Difference Between Human Red Blood Cell Na⁺-Li⁺ Countertransport and Renal Na⁺-H⁺ Exchange

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SUMMARY Several laboratories have reported that the activities of sodium-lithium countertransport are increased in red blood cells from patients with essential hypertension. Based on the many similarities between this transport system and the renal sodium-proton exchanger, a hypothesis has been put forth in the literature that increased red blood cell sodium-lithium countertransport activity may be a marker for increased sodium-proton exchange activity in the renal proximal tubule. The present studies were designed to test the hypothesis that sodium-lithium countertransport in red blood cells from humans or rabbits is mediated by the same transport mechanism that mediates sodium-proton exchange in the renal brush border from those species. Similar to what has been reported for the rabbit, the present studies show that an amiloride-sensitive sodium-proton exchanger is present in human renal brush border vesicles. However, Na⁺-Li⁺ countertransport in human and rabbit red blood cells, assayed under several different conditions, was not inhibited by amiloride. In agreement with what has been reported for humans, the present studies show that extracellular proton-stimulated sodium efflux is inhibited by amiloride in rabbit red blood cells. These data demonstrate a difference (amiloride sensitivity) between the red blood cell sodium-lithium countertransporter and the renal brush border sodium-proton exchanger in humans and rabbits. These experiments detract from the hypothesis that increased red blood cell sodium-lithium countertransport activity in patients with essential hypertension is a marker for increased sodium-proton exchange activity in the renal brush border. (Hypertension 9: 7-12, 1987)

KEY WORDS amiloride • brush border vesicles • rabbit red blood cells

MANY studies have examined whether an abnormality in red blood cell (RBC) sodium transport is linked to essential hypertension.1-6 An underlying hypothesis in these studies is that an abnormality in a specific RBC sodium transport system may be a marker for a similar abnormality in the same transport system located in another tissue. For instance, elevated activity of a specific sodium influx pathway in RBCs from patients with essential hypertension could be a marker for increased sodium influx by that same pathway across the luminal membrane of certain renal tubular cells or the sarcolemma of vascular smooth muscle. Such a transport defect in the cell membrane of these cell types could be related to the development or maintenance of hypertension.

The cell membrane of RBCs from humans and a variety of mammals mediates Na⁺-Li⁺ countertransport.7-9 This is an electroneutral transport system that can operate in a sodium-for-lithium or sodium-for-sodium exchange mode.7-9 Several laboratories have reported that the activities of this transport system are elevated in RBCs from patients with essential hypertension.2,5,6 Since lithium is only present in very low concentrations in humans, this RBC transport system, or its equivalent in other cell types, would be expected to mediate Na⁺-Na⁺ exchange.8 An increased activity of Na⁺-Na⁺ exchange would not alter net sodium transport in any tissue. Aronson10 and Funder et al.11 have independently suggested that RBC Na⁺-Li⁺ countertransport may be an operative mode of a sodium-proton exchange transport system. This hypothesis is based on the many similarities between RBC Na⁺-Li⁺ countertransport and renal brush border Na⁺-H⁺ exchange. Both transport systems are quinidine-inhibi-
table, 7, 12 electroneutral monovalent cation exchangers 8, 9, 13 with affinities for sodium and lithium, 7, 8, 9, 13-15 greater affinity for lithium than sodium, 7, 8, 14 and no affinity for choline, cesium, rubidium, or potassium 7, 13-15 Increased activity of Na+-Li+ countertransport in RBCs from hypertensive patients has been proposed as a marker for increased activity of renal brush border Na+-H+ exchange. 1, 10, 16 Such an abnormality in the brush border membrane could be responsible for increased proximal tubular sodium reabsorption, which could contribute to the hypertensive state. 17, 18 This is an attractive proposal that would be greatly strengthened if the aforesaid transport activities from both tissues were indeed mediated by the same system.

These studies were designed to test the hypothesis that Na+-Li+ countertransport in RBCs is mediated by the same transport system that mediates Na+-H+ exchange in the renal brush border membrane. The results demonstrate a difference between these two transport systems.

Materials and Methods

Human Brush Border Vesicles

Kidney tissue was obtained from fresh human kidneys immediately after surgical removal from patients with renal cancer. Brush border vesicles were prepared by a magnesium aggregation and differential centrifugation technique that was similar to the method used by this laboratory to prepare rat renal brush border vesicles. 19 Normal portions of human renal cortex were minced with a razor blade and homogenized with 10 strokes in a glass-teflon homogenizer in (in mM) 200 mannitol, 50 Tris, 80 HEPES, pH 7.5 (4 ml/g tissue). Then, MgSO4 was added to the cortical homogenate to a final concentration of 10 mM, and the homogenate was incubated on ice for 20 minutes. The homogenate was centrifuged at 4340 g for 10 minutes, and the supernatant was centrifuged at 27,000 g for 25 minutes. The resultant pellet was resuspended in homogenizing solution with 10 mM MgSO4 and centrifuged at 5900 g for 10 minutes, and the supernatant was centrifuged at 27,000 g for 25 minutes. The resultant pellet was resuspended in homogenizing solution with 10 mM MgSO4 and centrifuged at 7710 g for 10 minutes. The resultant supernatant was centrifuged at 27,000 g for 25 minutes, and the pellet was resuspended in homogenizing solution with 10 mM MgSO4 to a final protein concentration of 10 to 15 mg/ml. Protein was measured by the method of Lowry. 20 The activities of the brush border membrane marker alkaline phosphatase and the basolateral membrane marker Na+,K+-ATPase were determined for the cortical homogenate and final vesicles, as previously described. 19 Alkaline phosphatase activity in the vesicles was enriched ninefold relative to the cortical homogenate, while Na+,K+-ATPase activity was enriched by only 25%. Hence, the human renal vesicles represented primarily brush border membranes.

The vesicles were preincubated with the desired solutions for 90 minutes at 22°C before the sodium transport experiments. The timed uptake of 1 mM 22Na was measured in triplicate by a rapid Millipore (Bedford, MA, USA) filtration and washing technique, as previously described. 21 The composition of the preincubation and incubation media are given in the legend to Figure 1. This experiment was performed with at least three separate vesicle preparations.

RBC Cation Transport

To determine RBC cation transport, fresh blood was drawn into a heparinized syringe from the antecubital vein of a human volunteer or from the central ear artery of an adult male New Zealand White rabbit. The whole blood was centrifuged at 5000 g for 5 minutes at 4°C, and the plasma and buffy coat were removed. The efflux of lithium or sodium from human or rabbit RBCs was measured by methods modified from Canessa et al. 2 and are described in entirety as follows. One volume of packed RBCs was incubated in a shaking water bath for 3 hours at 37°C with five volumes of (in mM) 10 glucose, 10 Tris morpholinepropanesulfonic acid (MOPS), pH 7.4, and 150 LiCl or 150 NaCl plus 0.1 ouabain. The cells were centrifuged at 5000 g for 5 minutes at 4°C, and the packed cells were resuspended in ice-cold washing solution that contained (in mM) 75 MgCl2, 85 sucrose, 10 glucose, and 10 Tris MOPS, pH 7.4. This suspension was centrifuged at 5000 g for 5 minutes at 4°C, and five successive identical washing and centrifuging steps were performed to remove extracellular lithium or sodium. The hemotocrit of the washed suspension was measured. The efflux of lithium or sodium from the preloaded washed RBCs 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 centrifuge tubes. These tubes were immediately centrifuged at 5000 g for 3 minutes at 4°C, and the supernatants removed and stored in plastic tubes. The lithium or sodium concentrations of the supernatants were measured with a Corning 450 flame photometer (Corning Scientific Instruments, Medfield, MA, USA). The mean concentration of cation in the initial supernatants was subtracted from the mean concentrations in the subsequent supernatants, and the efflux of lithium or sodium into the different external media was calculated and expressed as millimoles per liter of RBCs.

The effect of Na+ on lithium efflux was measured by incubating lithium-loaded RBCs with 150, 25, or 0 mM NaCl and 0.1 mM LiCl and 0.1 mM ouabain, pH 7.4. The effect of Li+ on sodium efflux was measured by incubating sodium-loaded RBCs with 5, 1, 0.5, or 0 mM LiCl and 0.1 mM NaCl, pH 7.4. In all cases, choline chloride was added where necessary to maintain extracellular salt concentration at 150 mM. The effect of extracellular acidity on Na efflux was measured by incubating Na-loaded RBC with (in mM) 150 choline chloride, 0.1 mM ouabain, 10 Tris MOPS, pH 7.4, or 10 Tris morpholineethanesulfonic acid, pH 6.0. In addition, 4',4'-disothiocyanato-2,2'-disulfonic stilbene (DIDS) was present in the external media (0.125 mM) to help maintain the imposed pH gradients. All efflux experi-
ments were performed in the presence and absence of 1 mM amiloride. The exact composition of the external media is given in the legends to Figures 2 through 5. Each RBC transport experiment was performed on at least three separate occasions.

Materials
The $^{22}$Na (200 μCi/μg Na) was obtained from Amersham (Arlington Heights, IL, USA) and DIDS from Sigma Chemical (St. Louis, MO, USA). Amiloride HCl was a gift from Merck Sharp Dohme (West Point, PA, USA). All other chemicals were reagent grade.

Results

Brush Border Na$^+$-H$^+$ Exchange
In the presence of an outwardly directed proton gradient (pH$_{in}$ = 5.7, pH$_{out}$ = 7.4), the 10-second uptake of 1 mM $^{22}$Na in human brush border vesicles was stimulated sevenfold as compared with the absence of a pH gradient (pH$_{in}$ = pH$_{out}$ = 7.5). As shown in Figure 1, sodium uptake in the presence of an outwardly directed proton gradient was stimulated up to and including the 1-minute time point. At the 90-minute equilibrium time point, sodium uptake in control and acid-loaded vesicles was the same. When 1 mM amiloride was added to the incubation medium in the presence of an outwardly directed proton gradient, the stimulation of sodium uptake was inhibited. These data indicate that human renal brush border vesicles mediate Na$^+$-H$^+$ exchange and that amiloride can inhibit the Na$^+$-H$^+$ exchanger in these vesicles.

RBC Na$^+$-Li$^+$ Countertransport
The efflux of lithium from human RBCs into different external media was measured and plotted in Figure 2. The rates of lithium efflux into external media containing sodium were greater than the efflux rate into the sodium-free medium. The increment in lithium efflux rate caused by the presence of external sodium represents the activity of Na$^+$-Li$^+$ countertransport. The rates of lithium efflux into media containing 150, 25, or 0 mM NaCl were not affected by 1 mM amiloride. Thus, the activity of Na$^+$-Li$^+$ countertransport in human RBCs in the presence of 150 or 25 mM Na$_o$ was not inhibited by 1 mM amiloride.

RBC Li$^+$-Na$^+$ Countertransport
The Na efflux from rabbit RBCs was measured in the presence and absence of 1 mM amiloride and various concentrations of external lithium. The sodium efflux rate into lithium media minus the efflux rate into lithium-free media was taken as the activity of Li$^+$-Na$^+$ countertransport. As shown in Figure 4, the presence of 1 mM amiloride in the external media did not inhibit the activity of Li$^+$-Na$^+$ countertransport in the presence of 5, 1, or 0.5 mM external lithium.

Proton-Stimulated Na Efflux
Rabbit RBCs were preloaded with sodium and washed, and the efflux of sodium was measured in sodium-free and lithium-free external solutions at various pH concentrations, in the presence and absence of...
FIGURE 3. Effect of amiloride on Na\textsuperscript{+}-Li\textsuperscript{+} countertransport in rabbit RBCs. Lithium transport in rabbit RBCs was measured as described for human RBCs in the legend for Figure 2. Data presented are the results of a representative experiment.

1 mM amiloride. As shown in Figure 5, the efflux of sodium was stimulated when the external pH was lowered from 7.4 to 6.0. As also shown in Figure 5, the stimulation of sodium efflux by external protons was inhibited by 1 mM amiloride.

Discussion

In a number of studies, the activities of Na\textsuperscript{+}-Li\textsuperscript{+} countertransport in RBCs from patients with essential hypertension were elevated.\textsuperscript{2,3,6} The relation between these findings and elevated arterial pressure is obscure. It has been suggested that RBC Na\textsuperscript{+}-Li\textsuperscript{+} countertransport may be mediated by the same transport system as renal brush border Na\textsuperscript{+}-H\textsuperscript{+} exchange.\textsuperscript{1,10,16} If this were the case, increased RBC Na\textsuperscript{+}-Li\textsuperscript{+} countertransport activity could be a marker for increased Na\textsuperscript{+}-H\textsuperscript{+} exchange activity in the brush border membrane of the proximal tubule. Such an abnormality could cause increased proximal tubular reabsorption of salt and water, which could lead to a series of events resulting in elevated blood pressure.\textsuperscript{17,18} This attractive hypothesis would be strengthened if RBC Na\textsuperscript{+}-Li\textsuperscript{+} countertransport and renal brush border Na\textsuperscript{+}-H\textsuperscript{+} exchange were indeed mediated by the same system. Much circumstantial evidence gives credence to this possibility. The purpose of the present studies was to test the hypothesis that Na\textsuperscript{+}-Li\textsuperscript{+} countertransport in RBCs from humans and rabbits is mediated by the same transport system that mediates Na\textsuperscript{+}-H\textsuperscript{+} exchange in the renal brush border membrane from those species.

Data are presented that demonstrate that renal brush border vesicles from the human kidney mediate amiloride-sensitive Na\textsuperscript{+}-H\textsuperscript{+} exchange. Previous studies with renal brush border vesicles from the rabbit have reached the same conclusion.\textsuperscript{13,22} The present studies could not demonstrate the amiloride sensitivity of Na\textsuperscript{+}-Li\textsuperscript{+} countertransport in RBCs from humans or rabbits. In the rabbit RBCs, inhibition of countertransport by amiloride was not observed when the transport system operated in a Li\textsubscript{in} for Na\textsubscript{out} mode or a Na\textsubscript{in} for Li\textsubscript{out} mode. These findings suggest that RBC Na\textsuperscript{+}-Li\textsuperscript{+} countertransport and renal brush border Na\textsuperscript{+}-H\textsuperscript{+} exchange are different transport systems. Nevertheless, the same system may indeed be responsible for these
two transport activities, but amiloride is not able to effectively inhibit cation transport in the RBC membrane. This possibility seems unlikely since an amiloride-sensitive Na\(^+\)-H\(^+\) exchanger has been found in the RBC membrane from humans, dogs, and amphiuma.\(^{23-28}\) Although Na\(^+\)-H\(^+\) exchange is not demonstrable under normal conditions in these cells, an elevation in intracellular calcium\(^{29}\) or a decrease in cell volume\(^{23,28}\) clearly brings out this amiloride-sensitive cation translocation pathway. The present studies demonstrate that amiloride can also inhibit sodium transport in the rabbit RBC membrane. As shown in Figure 5, sodium efflux, which was stimulated by external protons, was abolished by 1 mM amiloride.

Amiloride is a competitive inhibitor of Na\(^+\)-H\(^+\) exchange in the renal brush border membrane.\(^{22}\) An inherent property of a competitive inhibitor is that a fixed concentration of inhibitor will become less effective as the substrate concentration is raised. It is important to consider whether the failure of 1 mM amiloride to inhibit RBC Na\(^+\)-Li\(^+\) countertransport in these studies was due to such kinetic restraints, rather than to an actual lack of sensitivity to amiloride. Indeed, amiloride is not an effective inhibitor of the Na\(^+\)-H\(^+\) exchanger in the presence of physiological concentrations of sodium.\(^{27}\) The ID\(_{50}\) of amiloride for the human RBC Na\(^+\)-H\(^+\) exchanger has been estimated to be about 17 \(\mu\)M.\(^{24}\) If human RBC Na\(^+\)-Li\(^+\) countertransport and human RBC Na\(^+\)-H\(^+\) exchange are mediated by the same process, then Michaelis-Menten inhibition kinetics predict that 1 mM amiloride should inhibit Na\(^+\)-Li\(^+\) countertransport by about 97% when the external sodium concentration equals its \(K_m\) value (25 mM).\(^{28}\) Figure 2 shows that amiloride did not inhibit human RBC Na\(^+\)-Li\(^+\) countertransport activity under these conditions. Figure 5 shows that about 90% of external proton-stimulated sodium efflux from rabbit RBCs was inhibited by 1 mM amiloride. Thus, the \(K_m\) of amiloride for this process is less than 0.11 mM. If rabbit RBC Na\(^+\)-Li\(^+\) countertransport and external proton-stimulated sodium efflux are mediated by the same process, then 1 mM amiloride should inhibit Na\(^+\)-Li\(^+\) countertransport by at least 82% when the external sodium concentration is less than its \(K_m\) value (50 mM).\(^{28}\) Figure 3 shows that amiloride did not inhibit it rabbit RBC Na\(^+\)-Li\(^+\) countertransport activity under these conditions. Thus, the failure of amiloride to inhibit RBC Na\(^+\)-Li\(^+\) countertransport probably was not due to kinetic restraints imposed by the external sodium and amiloride concentrations used in these studies. One must consider the possibility, however, that Na\(^+\)-H\(^+\) exchange and Na\(^+\)-Li\(^+\) countertransport are indeed mediated by the same system, but that the affinity for amiloride is absent or greatly reduced when the system operates in a sodium for lithium exchange mode. The present studies have not ruled out this possibility.

Jennings et al.\(^{28}\) recently reported studies designed to determine whether the rabbit RBC Na\(^+\)-Li\(^+\) countertransport system can also mediate Na\(^+\)-H\(^+\) exchange. These authors found that Na\(^+\)-Na\(^+\) exchange, an operative mode of the Na\(^+\)-Li\(^+\) countertransport system,\(^{9}\) occurred at a velocity over 10 mmol Na/L RBC/hr in rabbit RBCs. Despite this brisk transport rate, little stimulation of sodium efflux into sodium-free media was observed when extracellular pH was reduced from 7.5 to 6.9. These authors concluded that rabbit RBC Na\(^+\)-Na\(^+\) (or Na\(^+\)-Li\(^+\)) countertransport does not function appreciably in a Na\(^+\)-H\(^+\) exchange mode.\(^{28}\) The experimental conditions and results of the present study are different from those of Jennings et al.\(^{28}\) In the present study, amiloride-inhibitable sodium efflux from rabbit RBCs was stimulated by reducing extracellular pH from 7.4 to 6.0. The difference in the ability of external protons to stimulate sodium efflux in the present studies and those of Jennings et al.\(^{28}\) is not known, but it may be related to the larger increase in external proton concentration in the present study. Despite the differences in experimental conditions between the present study and that of Jennings et al.,\(^{28}\) the conclusions are the same: the RBC Na\(^+\)-Li\(^+\) countertransport system probably is not the Na\(^+\)-H\(^+\) exchanger.

The hypothesis that elevated Na\(^+\)-Li\(^+\) countertransport activity in RBCs from patients with essential hypertension is a marker for elevated Na\(^+\)-H\(^+\) exchange activity in the renal brush border membrane,\(^{10,16}\) would be a more attractive possibility if the two transport activities were mediated by the same system. The present studies detract from this hypothesis. Since Na\(^+\)-H\(^+\) exchange in sarcosomal vesicles from vascular smooth muscle is inhibitable by amiloride,\(^{29}\) the present data also detract from the recent suggestion by Funder et al.\(^{11}\) that elevated Na\(^+\)-Li\(^+\) countertransport activity may be a marker for increased vascular smooth muscle Na\(^+\)-H\(^+\) exchange activity.\(^{11}\) On the other hand, a physicochemical disturbance may exist in cell membranes throughout the bodies of patients with essential hypertension. Such a global membrane abnormality might increase the activities of both RBC Na\(^+\)-Li\(^+\) countertransport and renal brush border Na\(^+\)-H\(^+\) exchange, even if the two transport systems were distinct. If this were the case, RBC transport activity would be a marker for renal brush border transport activity. In support of this possibility, Weder\(^{30}\) recently has reported that RBC Na\(^+\)-Li\(^+\) countertransport activity was increased and renal fractional lithium clearance was decreased in patients with essential hypertension. The finding that renal lithium clearance was decreased suggests that proximal tubular sodium reabsorption by the brush border Na\(^+\)-H\(^+\) exchanger was increased in the hypertensive patients described by Weder.\(^{30}\)

Further studies are necessary to confirm the relation between RBC Na\(^+\)-Li\(^+\) countertransport and renal brush border Na\(^+\)-H\(^+\) exchange. The present studies demonstrate a difference between these two transport systems. The relation between RBC Na\(^+\)-Li\(^+\) countertransport and the hypertensive process remains obscure.

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