Increased monoester lipase activity in red blood cells during hyperthyroidism

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ABSTRACT

Human red blood cells (RBC) contain a monoester lipase (MEL) activity which is tightly associated with the cell membrane and has its active site externally oriented, as inferred from the ability of the intact cell to hydrolyse an exogenously added lipid substrate. Membrane-bound MEL activity was measured by a radiochemical assay in intact RBC from 29 untreated hyperthyroid patients. The mean (± s.d.) MEL level was higher ($P < 0.01$) in these patients ($1220 ± 212$ mu./$10^{12}$ RBC) than in the control group ($1010 ± 120$ mu./$10^{12}$ RBC). There was no difference between men and women. The increase in MEL values was associated with significantly ($P < 0.001$) decreased values of mean cellular volume and mean cellular haemoglobin content. A continued study of 13 patients, who became euthyroid with treatment, showed a normalization of the MEL values in RBC. The increased lipolytic potency of RBC represents a new characteristic of hyperthyroid patients. Further exploration of the possible diagnostic or prognostic implications of this enzymatic change seems warranted.


INTRODUCTION

We have recently identified a monoester lipase (MEL) activity in human red blood cells (RBC) (Boyer, Somma, Vérine et al. 1981). This MEL exhibits features typical of an ectoenzyme: it is tightly associated with the cell membrane and has its active site externally oriented, as inferred from the ability of intact RBC to hydrolyse an exogenously added lipid substrate. Under basal conditions a soluble MEL activity is measurable in human plasma at levels averaging 0·5 mu./ml, a value which represents less than 10% of the amounts of MEL activity found in RBC (Boyer et al. 1981). The physiological factors of MEL regulation are so far unknown. Since lipolytic enzymes have been shown to be sensitive to thyroid hormones (for review see Valdemarsson, 1983), we have searched for changes in MEL activity in RBC from hyperthyroid patients. The present study demonstrates, for the first time, that the lipolytic potency of intact RBC from non-anaemic hyperthyroid patients is increased.

MATERIALS AND METHODS

Twenty-nine hyperthyroid patients (28 with Graves’ disease and 1 with mononodular adenoma) were studied. The mean duration of hyperthyroidism was 4·5 months (range 1–24). There were 21 women and 8 men, aged 18–72 years (mean ± s.d. = 48 ± 14 years). Patients had not received therapy for thyroid disease for at least 6 months before the study. They were non-anaemic (blood levels of haemoglobin > 120 g/l) and had no history of blood disease or lipid abnormality. The control group included 28 healthy euthyroid women, aged 21–56 years (mean ± s.d. = 37 ± 10 years); none was taking steroid contraceptives. All participants in the study gave their informed consent.

Blood samples (5 ml) were taken, after an overnight fast, into siliconized tubes containing 0·5 ml sodium citrate (0·13 mol/l). The tubes were kept at 4 °C and processed within 48 h. A platelet- and leukocyte-free suspension of RBC was prepared from each sample by filtration on cellulose (Boyer et al. 1981). Assay of MEL activity was performed using a fraction (0·1 ml;
about $10^8$ RBC) of the purified RBC suspension as the enzyme source.

Monoester lipase activity was assayed using an emulsion of ethyl [9,10-$^3$H] oleate (2 μmol; about $2 \times 10^6$ c.p.m.) as the substrate, in a final volume of 1 ml at 37°C. The labelled ethyl ester was prepared and purified in the laboratory by conventional methods as previously reported (Boyer et al. 1981). The use of this substrate, totally water insoluble, establishes that MEL is a true lipase and not an esterase (Desnuelle, 1972). The ratio of ethyl oleate to mono-oleylglycerol lipase activities in human RBC has been shown to be 1:2:1 (Boyer et al. 1981); but mono-oleylglycerol, which is most probably the physiological substrate, has a slight haemolytic potency, and was not found to be a convenient substrate in vitro. Detailed technical data on this assay have been previously reported (Boyer et al. 1981). Results are expressed as μl. lipase activity/$10^{12}$ RBC; 1 μl. activity corresponds to the release of 1 nmol acid/min. Red cell counts, mean cellular volume (MCV) and mean cellular haemoglobin content (MCH) were determined using a Coulter counter model S (Coultronics France, 29 Av. Georges-Pompidou, 95580 Magency, France). Serum 3,5,3'-tri-iodothyronine ($T_3$) and thyroxine ($T_4$) were assayed by radioimmunoassay. Data from patient and control groups were compared using the unpaired t-test.

RESULTS

In the RBC of hyperthyroid patients the mean (± s.d.) level of MEL activity (1220±212 μl./$10^{12}$ RBC) was higher ($P<0.01$) than in the control group (1010±120 μl./$10^{12}$ RBC). In the hyperthyroid group there was no difference between the values in women ($1210\pm211$ μl./$10^{12}$ RBC; $n=21$) and in men ($1220\pm226$ μl./$10^{12}$ RBC; $n=8$), confirming earlier results in normal subjects (Boyer et al. 1981). The coefficient of variation for the 29 hyperthyroid subjects was relatively high (17.4 vs 11.9% in the control group), suggesting that their RBC population was rather heterogeneous for MEL activity. There was no correlation between MEL activity values and plasma thyroid hormone levels ($T_4$, $r=0.2$; $T_3$, $r=0.3$). Among the RBC parameters, mean values of which are comparable in men and women, hyperthyroid patients had a low MCV mean value ($83.7\pm4.9$ vs $89.9\pm5.2$ fl in normal subjects; $P<0.001$) as well as a low MCH value ($29.3\pm2.3$ vs $31.4\pm1.1$ pg in normal subjects; $P<0.001$).

We re-examined 14 of these patients between 3 and 26 months after the beginning of the treatment; eight patients had been treated by surgery, four by radioiodine and two by thionamides. Thirteen were clinically and biologically euthyroid and one was still hyperthyroid. For the 13 euthyroid patients (Fig. 1) the mean MEL values had fallen from 1280±223 before to 1000±137 μl./$10^{12}$ RBC after treatment ($P<0.001$); a single patient in this group had a value which increased slightly within the normal range.

DISCUSSION

The activity of lipolytic enzymes has been commonly found to be either normal or moderately increased during hyperthyroidism, whereas it is reduced during hypothyroidism (Valdemarsson, 1983). Comparison of data in human subjects and various animal species, however, demonstrates obvious qualitative and quantitative differences.

In hyperthyroid man, triester lipase activities have been found to be slightly increased, after heparin infusion, in plasma (Nikkilä & Kekki, 1972; Jubelin, Bettendorf & Boyer, 1974) and liver (Valdemarsson, Hansson, Hedner & Nilsson-Ehle, 1983); cholesterol ester hydrolase activity appeared to be also increased, 

\begin{figure}
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Changes in levels of monoester lipase (MEL) activity in red blood cells (RBC) of 13 hyperthyroid subjects before (○) and after (●) antithyroid drug or surgical treatment to make them euthyroid. The horizontal broken lines represent the mean (middle line) ± 2 s.d. for MEL values in 161 healthy adult subjects (Boyer et al. 1981).}
\end{figure}
particularly in liver, a possible explanation for enhanced metabolism and thereby low levels of cholesterol in blood (Severson & Fletcher, 1981). We have previously shown that an increase in MEL activity parallels that in triester lipase in plasma after heparin infusion (Jubelin et al. 1974). A similar increase in MEL activity is reported in RBC in the present study. This finding may be of interest since MEL activity in RBC, which is not releasable by heparin (Boyer et al. 1981), appears to be a lipolytic activity different from that assayed in plasma after heparin infusion. Also, MEL is the only lipolytic activity easily measurable in human RBC: these cells contain only very low levels of diester lipase activity (Somma, Arnaud & Boyer, 1983) and no detectable triacylglycerol (Boyer et al. 1981) or cholesterol ester hydrolase (J. Boyer, unpublished observations) activities.

The mechanism of the influence of thyroid function on MEL activity in RBC is unknown. Our previous findings of high levels of MEL activity in RBC from cord blood of normal newborn infants (Boyer et al. 1981) as well as in regenerative anaemias and acute proliferative blood disorders (Gastaut, Vérine, Carcassonne & Boyer, 1982) reflected, at least in part, an increased proportion of young RBC in the circulation. Apparently, the increase in MEL activity of RBC during hyperthyroidism might be consonant with such an interpretation. Thyroid hormones appear to stimulate erythropoietic activity by different mechanisms, such as increases in oxygen consumption (Meineke & Crafts, 1959), erythropoietin production (Fischer, 1972) and in the number of β-adrenergic receptors in precursor cells (Williams, Lefkowitz, Watanabe et al. 1977). The normal reticulocyte counts (1-2 ± 0-8%) in our non-anaemic hyperthyroid patients, however, seem to argue against this hypothesis, although young RBC may not all be counted as reticulocytes. Moreover, this hypothesis does not agree well with the microcytosis found in our, and other (Kuhn, Rieu, Rochette et al. 1983), hyperthyroid subjects, since normal young RBC are rather larger than the average mature cell. It therefore seems likely that the MEL increase does not simply reflect changes in the turnover of the RBC population.

Alternatively, we have suggested (Boyer et al. 1981) that MEL, besides its probable role in the turn-over of lipids in RBC membranes, might also act on circulating lipoproteins. Whereas lipoprotein lipase, the key regulatory enzyme in lipoprotein breakdown, is responsible for the degradation of tri- and diacylglycerols, MEL should play a major role in the further hydrolysis of blood monoaicylglycerol (Arnaud, Nobili & Boyer, 1979). The increased MEL activity found in RBC from hyperthyroid patients could then contribute to the low levels of acylglycerols in the plasma of these subjects and to their remarkable facility in clearing plasma triglyceride-rich very low-density lipoprotein (Abrams, Gremdy & Ginsberg, 1981).

The increased lipolytic potency of RBC represents a new biological characteristic of hyperthyroid patients. Its determination might represent a relatively easy way of evaluating the influence of thyroid hormone on a tissue parameter.

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REFERENCES


