Stimulation by Thyroid Hormone Analogues of Red Blood Cell Ca\textsuperscript{2+}-ATPase Activity in Vitro

CORRELATIONS BETWEEN HORMONE STRUCTURE AND BIOLOGICAL ACTIVITY IN A HUMAN CELL SYSTEM

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Human red blood cell membrane Ca\textsuperscript{2+}-ATPase activity is stimulated in vitro by physiological concentrations (10\textsuperscript{-10} M) of L-thyroxine (L-T\textsubscript{4}) and 3,5,3'-triiodothyronine (L-T\textsubscript{3}). This human cell system has been utilized to examine a series of iodothyronines and iodothyrosine analogues for structure-activity relationships. Analogue purity was verified by high pressure liquid chromatography. Analogues were studied at a concentration of 10\textsuperscript{-10} M and the stimulatory effect of each analogue was compared with that of L-T\textsubscript{3} in this system. Essential to Ca\textsuperscript{2+}-ATPase stimulation were occupation of the 3 and 5 phenyl positions by iodide, bromide, or methyl groups, the L-configuration of the alanine side chain, side chain length equal to that of alanine, and a perpendicular (skewed) conformation of the two rings. The 4'-hydroxyl group is not essential to Ca\textsuperscript{2+}-ATPase stimulation in this model system. T\textsubscript{3} was 76\% as active as T\textsubscript{3} in stimulating Ca\textsuperscript{2+}-ATPase activity. The stimulatory effect of 3,5-dimethyl-3'-isopropyl-L-thyronine and 3,5,3',5'-tetramethoxy-L-thyronine approximated that of L-T\textsubscript{3}. Selected tyrosine analogues also stimulated the enzyme. The bioactivities of hormone analogues in this human model of extranuclear thyroid hormone action differ in several ways from results obtained previously in other animal model systems in vitro and in vivo.

The desirability of a readily available human cell model in which to study the relationship between thyroid hormone structure and action has long been apparent. Evaluations of thyromimetic properties of hormone analogues in vitro have focused on several animal models, including the rat (1-3) and the tadpole (4, 5). Methods for examining structure-activity relationships in vitro have included extensive studies of nuclear binding of iodothyronines in animal pituitary (6), liver (7-9), and kidney (10). Rat liver mitochondrial binding studies have permitted limited analogue comparison (11). The binding of thyroid hormone and several analogues to rabbit red cell membranes has also been described but membrane binding has not correlated consistently with reported biologic activity in vitro or with nuclear binding studies (12). Thyroid hormone analogue binding to TBG, \textsuperscript{1} TBPA, and serum albumin has been extensively studied and reviewed (13, 14).

Uptake of \(\alpha\)-amino-isobutyrate and cycloleucine by isolated rat thymocytes is stimulated by thyroid hormone and the effects of thyroid hormone analogues in this system have been studied (15). The uptake of 2-deoxyglucose in the same cell system is also responsive to thyroid hormone, at a concentration closer to physiologic than used in many prior studies (16, 17). Recent studies of analogue binding to thymocyte membranes have shown direct correlation with the ability of the analogues to stimulate 2-deoxyglucose uptake (18). In the rat erythrocyte membrane, thyroid hormone can affect acetylcholinesterase (19) and \(Mg^{2+}\)-dependent adenylate triphosphatase (Ca\textsuperscript{2+}-ATPase) activity (20). The effect may be enzyme stimulation or inhibition, depending on the fatty acid composition of the membrane. Concentrations of hormone and analogues in these studies were between 10\textsuperscript{-8} and 10\textsuperscript{-9} M (20).

We have recently shown that the Ca\textsuperscript{2+}-ATPase activity of human red blood cell membrane is maximally stimulated in vitro by physiologic concentrations of thyroid hormone (10\textsuperscript{-9} M) (21). Stimulation of Ca\textsuperscript{2+}-ATPase by thyroid hormone is dependent upon hormone-binding to the red cell membrane (22) and upon the presence of calmodulin, a cytoplasmic activator protein for Ca\textsuperscript{2+}-ATPase (23). This red cell Ca\textsuperscript{2+}-ATPase assay system is a human cell model of extranuclear thyroid hormone action, the use of which in preliminary studies has indicated that D-amino and acetic acid analogues of iodothyronines do not stimulate Ca\textsuperscript{2+}-ATPase activity (21). We have now used this human cell Ca\textsuperscript{2+}-ATPase assay to investigate a wide variety of iodothyronine and iodothyrosine analogues and have characterized the importance of substituent groups to the stimulation of human red cell Ca\textsuperscript{2+}-ATPase activity.

MATERIALS AND METHODS

Hormones and Analogues—Hormones, hormone analogues, and their sources were as follows: L-T\textsubscript{4}, L-T\textsubscript{3}, D-T\textsubscript{4}, D-T\textsubscript{3}, tetrac, diiodothyronine; L-T\textsubscript{3}, 3,5,3',5'-triiodothyronine; D-T\textsubscript{3}, 3,5,3'-triiodothyronine; T\textsubscript{3}, 3,5,3',5'-tetrabromo-L-thyronine; T\textsubscript{3}, 3,5-bromo-L-thyronine; triac, triiodothyronine; triac, 3,5,3',5'-tetraiodothyroacetic acid; triac, 3,5,3',5'-triodothyroacetic acid; rT\textsubscript{3}, 3,3',5'-triiodothyronine; DMIT, 3,5-dimethyl-3'-isopropyl-L-thyronine; T\textsubscript{3}amine, 3,5,3'-triiodothyronamine; BSA, bovine serum albumin; EGTA, ethylene glycol bis(\(\alpha\)-aminoethyl)-\(N,N,N',N'\)-tetraacetic acid.

\textsuperscript{1} The abbreviations used are: TBG, thyroxine-binding globulin; TBPA, thyroxine-binding prealbumin; L-T\textsubscript{4}, L-thyroxine; D-T\textsubscript{4}, D-thyroxine; L-T\textsubscript{3}, 3,5,3'-triiodothyronine; D-T\textsubscript{3}, 3,5,3'-triiodothyronine; T\textsubscript{3}, 3,5-bromo-L-thyronine; T\textsubscript{3}, 3,5,3',5'-tetrabromo-L-thyronine; triac, triiodothyronine; triac, 3,5,3',5'-tetraiodothyroacetic acid; triac, 3,5,3',5'-triodothyroacetic acid; rT\textsubscript{3}, 3,3',5'-triiodothyronine; DMIT, 3,5-dimethyl-3'-isopropyl-L-thyronine; T\textsubscript{3}amine, 3,5,3'-triiodothyronamine; BSA, bovine serum albumin; EGTA, ethylene glycol bis(\(\alpha\)-aminoethyl)-\(N,N,N',N'\)-tetraacetic acid.
Thyroid Hormone and Ca\(^{2+}\)-ATPase

RESULTS

**L-T\(_3\) and L-T\(_4\) Stimulation of Red Cell Ca\(^{2+}\)-ATPase Activity in Vitro**—The mean basal Ca\(^{2+}\)-ATPase activity of red cell membranes in these experiments was 0.247 ± 0.023 (S.E.) μmol of P\(_i\)/mg of protein·90 min. With the addition of L-T\(_3\) (10\(^{-10}\) M), enzyme activity rose to 0.359 ± 0.036 μmol of P\(_i\)/mg·90 min resulting in an increase in activity of 0.112 ± 0.015 μmol of P\(_i\)/mg·90 min (p < 0.001) in a total of 14 experiments, utilizing membrane preparations from 10 donors. L-T\(_3\) (10\(^{-10}\) M) caused an increase in enzyme activity of 0.085 ± 0.008 μmol of P\(_i\)/mg·90 min in the same experiments (p < 0.001); this increase was 76% of that seen with T\(_3\), as indicated in Table I.

**Effect of Ring Iodination and Substitution with Bromide, Methyl, and Isopropyl Groups**—The effects of various iodothyronines and ring-substituted analogues on Ca\(^{2+}\)-ATPase activity are shown in Table I. L-T\(_3\) with iodination in the 3 and 5 positions on the inner ring, retained 77% of the effect of T\(_4\) in our assay system. In contrast, 3',5'-L-T\(_2\) had only 28% of the T\(_4\) effect, and 3,3'-L-T\(_2\) and 3'-monoiodo-L-thyronine were inactive. While L-T\(_3\) was 76% as active as T\(_3\), rT\(_3\) was inactive.

Full substitution of bromide for iodide in the 3,5,3'- and 5'-positions permitted virtually full retention of enzyme stimulating effect, and 3,5-dibromo-L-thyronine retained 50% of L-T\(_4\) action, as well as ¾ of the effect of 3,5-L-T\(_3\).

**Modification of Alanyl Side Chain: Acetic and Propionic Analogues, Decarboxylated Analogues, and Ethyl Esterification**—The deaminated analogues, tertac and triac, with one less carbon in the side chain than found in L-T\(_3\) and L-T\(_4\), were ineffective in this Ca\(^{2+}\)-ATPase system (Table II).

### Table I

<table>
<thead>
<tr>
<th>Analogue</th>
<th>% of T(_3) effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-T(_3)</td>
<td>100</td>
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<tr>
<td>L-T(_4)</td>
<td>76</td>
</tr>
<tr>
<td>rT(_3)</td>
<td>NS</td>
</tr>
<tr>
<td>3,5-L-T(_2)</td>
<td>77</td>
</tr>
<tr>
<td>3'-5'-L-T(_2)</td>
<td>28</td>
</tr>
<tr>
<td>3,3'-L-T(_3)</td>
<td>NS</td>
</tr>
<tr>
<td>3'-L-T(_3)</td>
<td>NS</td>
</tr>
<tr>
<td>3,5,3',5'-tetra-bromo-L-thyronine</td>
<td>98</td>
</tr>
<tr>
<td>3,5-dibromo-L-thyronine</td>
<td>50</td>
</tr>
<tr>
<td>N-acetyl DIMIT</td>
<td>NS</td>
</tr>
</tbody>
</table>

DIMIT, n-acetyl DIMIT; 4'-deoxy T\(_3\), 3'-bridged T\(_3\), and 3,5-di-methyl-DL-tyrosine were kindly provided by Dr. E. C. Jorgensen, San Francisco, CA; 3,3',5,5'-tetrabromo-L-thyronine was obtained from Glaxo Research Ltd. (London, United Kingdom); 3,5-diiodo-L-tyrosine and tyramine were prepared from Eastman Organic Chemicals (Rocheester, NY); 3,5'-L-T\(_3\), 3'-L-T\(_3\), and L-T\(_3\) ethyl ester were prepared from Henning Berlin GmbH (West Germany); 3-(p-hydroxyphenyl)propionic acid was obtained from Chemical Dynamics Corp., South Plainfield, NJ; 3-(3,5-diiodo-4-hydroxyphenyl)propionic acid and 3,5-diiodotyramine were synthesized in our laboratory from the noniodinated compounds; 3,5-dibromo-L-thyronine and T\(_3\) amine were the kind gifts of Drs. Rosalind Pitt-Rivers and R. D. Hesch, respectively. The purity of these compounds was verified by high pressure liquid chromatography in a 0.05 M KH\(_2\)PO\(_4\), 95% ethanol gradient system.

The majority of hormone analogues were dissolved in 4% propylene glycol, 0.4 N KOH prior to use. T\(_3\) amine was initially placed in propylene glycol/KOH with sufficient 95% ethanol added to dissolve the analogue completely; n-acetyl DIMIT was dissolved initially in 95% ethanol. Subsequent dilutions of analogues were made in 10 mM Tris buffer to a protein concentration of 1 mg/ml, as measured by high pressure liquid chromatography in a 0.05 M KH\(_2\)PO\(_4\), 95% ethanol gradient system.

Blood Cell Membranes—Red cell membranes were prepared hypotonically, as previously reported (21) from heparinized venous blood obtained from healthy euthyroid human volunteers. The research protocol was approved by the institutional Human Research Committee. The cells were pelleted by centrifugation and lysed in 10 volumes of hypotonic Tris buffer (10 mM) in the absence of 0.1 mM EDTA; the membranes were then washed twice in isotonic NaCl buffer, washed in 10 mM Tris buffer, and suspended in 10 mM Tris buffer to a protein concentration of 1 mg/ml, as measured by the method of Lowry et al. (24), using BSA as the standard. The membranes were washed free of endogenous thyroid hormone during this preparation (21). Membranes were stored in 10 mM Tris, pH 7.4, at −70 °C for 1-5 days until used.

**Incubation of Membranes Prior to Ca\(^{2+}\)-ATPase Assay**—Membranes (1 mg of protein/ml of buffer) were incubated at 37 °C for 60 min prior to ATPase assay, together with L-T\(_3\), L-T\(_4\), hormone analogue, or control diluent.

**Ca\(^{2+}\)-ATPase Activity**—Membrane enzyme activity was assayed by our previously described method (21, 23) in which hydrolysis of 1 mM ATP is quantified in the presence and absence of 0.15 mM Ca\(^{2+}\). The concentrations of Ca\(^{2+}\) in the assay system was 20 μM. The absolute increase in Ca\(^{2+}\)-ATPase activity by thyroid hormone analogues among cell donors. In each experiment, the absolute increase in Ca\(^{2+}\)-ATPase activity (μmol of P\(_i\)/mg·90 min) induced by L-T\(_3\) was determined and the effect of each analogue on enzyme activity was expressed as a per cent of activity stimulated by L-T\(_3\). The statistical analysis of significance of thyroid hormone and analogue stimulation of Ca\(^{2+}\)-ATPase activity was determined by paired t test. The enzyme activity of each sample incubated with hormone or analogue was compared with that of a simultaneous basal control sample that lacked hormone or analogue. The absolute increase in enzyme activity obtained with each analogue, if found to be significant (p < 0.05, and at least 25% of the L-T\(_3\) effect), was then compared with the L-T\(_3\)-associated Ca\(^{2+}\)-ATPase increase in the same experiments.

F. B. Davis and P. J. Davis, unpublished observations.
of significance were carried out as outlined in the legend to Table I.

**Effect of phenolic 4'-hydroxyl group, diphenyl ether linkage, and D-configuration on stimulation of red cell membrane Ca\(^{2+}\)-ATPase activity by thyroid hormone analogues**

Measurement of enzyme activity, analogue effect, and estimation of significance were carried out as indicated in the legend to Table I.

<table>
<thead>
<tr>
<th>Analogue</th>
<th>% of T(_4) effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3,5,3',5'-tetrafluorothyroxine acid</td>
<td>66</td>
</tr>
<tr>
<td>CH(_2)</td>
<td>H</td>
</tr>
<tr>
<td>CH(_2)</td>
<td>H</td>
</tr>
<tr>
<td>CH(_2)</td>
<td>H</td>
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<tr>
<td>CH(_2)</td>
<td>NH(_2)</td>
</tr>
<tr>
<td>CH(_2)</td>
<td>NH(_2)</td>
</tr>
</tbody>
</table>

**Effect of Tyrosine Analogues**—The effects of several ring-substituted tyrosine analogues on Ca\(^{2+}\)-ATPase activity are shown in Table IV. Iodinated, brominated, nitro-, and methyl-substituted analogues all possessed enzyme stimulatory activity, while tyrosine itself, at 10\(^{-9}\) M concentration, acted as an inhibitor of Ca\(^{2+}\)-ATPase activity. While the decarboxylated analogue, 3,5-diiodothyroxine, stimulated Ca\(^{2+}\)-ATPase activity, the deaminated analogue, 3,5-diiodo-4-hydroxyphenylpropiolic acid was nonstimulatory. The noniodinated parent compounds of these analogues were also inactive in this enzyme system.

**DISCUSSION**

The human erythrocyte membrane Ca\(^{2+}\)-ATPase assay used in the present study is an *in vitro* model of extraneural thyroid hormone action and affords the opportunity to define structure-activity relationships of thyroid hormone analogues in a human cell system. The enzyme assay is based on the method of Strittmatter et al. (26) which employs Tris buffer and generates enzyme activity levels which are lower than those reported by other methods (27-29). We have recently explored the effects of buffers on hormone-sensitive Ca\(^{2+}\)-ATPase (23) and have concluded that the imidazole buffer shown by Farrance and Vincenzi (30) and others (27) to yield high levels of enzyme activity inhibits thyroid hormone action on red cell membranes in a concentration-dependent manner. We have established elsewhere that stimulation of Ca\(^{2+}\)-ATPase by T\(_4\) is optimal at a hormone concentration of 10\(^{-8}\) M in both human erythrocyte (21,23) and rabbit sarcalamell (31) membranes. The observation that T\(_4\) is more effective than T\(_3\) in stimulating Ca\(^{2+}\)-ATPase activity is consistent with our previous studies and is not surprising.
Based on our analysis of Ca\textsuperscript{2+}-ATPase stimulation in the human red blood cell, we show that, at a physiologic concentration, thyroid hormone action requires a number of structural conditions. First, the inner ring 3 and 5 positions must be occupied by functional groups of a size comparable to that of iodine, if not iodine itself. While 3,5-T\textsubscript{2} was 77% as effective as T\textsubscript{3}, 3,5,5'-L-T\textsubscript{2} was minimally effective, and 3,3',5'-L-T\textsubscript{2} and 3',5'-monoiodo-L-thyronine not at all stimulatory. Reversely T\textsubscript{3}, lacking iodine in the 3-position, was inactive. The tetrabromo derivative of T\textsubscript{3} was equal to T\textsubscript{3} in Ca\textsuperscript{2+}-ATPase stimulation, and 3,5-di bromo-L-thyronine was 67% as effective as its iodinated counterpart, 3,5-T\textsubscript{2}. DIMIT, with 3,5-methyl substitutions and a 3'-isopropyl group, was fully as active as T\textsubscript{3} in this red cell system. DIMIT has received much attention as an in vivo thyromimetic analogue (3, 33, 34), and its activity in our in vitro system correlates well with its known in vivo activities. Acetylation of the amino group of DIMIT results in loss of Ca\textsuperscript{2+}-ATPase effect.

Second, it is necessary for the side chain to be of a critical length, at least propionyl, for some enzyme stimulating activity to be retained in deaminated hormone analogues. Both tetrac and triac, which contain one less side chain carbon than T\textsubscript{3} and T\textsubscript{3}, are inactive in the red cell Ca\textsuperscript{2+}-ATPase system. Addition of another carbon to the side chain of tetrac, forming diiodothyropropionic acid, results in enzyme stimulating activity which is 50% of the effect of T\textsubscript{3}. The decarboxylation of T\textsubscript{3} to form Tamine, leads to 50% retention of enzyme stimulating effect as compared with T\textsubscript{3} and 71% as compared with T\textsubscript{3}. Modification of the carbonyl group by esterification to T\textsubscript{3}-ethyl ester resulted in complete loss of Ca\textsuperscript{2+}-ATPase stimulation by the analogue.

Third, the perpendicular (skewed) conformation of two rings, conferred by the diortho substituted phenyl ether linkage, is necessary for maintenance of thyroid hormone stimulating effect. This is suggested by the observation that those analogues which are not sterically required to be skewed (e.g. rT\textsubscript{3}, 3,3',5'-T\textsubscript{3}, 3',5'-T\textsubscript{1}) have no stimulatory effect. Fourth, the L-configuration is also necessary for hormone action; both D-T\textsubscript{3} and D-T\textsubscript{3} were ineffective. Fifth, elimination of the 4'-hydroxyl group still permits stimulation of Ca\textsuperscript{2+}-ATPase activity.

Last, since tyrosine analogues with 3- or 3,5-substitutions by iodide, bromide, methyl, or nitro-groups retained the ability to stimulate red cell membrane Ca\textsuperscript{2+}-ATPase, it is apparent that these rings are not necessary for this hormone effect. A tyrosine derivative, lacking the side chain carbonyl group, retained stimulatory activity with iodide ions present in the 3,5-positions. With removal of the amino group, however, stimulatory activity was lost, even in the presence of 3- and 5-position iodination. Thus, the primary prerequisite for this stimulatory effect is recognition of a diortho-substituted tyrosyl moiety, followed by the stereospecifically oriented substituted diphenyl ether ring system. This is also the first observation of a hormonogenic response from selected single ring thyroid hormone analogues. Single ring analogues do not bind to the serum transport proteins, nor to the nuclear receptor.

Our findings with regard to the effect of thyroid hormone analogues are generally in agreement with in vitro and in vivo comparisons of biological potency carried out in animal systems, as summarized extensively by Jorgensen (13, 32) and Cody (14), except that in our assay system T\textsubscript{3} is more active than T\textsubscript{3}, and selected tyrosine analogues are stimulatory. Stimulation of rat thymocyte 2-deoxy-D-glucose uptake by L-isomers of rT\textsubscript{3}, 3,5-T\textsubscript{3}, and thyronine, as studied by Segal and Ingbar, has been demonstrated at analogue concentrations of 10\textsuperscript{-7} to 10\textsuperscript{-6} M (16) and is calcium-dependent (17). These authors have recently compared the effect of analogues on [\textsuperscript{125}I]T\textsubscript{3} binding to rat thymocytes and have shown that rT\textsubscript{3}, 3,5-T\textsubscript{3}, and thyronine had relative affinities for T\textsubscript{3}-binding sites on thymocyte membranes which correlated well with their relative stimulatory effect on 2-deoxy-D-glucose uptake (18). These findings contrast with our previous demonstration that tetrac, which binds to red cell membranes, displaces T\textsubscript{3}, also inhibits T\textsubscript{3} stimulation of Ca\textsuperscript{2+}-ATPase activity; tetrac has no intrinsic Ca\textsuperscript{2+}-ATPase stimulating effect (22). Our previous binding studies support the thesis that thyroid hormone stimulation of Ca\textsuperscript{2+}-ATPase in red cell membranes requires hormone binding to the membranes.

Studies of binding of thyroid hormone analogues to nuclei in a variety of animal cells (6-10) have shown that D-analogues as well as tetrac and triac are bound avidly despite the trivial metabolic activity of these compounds in intact animals (13). Rapid in vivo metabolism and excretion of D-analogues, as compared with L-isomers (13), and a relative decrease in plasma membrane transport of D-analogues (35) have been postulated to explain the occasional discrepancies between nuclear-binding and biological potency observed with the D-isomers. The avidity of the acetic acid analogues for nuclear and cell membranes is probably due to their lipophilic nature. Nuclear-binding studies have also shown that the 4'-hydroxyl group is critical, but the ether bridge not critical for binding (7). These findings are in contrast to ours, and probably reflect the existence of a variety of hormone receptor sites in different subcellular fractions, which recognize different portions of the analogue molecule.

Galo et al. (20) have examined the effect of thyroid hormone analogues on erythrocyte Ca\textsuperscript{2+}-ATPase in the rat. In their study, T\textsubscript{3} was 100-fold as effective as T\textsubscript{3} in stimulating Ca\textsuperscript{2+}-ATPase activity, but the hormones were stimulatory only in rats fed a diet containing lard as the principal fat source; with an increase in dietary saturated fatty acids leading to membrane lipid alterations, thyroid hormones became inhibitory to Ca\textsuperscript{2+}-ATPase activity. In these rat erythrocyte studies, D-isomers of T\textsubscript{3} and T\textsubscript{3} as well as tetrac, triac, and monio dothyronine were inactive in stimulating Ca\textsuperscript{2+}-ATPase activity in animals fed the lard-containing diet.

The extrapolation to man of structure-activity relationships described in animal models has some hazard. We have already reported that there are probably interspecies differences in susceptibility of red cell Ca\textsuperscript{2+}-ATPase activity to iodothyronine (36). It is also clear that thyroid hormone action on Na,K-dependent adenosine triphosphatase (Na,K-ATPase), a nucleus-dependent hormone effect, appears to be different in man and rat (37, 38).

Whether the Ca\textsuperscript{2+}-ATPase effect of thyroid hormone in the human red cell membrane applies to human cells of nonhematopoietic origin is not yet clear. We have found that the rabbit and human erythrocyte Ca\textsuperscript{2+}-ATPases are similar in terms of hormone responsiveness (36). We have recently determined that the sarcocellular Ca\textsuperscript{2+}-ATPases of rabbit myocardium is readily stimulated in vitro by 10\textsuperscript{-6} M L-T\textsubscript{3} and L-T\textsubscript{3}, but not stimulated by D-T\textsubscript{3}, tetrac, and triac (31). In the rabbit heart system, L-T\textsubscript{3} and L-T\textsubscript{3} are equipotent. In animal systems, the Ca\textsuperscript{2+}-ATPase in the red cell appears to be representative of plasma membrane Ca\textsuperscript{2+}-ATPase in a variety of tissues (39).

We have shown that stimulation of human red cell Ca\textsuperscript{2+}-ATPase activity by physiologic concentrations of T\textsubscript{3} and T\textsubscript{3} is accompanied by enhanced calcium efflux in vitro in the intact erythrocyte (40, 41), thus providing a functional correlation of the Ca\textsuperscript{2+}-ATPase studies in membrane vesicles. We
have extended the efflux studies to include DIMIT and D-T, at $10^{-3}$ m; the former stimulates Ca$^{2+}$ efflux, while the latter does not. Thus, we believe that the effect of iodothyronines on Ca$^{2+}$-ATPase activity in the human erythrocyte membrane is an index of biologic activity of thyroid hormone in the intact cell.

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Stimulation by thyroid hormone analogues of red blood cell Ca2+-ATPase activity in vitro. Correlations between hormone structure and biological activity in a human cell system.

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