Stimulation by Thyroid Hormone Analogues of Red Blood Cell Ca\(^{2+}\)-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\(^{2+}\)-ATPase activity is stimulated in vitro by physiological concentrations (10\(^{-10}\) M) of L-thyroxine (L-T\(_4\)) and 3,5,3'-triiodo-L-thyronine (L-T\(_3\)). This human cell system has been utilized to examine a series of iodothyronines and iodothyronine analogues for structure-activity relationships. Analogue purity was verified by high pressure liquid chromatography. Analogues were studied at a concentration of 10\(^{-10}\) M and the stimulatory effect of each analogue was compared with that of L-T\(_3\) in this system. Essential to Ca\(^{2+}\)-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'-hydroxy group is not essential to Ca\(^{2+}\)-ATPase stimulation in this model system. T\(_3\) was 76% as active as T\(_4\) in stimulating Ca\(^{2+}\)-ATPase activity. The stimulatory effect of 3,5-dimethyl-3'-isopropyl-L-thyronine and 3,5,3',5'-tetramethyl-L-thyronine approximated that of L-T\(_3\). 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 thyrmiometric 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, TBPA, and serum albumin has been extensively studied and reviewed (13, 14).

Uptake of a-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 Ca\(^{2+}\)-stimulable, Mg\(^{2+}\)-dependent adenosine triphosphatase (Ca\(^{2+}\)-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\(^{-9}\) and 10\(^{-8}\) M (20).

We have recently shown that the Ca\(^{2+}\)-ATPase activity of human red blood cell membrane is maximally stimulated in vitro by physiologic concentrations of thyroid hormone (10\(^{-10}\) M) (21). Stimulation of Ca\(^{2+}\)-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\(^{2+}\)-ATPase (23). This red cell Ca\(^{2+}\)-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\(^{2+}\)-ATPase activity (21). We have now used this human cell Ca\(^{2+}\)-ATPase assay to investigate a wide variety of iodothyronine and iodothyronine analogues and have characterized the importance of substituent groups to the stimulation of human red cell Ca\(^{2+}\)-ATPase activity.

MATERIALS AND METHODS

Hormones and Analogues—Hormones, hormone analogues, and their sources were as follows: L-T\(_4\), L-T\(_3\), D-T\(_3\), D-T\(_4\), T\(_3\), 3,5-diiodo-L-tyrosine, 3,5-dibromo-L-tyrosine, 3,5-dinitro-L-tyrosine, 3-monochloro-L-tyrosine, 3,5,3',5'-tetraiodothyroacetic acid; triac, 3,5,3',5'-triiodothyroacetic acid; rT3, 3,5,3',5'-triiodothyronine; 3,5,3',5'-tetraiodothyroacetic acid; DMIT, 3,5-dimethyl-3'-isopropyl-L-tyrosine; T\(_3\)amine, 3,5,3'-triiodothyronamine; BSA, bovine serum albumin; EGTA, ethylene glycol bis(2-aminoethyl)-N,N',N'-tetraacetic acid.

\(^{1}\) The abbreviations used are: TBG, thyroxine-binding globulin; TBPA, thyroxine-binding prealbumin; L-T\(_4\), L-thyroxine; D-T\(_4\), D-thyroxine; L-T\(_3\), 3,5,3'-triiodo-L-thyronine; D-T\(_3\), 3,5,3'-triiodo-D-thyronine; T\(_3\), 3,5-diiodo-L-thyronine; T\(_4\), 3,5,3',5'-tetraiodothyroacetic acid; triac, 3,5,3',5'-triiodothyroacetic acid; rT3, 3,5,3',5'-triiodothyronine; DMIT, 3,5-dimethyl-3'-isopropyl-L-tyrosine; T\(_3\)amine, 3,5,3'-triiodothyronamine; BSA, bovine serum albumin; EGTA, ethylene glycol bis(2-aminoethyl)-N,N',N'-tetraacetic acid.

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**Thyroid Hormone and Ca²⁺-ATPase**

**RESULTS**

**L-T₄ and L-T₃ Stimulation of Red Cell Ca²⁺-ATPase Activity in Vitro**—The mean basal Ca²⁺-ATPase activity of red cell membranes in these experiments was 0.247 ± 0.023 (S.E.) μmol of P/ mg of protein·90 min. With the addition of L-T₄ (10⁻¹⁰ M), enzyme activity rose to 0.359 ± 0.036 μmol of P/ mg·90 min resulting in an increase in activity of 0.112 ± 0.015 μmol of P/ mg·90 min (p < 0.001) in a total of 14 experiments, utilizing membrane preparations from 10 donors. L-T₃ (10⁻¹⁰ M) caused an increase in enzyme activity of 0.086 ± 0.008 μmol of P/ mg·90 min in the same experiments (p < 0.001); this increase was 76% of that seen with T₄, 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 Ca²⁺-ATPase activity are shown in Table I. L-T₄, with iodination in the 3 and 5 positions on the inner ring, retained 77% of the effect of T₄ in our assay system. In contrast, 3',5',L-T₂ had only 28% of the T₄ effect, and 3,3'-L-T₂ and 3'-monooiodo-L-thyronine were inactive. While L-T₃ was 76% as active as T₄, rT₃ 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-T₄ action, as well as ½ of the effect of 3,5-L-T₄.

**Modification of Alanyl Side Chain: Acetic and Propionic Acid Analogues, Decarboxylated Analogues, and Ethyl Esterification**—The deaminated analogues, tetraac and triac, with one less carbon in the side chain than found in L-T₄, and L-T₃, were ineffective in this Ca²⁺-ATPase system (Table II).

**TABLE I**

| Analogue | % of T₄ effect |
|----------|---------------|
| L-T₄     | 100           |
| L-T₃     | 76            |
| rT₃      | NS            |
| 3,5-L-T₂ | 27            |
| 3',5'-L-T₂| 28           |
| 3,3'-L-T₂| NS            |
| 3'-L-T₃ | NS            |
| 3,5,3',5'-tetrabromo-L-thyronine | 98 |
| 3,5-dibromo-L-thyronine | 50 |
| N-acetyl DIMIT | 87 |

*F. B. Davis and P. J. Davis, unpublished observations.*
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

**Table II**

| R\(_1\) | R\(_2\) | R\(_3\) | 5' Analogue | % of T\(_4\) effect |
|--------|--------|--------|-------------|-------------------|
| CH\(_2\) | H | CO\(_2\) | 1 | 3,5,3',5'-tetraiodothyropropionic acid | 56 |
| CH\(_2\) | H | CO\(_2\) | 1 | 3,5,3',5'-tetraiodothyroacetic acid | NS |
| CH\(_2\) | H | CO\(_2\) | H | 3,5,3',5'-tribromo-4-hydroxyphenylpropionic acid | 54 |
| CH\(_2\) | NH\(_2\) | CO\(_2\)H\(_2\) | H | L-T\(_3\) ethylester | NS |
| CH\(_2\) | NH\(_2\) | H | H | 3,5,3'-triiodothyronine | 54 |

Effect of Tyrosine Analogues—The effects of several ring-substituted tyrosine analogues on Ca\(^{2+}\)-ATPase activity are shown in Table IV. L-Tyrosine caused inhibition of enzyme activity, as indicated by the negative per cent of T\(_4\)-induced change in enzyme activity.

**Table IV**

| Analogue | % of T\(_4\) effect |
|----------|--------------------|
| I | COOH | NH\(_2\) | 3,5-diiodo-L-tyrosine | 46 |
| Br | Br | COOH | NH\(_2\) | 3,5-dibromo-L-tyrosine | 50 |
| Me | Me | COOH | NH\(_2\) | 3,5-dimethyl-L-tyrosine | 60 |
| NO\(_2\) | NO\(_2\) | COOH | NH\(_2\) | 3,5-dinitro-L-tyrosine | 41 |
| I | COOH | NH\(_2\) | 3-iodo-L-tyrosine | 69 |
| NO\(_2\) | H | COOH | NH\(_2\) | 3-nitro-L-tyrosine | 56 |
| H | COOH | NH\(_2\) | 1-tyrosine | 53 |
| I | H | NH\(_2\) | 3,5-diiodotyramine | 63 |
| H | H | NH\(_2\) | tyramine | 63 |
| I | I | COOH | H | 3-(3,5-diiodo-4-hydroxyphenyl)propionic acid | NS |
| H | H | COOH | H | 3-(p-hydroxyphenyl)propionic acid | NS |

In contrast, 3,5,3',5'-tetraiodothyropropionic acid had more than 50% of the stimulatory effect in enzyme activity seen with L-T\(_4\).

The decarboxylated analogue of L-T\(_3\), T\(_3\)-amine, was 54% as effective as L-T\(_4\), and 71% as effective as L-T\(_3\) in stimulating enzyme activity (Table II). Exhyl esterification of L-T\(_4\) resulted in loss of enzyme stimulatory action.

**Phenolic 4'-Hydroxyl Group Substitution**—The 4'-deoxy analogue of 3,5-L-T\(_2\) and 3,5-L-T\(_2\) itself were equally effective in stimulating Ca\(^{2+}\)-ATPase activity, as seen in Table III. In the absence of a phenolic group, a 4'-deoxy analogue (Table II) was effective in stimulating Ca\(^{2+}\)-ATPase activity, as seen in Table III. D-T\(_3\) and D-T\(_4\) were ineffective in stimulating Ca\(^{2+}\)-ATPase activity.

**Effect of Tyrosine Analogues**—The effects of several ring-substituted tyrosine analogues on Ca\(^{2+}\)-ATPase activity are shown in Table IV. L-Tyrosine caused inhibition of enzyme activity, as indicated by the negative per cent of T\(_4\)-induced change in enzyme activity.
Based on our analysis of $\text{Ca}^{2+}$-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$_3$ was 77% as effective as T$_3$, 3',5'-L-T$_3$ was minimally effective, and 3,3'-L-T$_2$ and 3'-monoiodo-L-thyronine not at all. Stimulatory T$_3$, lacking iodine in the 5-position, was inactive. The tetraiodo derivative of T$_3$ was equal to T$_3$ in Ca$^{2+}$-ATPase stimulation, and 3,5-dibromo-L-thyronine was 67% as effective as its iodinated counterpart, 3,5-T$_2$. DIMIT, with 3,5-methyl substitutions and a 3'-isopropyl group, was fully as active as T$_3$ 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$^{2+}$-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$_3$ and T$_2$, are inactive in the red cell Ca$^{2+}$-ATPase system. Addition of another carbon to the side chain of tetra- and triac, forming propiothyroproionic acid, results in enzyme stimulating ability which is 50% of the effect of T$_3$. The decarboxylation of T$_3$ to form T$_3$amine, leads to 50% retention of enzyme stimulating effect as compared with T$_3$ and 71% as compared with T$_2$. Modification of the carboxyl group by esterification to T$_3$-ethyl ester resulted in complete loss of Ca$^{2+}$-ATPase stimulation by the analogue.

Third, the perpendicular (skewed) conformation of two rings, conferred by the diorthosubstituted 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$_3$, 3',5'-L-T$_3$, 3'-T$_1$) have no stimulatory effect. Fourth, the $\text{L}$-conformation is also necessary for hormone action; both D-T$_3$ and D-T$_2$ were ineffective. Fifth, elimination of the 4'-hydroxyl group still permits stimulation of Ca$^{2+}$-ATPase activity. In these rat erythrocyte studies, D-analogue molecule.

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$^{2+}$-ATPase, it is apparent that two rings are not necessary for this hormone action. A tyramine derivative, lacking the side chain carboxyl 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$_3$ is more active than T$_3$, and selected tyrosine analogues are stimulatory. Stimulation of rat thymocyte $2\text{-deoxy-\text{D-glucose}}$ uptake by $L$-isomers of rT$_3$, 3,5-T$_3$, and thyrone, as studied by Segal and Inghar, has been demonstrated at analogue concentrations of 10$^{-7}$ to 10$^{-6}$ M (16) and is calcium-dependent (17). These authors have recently compared the effect of analogues on $[35\text{S}]\text{T}_3$ binding to rat thymocytes and have shown that rT$_3$, 3,5-T$_3$, and thyronine had relative affinities for T$_3$-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, displacing T$_3$, also inhibits T$_3$ stimulation of Ca$^{2+}$-ATPase activity; tetrac has no intrinsic Ca$^{2+}$-ATPase stimulating effect (22). Our previous binding studies support the thesis that thyroid hormone stimulation of Ca$^{2+}$-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$^{2+}$-ATPase in the rat. In their study, T$_3$ was 100-fold as effective as T$_3$ in stimulating Ca$^{2+}$-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$^{2+}$-ATPase activity. In these rat erythrocyte studies, D-isomers of T$_3$ and T$_2$ as well as tetrac, triac, T$_3$, and monoiodothyronine were inactive in stimulating Ca$^{2+}$-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 important interspecies differences in susceptibility of red cell Ca$^{2+}$-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$^{2+}$-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$^{2+}$-ATPases are similar in terms of hormone responsiveness (36). We have recently determined that the sarclemmal Ca$^{2+}$-ATPase of rabbit myocardium is readily stimulated in vitro by 10$^{-9}$ M T$_3$ and L-T$_3$, but not stimulated by D-T$_3$, tetrac, and triac (31). In the rabbit heart system, L-T$_3$ and L-T$_2$ are equipotent. In animal systems, the Ca$^{2+}$-ATPase in the red cell appears to be representative of plasma membrane Ca$^{2+}$-ATPase in a variety of tissues (39).

We have shown that stimulation of human red cell Ca$^{2+}$-ATPase activity by physiologic concentrations of T$_3$ and T$_2$ is accompanied by enhanced calcium efflux in vitro in the intact erythrocyte (40, 41), thus providing a functional correlate of the Ca$^{2+}$-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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REFERENCES
1. Gross, J., & Pitt-Rivers, R. (1953) Biochem. J. 53, 652-657
2. Baiker, S. B., Shimada, M. & Makuchi, M. (1965) Endocrinology 76, 115-121
3. Comise, F., Burrow, G. N. & Jorgensen, E. C. (1978) Endocrinology 102, 1670-1674
4. Tomita, K. & Lardy, H. A. (1966) J. Biol. Chem. 219, 595-604
5. Frieden, E. & Yoshizato, K. (1974) Endocrinology 95, 188-194
6. Samuels, H. H., Tsai, J. S. & Casanova, J. & Stanley, F. (1974) J. Clin. Invest. 54, 853-865
7. Koerner, D., Schwartz, H. L., Surks, M. I., Oppenheimer, J. H. & Jorgensen, E. C. (1975) J. Biol. Chem. 250, 6417-6423
8. DeGroot, L. J. & Torresani, J. (1975) Endocrinology 96, 357-369
9. Jorgensen, E. C., Bolger, M. B. & Dietrich, S. W. (1977) Proceedings of the International Congress of Endocrinology, 5th, 1976 Excerpta Med. Found. Int. Congr. Ser. No. 402, Vol. 1, pp. 117-120
10. Oppenheimer, J. H., Koerner, D., Schwartz, H. L. & Surks, M. I. (1972) J. Clin. Endocrinol. Metab. 35, 330-333
11. Sterling, K., Lazarus, J. H. & Milch, P. O. (1976) Endocrinology 98, (suppl.) (Abstr. 181)
12. Singh, S. P., Carter, A. C., Kydd, D. M. & Costanzo, R. R., Jr. (1976) Endocrinol. Res. Comm. 3, 119-131
13. Jorgensen, E. C. (1978) in Hormonal Proteins and Peptides (Li, C. H., ed) Vol. 6, pp. 297-294, Academic Press, New York
14. Cody, V. (1980) Endocrin. Rev. 1, 140-166
15. Goldfine, I. D., Smith, G. J., Simon, C. G., Ingbar, S. H. & Jorgensen, E. C. (1976) J. Biol. Chem. 251, 4235-4238
16. Segal, J. & Ingbar, S. H. (1979) J. Clin. Invest. 63, 507-515
17. Segal, J. & Ingbar, S. H. (1981) J. Clin. Invest. 68, 103-110
18. Segal, J. & Ingbar, S. H. (1982) J. Clin. Invest. 70, 919-926
19. De Mendoza, D., Moreno, H. & Farias, R. N. (1978) J. Biol. Chem. 253, 6255-6259
20. Gao, M. G., Uñates, L. E. & Farias, R. N. (1981) J. Biol. Chem. 256, 7113-7114
21. Davis, P. J. & Blas, S. D. (1981) Biochem. Biophys. Res. Commun. 109, 1073-1080
22. Davis, P. J., Davis, F. B. & Blas, S. D. (1982) Life Sci. 30, 675-682
23. Davis, F. B., Davis, P. J. & Blas, S. D. (1983) J. Clin. Invest. 71, 579-586
24. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
25. Fiske, C. H. & Subbarow, Y. (1925) J. Biol. Chem. 66, 375-400
26. Strittmatter, W. J., Hirats, F. & Axelrod, J. (1979) Biochem. Biophys. Res. Commun. 88, 147-153
27. Jarrett, H. W. & Penniston, J. T. (1978) J. Biol. Chem. 253, 4676-4682
28. Scharff, O. (1980) in Membrane Transport in Erythrocytes (Lassen, U. V., Ussing, H. H. & Weich, J. O., eds) Alfred Benzon Symposium 14, pp. 236-249, Munksgaard, Copenhagen
29. Vincenzi, F. F. & Larsen, F. L. (1980) Fed. Proc. 39, 2427-2431
30. Farrance, M. L. & Vincenzi, F. F. (1977) Experientia (Basel) 33, 865-866
31. Rudinger, A., Mylotte, K. M., Davis, P. J. & Davis, F. B. (1983) Clin. Res. 31, 215A
32. Jorgensen, E. C. (1976) PharmacoL Ther. Part B Gen. Syst. Pharmacol. 2, 661-662
33. Tamagn, E. I., Hershman, J. M. & Jorgensen, E. C. (1979) J. Clin. Endocrinol. Metab. 48, 196-200
34. Melmed, S., Spira, O., Gordon, A., Gross, J., Jorgensen, E. C. & Hershman, J. M. (1980) Endocrinology 107, 1050-1054
35. Samuels, H. H. (1983) in Molecular Basis of Thyroid Hormone Action (Oppenheimer, J. H. & Samuels, H. H., eds) pp. 35-65, Academic Press, New York
36. Davis, F. B., Kite, J. H., Jr., Davis, P. J. & Blas, S. D. (1982) Endocrinology 110, 297-298
37. Cole, C. H. & Waddell, R. W. (1976) J. Clin. Endocrinol. Metab. 42, 1056-1063
38. Smith, T. J. & Edelman, S. (1979) Fed. Proc. 38, 2150-2153
39. Wang, J. H., Edelman, I. S. & Oppenheimer, J. H. (1980) Curr. Top. Cell. Regul. 15, 47-107
40. Nieman, L. K., Davis, P. J., Davis, F. B. & Schoenl, M. (1982) Proceedings of the 38th Annual Meeting of the American Thyroid Association, Quebec City, Quebec, Canada, September, 1982
41. Nieman, L. K., Davis, F. B., Davis, P. J., Cunningham, E. E., Gutman, S., Blas, S. D. & Schoenl, M. (1983) Kidney Int., in press

3 P. J. Davis, F. B. Davis, and M. Schoenl, unpublished observations.
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