Na-K-dependent ATPase in Red Cells and Thyroid Status

TAMOTU SATO, SOHEI KAJIWARA, CHIAKI MIYAMORI
AND TAIZO KATO

Department of Pediatrics, School of Medicine, Kanazawa University
Takaramachi 13-1, Kanazawa City, 920

Abstract

The Relationship between ouabain-sensitive ATPase (Na-K ATPase) activity in erythrocytes and the thyroid status was studied in 36 patients with Graves’ disease and 58 patients receiving L-thyroxine (T4) replacement therapy. Forty normal children served as control. Total ATPase activity in 4 untreated hypothyroid patients was significantly reduced (11.0±4.6 vs 17.3±4.1 µg-P/h/mg-protein, P<0.01), and Na-K ATPase was undetectable, both of which were normalized after 4 weeks of L-T4 therapy. Na-K ATPase in hyperthyroid patients was also decreased (0.9±0.8 vs 4.0 ±2.7, P<0.01), but was gradually normalized after 3 months of euthyroid state.

Clinically euthyroid children treated with L-T4 were divided into 2 groups with regard to Na-K ATPase activity, normal and low. Analysis of the possible factors producing this difference revealed that, in primary hypothyroidism, the factor appeared to be the endogenous T4 level, while in patients with dwarfism, the secretory capacity of TSH or TSH-releasing hormone (TRH) was contributory. Thus Na-K ATPase activity in red cells remains within the normal range after L-T4 replacement in the presence of a severe degree of primary hypothyroidism or in association with secondary or tertiary hypothyroidism. Other factors such as the L-T4 dose, duration of the therapy, serum T4 and T3 concentrations, were not significantly different in the two groups. These results indicate that (1) Na-K ATPase in red cells is decreased in hyper- or hypothyroid state, (2) restoration of normal activity requires 1-3 months of euthyroid period, and (3) it is a sensitive index of peripheral thyroid status over the preceding few months.

Recently several studies on the alteration of sodium-potassium-dependent ATPase (Na-K ATPase) activity in erythrocytes from idiopathic obese subjects (Du Luise et al., 1980; Klimes et al., 1981) have been reported. Most investigators observed a reduced Na-K ATPase activity and ouabain-binding capacity in obesity, but an opposite effect has also been reported (Mir et al., 1981). The reason for this controversy and the cause of the alteration are still obscure at present. It is well documented that Na-K ATPase activity in tissues is dependent on thyroid status; hypothyroidism lowers the activity, while hyperthyroidism raises it (Ismail-Beigi & Edelman, 1971, 1973; Katz & Lindheimer, 1973; Lo et al., 1976; Asano et al., 1976). In erythrocytes from Graves’ patients, however, there is a functional impairment of Na-pump (Smith & Samuel, 1970; Goolden et al., 1971) and Na-K ATPase activity is reported to be reduced (Cole & Waddell, 1976). These observations prompted us to reevaluate Na-K ATPase.
activity in red cells obtained from non-obese children with various thyroid states, trying to find whether it can be used for the marker of peripheral thyroid hormone actions.

Materials and Methods

1) Patients and evaluation of their thyroid status.

Thirty-six patients with Graves' disease aged 6-18 yrs, 17 patients with cretinism aged 1m-20 yrs, 8 patients with chronic thyroiditis aged 5-23 yrs, and 5 children with constitutional short stature aged 2-12 yrs were selected. Forty normal children aged 4-18 yrs were chosen as a control group. None of them was obese and their body weight was below 120% of the ideal body weight. Most of them had been followed in our clinic for 1-6 yrs before this study. Physical growth and clinical signs had been examined monthly, including estimation of serum T4, T3 and TSH concentration, and evaluation of bone age was done once a year. The euthyroid state was defined as the normality of all these indices. All patients with Graves' disease had been treated with 100-300 mg/day of propylthiouracil (PTU). Six of them were in a hyperthyroid state, 20 were euthyroid with PTU therapy, and 10 were in complete remission after stopping the PTU treatment. Except for 4 untreated cretins, all patients with cretinism and those with chronic thyroiditis had received an appropriate dose of L-T4 and were in an euthyroid state at the time of the examination. In 34 patients with pituitary dwarfism and 5 constitutional dwarfs, the function of the pituitary-thyroid axis had been evaluated by the level of serum T4, T3 and TSH response to TSH-releasing hormone (TRH) before the therapy. Of 34 pituitary dwarfs, 5 were treated with GH alone, (6-8 U/w in 2 divided doses), whereas others were treated with 25-100 µg/day of L-T4 combined with hGH. Constitutional dwarfs were treated with 25-50 µg/day of L-T4 for several months. All of them showed normal response of TSH to TRH. Under these treatments, all patients were considered to be in an euthyroid state with normal serum T4 and T3 levels. From these patients, a blood sample(s) was obtained once or twice at various intervals. One patient with cretinism was examined at one week intervals after the L-T4 replacement therapy.

2) Preparation of red cell membranes.

Five ml of blood was drawn into a heparinized syringe from patients and control subjects. The blood was centrifuged immediately at 4°C for 5 min and the plasma and buffy coat were removed. The packed red cells were washed 3 times with saline buffered with 10 volumes of 5 mM Tris-HCl (pH 7.6) containing 0.1 mM EDTA. The hemolysates were obtained by sonification for 30 sec and the membranes were sedimentated by centrifugation for 20 min at 20,000 x g in a refrigerated centrifuge. The membranes were washed 3 times with 0.017 M NaCl containing 5 mM Tris (pH 7.6), and washed once more with 10 mM Tris buffer (pH 7.4). The membranes were finally suspended in 0.5 ml of the buffer and stored at -20°C until use. In most cases, ATPase assay was performed within 24 hrs after preparation of the samples.

3) ATPase assay.

The mixture containing 200 µl of membrane suspension, 1 mM disodium ATP (Sigma Chemical Co.), 25 mM KCl, 75 mM NaCl, 1 mM MgCl2, 0.1 mM EGTA and 25 mM Tris (pH 7.4) was incubated at 37°C for 60 min. Ouabain-sensitive ATPase (Na-K ATPase) activity was determined by the addition of 1 mM ouabain. The reaction was stopped by the addition of 200 µl of 20% trichloroacetic acid. After centrifugation at 2,500 rpm for 15 min at 0°C, the inorganic phosphorus (Pi) concentration in the supernatant was determined by Fiske-Subbarow's method (1925). The protein concentration of the membrane preparation was estimated by the method of Lowry et al. (1951). Na-K ATPase activity was defined as the difference in Pi concentration between the sample with and without ouabain, and expressed as µg-Pi/h/mg-protein. The intra-assay coefficient of variance of the enzyme assay was 4.8±3.7%. Statistical analysis was carried out by the Student's t-test or F-test.

Results

1) Na-K ATPase in Graves' disease.

Total ATPase activity in hyperthyroid patients was not significantly different from that in the control (Table 1). Na-K ATPase activity, however, was decreased in hyperthyroid patients as well as in euthyroid patients receiving PTU treatment, while those in complete remission showed normal enzyme activity. Although these results suggest a correlation between Na-K ATPase activity and thyroid status, no direct relation was found between serum T4 or T3 levels and Na-K ATPase activity (Fig. 1). This lack of correlation appears to be due to the
time lag in recovering normal Na-K ATPase activity in patients with Graves' disease (Fig. 2). The enzyme activity remained depressed during the first 3 months of the euthyroid period, and gradually rose to the normal level thereafter. This indicates that at least 3 months of euthyroid state are required for the restoration of normal activity of Na-K ATPase.

2) Na-K ATPase in primary hypothyroidism.

Four patients with cretinism had significantly decreased total and Na-K ATPase activity before L-T4 administration (Table 1), which rose to the normal range after 4 weeks of L-T4 replacement (Fig. 3-a). Other euthyroid patients with cretinism receiving L-T4 were divided into 2 groups in regard to enzyme activity, the normal and low groups (above or below the mean SD value of control group respectively). Patients with chronic thyroiditis treated with L-T4 also showed a reduced activity (Table 1). In these 3 groups, possible factors depressing Na-K ATPase activity were analyzed (Table 2). There was no significant difference in the L-T4 dose, serum T4, T3 and TSH levels, but the endogenous T4 level before therapy was definitely reduced in the normal group (0.7 ±0.5 vs 2.4±1.5 µg/100 ml, P<0.01). This
suggests that red cell Na-K ATPase may be reduced under an exogenous T4 excess, which is less frequently induced in patients with a severe degree of hypothryoidism when the L-T4 dose is apparently adequate.

3) *Na-K ATPase in dwarfism.*

Pituitary dwarfism receiving hGH therapy alone showed normal activity of the enzyme, whereas 13 patients with pituitary dwarfism and 5 with constitutional short stature exhibited a low Na-K ATPase activity during L-T4 treatment (Table 1). Again, L-T4 dose, duration of the therapy, serum T4, T3 and endogenous T4 levels were not significantly different between the normal and low Na-K ATPase groups. The most conspicuous difference between the 2 groups appears to be responsiveness of TSH to TRH before the therapy (Table 3). Most patients in the normal ATPase group had responded abnormally to the
TRH dose (blunted or delayed), suggesting the presence of pituitary or hypothalamic hypothyroidism (81%). Despite the smaller dose of L-T4 in the short stature group, all having normal TSH secretion, Na-K ATPase activity was markedly depressed. These facts indicate that the administration of L-T4 to children with a normal pituitary-thyroid axis produces more frequently an excessive state of T4 than in those with secondary or tertiary hypothyroidism.

4) Changes in Na-K ATPase in a 10 month period (Fig. 3-b).

Paired samples could be examined in 8 patients with cretinism and 13 patients with pituitary dwarfism at 10 month intervals. Generally, Na-K ATPase activity in an individual was maintained at a relatively constant level, but the administration or cessation of L-T4 suppressed or restored the activity. Undetectable Na-K ATPase activity in an untreated cretin was
normalized, while the enzyme activity in young infants with cretinism remained unchanged. In older patients, however, the activity tended to decrease during the course of the therapy, despite the same dosage of L-T4 throughout the period.

Discussion

Na-K ATPase activity in red cells is thought to be influenced by several factors, such as body weight, meals (Mir et al., 1982) and genetic disposition (Beutler et al., 1982), but the results of the present study revealed a close correlation with the thyroid status. Lin and Akera (1978) reported that the administration of T3 to rats resulted in an increased tissue concentration of Na-K ATPase in liver, kidney and skeletal muscle, but not in brain despite its highest affinity for ouabain-binding sites. This suggests that the responsiveness of Na-K ATPase to thyroid hormone differs with tissue. According to the observation by Cole and Waddell (1976), Na-K ATPase activity in the red cell membranes from Graves' patients was decreased, and this was associated with an elevated sodium content in the erythrocytes. They found a significant inverse correlation between serum T4 and Na-K ATPase. However, they could not reproduce this effect in vitro, concluding that Na-K ATPase activity in erythrocytes did not represent a direct effect of thyroid hormones on the matured red cell membrane. We also found a reduced enzyme activity in hyperthyroid patients, but we could not find a direct correlation between serum T4 and T3 levels and Na-K ATPase activity. This is probably due to the time lag required for the normalization of the enzyme activity, which is estimated to be approximately 3 months of the euthyroid period. Possibly the number of samples in Cole’s study might be too few to find out this time lag (n=10). A Recent study on Ca-Mg ATPase activity in red cells showed that an elevated enzyme activity in hypothyroid state required a 7–8 week period for normalization (Goswami & Rosenberg, 1981). It is conceivable that this time lag may be related to the erythrocyte's life span, since new protein synthesis may not be induced in the matured erythrocyte membrane. Therefore, reduced Na-K ATPase represents the thyroid status over the preceding few months. This is very analogous to the glycosilated hemoglobin concentration in the red cells from patients with diabetes mellitus (Gabbay et al., 1977).

Although the number of samples was limited, no detectable Na-K ATPase activity was found in the red cells from untreated cretinism. The line of evidence indicates that thyroidectomy produces a decrease in Na-K ATPase activity in tissues and the administration of thyroid hormones results in a proportional increase in the activity (Katz & Lindheimer, 1973; Lo et al., 1976; Asano et al., 1976). In red cell membranes also, Na-K ATPase activity of hypothyroid rats was reduced, although calmodulin-dependent Ca-Mg ATPase activity was enhanced (Goswami & Rosenberg, 1981). At present, the exact mechanism of the hypothyroidism-related changes in red cell membrane is obscure, but possible changes in phospholipid metabolism are postulated during maturation of the erythrocytes (Goswami & Rosenberg, 1981).

From these observations, it seems that Na-K ATPase may be an indicator of the peripheral thyroid status and may be useful as an index of the adequacy of the L-T4 replacement dose. In fact, approximately half of the patients receiving L-T4 therapy had low activity in the red cells. An analysis of possible factors reducing Na-K ATPase activity revealed that the major factor in cretinism is the degree of T4 deficiency at the time of diagnosis, whereas that in pituitary dwarfism is the state of
TSH and/or TRH secretory capacity. The patients with primary hypothyroidism and those with secondary or tertiary hypothyroidism maintained normal activity, whereas patients with mild hypothyroidism and dwarfs with normal TSH secretion tended to show reduced activity. This probably indicates a mild thyroid hormone excess in the latter and such conditions can not be checked by the level of serum thyroid hormone. Therefore, Na-K ATPase activity in red cells is a more sensitive indicator of the peripheral effect of thyroid hormone, when serum hormone levels are within the normal range. In addition, age of patients seems to be another possible contributory factor, because infants with cretinism maintained normal enzyme activity in the paired samples at 10 month intervals, while the activity declined in older patients (Fig. 3-b). In general, the thyroid hormone requirement tends to decrease with age (Oddie et al., 1966), and thyroid hormone-dependent changes in renal Na-K ATPase are modified by age in hyper- or hypothyroid rats (Gambert et al., 1981). The age-related change in Na-K ATPase activity in erythrocytes awaits further investigation. In summary, the results of the present study indicate that (1) Na-K ATPase activity in erythrocyte membranes is dependent on the thyroid status over the preceding several months and (2) it can be used as a sensitive index of peripheral thyroid hormone action in clinical practice.

Acknowledgement

This work is supported in part by a Grant from the Intractable Diseases Division, Public Health Bureau, Ministry of Health and Welfare in Japan.

References


