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REDUCED ACTIVITY OF ERYTHROCYTE Na, K-ATPase IN HYPOTHYROID CHILDREN*

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The maintenance of low intracellular sodium and high potassium concentrations is a function of the membrane bound enzyme Na,K-ATPase[1]. It has been postulated that the major role of the thyroid hormone is stimulation of Na,K-ATPase activity, probably mediated by synthesis of new enzyme units[2]. In the literature a study concerning the effects of thyroid status on the electrolyte distribution in rat tissues demonstrated that in both thyroidectomized and normal rats, the administration of thyroid hormone decreased intracellular Na^+ and increased K^+ concentrations of diaphragm and heart tissues[3]. The effect of thyroid hormone administration on erythrocyte Na^+ and K^+ was not significant. This result was explained with the absence of nucleus, ribosomes and mitochondria of mature rat erythrocyte and also with the short duration of the thyroid hormone administration. Most of the studies on this subject are experimental and the results are controversial. The effect of thyroidal status on the erythrocyte Na,K-ATPase and intracellular Na^+ and K^+ in children are discussed in this paper.

Material and Methods

This study was carried out on 24 hypothyroid children between the ages of 16 months and 18 years who were being followed by the Pediatric Endocrinology Unit at Hacettepe University Medical School. The diagnosis of hypothyroidism was made by case history, physical examination, bone-age determination and serum T_4 and T_3 assays. The control group consisted of 12 cases between 3 and 15 years of age. The hypothyroidic children were separated into two groups. The

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first group consisted of 9 hypothyroidic children with prominent hypothyroidic signs and symptoms who were diagnosed for the first time during the study. These patients were studied before thyroid hormone treatment was begun. The second group consisted of 15 hypothyroid children who were on long-term thyroid hormone treatment. These cases were studied during therapy and again 6-8 weeks after cessation of thyroid hormone therapy.

In addition to routine hemoglobin and hematocrit measurements, serum T_4 and T_3 concentrations were determined by radioimmunoassay. Erythrocyte Na^+ and K^+ levels were determined by Mayer and Starkey's method using an IL flame photometer[4]. Erythrocyte membranes were prepared from 10 ml venous blood in ACD (Acid citrate dextrose) by the method of Fortes et al[5]. They could be stored at −20 °C for 3 weeks without loss of activity. Na,K-ATPase activity of the erythrocyte membranes was determined by the spectrophotometric method of Barnet and Slack and expressed as nmol/hr/mg protein[6]. Protein was determined by the biuret method[7].

Results

In the first group, pretreatment erythrocyte Na,K-ATPase activity is significantly low compared to the post-treatment stage and the control group (Table I).

Although the increase in erythrocyte Na,K-ATPase activity after 6-8 weeks of thyroid hormone therapy was significant, this period was not long enough for it to

TABLE I

The Erythrocyte Na, K-ATPase Values* in Hypothyroid and Control Groups (Na, K – ATPase in nmol/hr/mg/protein)

	Group 1 (no = 9)		Group 2 (no = 15)	Control group (no = 12)
Before treatment	110.000 ± 6.83++	During treatment	292.00 ± 12.98+++	294.44 ± 13.00
During treatment	179.71 ± 6.46++	After cessation of treatment	253.00 ± 14.13++++	
P value	< 0.001		< 0.01	

* = Mean ± S.E.M.
++ = p<0.001
+++ = p>0.05
++++ = p<0.05
} In comparison with control group

reach the level of the control group. We also found a significant correlation between the percentage increase in erythrocyte Na,K-ATPase activity and the percentage increase in hemoglobin concentration after thyroid hormone therapy in the first group (Figure 1).

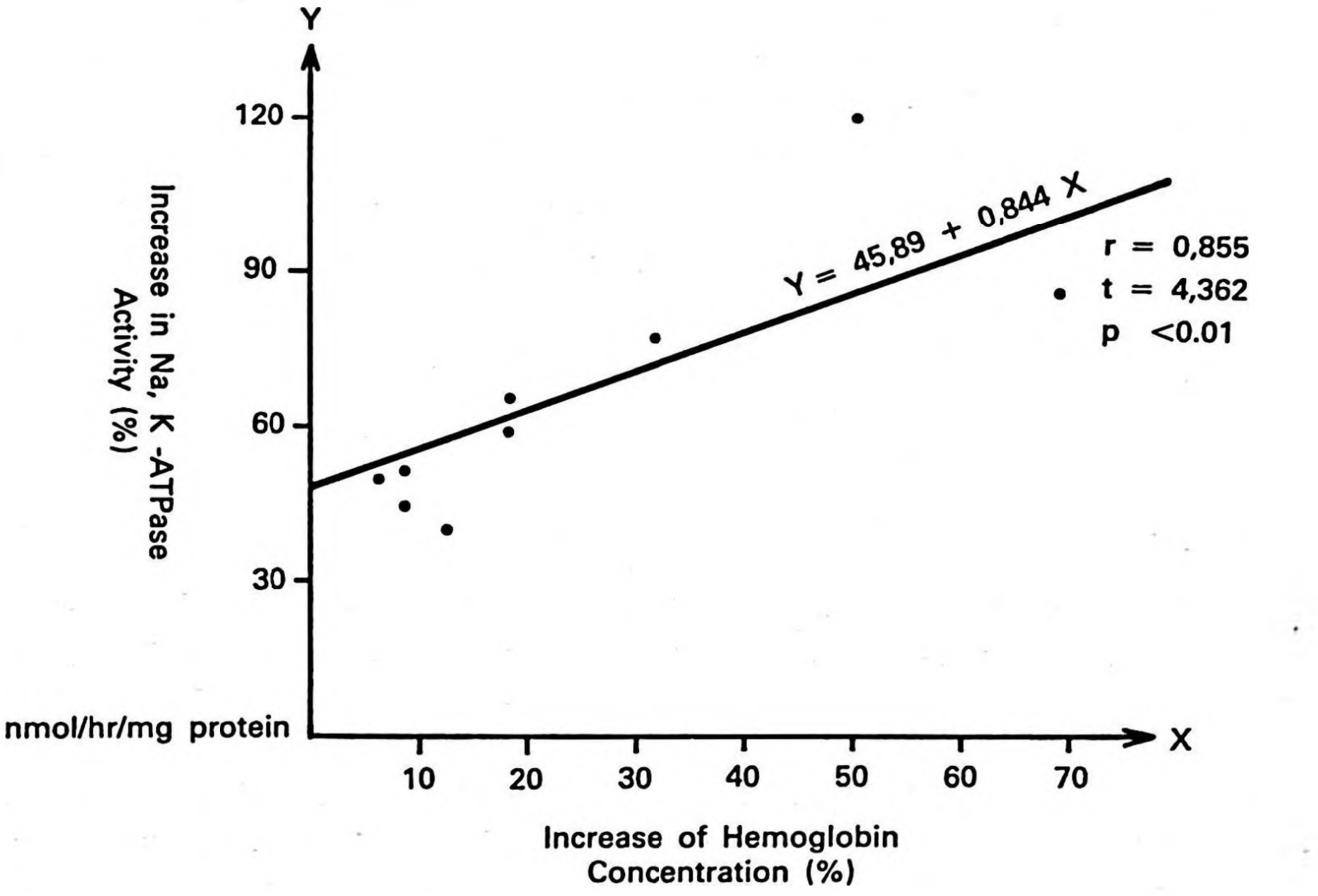

Figure 1

The regression line showing the correlation between the increase in hemoglobin and Na,K-ATPase activity.

With the second group, erythrocyte Na,K-ATPase activity during thyroid hormone therapy was no different from that of the control group. After cessation of thyroid hormone therapy for 6-8 weeks, the erythrocyte Na,K-ATPase activity decreased significantly, but this decrease of enzyme activity was not as low as with the first group (Table I). Intracellular Na^+ and K^+ values are shown in Table II. In both hypothyroid groups the intracellular Na^+ level during the hypothroid stage was significantly high when compared to the post-treatment stage and the control group. Further, there was not only an increase in the intracellular Na^+ during the hypothyroid condition there was also a decrease in intracellular K^+ (Table II).

TABLE II

Erythrocyte Na^+ and K^+ Concentrations* in Hypothyroid and Control Groups

	Group 1			Group 2		Control group	
	N^+	K^+		Na^+	K^+	Na^+	K^+
Before treatment	8.52 ± 0.21**	80.89 ± 0.87**	During treatment	6.42 ± 0.18	96.60 ± 1.25	6.14 ± 0.22	94.70 ± 1.59
During treatment	7.83 ± 0.12**	91.33 ± 1.62	After cessation of treatment	6.98 ± 0.12	92.70 ± 1.69		
	p<0.001	<0.001		<0.001	>0.05		

* Mean S.E.M.

** p<0.001
others p>0.05 In comparison with control group

Discussion

This study showed that in hypothyroidism the erythrocyte Na,K-ATPase activity is decreased and intracellular Na^+ concentration is increased. The hypothyroid patients were separated into two groups in order to demonstrate the effect of the lack of the thyroid hormone on erythrocyte Na,K-ATPase activity. In the first group, patients had been hypothyroid for a long time and no thyroid hormone had been prescribed before. In this group erythyrocyte Na,K-ATPase activity was very low. After 6-8 weeks of thyroid hormone therapy there was a significant increase in the enzyme activity, a concomitant decrease in the intracellular Na^+ concentration and an increase in intracellular K^+ concentration. Although the increase in enzyme activity after thyroid hormone therapy was significant ($p<0.001$), it did not reach the level of the control group. A correlation was found between the increase in the erythrocyte Na,K-ATPase activity and the increase in the hemoglobin concentration.

In the second group the hypothyroid patients had been on long-term thyroid hormone therapy. Their erythrocyte Na,K-ATPase activity and intracellular electrolyte concentrations did not differ from those of the control group. After cessation of thyroid hormone therapy for a 6-8 week duration erythrocyte Na,K-ATPase activity decreased. Although this decrease was significant ($p<0.01$) it was less than in the first group.

Beigi and Edelman in their study on thyroidectomized rats found an increased intracellular Na^+ and a decreased K^+ concentration in the heart and diaphragm tissues. After administration of the thyroid hormone, the intracellular Na^+

concentration decreased. However there was no change in the intra-erythrocytic electrolyte concentration[3].

We know that the thyroid hormone exerts its effect on Na,K-ATPase activity by increasing the synthesis of new pump sites. For such an effect a cell must have a nucleus and a mitochondria. As an erythrocyte has no nucleus and no mitochondria the effect of hypothyroidism on the erythrocyte membrane Na,K-ATPase activity must be on the nucleated erythrocyte precursor cells in the bone marrow. As the life span of an erythrocyte is 120 days a three-day duration of hypothyroidism in the rat in Edelman's[2] study could not have caused any change in the electrolyte concentration of the erythrocytes.

The presence of a correlation between the increase in erythrocyte Na,K-ATPase and the increase in hemoglobin concentration during thyroid hormone therapy strongly suggests that the thyroid hormone has an effect on the nucleated erythrocyte precursors in the bone marrow. It is also apparent that the effect of the thyroid hormone on the erythrocyte Na,K- ATPase activity does not make itself felt for at least 6-8 weeks.

Summary

Erythrocyte Na,K-ATPase enzyme activity and intracellular Na^+ and K^+ concentrations were determined in 9 hypothyroidic children with prominent hypothyroidic signs and symptoms. They were redetermined after 6-8 weeks of thyroid hormone treatment (group 1). Na,K-ATPase enzyme activity and intracellular Na^+ and K^+ concentrations were also determined in 15 hypothyroidic children who were on long-term thyroid hormone therapy. They were redetermined after discontinuing thyroid hormone therapy for 6-8 weeks (group 2).

In the first group Na,K-ATPase enzyme activity was found to be very low; after treatment with thyroid hormone it increased significantly but not enough to reach the level of the control group. In the pretreatment period the intracellular K^+ concentration was decreased and the Na^+ concentration was increased. After thyroid hormone therapy intracellular K^+ concentration increased while Na^+ concentration decreased. A correlation was found between the increase in the erythrocyte Na,K-ATPase activity and the increase in the hemoglobin concentration.

In the second group erythrocyte Na,K-ATPase activity during thyroid hormone therapy was no different from that of the control group. After the cessation of thyroid hormone therapy for 6-8 weeks, Na,K-ATPase activity decreased significantly, but this decrease in enzyme activity was not as low as in the first group.

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