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Rabbit skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase activity: stimulation in vitro by thyroid hormone analogues and bipyridines

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Sarcoplasmic reticulum-enriched membranes from rabbit skeletal muscle contained Ca^{2+}-ATPase activity which was significantly enhanced (26% increase, $P < 0.001$) in vitro by physiological concentrations (10^{-10} M) of L-thyroxine (T_4) and 3,3′,5-triiodo-L-thyronine (T_3). In contrast, the biologically inactive iodothyronine analogues D-T_4 and 3,3′,5,5′-tetraiodothyroacetic acid (Tetrac) (10^{-10} M) were without effect on enzyme activity. 3,5-Dimethyl-3′-isopropyl-L-thyronine (Dimit), a bioactive analogue, was highly effective as a Ca^{2+}-ATPase stimulator, increasing enzyme activity by 43% ($P < 0.02$ vs. T_4 effect). A bipyridine cardiac inotropic agent, milrinone, has been reported to be thyromimetic in a myocardial membrane Ca^{2+}-ATPase system, and in concentrations from 10^{-10} to 10^{-5} M enhanced skeletal muscle SR membrane Ca^{2+}-ATPase activity in vitro ($P < 0.001$). Milrinone analogues which have been previously shown to enhance rabbit myocardial membrane Ca^{2+}-ATPase activity, and which have a twist relationship of the pyridine rings, were also striated muscle Ca^{2+}-ATPase stimulators. We conclude that (1) striated muscle is a mammalian tissue in which physiological levels of biologically relevant thyroid hormone analogues, particularly Dimit, stimulate Ca^{2+}-ATPase activity in vitro by a non-genomic mechanism; (2) cardiac bipyridine analogues which are thyromimetic in vitro in rabbit heart, and which have structural homologies with thyroid hormone, are stimulators of rabbit striated muscle sarcoplasmic reticulum Ca^{2+}-ATPase activity.

Introduction

In skeletal muscle, contraction and relaxation are regulated, respectively, by the rapid release of Ca^{2+} from sarcoplasmic reticulum (SR) and re-uptake of Ca^{2+} by SR [1]. Re-uptake is a function of SR Ca^{2+}-ATPase (calcium pump) activity. While it has been suggested that SR Ca^{2+}-ATPase is also involved in Ca^{2+} release from SR [2], it is likely that excitation-contraction coupling depends upon other mechanisms for opening the Ca^{2+} release channel of SR [3]. Our laboratory has reported that thyroid hormone stimulates the activity of human and animal red cell membrane Ca^{2+}-ATPase [4,5], a calmodulin-regulated enzyme. The mechanism of this extranuclear action of the hormone in the red cell is incompletely understood, but appears to involve release from erythrocyte membranes of a calmodulin-enhancing factor [6]. Hormone action on red cell Ca^{2+}-ATPase activity may be modulated by α-adrenergic receptor agonism [7] and by specific inositol phosphates [8]. The latter may also be involved in the non-genomic action of thyroid hormone on bone [9]. We have also observed that thyroid hormone enhances Ca^{2+}-ATPase activity in vitro in rabbit myocardial sarcolemmal membranes [10]. The actions of iodothyronines on the red cell and myocardial membranes occur at physiological concentrations of thyroid hormone. The effects of thyroid hormone in vivo on striated muscle function are documented [11,12]. We have examined the susceptibility of rabbit skeletal muscle SR Ca^{2+}-ATPase to stimulation in vitro by thyroid hormone analogues.

We were also interested in determining whether a thyromimetic bipyridine, milrinone [13] (Fig. 1), and several of its analogues, were capable of stimulating skeletal muscle SR Ca^{2+}ATPase, comparable to its in vitro action on myocardial membrane Ca^{2+}-ATPase activity [14]. We have reported that milrinone shares structural homologies with thyroid hormone, as deter-

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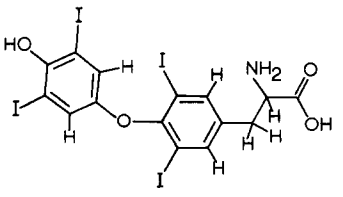
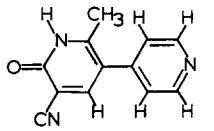


Thyroxine



Milrinone

Fig. 1. Molecular structures of T_4 and milrinone.

mined by X-ray crystallographic analysis [14], and others have shown that milrinone mimics thyroid hormone action in rat bone cells [15].

Materials and Methods

Reagents and hormones

Na_2ATP, 3,5,3′,5′-tetraiodo-L-thyronine (L-thyroxine, T_4), D-T_4, 3,5,3′-triiodo-L-thyronine (T_3), 3,5,3′,5′-tetraiodothyroacetic acid (Tetrac) and 3,5,3′-triiodothyroacetic acid (Triac) were obtained from Sigma (St. Louis, MO). 3,3′,5′-L-Triiodothyronine (reverse T_3, rT_3) was obtained from Warner-Lambert (Philadelphia, PA), and 3,5-dimethyl-3′-isopropyl-L-thyronine (Dimit) from the late Dr. E.C. Jorgensen (San Francisco, CA). Milrinone (2-methyl-5-cyano-[3,4′-bipyridin]-6(1*H*)-one) and its analogues were graciously provided by Sterling-Winthrop Research Institute (Rensselaer, NY). The analogues were as follows: 2-methyl-5-bromo-[3,4′-bipyridin]-6(1*H*)-one (WIN 47817); 2-methyl-5-amino-[3,4′-bipyridin]-6(1*H*)-one (WIN 47170); 2,3-methyl-5-cyano-[3,4′-bipyridin]-6(1*H*)-one (WIN 48179); 5-cyano-[3,4′-bipyridin]-6(1*H*)-one (WIN 37582); 2-methyl-[3,4′-bipyridin]-6(1*H*)-one (WIN 47623). [^{125}I]T_4 was obtained from New England Nuclear (Boston, MA), and had a specific activity of 1250 μCi/μg.

Preparation of striated muscle microsomes

Striated muscle microsomes were prepared from rabbit (New Zealand White) back muscle by the method of Sumida et al. [16] with slight modification. Muscle (75–100 g) was cleaned of fat and connective tissue, minced and homogenized in 300 ml of 10 mM Tris maleate (pH 6.8) with 3 thirty-second bursts in a Sorvall Omnimixer at speed 8. The homogenate was centrifuged at 5000 × g for 15 min. The supernatant was passed through four layers of cheesecloth and the filtrate was centrifuged at 15000 × *g* for 20 min. The resultant supernatant was filtered through four layers of cheesecloth and this filtrate centrifuged at 143000 × *g* for 30 min. The pellet was suspended in 100–150 ml of 0.6 M KCl/10 mM Tris maleate buffer and then centrifuged at 143000 × *g* for 45 min. The final pellet was suspended by hand in 6–12 ml of 10 mM Tris (pH 7.4), then frozen quickly and stored in liquid nitrogen. Enzyme activity was assayed within 1–10 days of microsome preparation. Yield of microsomes was approximately 1 mg protein/g skeletal muscle. There was no latent Na,K-ATPase activity unmasked by SDS, indicating that the preparation consisted primarily of SR membranes [17].

Ca^{2+}-ATPase assay

Mg^{2+}-dependent, Ca^{2+}-stimulable ATPase activity was assayed by our previously published method [4,5,10] in which the hydrolysis of ATP is measured in the presence and absence of Ca^{2+}. The reaction mixture contained 5 μg membrane protein, 25 mM Tris (pH 7.4), 0.1 mM EGTA, 1 mM $MgCl_2$, 75 mM NaCl, 25 mM KCl and 1 mM ATP, with or without $CaCl_2$, in a total volume of 2.5 ml. The assay began with the addition of the $MgCl_2$ and ATP. Maximum enzyme activity was achieved at a total calcium concentration of 0.15 mM, which provided a free calcium concentration of 12 μM as determined by ion-specific electrode. Using 5 μg protein per assay sample, enzyme activity was linear for 60 min at 37°C. Enzyme activity was expressed as μmol inorganic phosphate (P_i) liberated per mg membrane protein per min. 'Basal' enzyme activity referred to Ca^{2+}-ATPase activity in samples with no added hormone, bipyridine or analogue. Thyroxine, milrinone and associated analogues were added just prior to the start of the assay period and the effects were determined by measurement of the difference between enzyme activity in the presence and absence of these factors; control samples contained hormone or milrinone diluent (0.04 N KOH/4% propylene glycol diluted 1:10^7 in 10 mM Tris, or 1% DMSO in 10 mM Tris, respectively). Neither diluent affected enzyme activity.

Thyroid hormone binding to skeletal muscle SR membranes

Tracer [^{125}I]T_4, $4.3 \cdot 10^{-12}$ M, was incubated with 200 μg of skeletal muscle SR membranes and varying concentrations of unlabelled T_4 or milrinone, in 1 ml of 10 mM Tris, pH 7.45, at 37°C for one hour. Separation of bound and free hormone was accomplished by centrifugation at 20000 × g for 10 min. Nondisplaceable binding of labelled T_4 accounted for 67% of total binding, at a T_4 concentration of 10^{-6} M, and was subtracted from the total binding at each T_4 and milrinone concentration.

Statistical analysis

Each experiment was performed in duplicate on membranes from a single preparation. The results pre-

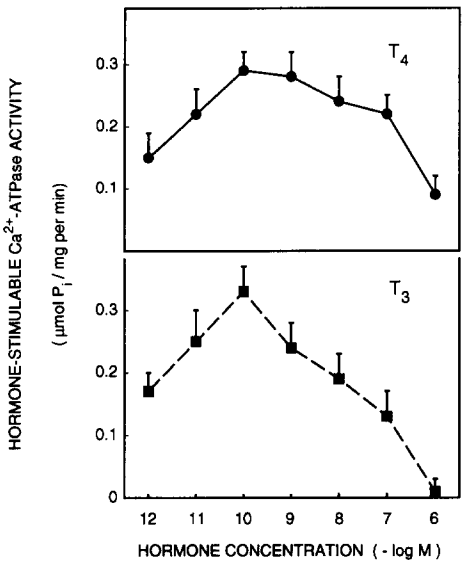


Fig. 2. Effect of T_4 (upper panel) and T_3 (lower panel) on skeletal muscle sarcoplasmic reticulum (SR) Ca^{2+}-ATPase activity in vitro. Hormone concentrations of 10^{-12} to 10^{-6} M were used. The ordinate shows the parabolic change in enzyme activity observed with the addition of iodothyronine, which was significant overall ($P < 0.001$, ANOVA). With both T_4 and T_3, stimulation was maximal at 10^{-10} M.

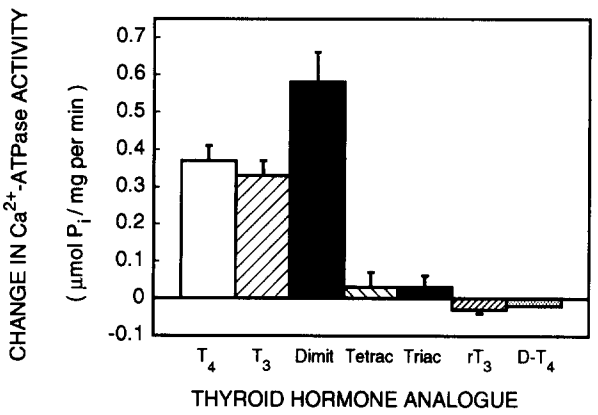


Fig. 3. Effect of thyroid hormone and analogues on skeletal muscle SR Ca^{2+}-ATPase activity. The ordinate indicates the changes in enzyme activity with the addition of T_4, T_3 or analogue at a concentration of 10^{-10} M. T_4, T_3 and Dimit each stimulated enzyme activity ($P < 0.001$, paired t-test, comparing activity with and without hormone analogue), while Tetrac, Triac, rT_3 and D-T_4 were inactive. The Dimit effect was significantly greater than that due to T_4 ($P < 0.02$, paired t-test).

sented are means ± standard error of means (SEM) of three or more experiments. Statistical analysis was carried out by one-way analysis of variance (ANOVA) or by Student's paired t-test. Intra-assay and interassay coefficients of variation for the Ca^{2+}-ATPase assay were 1.6% and 18.0%, respectively.

Results

Effect of T_4 and T_3 on Ca^{2+}-ATPase activity

Skeletal muscle Ca^{2+}-ATPase activity in the absence of T_4 or T_3 (i.e., basal activity) was 1.12 ± 0.08 µmol P_i per mg membrane protein per min in six duplicated experiments. T_4 stimulated Ca^{2+}-ATPase activity in concentrations from 10^{-12} to 10^{-6} M ($P < 0.001$, ANOVA) with maximal T_4-stimulable Ca^{2+}-ATPase of 0.29 ± 0.03 µmol P_i at an optimal T_4 concentration of 10^{-10} M (Fig. 2, upper panel, $P < 0.001$, paired t-test). The previously reported optimal T_4 concentration for Ca^{2+}-ATPase stimulation in human and rabbit red cell membranes [4,5], rabbit reticulocytes [18] and rabbit cardiac membranes [10] has been 10^{-10} M. T_3 was equal to T_4 in stimulating Ca^{2+}-ATPase activity with maximal effect seen at 10^{-10} M T_3 (Fig. 2, lower panel, $P < 0.001$, paired t-test).

Specificity of thyroid hormone effect

Other thyroid hormone analogues were then studied at a concentration of 10^{-10} M in this assay system to determine specificity of the hormone effect. As shown in Fig. 3, Dimit, a bioactive thyroid hormone analogue thought to exert its effect via extranuclear actions [19], was significantly more active than T_4 in stimulating the Ca^{2+}-ATPase ($P < 0.02$), with an increase of 0.58 ± 0.08, as compared with a T_4 effect of 0.37 ± 0.04 µmol P_i in the same experiments. The maximal Dimit effect was seen with a concentration of 10^{-10} M (results not shown). Tetrac, reverse T_3 and D-T_4, all biologically inactive thyroid hormone analogues [20], had no significant effect on basal Ca^{2+}-ATPase activity. Triac, which has thyromimetic activity in vivo in man [21], was inactive in the present enzyme assay system.

Effect of milrinone on SR Ca^{2+}-ATPase activity in vitro

Milrinone stimulated skeletal muscle SR Ca^{2+}-ATPase activity (Fig. 4) in concentrations from 10^{-10} M to 10^{-5} M ($P < 0.001$, ANOVA). The minimum concentration of milrinone necessary for significant stimulation of enzyme activity was 10^{-10} M ($P < 0.01$,

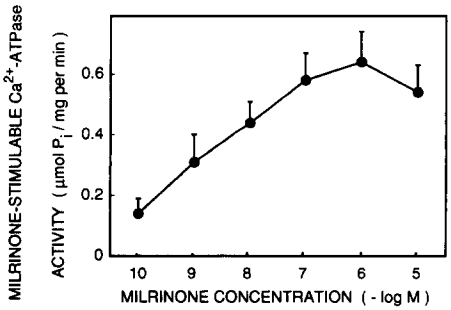


Fig. 4. Stimulation by milrinone of skeletal muscle SR Ca^{2+}-ATPase activity. The ordinate shows the increase in enzyme activity with the addition of milrinone; stimulation was significant at 10^{-10} M ($P < 0.002$, paired t-test) and maximal at 10^{-6} M.

paired t-test), and the maximal milrinone effect was seen at a concentration of 10^{-7} to 10^{-6} M. Other compounds known to stimulate Ca^{2+}-ATPase activity, such as flavonoids at low concentrations [22] and thyroid hormone [4,5,10,18], have a similar parabolic dose-response curve (see present Fig. 2).

Effect of milrinone analogues on Ca^{2+}-ATPase activity

To further elucidate structure-activity characteristics of milrinone, other bipyridines were tested in this assay system at a concentration of 10^{-7} M (Fig. 5). The 2-methyl-5-bromo analogue of milrinone was more active than 10^{-7} M milrinone in stimulating the Ca^{2+}-ATPase ($P < 0.001$). The C2 methyl group has been postulated to enhance thyromimetic activity of milrinone in sarcolemma-enriched membranes by forcing the two pyridine rings to adopt a more twisted conformation [14]. The 2-methyl-5-amino analogue was a weaker but significant stimulator of the enzyme; the 5-amino group does not occupy the same volume or have the same electrochemical potential as the cyano group of milrinone. Further studies showed that 10^{-6} and 10^{-7} M were the optimal concentrations for maximal stimulation by the 5-bromo and 5-amino analogues, respectively (data not shown).

The 5-cyano bipyridine analogue, lacking a methyl group in the C2 position, was weakly inhibitory in the skeletal muscle Ca^{2+}-ATPase system, and the 2-methyl analogue, lacking a substituent in the C5 position,

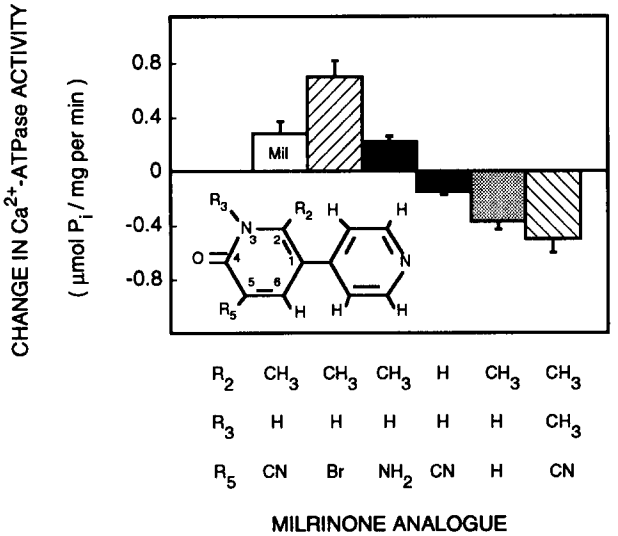


Fig. 5. Change in Ca^{2+}-ATPase activity of skeletal muscle membranes with addition of milrinone (Mil) and selected milrinone analogues. The ordinate indicates the increase or decrease in activity with a 10^{-7} M concentration of analogue. The ring substitutions of the analogues, including milrinone, are indicated below the graph. Necessary for enzyme stimulation are a C2 methyl group, a C5 substituent of a size similar to CN, Br or NH_2, and an unblocked nitrogen in the 3 position.

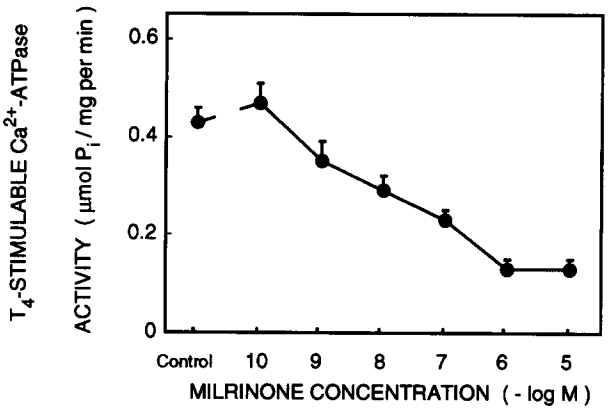


Fig. 6. Inhibition by milrinone of T_4-stimulable Ca^{2+}-ATPase activity in rabbit striated muscle SR. The increase in enzyme activity with 10^{-10} M T_4 is shown on the ordinate. The control value indicates T_4-stimulable enzyme activity in the absence of milrinone. Milrinone, 10^{-9} to 10^{-5} M, progressively inhibited the T_4 effect ($P < 0.001$, ANOVA).

significantly inhibited the Ca^{2+}-ATPase (Fig. 5). A milrinone analogue with a methyl group substituted on the 3 N position was also inhibitory in its action on Ca^{2+}-ATPase activity in vitro.

Inhibition of thyroid hormone-stimulable Ca^{2+}-ATPase activity by milrinone

The stimulatory effect of T_4 (10^{-10} M) in vitro on skeletal muscle SR Ca^{2+}-ATPase in the absence and presence of various concentrations of milrinone is shown in Fig. 6. Increasing the concentration of milrinone progressively and significantly inhibited the stimulatory effect of T_4 at concentrations of 10^{-9} M milrinone and greater ($P < 0.05$).

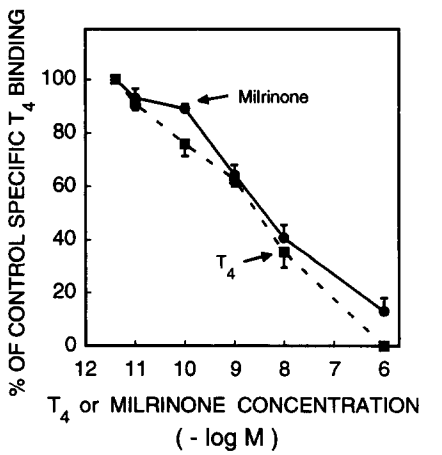


Fig. 7. Effect of milrinone and T_4 on binding of [^{125}I]T_4 to rabbit skeletal muscle SR membranes. Results are expressed as mean ± SEM of the percent of specific T_4 binding with tracer, alone ($4.3 \cdot 10^{-12}$ M). Specific binding was 31% of total binding. There are progressive and similar reductions in [^{125}I]T_4-binding seen with both unlabelled T_4 and milrinone.

Effect of milrinone on binding of T_4 to skeletal muscle SR membranes

Studies of radiolabelled T_4 binding to SR membranes revealed that milrinone is as effective as unlabelled T_4 at displacing T_4 from these membranes. Shown in Fig. 7 is progressive displacement of tracer hormone by either T_4 or milrinone, such that 50% inhibition of specific, displaceable binding was achieved by $4 \cdot 10^{-9}$ M T_4 or milrinone.

Discussion

In the present studies, T_4 and T_3 (10^{-12} to 10^{-6} M) stimulated Ca^{2+}-ATPase activity in vitro in rabbit striated muscle membranes. The latter was an SR-enriched preparation [16] free of myofibrils and attendant myosin-associated Ca^{2+}-ATPase, and free of sarcolemmal Na,K-ATPase. Thus, we conclude that SR Ca^{2+}-ATPase is stimulable in vitro by thyroid hormone, like the Ca^{2+}-ATPases of erythroid cells [4,5,18] and myocardial membranes [10,14], by a process independent of the cell nucleus. Skeletal muscle SR protein binding sites for T_4 and T_3 have previously been described [23,24].

Structure-activity studies of thyroid hormone analogues showed that several analogues regarded as relatively inactive biologically – such as tetraiodothyroacetic acid (Tetrac), D-T_4 and rT_3 – failed to stimulate striated muscle SR Ca^{2+}-ATPase activity. On the other hand, Dimit, for which the nuclear iodothyronine receptor has little or no affinity [19], was more active than T_4 and T_3 in the muscle Ca^{2+}-ATPase model. This noniodinated analogue has also been shown to stimulate erythroid cell Ca^{2+}-ATPase in vitro [25] and is bioactive in intact animals [26]. Triac, which is also bioactive in vivo, although in pharmacologic concentrations [21], had no effect on skeletal muscle SR Ca^{2+}-ATPase activity, nor on erythrocyte Ca^{2+}-ATPase activity [25], at a concentration of 10^{-10} M. These findings support biologic relevance of the membrane Ca^{2+}-ATPase model.

The mechanism by which thyroid hormone stimulates skeletal muscle SR membrane Ca^{2+}-ATPase activity in vitro is not yet known. Although mammalian striated muscle SR Ca^{2+}-ATPase has been reported to be unresponsive to calmodulin [27], human muscle Ca^{2+}-ATPase activity has been shown to be stimulated in vitro by calmodulin [28] and we have shown avian [29] and mouse [30] SR Ca^{2+}-ATPase activities to be activated by calmodulin. Thus, it is possible that thyroid hormone action on skeletal muscle Ca^{2+}-ATPase may involve the liberation of a calmodulin-enhancing factor, such as we have described in the human red cell [6]. The possibility that thyroid hormone might increase permeability of vesicles in the SR preparation to Ca^{2+}, and thus enhance Ca^{2+}-ATPase activity, appears unlikely. We have excluded a Ca^{2+} ionophore effect of thyroid hormone in red cell vesicles [8] and an effect of the hormone on cell membrane K^+ permeability has been shown to be nucleus-mediated [31], rather than a direct effect on the plasma membrane.

Studies of milrinone, a bipyridine compound whose substituted ring shares structural homologies with the phenolic ring of thyroxine by X-ray crystallographic analysis, revealed that it stimulated rabbit skeletal muscle SR Ca^{2+}-ATPase activity. Milrinone has been shown to be thyromimetic in its stimulatory action on sarcolemma-enriched rabbit myocardial membrane Ca^{2+}ATPase in vitro [14] and to compete with thyroid hormone for binding sites on human serum transthyretin (TBPA) [32]. Others have shown that milrinone has thyromimetic effects on rat bone cells [15], at least some of which are non-genomic [9]. Our structure-activity studies of milrinone analogues in the in vitro skeletal muscle Ca^{2+}-ATPase model revealed that the C2 methyl group was necessary for stimulation of the enzyme; compounds lacking this substituent were inactive. The C2 substitution forces the two pyridine rings to adopt a more twisted conformation similar to that of T_4 and T_3. A C5 substituent was necessary to promote stimulatory activity, with Br substitution being more potent than CN substitution, which in turn was more potent than NH_2 substitution. Amrinone, a 5-amino bipyridine analogue lacking both the C2 methyl and the C5 cyano groups of milrinone, has been studied in the myocardial membrane Ca^{2+}-ATPase system, and had no effect on enzyme activity [14]. Thus, the skeletal muscle SR model is useful for analysis of structure-activity relationships among thyromimetic compounds.

The nature of the binding sites that account for the action of iodothyronines in vitro on muscle SR Ca^{2+}-ATPase activity in the present studies is not clear. A major multifunctional T_3-binding protein (T_3BP) previously described in muscle [23,24] has been identified as protein disulfide isomerase (PDI) [24]. However, the K_d for the binding of thyroid hormone by this T_3BP/PDI is in the micromolar range [23], whereas we obtained 50% displacement of tracer T_4 in our studies of muscle SR at nanomolar concentrations of unlabelled T_4. The hormonal effect on Ca^{2+}-ATPase we describe, like hormone actions in general, is one of amplification – rather than a direct effect of hormone on Ca^{2+}-ATPase – given the stoichiometry of subnanomolar concentrations of circulating iodothyronines and the role of Ca^{2+}-ATPase as the predominant protein in SR.

Studies of thyroid hormone-binding to skeletal muscle membranes in the presence of various concentrations of milrinone revealed competition of the bipyridine and iodothyronine for the same site(s), as is the case with binding to transthyretin [32]. While these

binding studies provide a mechanism, at least in part, for milrinone's antagonism of the action of thyroid hormone (10^{-10} M) on skeletal muscle membrane Ca^{2+}-ATPase activity (Fig. 6), they must be reconciled with the ability of milrinone to stimulate enzyme activity in the absence of thyroxine. Additive actions on Ca^{2+}-ATPase activity of milrinone and thyroid hormone might be predicted, for example. It should be noted that both iodothyronines and milrinone have parabolic concentration-response curves in muscle Ca^{2+}-ATPase models (Figs. 2, 4; Ref. 14) and that at a 10^{-10} M concentration, thyroxine is maximally effective in stimulating the enzyme. We believe that progressive additions of milrinone in the presence of T_4 reveal the downward slope of the concentration-enzyme response relationship. Tetrac has also been shown to inhibit thyroid hormone's stimulation of membrane Ca^{2+}-ATPase and to displace T_4 from membrane sites [33]; interestingly, however, Tetrac has no enzyme stimulatory activity of its own. The basis for the parabolic dose-response relationship of thyroid hormone and membrane Ca^{2+}-ATPase activity is not known, but the same parabolic concentration-response has been shown by Baskurt et al. [34] for the regulation by thyroid hormone in vitro of intracellular calcium concentration in the human red cell. Consistent with the parabolic dose-response pattern of thyroid hormone's action on Ca^{2+}-ATPase activity which we have shown, extraordinarily high concentrations ($> 10^{-5}$ M) of thyroid hormone have been shown to inhibit Ca^{2+} accumulation by isolated SR vesicles from skeletal muscle [23], as well as SR Ca^{2+}-ATPase activity. This inhibition, however, was not stereospecific.

The physiological implications of the current observations are speculative. Since stimulation of SR Ca^{2+}-ATPase activity underlies increased SR re-uptake of Ca^{2+} [1], an action of thyroid hormone on this enzyme would enhance the rate of relaxation of striated muscle. This phenomenon is observed clinically in hyperthyroidism, but because thyroid hormone has both nucleus-mediated [35] and direct effects, shown here, on SR Ca^{2+}-ATPase activity, either or both influences could be implicated.

Studies by Sayen, Rohrer and Dillmann [35] have shown that the nucleus-dependent actions of T_3 on rat striated muscle SR Ca^{2+}-ATPases are complex. These effects are enzyme isoform-specific (sarcoplasmic endoplasmic reticulum Ca^{2+}-ATPase type 1 [SERCa1] and type 2 [SERCa2]), as well as time-specific, i.e., related to acute or chronic administration of the hormone [35]. In slow soleus muscle, where SERCa2 predominates, the abundance of SERCa2 mRNA decreased in established hyperthyroidism, whereas SERCa1 mRNA increased. SERCa1 mRNA was unaffected by hyperthyroidism in fast muscle. It is thus difficult at this point to attribute a consistent clinical finding of hyperthyroidism, such as increased rate of relaxation of skeletal muscle, to action of thyroid hormone on Ca^{2+}-ATPase genes. It is speculated that a direct extranuclear effect of the hormone on enzyme activity may dominate in that setting. In contrast, hypothyroidism in the rat is associated with frank decreases in mRNA abundance for both SERCa1 and SERCa2 in soleus [35], and this observation may explain delayed muscle relaxation.

Acknowledgments

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References

1 Martonosi, A.N. (1986) in Myology, Basic and Clinical (Engel, A.G. and Banker, B.Q., eds.), pp. 521–562, McGraw-Hill, New York.
2 McWhirter, J.M., Gould, G.W., East, J.M. and Lee, A.G. (1987) Biochem. J. 245, 713–722.
3 Caswell, A.H. and Brandt, N.R. (1989) Trends Biochem. Sci. 14, 161–165.
4 Davis, P.J. and Blas, S.D. (1981) Biochem. Biophys. Res. Commun. 99, 1073–1080.
5 Davis, F.B., Kite, J.H., Jr., Davis, P.J. and Blas, S.D. (1982) Endocrinology 10, 297–298.
6 Lawrence, W.D., Deziel, M.R., Davis, P.J., Schoenl, M., Davis, F.B. and Blas, S.D. (1993) Clin. Sci. 84, 217–223.
7 Sundquist, J., Blas, S.D., Hogan, J.E., Davis, F.B. and Davis, P.J. (1992) Cell Signal. 4, 795–799.
8 Davis, P.J., Davis, F.B. and Lawrence, W.D. (1989) Endocr. Res. 15, 651–682.
9 Lakatos, P. and Stern, P.H. (1991) Acta Endocrinol. 125, 603–608.
10 Rudinger, A., Mylotte, K.M., Davis, P.J., Davis, F.B. and Blas, S.D. (1984) Arch. Biochem. Biophys. 229, 379–385.
11 Gold, H.K., Spann, J.F., Jr. and Braunwald, E. (1970) J. Clin. Invest. 49, 849–854.
12 Argov, Z., Renshaw, P.F., Boden, B., Winokur, A. and Bank, W.J. (1988) J. Clin. Invest. 81, 1695–1701.
13 Baim, D.S., McDowell, A.V., Cherniles, J., Monrad, E.S., Parker, J.A., Edelson, J., Braunwald, E. and Grossman, W. (1983) N. Engl. J. Med. 309, 748–756.
14 Mylotte, K.M., Cody, V., Davis, P.J., Davis, F.B., Blas, S.D. and Schoenl, M. (1985) Proc. Natl. Acad. Sci. USA 82, 7974–7978.
15 Krieger, N.S., Stappenbeck, T.S. and Stern, P.H. (1987) J. Clin. Invest. 79, 444–448.
16 Sumida, M., Wang, T., Mandel, F., Froehlich, J.P. and Schwartz, A. (1978) J. Biol. Chem. 253, 8772–8777.
17 Jones, L.R., Besch, H.R., Jr., Fleming, J.W., McConnaughey, M.M. and Watanabe, A.M. (1979) J. Biol. Chem. 254, 530–539.
18 Lawrence, W.D., Davis, P.J., Blas, S.D. and Schoenl, M. (1984) Arch. Biochem. Biophys. 235, 78–85.
19 Koerner, D., Schwartz, H.L., Surks, M.I., Oppenheimer, J.H. and Jorgensen, E.C. (1975) J. Biol. Chem. 250, 6417–6423.
20 Cody, V. (1980) Endocr. Rev. 1, 140–166.
21 Beck-Peccoz, P., Piscitelli, G., Cattaneo, M.G. and Faglia, G. (1983) J. Endocrinol. Invest. 6, 217–223.
22 Davis, F.B., Middleton, E., Jr., Davis, P.J. and Blas, S.D. (1983) Cell Calcium 4, 71–81.
23 Shoshan-Barmatz, V. and Shainberg, A. (1991) Biochim. Biophys. Acta 1065, 82–88.

24 Fliegel, L., Newton, E., Burns, K. and Michalak, M. (1990) J. Biol. Chem. 265, 15496–15502.
25 Davis, F.B., Cody, V., Davis, P.J., Borzynski, L.J. and Blas, S.D. (1983) J. Biol. Chem. 258, 12373–12377.
26 Bachrach, L.K., Dibattista, D., Burrow, G.N. and Holland, F.J. (1983) Endocrinology 112, 2021–2024.
27 Chiesi, M. and Carafoli, E. (1982) J. Biol. Chem. 257, 984–991.
28 Niebroj-Dobosz, I., Kornguth, S., Schutta, H.S. and Siegel, F.L. (1989) Neurology 39, 1610–1614.
29 Galindo, J., Jr., Hudecki, M.S., Davis, F.B., Davis, P.J., Thacore, H.R., Pollina, C.M., Blas, S.D. and Schoenl, M. (1988) Biochemistry 27, 7519–7524.
30 Zapalowski, C., Hudecki, M.S., Davis, F.B., Davis, P.J., Pollina, C. and Blas, S.D. (1989) Clin. Res. 37, 464A.
31 Haber, R.S., Ismail-Beigi, F. and Loeb, J.N. (1988) Endocrinology 123, 238–247.
32 Davis, P.J., Cody, V., Davis, F.B., Warnick, P.R., Schoenl, M. and Edwards, L. (1987) Biochem. Pharmacol. 36, 3635–3640.
33 Davis, P.J., Davis, F.B. and Blas, S.D. (1982) Life Sci. 30, 675–682.
34 Baskurt, O.K., Levi, E., Temizer, A., Ozer, D., Caglayan, D., Dikmenoglu, N. and Andac, S.O. (1990) Life Sci. 46, 1471–1477.
35 Sayen, M.R., Rohrer, D.K. and Dillmann, W.H. (1992) Mol. Cell. Endocrinol. 87, 87–93.