Erythrocyte Ouabain Binding Capacity as a Possible Cellular Index of Hyperthyroid Status

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Abstract

The maximum ouabain binding capacity in erythrocytes from 8 normal subjects and 14 patients with hyperthyroidism was assessed by measuring [³H]-ouabain binding. The mean value for maximum ouabain binding capacity was significantly lower in the patients than in normals (0.422±0.084 vs 0.671±0.095 pmol per 10⁹ cells, p < 0.001). Furthermore, a close inverse correlation was found between the ouabain binding capacity and serum T₃ (r=−0.766; p < 0.01) or T₄ (r=−0.870; p < 0.001) levels. These results suggest that the maximum ouabain binding capacity in erythrocytes may provide a useful index of the peripheral effect of thyroid hormone.


Very little is known about the thyroidal regulation of this enzyme activity in human tissues. Reduced activity of ouabain-sensitive ATPase in erythrocyte ghost from patients with hyperthyroidism was reported by Cole and Waddell (1976). Recently Arnott et al. (1982) showed that lymphocyte ouabain binding capacity assessed by [³H]-ouabain binding was reduced in hypothyroid patients compared with normal subjects. However, these workers could not observe any change in this enzyme in lymphocytes from patients with hyperthyroidism. Thus, the effect of thyroid hormones on Na, K, ATPase activity in human tissues remains to be elucidated.

In this paper we present data on the maximum ouabain binding capacity of erythrocytes from hyperthyroid patients. Other workers (Schmalzing et al. 1980; Gelbert and Goldman 1977) have repeatedly shown that the number of ouabain binding sites
reflects the activity of Na, K, ATPase.

Materials and Method

Subjects

Fourteen patients with hyperthyroidism were selected from the out-patient clinic of the Division of Endocrinology and Endocrine Research Laboratory, Toranomon Hospital. Their ages ranged from 28 to 60 years (Table 1). Some of the patients were under treatment with antithyroid drugs. Their thyroid status were evaluated by the clinical signs and symptoms and also by the results of T₃ and T₄ radioimmunoassays. These patients were normotensive, and showed no abnormalities in serum electrolytes, creatinine, blood urea nitrogen or in the complete blood count. The control subjects were laboratory personnel ranging in age from 29 to 57 years. They were normotensive, and none of them was obese.

[³H]-ouabain binding assay

Venous blood was drawn into a heparinized syringe after an overnight fast for all subjects. Erythrocytes were prepared according to the procedure described by Gambhir et al. (1977) by using Ficoll-Paque (Pharmacia Fine Chemicals), and were washed twice with 4 volumes of assay buffer, which contained 150 mM sodium chloride, 30 mM Hepes and 10 mM glucose, pH 7.5. Ouabain binding to erythrocytes was measured by the method described by De Luise et al. (1980). Briefly, 200 µl of cell suspension (4 x 10⁸ erythrocytes) was incubated with 0.5 pmol of [³H]-ouabain (New England Nuclear, Specific Activity 14.0–18.0 Ci/µmol) and unlabeled ouabain ranging in concentration from 1 nM to 100 µM in a total volume of 250 µl. Incubation was carried out for 3 hr at 37°. The cells were then washed twice with 2 ml of ice-cold 140 mM choline chloride and the cell-associated radioactivity was eluted with 300 µl of 5% TCA and counted in 4 ml of ACS-II (Amersham) in a Packard liquid scintillation spectrometer. Approximately 10 per cent of the [³H]-ouabain bound to erythrocytes when no unlabeled ouabain was added and nonspecific binding determined in the presence of 100 µM unlabeled ouabain was less than 0.5 per cent of total binding. Maximal ouabain binding capacity was calculated by the method described by De Luise et al. (1980) using a SEIKO 8500 computer. Within- and between-assay variation in this laboratory were 6.9 and 7.8 per cent in terms of coefficient of variation, respectively.

Table 1. Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Methimazol 30 mg/d</th>
<th>Propranolol 60 mg/d</th>
<th>T₃ (ng/dl)</th>
<th>T₄ (µg/dl)</th>
<th>T₄ uptake (%)</th>
<th>TBG (µg/ml)</th>
<th>Cholesterol (mg/dl)</th>
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<td>37</td>
<td>18.3</td>
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<tr>
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</table>
Others

Serum T₃, T₄ and TBG were determined by a standard radioimmunoassay technique by using commercially available kits.

Results

Individual values for maximum ouabain binding capacity of cells from normal subjects and patients with hyperthyroidism are shown in Fig. 1. The mean value for 14 hyperthyroid patients was 0.422 ± 0.084 (SD) pmol per 10⁹ cells, and this was significantly lower (p < 0.001) than the value of 0.671 ± 0.095 pmol per 10⁹ cells for 8 controls. The latter is in accordance with the normal value reported by De Luise et al, (1980). Other than listed in Table 1 and Figures, we measured ouabain binding capacity in 21 normal subjects. The values (0.616 ± 0.68 pmol per 10⁹ cells) were essentially the same as those obtained with 8 normals.

Figure 2 shows the relationship between circulating thyroid hormone levels and maximum ouabain binding capacity in erythrocytes from patients and controls. As can be seen, a close inverse correlation was found between these variables (r = -0.766; p < 0.001 for T₃ or r = -0.870; p < 0.001 for T₄).

The correlation coefficients for the relationship between hormones and serum total cholesterol which was determined in 20 out of 22 subjects were -0.342 for T₃ (ns) and -0.302 for T₄ (ns). The correlation between...
thyroid hormone and ouabain binding capacity in erythrocytes was much closer than that between hormones and serum total cholesterol levels which were conventionally in use as an index of thyroid status.

**Discussion**

The present study first demonstrated that the maximum ouabain binding capacity assessed by ouabain binding assay had a close inverse correlation with serum T3 \((r = -0.778; p<0.001)\) or T4 \((r = -0.896; p<0.001)\) levels, and suggests that the Na, K, ATPase activity in human erythrocytes is regulated by circulating thyroid hormones. Despite the indirect nature of ATPase assay, the number of ouabain binding sites assessed by specific binding of ouabain has been repeatedly shown to correlate with the activity of this enzyme (Schmalzing et al. 1981; Gelbert et al. 1977). In accordance with the present data, the Vmax value of ouabain-sensitive (Na, K, ATPase-mediated) rubidium uptake by erythrocytes from hyperthyroid patients was found to be lower than the value in normals in our preliminary experiment (unpublished observation).

An increase in the activity of the erythrocyte sodium pump has been described in diseases such as a certain type of morbid obesity (De Luise and Flier 1982), hereditary hemolytic syndromes (Wiley 1971; Wiley et al. 1975), protein-calorie malnutrition (Kaplay 1978) and glucocorticoid excess (Kaji et al. 1981). Patients with obesity (De Luise et al. 1980; De Luise et al. 1982; Klimes et al. 1982), myotonic muscular dystrophy (Lomber et al. 1981) and uremia (Smith and Welt 1976) were shown to have fewer pump units than controls. In hyperthyroid patients, reduced activity of the erythrocyte sodium pump has been suggested by several investigators because of an elevated concentration of sodium within their erythrocytes (Boeckelman 1958; Goolden et al. 1971). Cole and Waddell (1976) confirmed these findings by measuring ouabain-sensitive ATPase activity in erythrocyte membranes from hyperthyroid patients. Although these workers noted a close inverse correlation between this enzyme activity and serum T4 level in a course of treatment with antithyroid drug or radioactive iodine in one patient, they could not observe a significant correlation between these variables in other patients they had examined.

In contrast with the present data, thyroid hormones are known to enhance Na, K, ATPase activity in rodent tissues including heart (Philipson and Edelman 1977a and b), liver (Ismail-Beigi and Edelman 1970; Ismail-Beigi and Edelman 1971; York et al. 1978; Gambert et al. 1981), skeletal muscle (Asano et al. 1976) and kidney (Ismail-Beigi and Edelman 1970; Ismail-Beigi and Edelman 1971; Gambert et al. 1981; Lo and Edelman 1976a and b). It is not clear whether these difference are due to a species or tissue specificity of thyroid hormone effect. The latter explanation is favored by the finding that erythrocyte from T3 treated rats showed a significant elevation of intracellular sodium concentration (Ismail-Beigi and Edelman 1973). The data imply that the activity of Na, K, ATPase may be reduced in erythrocytes from hyperthyroid rats, despite the fact that the increase in the activity of this enzyme was shown in homogenates of liver and kidney in a parallel experiment.

The mechanism by which thyroid hormone reduces the number of sodium pump units in erythrocytes is unknown. It is generally accepted that the thyroid hormone action is initiated by its binding to nuclear receptor of the thyroid hormone (Oppenheimer et al. 1972; Samuels et al. 1973). Therefore, it can be speculated that the hormone reduces the number of erythrocyte sodium pump units in a stage of erythropoiesis, rather than in a stage of nonnucleated matured cell. It has recently been shown, however, that specific binding sites
also exist on the plasma membranes of rat liver (Pliam and Goldfine 1977). Thus, the direct action of thyroid hormone on the plasma membranes of matured erythrocytes cannot be excluded.

Whatever the mechanism of this change is, the maximum ouabain binding capacity in erythrocytes may provide a reliable cellular index of thyroid hormone action in hyperthyroid patients, if other causes of a change in sodium pump activity are excluded. The relationship between circulating thyroid hormones and ouabain binding capacity was much closer than that observed between hormones and serum total cholesterol levels which were in use as a convenient cellular index of thyroid status. Further studies on the erythrocyte sodium pump activity in hypothyroidism and also the effect of treatment of hypo- and hyperthyroidism on this enzyme activity are being carried out in this laboratory.

Note Added in Proof.

Since this manuscript was submitted, De Luise et al. (1983) have presented data showing that the erythrocyte ouabain binding capacity was reduced in hyperthyroidism. These authors, however, could not observe a significant correlation between the extent of the reduction in erythrocyte ouabain binding capacity and serum T₃ or T₄ levels. While these results are apparently different from ours, the cause of the discrepancy is obscure.

Acknowledgment

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References


