

Abnormal Membrane Sodium Transport in Liddle's Syndrome

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ABSTRACT We have documented the presence of abnormal sodium transport in Liddle's syndrome by measuring sodium concentration, sodium influx, and fractional sodium outflux in vitro in erythrocytes from normal subjects, two patients with Liddle's syndrome, and one patient with primary hyperaldosteronism. Sodium influx and fractional sodium outflux, but not sodium concentration, were significantly increased in patients with Liddle's syndrome. Sodium outflux in a patient with primary hyperaldosteronism did not differ significantly from normal. These alterations of sodium transport in erythrocytes from patients with Liddle's syndrome were not attributable to circulating levels of aldosterone, renin, angiotensin, or serum potassium. Furthermore, changes in aldosterone secretory rate and levels of circulating renin produced by varying dietary sodium intake, did not alter sodium influx or fractional sodium outflux in either patients with Liddle's syndrome or normal subjects. The response of fractional sodium outflux and sodium influx to ouabain, ethacrynic acid, and to changes in the cation composition of the incubation medium suggests that the increased sodium fluxes in Liddle's syndrome do not result solely from a quantitative increase in those components of sodium transport which occur in normal human erythrocytes. Instead, at least a portion of the increased erythrocyte sodium transport in Liddle's syndrome represents a component of sodium transport which does not occur in normal human erythrocytes.

INTRODUCTION

The primary defect in Liddle's syndrome (1) (hypertension, hypoaldosteronism, hypokalemia, decreased renin, and angiotensin) is unknown. A primary ab-

normality of renal sodium transport has been postulated (2), and the syndrome has been suggested to be a familial disorder affecting individuals of both sexes and of successive generations (1). If this syndrome reflects a generalized, inherited abnormality of sodium transport, other tissues may be involved. To explore this possibility we have measured sodium influx and fractional sodium outflux in erythrocytes from two patients with Liddle's syndrome. We have also studied the effects of ouabain, ethacrynic acid, and of altering the sodium and potassium concentrations in the external medium on bidirectional sodium fluxes.

METHODS

Erythrocytes from the following subjects were studied: 2 Caucasian sisters (G. L. age 9 and J. L. age 16) both of whom had hypoaldosteronism, hypokalemia, and hypertension with decreased circulating levels of renin and angiotensin (3), 17 normal females (age 8-30 yr), 15 normal males (age 17-34), and a 47 yr old male with primary hyperaldosteronism (hypokalemia, hypertension, decreased renin, and angiotensin) whose diagnosis was confirmed by subsequent surgical removal of an adrenal adenoma. All of these subjects had taken no medication for at least 3 wk before the time that they were studied.

Heparinized blood was obtained by venipuncture. The cells were separated by centrifugation at 3000 *g* for 5 min and the plasma and buffy coat removed by aspiration. The cells were then washed three times with isosmotic NaCl, divided into two portions and placed in identical preincubation solutions at approximately 10% hematocrit. ^{24}Na was added to the preincubation mixture containing erythrocytes which were subsequently used for sodium outflux determinations. At the end of 2 hr, samples were taken for determination of hematocrit and hemoglobin concentration. The cells were then separated by centrifugation and washed three times with isosmotic choline chloride. A portion of the washed cells was hemolyzed and diluted for the determination of sodium, potassium, and hemoglobin concentration. Hemoglobin was measured using the cyanmethemoglobin method (4). Sodium and potassium concentrations were measured with an Instrumentation Laboratory model 143 flame photometer (Instrumentation Laboratory,

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Inc, Lexington, Mass.). Erythrocyte sodium concentration was calculated after the method of Sachs and Welt (5).

Sodium outflux. The cells which were preincubated in the medium containing ^{24}Na were added to various incubation solutions (prewarmed to 37°C) at an hematocrit less than 4%. After mixing thoroughly, samples were taken at time "zero" and various intervals thereafter. The samples were chilled, the cells separated by centrifugation at 3000 *g* for 2 min, and a portion of the supernatant was taken for measurement of radioactivity. At some time during the experiment, a portion of the incubation mixture (i.e., cells plus medium) was sampled and its radioactivity measured. Initially, samples were taken at 0, 15, 30, 45, and 60 min; however, since the fractional sodium outflux was found to be constant over this period, fractional sodium outflux was calculated from samples taken at 0 and 30 min. Radioactivity was determined with a Packard model 3320 liquid scintillation spectrometer (Packard Instrument Co, Downers Grove, Ill.) or a Nuclear-Chicago model DS5 crystal scintillation spectrometer (Nuclear-Chicago Corp, Des Plaines, Ill.).

Fractional sodium outflux (*k*) was calculated from the following equation (6) :

$$k = \frac{-\ln (A_{30}/A_0)}{t}$$

k = fractional sodium outflux (hr^{-1})

A_0 and A_{30} = amount of radioactivity in a portion of cells at 0 and 30 min

t = time (hr).

Sodium influx. The cells which were preincubated in the nonradioactive medium were added to various prewarmed incubation solutions which contained ^{24}Na but were otherwise identical in composition with those used for outflux determination. The cells were mixed thoroughly and samples were taken at 0 and 30 min. The samples were chilled and the cells washed three times by alternate centrifugation and resuspension in cold isosmotic choline chloride. Radioactivity was measured with a Nuclear-Chicago model DS5

crystal scintillation spectrometer. After counting, the cells were hemolyzed, diluted to 50 ml, and the hemoglobin concentration of the hemolysate was measured. The volume of cells counted was calculated from the hemoglobin content of the hemolysate and the previously measured hemoglobin content per volume of cells. Changes in cell volume (determined gravimetrically) during the 30-min incubation period were negligible.

Sodium influx (J_{in}) was calculated from the following equation (6).

$$J_{\text{in}} = \left[\frac{kU}{1 - e^{-kt}} \right] / \text{SA}$$

J_{in} = sodium influx (millimoles/liter cells per hour)

U = uptake of radioactivity per liter of cells at time "t"

SA = specific activity of the incubation medium

t = time (hr).

The standard preincubation and incubation solutions had the following composition (mM): NaCl, 146; KCl, 4.0; K_2HPO_4 , 1.0; tris buffer (pH=7.4), 18; glucose, 11.1. Whenever the concentration of sodium or potassium was decreased, an equimolar amount of choline was substituted. Ouabain and ethacrynic acid were added at concentrations of 0.1 mM and 1.0 mM respectively.

Radioactivity was usually such that the standard deviation of the observed counts was 1% or less and all counts were corrected for decay. Liquid scintillation counting was performed using 10 ml of solution composed of 15 parts toluene (J. T. Baker Chemical Co., Phillipsburg, N. J.), 5 parts Triton X-100 (New England Nuclear Corp., Boston, Mass.) and 1 part Liquifluor (New England Nuclear Corp.).

RESULTS

Sodium concentration in erythrocytes from patients with Liddle's syndrome (6.35 ± 0.21 mmoles/liter cells) did not differ significantly from that of normal females (6.59 ± 0.84). Table I summarizes the data for fractional sodium outflux in normal female subjects and the two patients with Liddle's syndrome. In each of the four different incubation media studied, fractional sodium outflux in erythrocytes from patients with Liddle's syndrome was significantly greater than that from control erythrocytes. The decrease in fractional sodium outflux produced by removing potassium from a high-Na medium ($\text{Na} = 146$ mM) was significantly greater in erythrocytes from patients with Liddle's syndrome. The changes in fractional sodium outflux produced by removing potassium from a low-Na medium ($\text{Na} = 5$ mM) or those produced by removing sodium were not significantly different when erythrocytes from patients with Liddle's syndrome were compared with controls.

In a high-Na; high-K medium, fractional sodium outflux in erythrocytes from the patient with primary hyperaldosteronism (0.471/hr) did not differ significantly from fractional sodium outflux in 15 normal males (0.486 ± 0.039).

Table II summarizes the data for sodium influx in normal female subjects and the two patients with Liddle's

TABLE I
Effects of Altering External Sodium and Potassium
Concentrations on Fractional Sodium
Outflux, hr^{-1}

Incubation medium	Normal females (17)	Patient G. L.	Patient J. L.
<i>mM</i>			
1) Na = 146; K = 6	$0.452 \pm 0.048^*$	0.564	0.610§
2) Na = 146; K = 0	0.294 ± 0.032	0.361	0.411§
3) Na = 5; K = 6	0.489 ± 0.035	0.589	0.619§
4) Na = 5; K = 0	0.162 ± 0.020	0.258	0.294§
1)-2)	$0.158 \pm 0.020^\dagger$	0.203	0.199§
3)-4)	0.327 ± 0.038	0.331	0.325
1)-3)	-0.037 ± 0.034	-0.025	-0.009
2)-4)	0.131 ± 0.021	0.103	0.117
(1-2)-(3-4)	-0.169 ± 0.037	-0.128	-0.126§

Number of subjects in parentheses.

* Values expressed as mean ± 1 SD.

† Mean of paired differences ± 1 SD.

§ Both patients significantly different from normal females ($P < 0.02$) by Mann-Whitney U test (7).

The values for each patient with Liddle's syndrome were determined in triplicate.

TABLE II
Effects of Altering External Sodium and Potassium
Concentrations on Sodium Influx,
millimoles/liter cells per hour

Incubation medium	Normal females (17)	Patient G. L.	Patient J. L.
<i>mM</i>			
1) Na = 146; K = 6	1.01 ±0.18*	1.80	1.72§
2) Na = 146; K = 0	1.58 ±0.23	2.19	2.01§
3) Na = 5; K = 6	0.064 ±0.016	0.110	0.120§
4) Na = 5; K = 0	0.062 ±0.012	0.079	0.081§
1)-2)	-0.57 ±0.08‡	-0.39	-0.29§
3)-4)	0.002 ±0.01	0.031	0.039§
1)-3)	0.95 ±0.16	1.69	1.60§
2)-4)	1.52 ±0.22	2.11	1.93§
(1-2)-(3-4)	-0.57 ±0.08	-0.42	-0.33§

Number of subjects in parentheses.

* Values expressed as mean ±1 SD.

‡ Mean of paired differences ±1 SD.

§ Both patients significantly different from normal females ($P < 0.02$) by Mann-Whitney U test (7).

The values for each patient with Liddle's syndrome were determined in triplicate.

syndrome. Sodium influx was significantly greater in erythrocytes from patients with Liddle's syndrome in each of the four different incubation media studied. The increase in sodium influx produced by removing potassium from a high-Na medium was significantly less in erythrocytes from patients with Liddle's syndrome compared with controls. Removing potassium from a low-Na medium produced no change in sodium influx in control erythrocytes, but produced a significant decrease in sodium influx in erythrocytes from patients with Liddle's syndrome. The decrease in sodium influx, produced by removing sodium from a high-K ($K = 6$ mM) and a low-K medium ($K = 0$ mM), was significantly greater in erythrocytes from patients with Liddle's syndrome.

In a high-Na; high-K medium, sodium influx in erythrocytes from the patient with primary hyperaldosteronism (1.18 mmoles/liter cells per hr) did not differ significantly from sodium influx in 15 normal males (1.15 ±0.23).

In the patients with Liddle's syndrome there was no significant alteration in erythrocyte sodium concentration, fractional sodium outflux, or sodium influx when changes in aldosterone secretory rate and circulating renin levels were produced by varying dietary sodium intake. Similarly, in three normal adult males, ingestion of a high salt diet (350 mEq sodium daily for 6 wk) produced no significant change in erythrocyte sodium concentration, fractional sodium outflux, or sodium influx.

The increased fractional sodium outflux in Liddle's syndrome was reduced but not abolished by the addition of ouabain or ethacrynic acid to the incubation medium (Table III). Addition of either agent produced a significantly greater decrease in fractional sodium outflux

in Liddle's syndrome than in normals. The decrease in fractional outflux produced by adding ethacrynic acid to a medium containing ouabain was significantly greater in Liddle's syndrome. In contrast, the decrease in fractional outflux produced by adding ouabain to a medium containing ethacrynic acid was not significantly greater in Liddle's syndrome. Neither ouabain nor ethacrynic acid nor both abolished the increased fractional outflux in Liddle's syndrome since in each of the four different incubation media studied, outflux in erythrocytes from the two patients was significantly greater than that in normal erythrocytes.

The effects of ouabain and of ethacrynic acid on sodium influx were similar to their effects on outflux in that the addition of either or both of these agents reduced but did not abolish the increased sodium influx in Liddle's syndrome (Table IV). In normal erythrocytes, neither ouabain nor ethacrynic acid significantly altered sodium influx; in Liddle's syndrome each agent decreased sodium influx. In the two patients, adding ethacrynic acid to a medium containing ouabain decreased sodium influx, but adding ouabain to a medium containing ethacrynic acid did not alter influx. Neither ouabain nor ethacrynic acid nor both abolished the increased sodium influx in Liddle's syndrome since in each of these four different incubation media studied, sodium influx in erythrocytes from the two patients was significantly greater than that in normal erythrocytes.

DISCUSSION

The alterations in sodium influx and outflux in erythrocytes from two patients with Liddle's syndrome document the presence of abnormal sodium transport in non-

TABLE III
Effects of Ouabain and Ethacrynic Acid
on Fractional Sodium Outflux, hr⁻¹

Incubation medium	Normal females (17)	Patient G. L.	Patient J. L.
<i>mM</i>			
1) Na = 146; K = 6	0.452 ±0.048*	0.564	0.610§
2) + Ouab.	0.111 ±0.021	0.196	0.197§
3) + Eta.	0.238 ±0.022	0.279	0.296§
4) + Ouab. + Eta.	0.054 ±0.014	0.114	0.101§
1)-2)	0.341 ±0.049‡	0.368	0.413§
1)-3)	0.214 ±0.045	0.285	0.314§
2)-4)	0.057 ±0.023	0.082	0.086§
3)-4)	0.184 ±0.022	0.165	0.195
(1-2)-(3-4)	0.157 ±0.018	0.203	0.218§

Ouab., ouabain 0.1 mM; Eta., ethacrynic acid 1.0 mM.

Number of subjects in parentheses.

* Values expressed as mean ±1 SD.

‡ Mean of paired differences ±1 SD.

§ Both patients significantly different from normal females ($P < 0.02$) by Mann-Whitney U test (7).

The values for each patient with Liddle's syndrome were determined in triplicate.

TABLE IV
Effects of Ouabain and Ethacrynic Acid on Sodium
Influx, millimoles/liter cells per hour

Incubation medium	Normal females (17)	Patient G. L.	Patient J. L.
<i>mM</i>			
1) Na = 146; K = 6	1.01 ± 0.18*	1.80	1.72§
2) + Ouab.	0.98 ± 0.22	1.52	1.50§
3) + Eta.	1.04 ± 0.06	1.32	1.34§
4) + Ouab. + Eta.	1.06 ± 0.08	1.32	1.33§
1)-2)	0.03 ± 0.09‡	0.28	0.22§
1)-3)	-0.03 ± 0.17	0.48	0.38§
2)-4)	-0.08 ± 0.21	0.20	0.17§
3)-4)	-0.02 ± 0.07	0.00	0.01
(1-2)-(3-4)	0.05 ± 0.19	0.28	0.21§

Ouab., ouabain 0.1 mM; Eta., ethacrynic acid 1.0 mM.

Number of subjects in parentheses.

* Values expressed as mean ± 1 SD.

‡ Mean of paired differences ± 1 SD.

§ Both patients significantly different from normal females ($P < 0.02$) by Mann-Whitney U test (7).

The values for each patient with Liddle's syndrome were determined in triplicate.

renal tissue in this clinical disorder. The abstract of Helbock and Reynolds (8) reporting increased erythrocyte sodium uptake in a patient with Liddle's syndrome indicates that the altered erythrocyte sodium transport which we have observed in two sisters is related to the other abnormalities in Liddle's syndrome (1, 2) and does not represent an unrelated, independently inherited abnormality. The following observations suggest that these alterations represent an intrinsic abnormality of the erythrocyte rather than a secondary effect resulting from altered levels of some circulating substance. Hypokalemia can be excluded as an etiologic factor since we and others (9, 10) have demonstrated that decreasing external potassium increases sodium influx, decreases sodium outflux, and tends to increase the intracellular sodium concentration. In erythrocytes from Liddle's syndrome, sodium influx was elevated, fractional sodium outflux was increased, and the intracellular sodium concentration was normal. Chronic hypokalemia can be excluded as the cause of the altered erythrocyte sodium transport in Liddle's syndrome, since studies on erythrocytes from patients with chronic hypokalemia (9) indicate that the effects of chronically lowered external potassium can be rapidly reversed in vitro by adding potassium to the external medium.

The decreased levels of circulating renin and angiotensin found in patients with Liddle's syndrome can also be excluded as etiologic factors since erythrocyte sodium concentration, sodium outflux, and sodium influx in our patient with primary hyperaldosteronism (who also had decreased levels of renin and angiotensin) did not differ significantly from normal.

We¹ and others (11) found no effect of aldosterone on sodium fluxes in human erythrocytes in vitro. Spach and Streeten (12) reported that aldosterone decreased sodium exchange in canine erythrocytes; however, canine erythrocytes differ from human erythrocytes in that they have high intracellular sodium, low intracellular potassium, and no detectable Na⁺, K⁺-dependent ATPase (13). Since the effect of aldosterone on ion transport is thought to be secondary to its effect on protein synthesis (14), it is possible that aldosterone might alter erythrocyte sodium transport by acting on nucleated erythrocyte precursors. The normal values for fractional sodium outflux found in our patient with primary hyperaldosteronism argue against this possibility. It is also possible that the maximum effect of aldosterone on human erythrocyte sodium transport occurs at relatively low concentrations, and that only by lowering the circulating level of aldosterone can one detect an effect of this hormone on erythrocyte sodium transport. The normal values for sodium influx and sodium outflux obtained in erythrocytes from three normal subjects who had been salt-loaded for 6 wk, argue against this possibility. Furthermore, elevating the levels of circulating aldosterone in the patients with Liddle's syndrome produced no significant change in sodium outflux or sodium influx.

We cannot exclude unequivocally the presence in Liddle's syndrome of a circulating substance which might produce the observed effects on erythrocyte sodium transport. Using mannitol-¹⁴C as a marker for retained plasma, we observed that less than 1×10^{-7} of the original plasma was present at the beginning of the preincubation. When the additional dilution produced by the preincubation solution and the three washes before the incubation are considered, it is unlikely that any traces of plasma were present during the incubation. Thus, if such a circulating substance does exist it would have to be bound or concentrated by the erythrocyte, or its effect would have to persist for at least 3 hr after the erythrocytes were separated from the plasma. At least 40% of the inhibitory activity of cardiac glycosides (which are bound to the human erythrocyte membrane) (15) can be removed by repeated washing over a 2-hr period (11), and the effect of cardiac glycosides on rabbit erythrocytes is even more readily reversible (16).

Several observations suggest that at least part of the increased sodium fluxes observed in erythrocytes from patients with Liddle's syndrome result from a new component of sodium transport having characteristic responses to ouabain, to ethacrynic acid, and to alterations in the cation composition of the incubation medium rather than from an enhancement of the com-

¹ Unpublished data.

ponents of sodium transport which occur in normal human erythrocytes in vitro.

The major portion (approximately 80%) of the increased fractional sodium outflux in Liddle's syndrome was independent of the sodium or potassium concentration in the incubation medium. The remaining 20% was dependent on the presence of external potassium since removing potassium from a high-Na medium produced a greater decrease in fractional sodium outflux in erythrocytes from Liddle's syndrome. This portion was also dependent on the presence of external sodium since removing potassium from a low-Na medium produced an equivalent decrease in sodium outflux in Liddle's syndrome and controls. Others (6, 17) have demonstrated that when potassium is removed from the medium bathing human erythrocytes in vitro, the sodium-potassium exchange is partially replaced by a sodium-sodium exchange, and that the latter exchange can be abolished by reducing the external sodium concentration to approximately 5 mM. As is indicated by the data in Table I, the increased fractional sodium outflux observed in Liddle's syndrome is not attributable to an increase in sodium-potassium exchange (3-4) or to an increase in sodium-sodium exchange (2-4).

In erythrocytes from patients and controls, removing potassium from a high-Na medium produced an increase in sodium influx; however, removing potassium from a low-Na medium decreased sodium influx in Liddle's syndrome but produced no significant change in influx in normal erythrocytes. Our confidence about the absence of an effect of potassium on sodium influx in control erythrocytes incubated in a low-Na medium is somewhat limited by the small magnitude of the values for sodium influx; however, our precision is such that we should have been able to detect readily a 50% change in influx.

The effects of ethacrynic acid and of ouabain on sodium influx also suggest that at least a portion of the increased sodium fluxes in Liddle's syndrome represent a component of sodium transport which is not present in normal erythrocytes since neither ouabain nor ethacrynic acid significantly altered sodium influx in normal erythrocytes; however each agent decreased sodium influx in erythrocytes from patients with Liddle's syndrome. Furthermore, ouabain and ethacrynic acid had quantitatively similar effects on both the increased fractional outflux and the increased influx in Liddle's syndrome. Approximately 25% of the increase in both influx and fractional outflux was sensitive to ethacrynic acid. Another 35% of the increase was sensitive to both ethacrynic acid and to ouabain. There was no detectable portion of the increase in either influx or frac-

tional outflux which was sensitive to ouabain but not to ethacrynic acid. Approximately 40% of the increased sodium transport in Liddle's syndrome was not inhibitable by ouabain or by ethacrynic acid.

Our studies of erythrocyte sodium transport in Liddle's syndrome are not sufficient to exclude an alteration in cellular energy metabolism or in the process by which energy is coupled to sodium transport as the basic defect. We are also unable to specify whether the alterations which we have detected in Liddle's syndrome are present in all circulating erythrocytes or are restricted to a certain fraction of the total erythrocyte population. However, the purpose of this report is to document the presence of abnormal sodium transport in nonrenal tissue in Liddle's syndrome and to characterize this abnormality in terms of the effects of ouabain, ethacrynic acid, and altering the cation composition of the incubation medium on bidirectional sodium fluxes.

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