.

Relation Between Na^+-H^+ and Li^+-H^+ Exchange in Sarcolemmal Vesicles

The data presented thus far with bovine sarcolemmal vesicles indicate that Na^+-H^+ exchange and Na^+-Li^+ exchange are mediated via a single cation exchange transport system with affinity for Na^+, Li^+, and protons. If this is the case, Li^+-H^+ exchange, as well as Na^+-H^+ exchange, should be demonstrable, and the efflux of protons that is stimulated by high external concentrations of Li^+ or Na^+ should not be additive. These predictions were tested in the following experiment. Vesicles were preloaded to pH_{in} 5.0 and rapidly mixed with external media at pH 7.5 containing 100 mM NMG^+, 50 mM Na^+ plus 50 mM NMG^+, 50 mM Li^+ plus 50 mM NMG^+, or 50 mM Na^+ plus 50 mM Li^+. The dissipation of the outwardly directed proton gradient was measured by monitoring the fluorescence quenching of the pH probe, acridine orange. As shown in Figure 8, in the presence of external NMG^+ (control), the fluorescence signal gradually rose, indicating a leak of protons from the vesicles. The first-order rate constant for this process (k) was 0.011 ± 0.0005 sec^{-1} ($n=4$). When the external media contained Na^+, the inside acidic pH gradient collapsed faster, indicating the presence of Na^+-H^+ exchange ($k=0.018\pm0.001$ sec^{-1}, $n=4$) ($p<0.01$). When the external media contained Li^+, proton efflux was also faster than under control conditions, indicating the presence of Li^+-H^+ exchange ($k=0.019\pm0.001$ sec^{-1}, $n=4$) ($p<0.01$). When external media contained both Na^+ and Li^+, the efflux of protons was not faster than efflux in the presence of external Na^+ or Li^+ alone ($k=0.018\pm0.001$ sec^{-1},

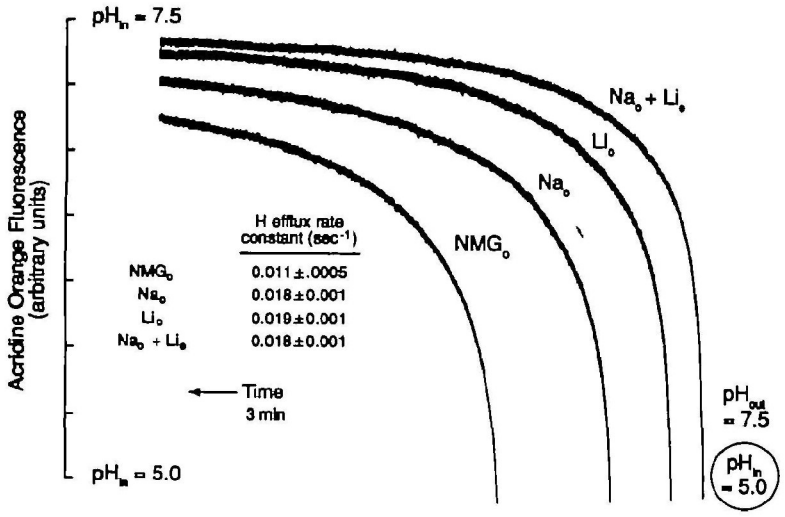

FIGURE 8. *Tracings showing effects of external Na^+ and Li^+ on proton efflux from bovine sarcolemmal vesicles. Vesicles were preincubated for 90 minutes at 22° C with (mM) mannitol 181, MES 40, Tris 2, and HEPES 3.2, pH 5.0. Ten microliters of vesicles (about 100 μg protein) were rapidly mixed with 1 ml solution containing (mM) acridine orange 0.006, Tris 10, and HEPES 16, pH 7.5, plus NMG chloride 100, or NaCl 50 and NMG chloride 50, or LiCl 50 and NMG chloride 50, or NaCl 50 and LiCl 50. Fluorescence was measured over time (right to left) immediately after mixing by activating at 493 nm and recording the emission at 530 nm. Data are from a representative experiment. pH_{in}, internal pH; NMG_o, external NMG; Na_o, external sodium; Li_o, external lithium; pH_{out}, external pH.* Inset: *The first-order rate constants for proton gradient dissipation were calculated as described in "Materials and Methods." Data represent the mean±SEM from four separate experiments.*

n=4) (p<0.01 vs. control; p=NS vs. Na^+_{out} or Li^+_{out}). These data indicate that the vesicles can mediate Na^+-H^+ exchange and Li^+-H^+ exchange. They also indicate that Na^+-H^+ exchange was saturated at 50 mM Na^+_{out}, that Li^+-H^+ exchange was saturated at 50 mM Li^+_{out}, and that Na^+ and Li^+ occupy the same external transport site when exchanging for internal protons.

Inhibitor Studies

As a final test to determine whether Na^+-H^+ exchange and Na^+-Li^+ exchange are mediated by the same transport system in bovine sarcolemmal vesicles, we tested whether ethylisopropylamiloride would inhibit both transport processes with identical potency. Na^+-H^+ exchange and Na^+-Li^+ exchange activities were taken as the proton gradient– and Li^+ gradient–stimulated components of Na^+ uptake, respectively, and were measured under identical *cis* conditions. These measurements were made in the absence or presence of 10^{-3}, 10^{-4}, or 10^{-5} M ethylisopropylamiloride. As shown in Figure 9, the drug had exactly the same dose-response effect for inhibiting Na^+-H^+ exchange and Na^+-Li^+ exchange. These data provide further evidence that both sarcolemmal transport functions are mediated by the same mechanism.

Discussion

Several laboratories have demonstrated that Na^+-Li^+ countertransport is increased in red blood cells from patients with essential hypertension.[2,5,6] In the absence of Li^+, this transport system mediates Na^+-Na^+ exchange.[7] Increased Na^+-Na^+ exchange activity would not affect net Na^+ transport in the red blood cell or any other tissue. Thus, the relation between increased red blood cell Na^+-Li^+ countertransport activity and the hypertensive process is obscure.

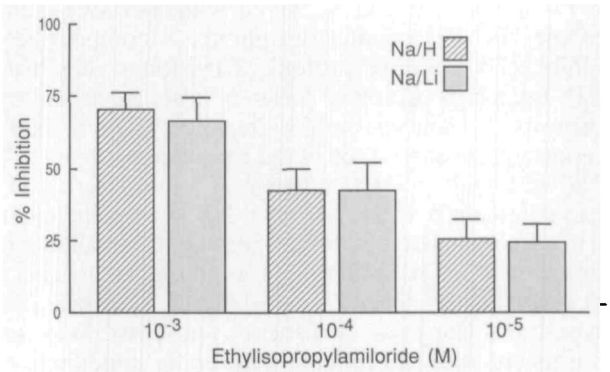

FIGURE 9. *Bar charts showing effects of ethylisopropylamiloride on Na^+-H^+ exchange and Na^+-Li^+ exchange in bovine sarcolemmal vesicles. Vesicles were preincubated with buffer at pH 7.5 or 5.0, as described in the legend of Figure 2A, and with buffer containing 25 mM choline chloride or LiCl as described in the legend of Figure 2B. In all cases, the 30-second uptake of 1 mM $^{22}Na^+$ was assayed in the presence of (mM) choline$^+$ 5, Li^+ 5, Cl^- 11, mannitol 84, MES 8, Tris 47, and HEPES 65, pH 7.5, with or without the indicated concentrations of ethylisopropylamiloride. In the presence of a given concentration of inhibitor, Na^+-H^+ exchange and Na^+-Li^+ exchange activities were calculated as the differences in $^{22}Na^+$ uptake between vesicles preincubated with solution at pH 5.0 and 7.5 and between vesicles preincubated with solution containing 25 mM Li^+ or choline$^+$, respectively. Na^+-H^+ and Na^+-Li^+ exchange in the presence of ethylisopropylamiloride are expressed as percent inhibition relative to these transport rates in the absence of inhibitor. Data represent the mean±SEM of six separate experiments.*

Aronson[8] and Funder et al[9] have proposed that red blood cell Na^+-Li^+ countertransport may be an operative mode of a Na^+-H^+ exchanger. This was based on the many similarities between human red blood cell Na^+-Li^+ countertransport and rabbit renal brush border Na^+-H^+ exchange. Both are quinidine inhibitable,[18,31] electroneutral monovalent cation exchangers[7,30,32] with affinities for Na^+ and Li^+,[7,18,30,32-34] greater affinity for Li^+ than Na^+,[32,33] and no affinity for K^+, Rb^+, Cs^+ or choline$^+$.[7,18,30,32-34] It is an attractive hypothesis that the elevated Na^+-Li^+ countertransport activity in red blood cells from hypertensive patients may be a marker for increased Na^+-H^+ exchange activity in renal proximal tubular or vascular smooth muscle cells.[9,10] Increased Na^+-H^+ exchange activity in the kidney could lead to enhanced reabsorption of filtered salt and water, thereby contributing to the generation or maintenance of hypertension.[35,36] Increased Na^+-H^+ exchange activity in vascular smooth muscle could lead to increased intracellular Na^+ concentration or cell pH. Both of these abnormalities have been linked to increased vascular smooth muscle tone.[37,38]

It is well known that the Na^+-H^+ exchanger from several different sources has affinity for Li^+.[26-29] It is controversial, however, whether the red blood cell Na^+-Li^+ countertransporter can mediate Na^+-H^+ exchange. Funder et al[9] found that Na^+-Na^+ exchange in bovine red blood cells, which is an operative mode of the Na^+-Li^+ countertransporter, is competitively inhibited by external protons. They found that Na^+-Li^+ exchange in these cells is also inhibited by protons.[21] Canessa and colleagues[16,17,39-43] have reported that human red blood cells mediate Na^+-Li^+, Na^+-H^+, and Li^+-H^+ exchange and that internal H^+ can stimulate both Na^+-H^+ and Na^+-Li^+ exchange in a manner consistent with its operation as an allosteric modifier.[39,42,43] A similar role for internal H^+ has been described for Na^+-H^+ exchangers in other cell types.[44,45] The data of Canessa and coworkers are consistent with the concept that, under conditions of pH equilibrium higher than 7.0, human red blood cell Na^+-H^+ exchange is in a different conformational state that promotes amiloride-insensitive Na^+-Li^+ exchange.[43] On the other hand, Jennings et al[46] reported that Na^+-Na^+ exchange in rabbit red blood cells could not function appreciably in a Na^+-H^+ exchange mode. In addition, Na^+-Li^+ countertransport can be easily demonstrated in human red blood cells under control conditions,[2,6,7] but Na^+-H^+ exchange can only be seen under special experimental conditions, such as raising intracellular Ca^{2+} concentration.[16,17] Additional studies have shown that Na^+-Li^+ countertransport in human red blood cells is insensitive to amiloride,[18] whereas this drug readily inhibits Na^+-H^+ exchange activity in the red blood cell[17] and other tissues.[47]

To determine whether Na^+-Li^+ countertransport in the red blood cell might be a marker for a similar transport system in vascular smooth muscle, we previously demonstrated Na^+-Li^+ exchange in sarcolemmal vesicles from canine superior mesenteric artery.[19] This canine sarcolemmal Na^+-Li^+ exchange process has several features in common with the human red blood cell Na^+-Li^+ countertransport system. Both are ouabain-insensitive electroneutral transport mechanisms,[18,19,32] demonstrate half-maximal activity at about 2 mM Li^+,[19,22] and are inhibited by phloretin and quinidine.[7,18,19] We have also previously shown that the Na^+-H^+ exchanger in sarcolemmal vesicles from canine superior mesenteric artery is inhibited by phloretin and quinidine[19] and that proton gradient–stimulated Na^+ efflux and Li^+ gradient–stimulated Na^+ efflux from these vesicles are not additive.[19] Finally, we showed that Na^+-H^+ and Na^+-Li^+ exchange are inhibited by ethylisopropylamiloride in canine vesicles.[19] Unfortunately, the relative effects of the drug on the two modes of Na^+ transport could not be determined in those experiments because Na^+ uptake was not assayed under the same *cis* conditions. Nevertheless, these previous studies suggested that Na^+-Li^+ exchange in canine sarcolemmal vesicles might be homologous with the human red blood cell Na^+-Li^+ countertransporter and that sarcolemmal Na^+-Li^+ and Na^+-H^+ exchange might be mediated by a common transport system.

To see whether the activity of the canine sarcolemmal Na^+-Li^+ exchanger might reflect the activity of a putative Na^+-Li^+ countertransport system in the red blood cell from this species, we attempted to demonstrate the latter by determining whether an inwardly directed Na^+ gradient would stimulate Li^+ efflux from canine red blood cells. As opposed to results with human and rabbit red blood cells, an inwardly directed Na^+ gradient failed to stimulate Li^+ efflux from canine red blood cells (Figure 1). These results agreed with previous studies that demonstrated the presence of Na^+-Li^+ countertransport in red blood cells from humans[2,5-7,18,32,34] and rabbit.[7,46] The present studies are also in accord with those of Parker,[48,49] who was unable to demonstrate *trans*stimulation of Na^+ or Li^+ efflux by Na^+_{out} in canine red blood cells. It would seem, then, that either canine red blood cells do not contain a Na^+-Li^+ countertransport system or that the system is not operational under conditions where Na^+-Li^+ countertransport is readily demonstrable in red blood cells from other species.

Clearly, if the activity of a given Na^+ transport system in the red blood cell is to be a reflection of the activity of that transport system in vascular smooth muscle, both tissues must possess the transport system in question. The cow, unlike the dog, has an easily demonstrable red blood cell Na^+-Li^+ countertransport system.[7,9,20,21] In addition, we have previously demonstrated that sarcolemmal vesicles from bovine superior mesenteric artery can mediate Na^+-H^+ exchange.[22] We therefore sought to demonstrate Na^+-Li^+ exchange in these vesicles. In addition, due to the confusion over whether Na^+-Li^+ exchange is an operative mode of Na^+-H^+

exchange in any tissue, we wished to determine whether Na^+-Li^+ exchange and Na^+-H^+ exchange in bovine sarcolemmal vesicles are mediated by the same transport system.

In agreement with previous results,[22] the present studies showed that bovine sarcolemmal vesicles contain a Na^+-H^+ exchanger (Figures 2A, 5, 7, and 8). The present studies also showed that a Li^+ gradient directed in one direction stimulated the transport of Na^+ in the opposite direction (Figures 2B, 3, 6, and 7). Li^+ gradient–stimulated Na^+ uptake could not be explained by an alteration in membrane potential (Figure 3) or by indirect coupling between the outwardly directed Li^+ gradient and Na^+ uptake via intravesicular acidification (Figure 4). Thus, the data suggest that the sarcolemmal vesicles contain a Na^+-Li^+ exchange transport system. Since a change in membrane potential did not affect the Li^+ gradient–stimulated component of Na^+ uptake (Figure 3), the data are consistent with electroneutral operation of Na^+-Li^+ exchange in these vesicles.

We have demonstrated that Na^+-H^+ exchange activity is inhibited by Li^+_{out} (Figure 5) and that Na^+-Li^+ exchange activity is inhibited by external H^+ (Figure 6). The present studies have shown that Na^+ efflux via Na^+-Li^+ exchange and via Na^+-H^+ exchange are not additive (Figure 7). We have shown that Li^+-H^+ exchange is demonstrable in these vesicles and that proton efflux via Na^+-H^+ exchange and via Li^+-H^+ exchange are not additive (Figure 8). Taken together, the data strongly suggest that Na^+-H^+ exchange and Na^+-Li^+ exchange in bovine sarcolemmal vesicles are mediated via a single cation exchanger with affinity for Na^+, Li^+, and protons. Finally, we have obtained additional evidence to support this possibility by showing that ethylisopropylamiloride has identical potency for inhibiting Na^+-H^+ exchange and Na^+-Li^+ exchange (Figure 4).

It is noteworthy that in the absence of a pH gradient, an outwardly directed Li^+ gradient did not measurably acidify sarcolemmal vesicles (Figure 4). Although the data in Figure 8 support the presence of Li^+-H^+ exchange activity in bovine sarcolemmal vesicles, the data in Figure 4 do not demonstrate it. This apparent discrepancy may be explicable on the basis of relatively low Li^+-H^+ exchange activity in sarcolemmal vesicles, effective buffering of the intravesicular space, and a relatively large proton leak rate. As a result, Li^+-H^+ exchange activity was not able to generate a measurable pH gradient.

It should be pointed out that the apparent K_i of ethylisopropylamiloride for inhibiting Na^+-H^+ or Na^+-Li^+ exchange in these studies was about 10^{-4} M. This value is over three orders of magnitude higher than the apparent K_i of this drug for inhibiting Na^+-H^+ exchange activity in other tissues.[50,51] The reason for the relatively low sensitivity of Na^+-H^+ and Na^+-Li^+ exchange to ethylisopropylamiloride in these studies is not known. Although Na^+-H^+ and Na^+-Li^+ exchange activities were clearly inhibited by ethylisopropylamiloride, the drug lacks specificity in other tissues when used at these concentrations.[52]

It would seem reasonable to conclude from previous studies that either the red blood cell Na^+-Li^+ exchanger is distinct from the Na^+-H^+ exchanger or that both transport functions are mediated by a common carrier but are observable under different experimental conditions and have different inhibitor sensitivities.[16–18,39–43] The present studies, on the other hand, demonstrate that in at least one tissue both Na^+-H^+ exchange and Na^+-Li^+ exchange are mediated by a common carrier, are observable under similar experimental conditions, and have the same sensitivity to at least one inhibitor.

Whether Na^+-Li^+ exchange and Na^+-H^+ exchange are mediated by the same transport system in bovine red blood cells or whether bovine red blood cell Na^+-Li^+ countertransport activity is indeed a marker for Na^+-Li^+ (and Na^+-H^+) exchange activity in vascular smooth muscle from this species is not currently known. Additional studies are needed to answer these questions and to determine whether increased vascular smooth muscle Na^+-H^+ exchange activity could, in fact, give rise to increased vascular smooth muscle tone.

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KEY WORDS • red blood cell Na^+-Li^+ countertransport • ethylisopropylamiloride • acridine orange • vascular smooth muscle

exchange in any tissue, we wished to determine whether Na^+-Li^+ exchange and Na^+-H^+ exchange in bovine sarcolemmal vesicles are mediated by the same transport system.

In agreement with previous results,[22] the present studies showed that bovine sarcolemmal vesicles contain a Na^+-H^+ exchanger (Figures 2A, 5, 7, and 8). The present studies also showed that a Li^+ gradient directed in one direction stimulated the transport of Na^+ in the opposite direction (Figures 2B, 3, 6, and 7). Li^+ gradient–stimulated Na^+ uptake could not be explained by an alteration in membrane potential (Figure 3) or by indirect coupling between the outwardly directed Li^+ gradient and Na^+ uptake via intravesicular acidification (Figure 4). Thus, the data suggest that the sarcolemmal vesicles contain a Na^+-Li^+ exchange transport system. Since a change in membrane potential did not affect the Li^+ gradient–stimulated component of Na^+ uptake (Figure 3), the data are consistent with electroneutral operation of Na^+-Li^+ exchange in these vesicles.

We have demonstrated that Na^+-H^+ exchange activity is inhibited by Li^+_{out} (Figure 5) and that Na^+-Li^+ exchange activity is inhibited by external H^+ (Figure 6). The present studies have shown that Na^+ efflux via Na^+-Li^+ exchange and via Na^+-H^+ exchange are not additive (Figure 7). We have shown that Li^+-H^+ exchange is demonstrable in these vesicles and that proton efflux via Na^+-H^+ exchange and via Li^+-H^+ exchange are not additive (Figure 8). Taken together, the data strongly suggest that Na^+-H^+ exchange and Na^+-Li^+ exchange in bovine sarcolemmal vesicles are mediated via a single cation exchanger with affinity for Na^+, Li^+, and protons. Finally, we have obtained additional evidence to support this possibility by showing that ethylisopropylamiloride has identical potency for inhibiting Na^+-H^+ exchange and Na^+-Li^+ exchange (Figure 4).

It is noteworthy that in the absence of a pH gradient, an outwardly directed Li^+ gradient did not measurably acidify sarcolemmal vesicles (Figure 4). Although the data in Figure 8 support the presence of Li^+-H^+ exchange activity in bovine sarcolemmal vesicles, the data in Figure 4 do not demonstrate it. This apparent discrepancy may be explicable on the basis of relatively low Li^+-H^+ exchange activity in sarcolemmal vesicles, effective buffering of the intravesicular space, and a relatively large proton leak rate. As a result, Li^+-H^+ exchange activity was not able to generate a measurable pH gradient.

It should be pointed out that the apparent K_i of ethylisopropylamiloride for inhibiting Na^+-H^+ or Na^+-Li^+ exchange in these studies was about 10^{-4} M. This value is over three orders of magnitude higher than the apparent K_i of this drug for inhibiting Na^+-H^+ exchange activity in other tissues.[50,51] The reason for the relatively low sensitivity of Na^+-H^+ and Na^+-Li^+ exchange to ethylisopropylamiloride in these studies is not known. Although Na^+-H^+ and Na^+-Li^+ exchange activities were clearly inhibited by ethylisopropylamiloride, the drug lacks specificity in other tissues when used at these concentrations.[52]

It would seem reasonable to conclude from previous studies that either the red blood cell Na^+-Li^+ exchanger is distinct from the Na^+-H^+ exchanger or that both transport functions are mediated by a common carrier but are observable under different experimental conditions and have different inhibitor sensitivities.[16–18,39–43] The present studies, on the other hand, demonstrate that in at least one tissue both Na^+-H^+ exchange and Na^+-Li^+ exchange are mediated by a common carrier, are observable under similar experimental conditions, and have the same sensitivity to at least one inhibitor.

Whether Na^+-Li^+ exchange and Na^+-H^+ exchange are mediated by the same transport system in bovine red blood cells or whether bovine red blood cell Na^+-Li^+ countertransport activity is indeed a marker for Na^+-Li^+ (and Na^+-H^+) exchange activity in vascular smooth muscle from this species is not currently known. Additional studies are needed to answer these questions and to determine whether increased vascular smooth muscle Na^+-H^+ exchange activity could, in fact, give rise to increased vascular smooth muscle tone.

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