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Reduced Na^+,K^+-ATPase Activity in Patients with Pseudohypoaldosteronism

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ABSTRACT. Pseudohypoaldosteronism is a hereditary salt-wasting syndrome usually seen in infancy with weight loss, dehydration, and failure to thrive. The pathophysiologic origin of pseudohypoaldosteronism is unknown. The defect could be related to the unresponsiveness of target organs to mineralocorticoids resulting in hyponatremia, hyperkalemia, and markedly elevated plasma aldosterone and renin levels. Red blood cell Na^+,K^+-ATPase activity was measured in a pair of twins with pseudohypoaldosteronism, in an unrelated child with hypoaldosteronism, and in an age-matched group of 50 healthy infants and young children. The enzyme was assayed by a method that couples ATP hydrolysis with NADH oxidation. Plasma renin and aldosterone levels were measured by RIA. Red blood cell Na^+,K^+-ATPase activity in the twins with pseudohypoaldosteronism was very low at the time of diagnosis (3 wk). In both twins a time-related gradual increase in enzyme activity was observed during the 1st mo of life, reaching control values between 6 and 8 mo of age. This increase was associated with both a reduction in salt requirement and clinical improvement. Plasma renin activity and aldosterone levels were very high at the time of diagnosis. Plasma renin activity reverted gradually to normal values, whereas aldosterone levels remained high throughout the follow-up period. The child with hypoaldosteronism had normal Na^+,K^+-ATPase activity at diagnosis and during follow-up. (*Pediatr Res* 35: 372–375, 1994)

Abbreviations

PHA, pseudohypoaldosteronism
RBC, red blood cell
HA, hypoaldosteronism
PRA, plasma renin activity

PHA is a syndrome usually seen in infancy, and it is characterized by salt wasting, dehydration and failure to thrive. The prominent metabolic manifestations are natriuresis and hyponatremia, blunted renal excretion of potassium and hyperkalemia, and elevated serum renin and aldosterone levels (1). The degree of the clinical manifestations varies significantly, ranging from severely affected patients who die in infancy (2, 3), to patients without symptoms (4–6). Treatment consists of sodium supplementation, usually up to the age of 2 y (7, 8). HA is an inborn error in aldosterone biosynthesis found mainly among Jews of Iranian origin (9, 10). The clinical expression of this salt-wasting syndrome varies widely with age and is particularly severe in infancy. Spontaneous improvement in sodium conservation occurs as the individuals become older. Aldosterone deficiency in the face of overproduction of zona glomerulosa 18-hydroxycorticosterone (10) was found, an abnormality for which the term type 2 corticosterone methyl-oxidase defect was coined (11). Treatment with mineralocorticoids and high-sodium diet reduces the secretion of the aldosterone precursor, restores electrolyte balance, and normalizes the growth rate (3).

Little information has been added in regard to the pathophysiologic expression of PHA since its original description by Cheek and Perry (12) in 1958. It is believed that patients with PHA represent a blunted target organ responsiveness to aldosterone either because of a reduced number in mineralocorticoid (type 1) receptors (13) or a low receptor affinity to aldosterone (14). A defect in sodium reabsorption along the proximal tubule and the loop of Henle has also been suggested (15). Low Na^+,K^+-ATPase activity in both proximal and distal tubular segments in a child with PHA has been demonstrated previously (16). No further data substantiating these findings have been published. In the present study we investigated RBC Na^+,K^+-ATPase activity in a pair of twins with PHA and in an infant with HA to clarify the pathophysiologic expression of PHA.

PATIENTS AND METHODS

Patients. The patients with PHA were dizygous twins, female and male, born to unrelated white Jewish parents of North African origin. Family history did not reveal neonatal deaths or symptoms suggestive of PHA during infancy. The pregnancy was complicated by diabetes, which was treated by dietary restriction. The twins were born at 35 wk of gestation weighing 2.3 and 2.2 kg, respectively. At the age of 3 wk, they were hospitalized because of vomiting, lethargy, and loss of appetite and weight. Laboratory evaluation established the diagnosis of PHA (1).

The male infant with HA was born at term after an uncomplicated pregnancy and delivery to unrelated white Jewish parents of Turkish origin. His birth weight was 2.6 kg. He was the 1st-born child. Family history did not reveal neonatal deaths or symptoms suggestive of a salt-wasting disorder. At the age of 2 mo he was admitted because of vomiting and failure to thrive. Laboratory evaluation established the diagnosis of HA (3).

Samples. Preparation of RBC ghosts (17) was performed on blood collected on Na EDTA and centrifuged at 600 × *g* for 30 min (4°C). Packed RBC were washed three times in 0.9% NaCl with a 5-min centrifugation after each wash. RBC were hemolyzed in 10 mM Tris/1 mM EDTA, and ghosts were sedimented at 27 000 × *g* for 20 min (4°C). The pellet was washed two to four times in 10 mM Tris (pH 7.4) with a 10-min centrifugation at 27 000 × *g* (4°C) after each wash. Membranes were resuspended in 20 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (pH 7.4)/2 mM DTT.

Total and ouabain-sensitive ATPase activities were measured

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spectrophotometrically at 37°C in a thermostated Gilford 2600 spectrophotometer (Gilford Instruments Lab. Inc., Oberlin, OH) with a method that couples ATP hydrolysis with NADH oxidation (18). Na^+,K^+-ATPase activity was calculated by subtracting ouabain-sensitive ATPase activity from total ATPase activity. The final composition of the reaction mixture was as follows: 50 mM Tris-HCl (pH 7.4); 130 mM NaCl; 5 mM KCl; 3 mM $MgCl_2$; 3 mM vanadium-free Tris-ATP; 1.5 mM NADH; 0.83 mM phosphoenolypyruvate; 1 IU/mL pyruvate kinase; and 1 IU/mL lactate dehydrogenase. Ouabain concentration, when present, was 1 mM. The reaction was started by the addition of 50- to 75-μg membrane protein and run in triplicates. Results were expressed as nanomoles of NADH oxidized per minute per milligram of protein. Analysis of variance for precision within and between assays yielded p values for the F ratios that were >0.5. The lower limit of detectability was 0.2 nmol NADH/min/mg protein. The amount of membrane proteins was measured by a modification of the method of Lowry *et al.* (19) with a commercial kit (Sigma Chemical Co., St. Louis, MO; procedure no. P5656).

RBC Na^+,K^+-ATPase activity was determined in the twins with PHA and their parents, in the infant with HA and his parents, and in 50 healthy infants of both sexes ages 1 to 18 mo. Informed consent approved by the Helsinki Committee of our hospital was obtained from the parents.

Hormones were determined by RIA commercial kits: aldosterone, 17-hydroxyprogesterone, dehydroepiandrosterone sulfate, cortisol (Diagnostic Products Co., Los Angeles, CA); renin (New England Nuclear, Boston, MA); and testosterone (Zer Sci Based Industries Ltd., Jerusalem). Sweat samples were collected by pilocarpine iontophoresis, and saliva was collected from the sublingual region.

Statistics. Results of RBC Na^+,K^+-ATPase activity in controls are presented as the mean ± SD. The statistical significance between patients and controls was assessed by t test for unpaired series. The p values < 0.05 were considered significant. The relationship between RBC Na^+,K^+-ATPase activity and plasma renin and aldosterone, respectively, in the two patients was assessed by linear regression analysis.

RESULTS

Initial laboratory evaluation performed on admission at the age of 3 wk in the twins with PHA revealed marked hyponatremia and hyperkalemia. Urine sodium concentrations were high, and urine potassium levels were low. Sweat sodium and chloride and saliva chloride concentrations were normal (Table 1). Plasma levels of 17-hydroxyprogesterone, cortisol, testosterone, and dehydroepiandrosterone sulfate were normal, thus excluding congenital adrenal hyperplasia. Ultrasonography of the adrenals demonstrated normal-sized glands.

Treatment with a high-sodium diet (8 g/d) was commenced. Serum sodium and potassium values reverted to normal within 10 d and remained so during the 18 mo of follow-up. Sodium intake was gradually tapered, and both children, at the age of 18 mo, have been maintained on 2 g/d of salt (3 mEq/kg/d). Physical and psychomotor development was normal in both.

PRA, initially high, reverted gradually to normal values within 6 to 8 mo in both patients (Table 2). Plasma aldosterone values were markedly elevated in both twins. Although a gradual time-related decrease was observed, values were still significantly high at the age of 18 mo (Table 2). Both parents had normal PRA levels. However, plasma aldosterone levels were significantly high (2180 and 2400 pmol/L and 2208 and 2050 pmol/L for the father and mother, respectively; normal, 55–388 pmol/L).

Laboratory evaluation of the child with HA performed on his admission at the age of 2 mo revealed hyponatremia (122 mEq/L), hyperkalemia (7.0 mEq/L), high urinary sodium concentration (120 mEq/L) and low urinary potassium level (17 mEq/L). PRA was high [37 ng/(L·s)], but plasma aldosterone value was very low (<137 pmol/L). Hormonal studies excluded congenital adrenal hyperplasia. Both parents had normal PRA and aldosterone levels. Treatment with high-sodium diet (10 g/d) and 9α-fluorohydrocortisone (Florinef, 0.05 mg/d) was started. Serum electrolytes normalized within 2 wk and remained so during the 14 mo of follow-up. Although a gradual time-related decrease of PRA was observed, the level at the age of 6 mo was still high [4.4 ng/(L·s); normal, <2.8], and normal [0.8 ng/(L·s)] at the age of 12 mo. Plasma aldosterone values during the follow-up were within the normal range. The child has been growing well, and no salt-wasting crises have been observed.

Compared with control values, RBC Na^+,K^+-ATPase activity was very low in the twins with PHA when admitted at the age of 3 wk. A gradual age-related increase of enzymatic activity was observed in both patients, reaching values similar to the control group at the age of 6 to 8 mo (Table 3). In the control group a mild but insignificant reduction in RBC Na^+,K^+-ATPase activity with age was observed (Table 3).

The child with HA had normal Na^+,K^+-ATPase activity at diagnosis and at the age of 6 and 12 mo (7.4, 8.6, and 7.1 nmol NADH/min/mg protein, respectively).

A significant inverse correlation between RBC Na^+,K^+-ATPase and both PRA and plasma aldosterone was observed in both twins with PHA. The correlation coefficients were 0.91 and 0.95 for the girl ($p < 0.02$) and 0.75 and 0.88 for the boy ($p < 0.05$). Normal RBC Na^+,K^+-ATPase activity was observed in both

Table 1. *Initial clinical and biochemical data in two patients with PHA*

Characteristic	Girl	Boy	Normal
Weight (kg)	2.1	1.9	
Serum sodium/potassium (mmol/L)	122/9.1	120/8.5	135–146; 3.5–5.0
Urinary sodium/potassium (mmol/L)	65/5	37/4	Ratio <2
Sweat sodium/chloride (mmol/L)	20/22	24/20	<40
Saliva chloride (mmol/L)	10	12	<30

Table 2. *PRA and plasma aldosterone concentrations in twin infants with PHA by age**

	Girl		Boy	
Age (mo)	PRA [ng/(L·s)]	Ald (pmol/L)	PRA [ng/(L·s)]	Ald (pmol/L)
0.75	154	19 700	91	32 140
4	6.8	12 302	7.3	30 304
6	4.3	11 919	1.7	13 055
8	1.2	10 987	1.6	10 768
10	1.0	10 768	0.9	10 494
18	1.3	2 400	1.1	4 072

* The PRA values in healthy children age 7 d to 1 y were up to 2.8 ng/(L·s). Plasma aldosterone levels in healthy infants ages 7 d to 3 mo were up to 3000 pmol/L, and the levels in those ages 4 mo to 1 y were up to 900 pmol/L. Ald, plasma aldosterone.

Table 3. *RBC Na^+,K^+-ATPase activity in twin infants with PHA and control infants by age**

Age (mo)	Girl	Boy	Control†	Range
0.75	<0.20	<0.20	6.3 ± 0.99 (6)	4.7–7.2
4	2.95	1.53	5.3 ± 1.2 (7)	4.1–7.2
6	3.84	2.27	5.12 ± 1.21 (17)	4.0–7.5
8	5.17	4.30	4.6 ± 1.0 (8)	3.4–7.4
10	5.23	4.97	4.4 ± 0.7 (6)	3.8–5.9
18	5.48	5.27	4.7 ± 1.1 (6)	3.8–6.9

* Activity measured in nanomoles of NADH per minute per milligram of protein.

† Number in parentheses signifies number of control children studied.

parents of the twins with PHA (8.5 in the father and 7.2 nmol NADH/min/mg protein in the mother) and in the parents of the child with HA (father, 5.4 and mother, 5.9 nmol NADH/min/mg protein).

DISCUSSION

PHA is a genetic syndrome characterized by severe disturbances in sodium, potassium, and water balance and by elevated renin and aldosterone levels. It is considered to be a self-limited condition, and clinical remission after apparent normalization in electrolyte balance and PRA occurs usually within the 1st few years of life (3). In contrast, elevated serum aldosterone levels persist into adulthood (3–6, 13, 20). Adults with PHA but without symptoms seem to be more prone to have electrolyte disturbances under stressful conditions (20). These findings suggest that the basic defect, compensated to some degree, persists throughout life.

The pathogenesis of PHA is still unclear. A decrease in mineralocorticoid (type 1) receptors has been reported in the monocytes of both children with PHA and symptoms and also in adults after complete clinical remission (13). A decrease in mineralocorticoid receptors is observed also in patients with primary hypoaldosteronism (21). Thus, the reduced number of receptors may reflect a down-regulation of the receptors as a result of the persistently elevated aldosterone levels in PHA (14, 20) and may not be the primary pathogenetic mechanism.

In 1976, Bierich and Schmidt (16) failed to detect any Na^+,K^+-ATPase activity in the proximal and distal nephron segments of a child with PHA. Recently, Cugini *et al.* (22) observed low Na^+,K^+-ATPase activity and reduced Na^+,K^+ transmembrane flux in RBC of a child with PHA. Similar findings, although less pronounced, were observed in the child's mother as well, who did not have symptoms. In the twins we studied, RBC Na^+,K^+-ATPase activity was significantly reduced at the age of 3 wk and increased gradually to normal levels within several months. No immunologic methods for the determination of the enzyme were performed. Therefore, the reduced activity found by us and by others (16, 22) may not necessarily reflect a true reduction in the number of enzyme units. A circulating endogenous inhibitor reducing its activity could also be considered, but were it so, we have to assume its persistence into adulthood. The change in RBC enzyme values correlated with gradual clinical improvement, reduction in salt intake, and normalization of PRA levels. It is therefore tempting to assume that a similar increase in Na^+,K^+-ATPase occurring in the renal tubules as well may have contributed to the improvement in water and electrolyte balance.

Aldosterone modulates Na^+,K^+-ATPase activity by an increase in net sodium and potassium transport by alterations in conductance of these electrolytes in the apical membrane and tight junction of the cortical collecting duct (23, 24). The very low Na^+,K^+-ATPase activity observed by us and by others (16, 22) may either result from a relative aldosterone unresponsiveness causing a reduction in transmembraneal electrolyte fluxes or reflect a primary defect in the activity of the enzyme. The normal Na^+,K^+-ATPase activity found in the child with HA despite very low levels of plasma aldosterone favors the idea of a primary defect in Na^+,K^+-ATPase in children with PHA. Previous reports (14, 25) have demonstrated a lack of response in intracellular Na^+ and K^+ concentrations to aldosterone in mononuclear leukocytes of patients with PHA. This lack of response may represent the net result of the effect of aldosterone on membrane permeability of Na and its direct stimulation of the number of Na-K pump sites (26), resulting in an increase in sodium extrusion from the cells.

Aldosterone probably does not affect Na-K fluxes in the mature RBC of normal individuals, a finding possibly reflecting the lack of aldosterone receptors in RBC (27). Thus, we suggest that the alterations in mature RBC Na^+,K^+-ATPase activity observed by us and by others (22) may reflect its action on the nucleated precursor cells during the early erythropoietic phase. In the children with PHA whom we studied, aldosterone levels decreased as RBC Na^+,K^+-ATPase activity increased. Therefore, we have to invoke a time-related increase in responsiveness to aldosterone by the erythropoietic cells.

In both twins with PHA and in the infant with HA, serum sodium and potassium concentrations reverted to normal values within 10–14 d. The RBC Na^+,K^+-ATPase activity in the twins with PHA increased to normal values within 6–8 mo, whereas the infant with HA had persistently normal values. Therefore, it seems unlikely that the difference in Na^+,K^+-ATPase activity between the twins with PHA and infant with HA reflected differences in serum electrolytes concentration.

Recently, it has been suggested that PHA includes two clinically and genetically distinct entities with either renal alone or multiple target organ defects (20). The renal type is characterized by an autosomal dominant transmission; clinically it is self-limited, but elevated aldosterone levels tend to persist, whereas PHA affecting multiple organs is inherited as an autosomal recessive disease. The biochemical and hormonal perturbations in our patients with PHA and the elevated aldosterone levels in their parents are compatible with the renal form of the disease (20).

Despite the increased Na^+,K^+-ATPase activity with age found in our patients, the aldosterone levels remain high in patients with PHA, and even in the adults without symptoms (3–6, 13, 20). Chronic salt depletion and the resultant hyperreninemia could stimulate zona glomerulosa leading to hypertrophy of the zone and secondary hyperaldosteronism. Additionally, the possibility that target organ defects may cause stimulation of aldosterone cannot be excluded.

We suggest that a reduced Na^+,K^+-ATPase activity may play a primary role in the cause of PHA. It may well be that the gradual normalization of enzyme levels associated with clinical remission is possibly achieved and maintained throughout life only by persistently excessive production of aldosterone by its effects on sodium and potassium cellular transmembrane conductance or its direct effect by increasing the number of Na^+,K^+-ATPase pump sites as previously suggested (26).

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