ABNORMAL ERYTHROCYTE NA, K-ATPASE ACTIVITY IN A NORTHEASTERN THAI POPULATION

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Abstract. We studied the cellular membrane enzyme responsible for potassium transport in different Thai populations. We measured plasma and intraerythrocytic concentrations of sodium and potassium, activities of erythrocytic membrane Na, K-activated adenosine triphosphatase (Na, K-ATPase), ouabain-insensitive ATPase, total ATPase and the activity ratio of Na, K-ATPase/total ATPase in 25 healthy blood donors at Khon Kaen University Hospital, Khon Kaen (group 1), and in 32 donors at the National Blood Center, Thai Red Cross Society, Bangkok (group 2). Group 1 subjects had significantly higher concentrations of erythrocyte sodium ($p < 0.001$) and lower activity of Na, K-ATPase ($p < 0.001$) than group 2. When data of these 2 groups were combined, erythrocyte Na^+ correlated inversely with Na, K-ATPase and the activity ratio of Na, K-ATPase/total ATPase. Our study suggests that there is a defect in membrane transport enzymes for sodium/potassium in certain northeast Thai populations.

INTRODUCTION

Sodium and potassium-activated ATPase (Na, K-ATPase, EC 3.6.1.3) is a major ATP-dependent enzyme of cell membranes. It couples outward transport of $3Na^+$ with inward transport of $2K^+$ per 1 ATP. Abnormalities in number or function of Na, K-ATPase on red cell membranes have been reported in various diseases such as hypertension (Rahman *et al,* 1986; Quintanilla *et al,* 1988), hyperthyroidism (Cok and Waddel, 1976; Dasmahapatra *et al,* 1985), diabetes (Finotti and Palatini, 1986), chronic renal failure (Lok, 1973), liver disease (Alam *et al,* 1978), and sickle cell anemia (Izumo *et al,* 1987). Ethnic and gender disparity in activity of the enzyme has also been observed (Lasker *et al,* 1985).

We previously reported that healthy controls as well as patients with renal stone disease from northeast Thailand had a high incidence of hypokalemia associated with low urinary potassium excretion. This could be reversed by potassium supplementation (Sriboonlue *et al,* 1991). Moreover, intraerythrocytic potassium concentration (RBC K) was lower during the hot season than during the cool season (Tungsanga *et al,* 1990). The data indirectly suggest a potassium-depleted state among residents of northeast Thailand. There might be an additional abnormality in membrane transport of potassium. In this communication we describe our studies of intraerythrocyte sodium concentration (RBC Na), RBC K and activities of erythrocytic membrane total ATPase (TATPase) and Na, K-ATPase of controls from Bangkok, central Thailand, and Khon Kaen in northeast Thailand.

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MATERIALS AND METHODS

The study was carried out in 2 population groups. Group 1 consisted of 25 healthy blood donors at the Blood Center, Khon Kaen University Hospital (400 km northeast of Bangkok). All were ethnic Thai-Lao. Group 2 consisted of 32 healthy donors at the National Blood Center, Thai Red Cross Society, Bangkok. They were Central Thai or Thai-Chinese. All subjects were male with ages ranging from 20-46 years (30 ± 2) and 20-45 years (34 ± 1) respectively. They had normal general physical examinations and urinalyses.

Plasma concentrations of sodium and potassium were measured by flame photometry. Red cells

were separated from heparinized blood by refrigerated centrifugation and processed as previously described by Hanahan and Eckholm (1978). They were washed thrice with 112 mM $MgCl_2$. An aliquot of the cell suspension was kept for measuring intracellular RBC Na and RBC K by the method of Mayer and Starkey (1977). The remaining part was washed thrice with ice-cooled 155 mM NaCl buffered with 0.003 mM histidine, pH 7.5, and hemolysed with 0.1 mg/ml saponin in the same buffer. After centrifugation at 30,000*g* for 30 minutes, the membrane was washed and resuspended in NaCl-histidine buffer. It was stored at -20°C until use within 1-3 days.

The membrane suspension was used immediately after thawing. The mean protein concentration of the suspension as measured by the method of Lowry *et al* (1951) was 4 mg/ml. A 0.1 ml aliquot of membrane suspensions was incubated at 37°C, for 90 minutes in a 0.4 ml solution containing 100 mM NaCl, 20 mM KCl, 1 mM ethylene glycol-bis (beta-aminoethyl ether) N, N, N', N'-tetraacetic acid (EGTA), 2 mM $MgCl_2$, 2 mM ATP and 100 mM Tris, pH 7.5 (DeLuise and Charles, 1982). Another aliquot of the membrane suspension was incubated under the same conditions with 1 mM ouabain added to inhibit Na, K-ATPase. The reaction was stopped by addition of trichloroacetic acid. Phosphorus release was quantitated by the method of Lawrence (1974). Total ATPase activity was expressed as nmoles inorganic phosphorus (Pi) release/mg protein/hour (nmol Pi/mg/hour), ouabain-insensitive ATPase activity as the amount of Pi released after incubation with ouabain, and ouabain-sensitive ATPase activity as the difference between the two values. Intraassay variation of Na, K-ATPase activity was less than 4.9% and interassay variation after refrigeration at -20°C for 5 days was less than 6.5%.

Data were expressed as mean ± SEM. Statistical significance of the values was analysed by unpaired *t*-test and linear regression using the microcomputer SPSS/pc$^+$ program (Norusis, 1986).

RESULTS

Plasma sodium, plasma potassium, and RBC K of groups 1 and 2 did not differ significant (Table 1). The RBC Na was significantly higher in group 1 than in group 2 ($p < 0.001$). Using a mean + 2SD value of RBC Na of Bangkok blood donors (group 2) as an upper normal limit, about 80% of group 1 cases had high RBC Na.

Activities of erythrocytic membrane TATPase, ouabain-insensitive ATPase and Na, K-ATPase of group 1 and groups 2 subjects are shown in Fig 1. There was no significant difference in TATPase enzyme activity between the 2 groups. However, group 1 had higher ouabain-insensitive ATPase and lower Na, K-ATPase activities than group 2 (95 ± 5 vs 68 ± 4 nmol Pi/mg/hours, $p = 0.001$; and 52 ± 4 vs 98 ± 5 nmol Pi/mg/hour, $p < 0.001$, respectively).

The activity ratio of erythrocytic membrane Na, K-ATPase/TATPase was significantly lower in group 1 than in group 2 (36 ± 2% vs 59 ± 2%, $p < 0.001$, Fig 2). Using a mean minus 2SD value of the Na, K-ATPase/TATPase activity ratio of group 2 as a lower limit of normal, about 80% of group 1 subjects had the ratio below the normal range.

Table 1

Levels of Na and K concentration in plasma and in erythrocytes in group 1 (Khon Kaen) and group 2 (Bangkok) subjects (mean ± SEM).

Subjects	Plasma(mmol/l)		Erythrocytes (mmol/l)	
	Na	K	Na	K
Group 1 mean	139 ± 4	4 ± 0.3	13.6 ± 3.1	99.4 ± 7.8
range	(129 - 148)	(3.4 - 4.9)	(7.2 - 20.8)	(75 - 110)
Group 2 mean	139 ± 1	4 ± 0.3	8.1 ± 1.4	92.4 ± 8.9
range	(129 -156)	(3.5 - 4.6)	(5.5 - 10.9)	(81 - 113)
p-value	NS	NS	<0.001	NS

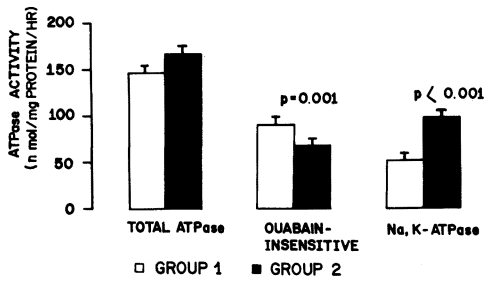

Fig 1—Mean (+ SEM) erythrocyte membrane total ATPase, ouabain-insensitive ATPase and Na^+, K^+-ATPase activities (nmol/mg protein/hour) in group 1 (open bars) and group 2 (closed bars).

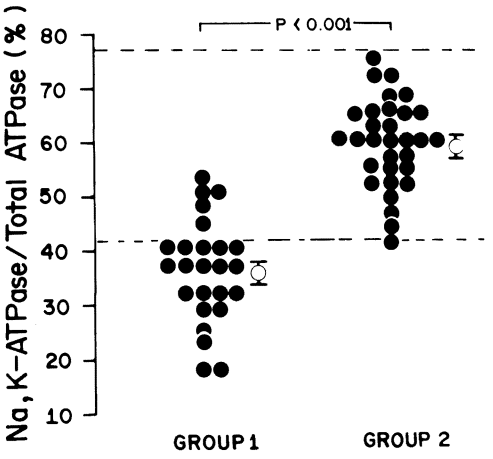

Fig 2—Distribution of activity ratios of Na, K-ATPase/total ATPase of erythrocytic membrane in grops 1 and 2. The open circles and vertical lines denote mean ± SEM. The hatched lines denote mean ± 2SD range of group 2 data.

There was no correlation between RBC Na or RBC K and activities of erythrocyte ATPases in each group. However, when data from groups 1 and 2 were combined, there was good correlation between RBC Na and erythrocyte Na, K-ATPase ($r = 0.457$, $p = 0.0003$) and the activity ratio of Na, K-ATPase/TATPase ($r = 0.592$, $p = 0.0001$) (Fig 3).

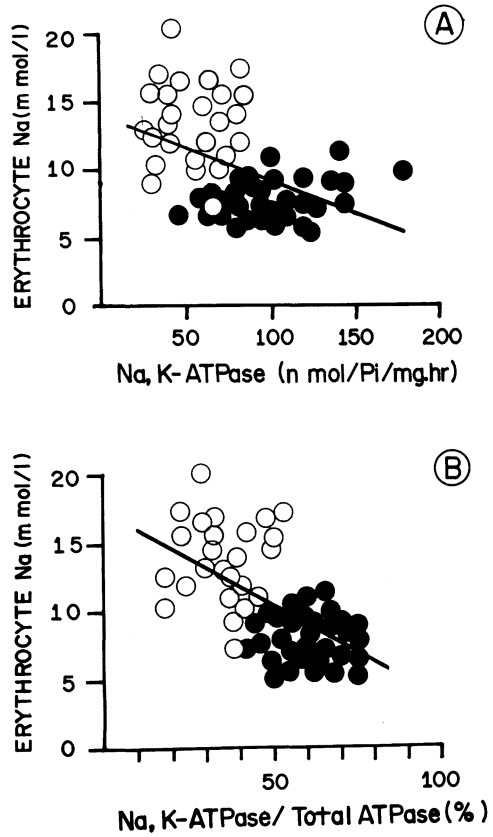

Fig 3—Correlations between erythrocyte sodium (RBC Na) and (A) ouabain-sensitive ATPase (Na, K-ATPase), ($r = 0.457$, $p = 0.0003$) or (B) activity ratio of Na, K-ATPase/total ATPase ($r = 0.592$, $p = 0.0001$) in combined group 1 (open circles) and group 2 (closed circles) subjects.

DISCUSSION

Increased intracellular sodium usually stimulates membrane Na, K-ATPase activity (Clausen and Kjeldsen, 1987). Thus, the greater the intracellular sodium, the greater the activity of membrane Na, K-ATPase. In this study the inverse relationships between RBC Na and activity of erythrocyte Na, K-ATPase or the activity ratio of Na, K-ATPase/TATPase as seen in Fig 3 appear contradictory. Despite high RBC Na in group 1 subjects, erythrocytic membrane Na, K-ATPase activity was low, whereas it would be expected to be elevated. This suggests a quantitative or qualitative diminution

in the function of Na, K-ATPase causing secondary retention of intracellular sodium. However, a primary abnormality in membrane permeabiltiy for the sodium influx causing increased RBC Na could not be ruled out.

We have no explanation for the lower activity of erythrocyte Na, K-ATPase and higher activity of ouabain-insensitive ATPase in group 1 than in group 2 subjects. Low activity of the Na, K-ATPase enzyme has been observed in various clinical disorders (Rahman *et al,* 1986; Quintanilla *et al,* 1987; Cok and Waddel, 1976; Dasmahapatra *et al,* 1985; Finotti and Palatini, 1986; Lok, 1973; Alam *et al,* 1978; Izumo *et al,* 1987). None of these were present in our subjects. Difference in nutrient intake between these 2 groups might be one explanation. Potassium depletion can increase erythrocytic membrane Na, K-ATPase activity, numbers of the Na, K-ATPase enzyme and membrane permeability for sodium influx (Chan and Sanstone, 1969; Clausen and Kjeldsen, 1987; Hoffmann and Smith, 1970). A potassium-depleted state has been found in healthy populations of northeast Thailand (Sriboonlue *et al,* 1991). However, one may argue that potassium deficiency was unlikely to be responsible for a decrease in Na, K-ATPase function in group 2 subjects because plasma potassium and RBC K were comparable between groups 1 and 2, and the Na, K-ATPase activity of group 2 was found to be decreased rather than increased. High salt intake also can lower membrane Na, K-ATPase activity (Quintanilla *et al,* 1988). However, the average salt intake among healthy residents in northeast Thailand is as low as 73 ± 2 mEq/day as opposed to 200 ± 10 mEq/day among Bangkok citizens (Puwastien *et al,* personal communication). These data do not support a role for high salt intake as the cause of low activity of the Na, K-ATPase in group 1. An influence from other nutrients or from genetic predisposition can not be ruled out.

Whatever the explanation, our findings may have potential clinical implications because low activity of cell membrane Na, K-ATPase and/or increased cell sodium may affect cell pH, cell volume and resting membrane potential. Alteration of other organ function may develop if this derangement reaches a critical point. We recently have proposed an association between defective membrane ATPase enzymes and various clinical disorders commonly observed in northeast Thailand such as renal stone disease, the sudden unexplained death syndrome, and renal tubular acidosis (Sitprija *et al,* 1991). Further studies for the Na, K-ATPase enzyme function in these diseases may give us a better understanding of their pathophysiologic mechanisms and clinical significance.

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