Biochimica et Biophysica Acta, 1065 (1991) 82–88
© 1991 Elsevier Science Publishers B.V. 0005-2736/91/$03.50
ADONIS 000527369100225X

BBAMEM 75247

Inhibition of Ca^{2+} accumulation in isolated sarcoplasmic reticulum by thyroid hormones

Varda Shoshan-Barmatz [1] and Asher Shainberg [2]

[1] *Department of Biology, Ben-Gurion University of the Negev, Beer Sheva (Israel) and* [2] *Department of Life Sciences, Bar-Ilan University, Ramat-Gan (Israel)*

(Received 20 September 1990)

Key words: Sarcoplasmic reticulum; Thyroxine; Calcium ion accumulation

Thyroid hormones inhibit Ca^{2+} accumulation and ATPase activity of isolated sarcoplasmic reticulum vesicles. Half-maximal inhibition was obtained by about 2.5 μM. The ATP hydrolysis activity of the purified (Ca^{2+} + Mg^{2+})-ATPase or of the SR vesicles, in the presence of the Ca^{2+} ionophore A23187, is not inhibited by T_3 or T_4. Modification of T_3 or T_4 in the ring portion, but not in the amino portion, of the molecules results in T_4 and T_3 analogues which are unable to inhibit Ca^{2+} accumulation. T_3 and T_4 have no significant effect on various partial reactions of the transport cycle such as: the binding of ATP and Ca^{2+}, or ADP–ATP exchange and E-P formation from ATP, but they inhibit the E-P formation from inorganic phosphate (P_i) and ATP–P_i exchange. The inhibition of both Ca^{2+} accumulation and ATPase activity by T_3 or T_4 is increased in the presence of P_i. Binding sites for [^{125}I]T_3 and for [^{125}I]T_4 in SR proteins were demonstrated using either equilibrium dialysis or gel overlay techniques. The results suggest that the thyroid hormones inhibit the ATP-dependent Ca^{2+} accumulation, probably by inhibiting the transport of anions which act as the Ca^{2+} precipitating anion.

Introduction

Thyroid hormones (TH) regulate differentiation and development and are important for the regulation of metabolic homeostasis [1]. Some of these actions are believed to be mediated through the interaction of TH with nonhistone chromatin-associated nuclear receptors [2]. In cardiac muscle, TH were shown to regulate Ca^{2+}-ATPase expression both *in vivo* [3] and *in vitro* [4]. Extranuclear binding sites for TH, however, have also been detected in other cellular components including the plasma membranes, mitochondria, cytosol, and the nuclear envelope [5].

Skeletal muscle is one of the major target organs of thyroid hormones and there is considerable clinical evidence to show that skeletal muscle can be affected by hyper- or hypo-thyroid function [6]. Studies on hyperthyroid pathogenesis have failed to clarify the mechanism underlying the abnormalities of muscle tissue. It was shown that administration of thyroxine (T_4) to animals gave rise to increased levels of Ca^{2+} in the myoplasm [7,8]. This increased intracellular Ca^{2+} level might be the reason for a net catabolic state which is manifested in muscle atrophy in thyrotoxic animals. Indeed, the ability of Ca^{2+} to alter rates of protein turnover of skeletal muscle has been reported [9,10].

The ATP-dependent uptake of Ca^{2+} by the sarcoplasmic reticulum (SR), is generally regarded as being the regulator of the intracellular calcium concentration which, in turn, controls the contraction/relaxation cycle of the muscle [11]. It is well established that the (Ca^{2+} + Mg^{2+})-ATPase of the sarcoplasmic reticulum is the enzyme that catalyzes Ca^{2+} transport at the expense of ATP hydrolysis [12]. The ATP-dependent Ca^{2+} transport activity is believed to involve several steps including: the binding of Ca^{2+} and ATP, formation of a covalently linked phosphoenzyme intermediate, Ca^{2+} translocation and, finally, breakdown of the phosphorylated intermediate [13,14]. The overall reaction results in the translocation of 2 Ca^{2+} from outside to inside the vesicles and in the splitting of 1 molecule of ATP [13]. In the reverse direction, the Ca^{2+} pump catalyzes the synthesis of ATP coupled to the release of Ca^{2+} [15].

Since the SR is responsible for maintaining a low

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; SR, sarcoplasmic reticulum; T_4, thyroxine; T_3, triiodothyronine.

Correspondence: V. Shoshan-Barmatz, Department of Biology, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel.

cytosolic Ca^{2+} level in the muscle, it is speculated that it might be damaged in hyperthyroid animals. Of relevance is the observation that the uptake of Ca^{2+} by SR isolated from T_4-treated animals is reduced, whereas ATP hydrolysis activity is not affected [16]. Furthermore, it was recently shown that TH cause an inhibition of ATP-dependent Ca^{2+} accumulation in SR of skinned skeletal muscle fibers [17]. In contrast, Kim et al. [18] and Simonides and Van Hardeveld [19] have reported that in thyrotoxic animals, Ca^{2+} uptake by the SR of slow muscle is increased while the kinetics of Ca^{2+} uptake by the fast muscle are not altered. Because of the controversy in the literature, the objectives of the present communication are to elucidate the interaction of TH with isolated SR and the mechanism by which the hormone exerts its effect.

Materials and Methods

Materials. ATP, ADP, D-thyroxine, L-thyroxine (T_4), 3,3′,5-triiodo-L-thyronine (T_3), 3,3′,5-triiodo-D-thyronine, 3,3′,5-triiodo-L-thyronine propionic acid, 3,5-diiodo-L-thyronine and 3′,5′,3-triiodo-L-thyronine (reverse T_3) were obtained from Sigma Chemical Co. and were dissolved in 0.1 M NaOH and used immediately. [^{32}P]Phosphate from Amersham and [8-^{14}C]ADP, [8-^{14}C]ATP, $^{45}CaCl_2$, [^{125}I]T_3 and [^{125}I]T_4 were purchased from New England Nuclear. [γ-^{32}P]ATP was obtained from the Nuclear Research Center, Negev, Israel.

Protein preparations. Preparation of SR vesicles and the purified (Ca^{2+} + Mg^{2+})-ATPase was carried out as described previously [20]. Protein concentration was determined according to Lowry et al. [21].

Assays. Active $^{45}CaCl_2$ uptake by SR vesicles was determined by Millipore filtration [22]. The basic reaction mixture contained 20 mM Mops (pH 6.8), 100 mM KCl, 3 mM $MgCl_2$, 3 mM ATP, 0.5 mM $CaCl_2$ (containing [^{45}Ca] about 10^6 cpm/μmol), 0.5 mM EGTA, and 50 mM KP_i. The uptake was initiated by the addition of SR, 100 μg/ml. After incubation for 1 min or other indicated time at 30°C, 0.18 ml samples were filtered through nitrocellulose filters (0.45 μm pore) and washed with 5 ml of 0.15 KCl. Radioactivity on the filters was measured in a liquid-scintillation counter. ATPase activity was assayed under conditions similar to those used for Ca^{2+} uptake except that $^{45}CaCl_2$ was omitted and [γ-^{32}P]ATP ($5 \cdot 10^4$ cpm/μmol) was added to the reaction mixture. [^{32}P]P released from [γ-^{32}P]ATP was extracted as phosphomolybdate into isobutanol/xylene (1 : 1, v/v) according to Avron [23] and measured by liquid scintillation counting. ATP–P_i exchange, phosphorylation by P_i or ATP and ADP–ATP exchange were assayed as described [24].

Binding of Ca^{2+}, ATP, T_3 and T_4. The binding of ^{45}Ca, or [^{14}C]ATP to the purified enzyme was measured by the filtration method of Meissner [25], except that the radioactivity remaining on the 0.22 μm Millipore filter (GSW 02500) was counted. Parallel samples without protein were filtrated in order to measure non-specific binding. Binding of [^{125}I]T_3 or [^{125}I]T_4 to the purified enzyme or to the proteolipid was measured by the equilibrium dialysis procedure [25] except that dialysis was carried out at 22°C for 16 h using spectra-pure 3 dialysis bags. The purity of [^{125}I]T_3 and [^{125}I]T_4 was analysed by thin-layer chromatography on silica gel with chloroform/methanol/water (65 : 35 : 2, v/v) as a solvent.

Results

Effect of thyroid hormones on Ca^{2+}-dependent ATP hydrolysis and Ca^{2+} accumulation

The effects of T_4, T_3 and of several analogues of T_4 and T_3 on Ca^{2+} accumulation by isolated SR vesicles is summarized in Table I. T_4 and T_3 both inhibited the Ca^{2+} accumulation but the degree of inhibition by T_3 was higher than that of T_4. Modification of both hormones in the amino acid portion of the molecules had only a slight effect on the inhibitory effect of T_4 and T_3. In contrast, modification in the ring portion of the molecules resulted in T_4 and T_3 analogues which were unable to inhibit Ca^{2+} accumulation.

The inhibitory effect of T_4 was immediately evident and was the same regardless of whether the hormone was dissolved in NaOH, ethanol or dimethyl sulfoxide (DMSO). The inhibition of Ca^{2+} accumulation by T_3 or T_4 was irreversible. We found that it was impossible to restore Ca^{2+} accumulation activity by ultracentrifugation or by passing the membranes through a Sephadex G-50 column (data not shown).

The effect of thyroxine on Ca^{2+}-dependent ATP hydrolysis and the coupled Ca^{2+} uptake is shown in

TABLE I

Effect of thyroxine and its analogues on Ca^{2+} accumulation

Ca^{2+} accumulation was assayed as described under Materials and Methods, in both the absence and the presence of the indicated concentrations of the hormone or its analogues.

Component added	Concn. (μM)	Ca^{2+} accumulation (μmol/mg protein)	Percent of control
None	–	1.545	100
L-Thyroxine (T_4)	20	0.362	23.4
D-Thyroxine	20	0.499	32.3
3,3′,5-Triiodo-L-thyronine (T_3)	20	0.251	14.6
3,3′,5-Triiodo-D-thyronine	20	0.213	13.9
3,3′,5-Triiodo-propionic acid	20	0.311	19.9
3,5-Diiodo-L-thyronine	20	1.487	96.2
3,5′,3-Triiodo-L-thyronine (reverse T_3)	20	1.376	87.9

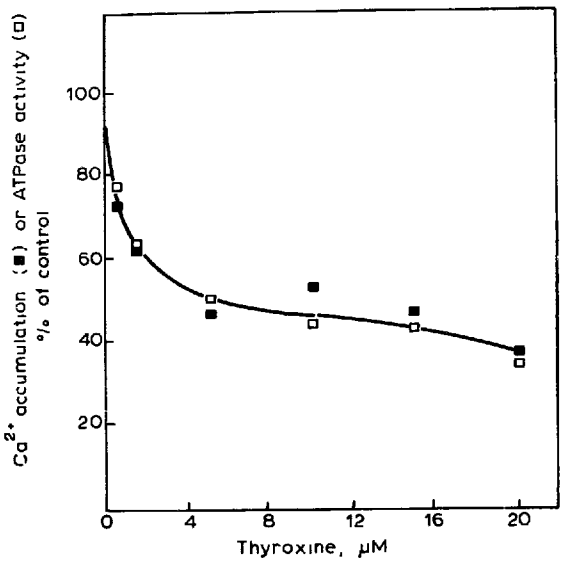

Fig. 1. Inhibition of Ca^{2+} accumulation and ATPase activity by T_4. Ca^{2+} accumulation (■) and ATPase activity (□) were assayed for 1 min at 24°C as described under Materials and Methods, except that the indicated concentration of T_4 was included in the assay medium. Control activity (100%) was 1.55 and 0.97 μmol/mg protein per min for Ca^{2+} accumulation and ATPase activity, respectively.

Fig. 1. Both ATPase activity and the Ca^{2+} accumulation were inhibited by T_4, with half-maximal inhibition occurring at about 2.5 μM, and maximal inhibition of about 65% obtained at about 20 μM of T_4. These results may suggest that the inhibition of both the ATPase activity and Ca^{2+} transport results from the same interaction of T_4 with the SR.

In Table II, the inhibition by T_3 and T_4 of the ATP hydrolysis by SR vesicles and by the purified ATPase is compared. While an inhibition of about 65% was obtained with the SR, an inhibition of only about 13–20% of the ATPase activity of purified protein was observed.

Table III shows that the inhibition of Ca^{2+}-dependent ATP hydrolysis by T_3 is noticeably reduced in the presence of the Ca^{2+} ionophore A23187. The conclusions which can be drawn from Table II and Table III may suggest that the inhibition of ATP hydrolysis by T_3

TABLE II

Effect of T_3 and T_4 on ATPase activity of sarcoplasmic reticulum and purified enzyme

ATPase activity of SR vesicles or purified $(Ca^{2+} + Mg^{2+})$-ATPase was assayed as described under Materials and Methods.

Additions	ATPase activity (μmol/mg protein per min)	
	purified ATPase	SR-ATPase
None	6.97	0.97
T_3, 10 μM	6.12	0.45
20 μM	6.06	0.34
T_4, 10 μM	5.73	0.55
20 μM	5.68	0.41

TABLE III

Effect of A23187 on ATP hydrolysis activity alone and in the presence of T_3

Ca^{2+}-ATPase activity of SR vesicles was assayed in the presence of 50 mM KP_i as described under Materials and Methods except that the indicated compounds were added to the assay medium.

Assay conditions	ATPase activity (μmol P_i release/ mg protein per min)
Control	0.96
A23187, 5 μM	2.36
T_3, 11 μM	0.44
T_3 + A23187, 5 μM	1.86

or T_4 is not attributable to direct interaction with $(Ca^{2+} + Mg^{2+})$-ATPase.

Effect of T_4 on the partial reactions of ATP-dependent Ca^{2+} transport

In order to examine the possibility that a defined state of the enzyme and/or step(s) in ATP-dependent Ca^{2+} transport is affected by thyroxine, we determined the effect of T_4 or T_3 on several reactions of the forward and reverse modes of the transport system. T_3 or T_4 have no significant effect on either $^{45}Ca^{2+}$ or [^{14}C]ATP binding to the purified Ca^{2+}-ATPase (data not shown). This suggests that the inhibitory effect of T_3 or of T_4 is not due to their interaction(s) with the Ca^{2+} or ATP binding sites.

The ADP–ATP exchange, catalyzed by the Ca^{2+}-dependent ATPase, was not affected by T_3 even at high concentrations, such as 26 μM and 52 μM (Table IV, Expt. I). Since this reaction represents the formation of $E_1 \sim P$ from ATP and the resynthesis of ATP from exogenous ADP and the phosphoprotein, and since T_3 did not affect this reaction, it is possible that T_3 has no effect on the reactions involving the E_1 form of the enzyme. This possibility is supported by the results shown in Table IV (Expt. II), in which the effect of T_3 on the steady-state formation of a trichloroacetic acid-stable phosphorylated intermediate from ATP in the presence of Ca^{2+}, and from Pi in the presence of Mg^+, were tested. As can be seen, T_3 had no effect on the total amount of the phosphoenzyme formed from ATP, but it strongly inhibited the formation of $E \sim P$ from P_i (Table IV).

The effect of T_3 on the reversal of the Ca^{2+} pump was examined using the ATP–P_i exchange. Fig. 2 shows that 14 μM of T_3 inhibited about 50% of the exchange activity observed during the period of net Ca^{2+} accumulation and about 74% in the steady-state period of Ca^{2+} accumulation. Since the ATP–P_i exchange is coupled to the Ca^{2+} exchange between the pools of Ca^{2+} located separately in the vesicles and in the assay medium [26], the low exchange activity observed in the

TABLE IV

Effect of T_3 on ADP–ATP exchange and on the formation of phosphoprotein from ATP and from P_i

In experiment I (Expt. I) SR membranes (20 μg protein) were incubated for 4 min in 0.30 ml of medium containing 60 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 6 mM ATP, 1.5 mM [^{14}C]ADP (containing $4 \cdot 10^5$ cpm/μmol) and 60 μM $CaCl_2$. The reaction was stopped by the addition of EGTA to a final concentration of 5 mM. ADP and ATP were separated by chromatography on a polyethyleneimine cellulose column as described [38]. In Experiment II (Expt. II) the assay medium composition for E-P formation from ATP was: 30 mM Tris-maleate (pH 6.8), 100 mM KCl, 20 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.2 mM [γ-^{32}P]ATP (containing 10^7 cpm/μmol) and for E-P formation from P_i was: 30 mM Tris-maleate (pH 6.8), 100 mM KCl, 20 mM $MgCl_2$, 1 mM EGTA and 6 mM [^{32}P]P_i (containing $4 \cdot 10^7$cpm/μmol). In both reactions SR vesicles were 0.5 mg/ml and the final volume was 0.5 ml. The reaction was carried out at 25°C and was quenched after 30 s by the addition of an ice-cold solution of trichloroacetic acid (TCA) to a final concentration of 5%. Two aliquots of 0.2 ml were filtered through nitrocellulose filters (0.45 μm) which were washed three times with 5 ml of 5% TCA containing 0.1 mM ATP or 2 mM P_i for E-P formation from ATP or P_i, respectively, and counted for radioactivity.

T_3, μM	ATP–ADP exchange (μmol [^{14}C]ATP formed/mg protein)	
Expt. I		
None	11.9	
26 μM	12.9	
52 μM	12.5	
T_3, μM	E-P formation (nmol/mg protein)	
	from ATP	from P_i
Expt. II		
None	5.8	3.3
7.4	5.2	1.8
14.4	5.7	1.4
28.6	4.9	–
35.0	–	0.7

presence of T_3 is a result of the low Ca^{2+} accumulated in the vesicles. However, the formation of E-P from P_i is an intermediate in this reaction; therefore, the inhibition of its formation (Table IV, Expt. II) could be responsible (at least partially) for the inhibition of the ATP–P_i exchange. Thus, it appears that T_3 and T_4 affected only the partial reactions involving E-P formation from P_i. It is therefore possible that the inhibition of Ca^{2+} accumulation and of the coupled ATP hydrolysis by T_3 and T_4 resulted from an indirect effect such as the inhibition of a co-transported anion (e.g. P_i).

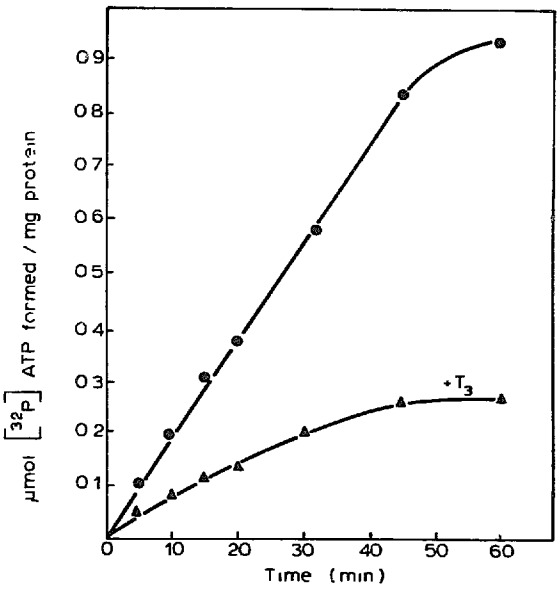

Fig. 2. Effect of T_3 on ATP–P_i exchange activity of SR membranes. ATP–P_i exchange was assayed for the indicated time periods. The assay medium contained 30 mM Tris-maleate (pH 7.0), 20 mM $MgCl_2$, 0.1 mM $CaCl_2$ 6 mM [^{32}P]phosphate (containing $2 \cdot 10^6$ cpm/μmol), 4 mM ATP, 0.5 mM ADP and 50 μg/ml SR membranes. T_3 concentration was 14 μM. The [γ-^{32}P]ATP formed was measured as described in Ref. 23.

P_i-dependence of T_3 inhibition of Ca^{2+} accumulation and ATPase activity

Fig. 3A shows that the rate of Ca^{2+} accumulation measured in the absence and in the presence of T_3 was

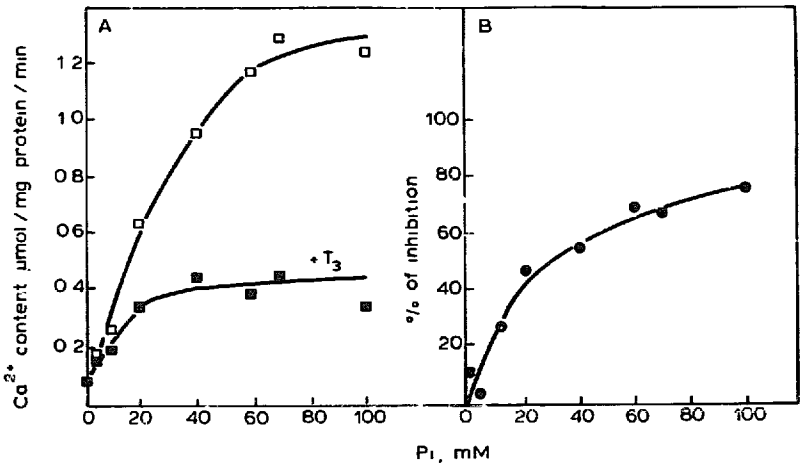

Fig. 3. Effect of P_i on the inhibition of Ca^{2+} accumulation by T_3. Ca^{2+} accumulation was assayed for 1 min as in Fig. 1, except that the P_i concentration was varied as indicated. T_3 concentration was 13 μM.

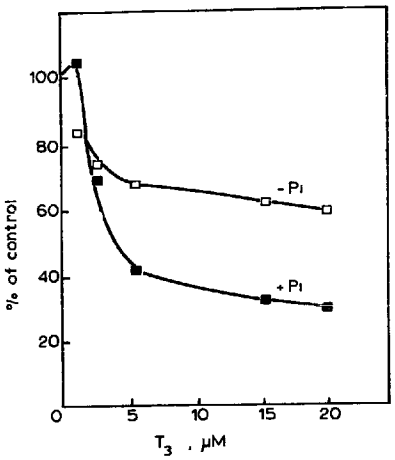

Fig. 4. Effect of T_3 on ATPase activity of SR vesicles assayed in the absence and in the presence of inorganic phosphate. ATPase activity of SR vesicles was assayed in the presence of the indicated concentration of T_3, and in the absence (□) and in the presence (■) of 50 mM KP_i, as described in Fig. 1.

higher when the P_i concentration was increased. However, the results clearly show that the inhibition of Ca^{2+} accumulation by T_3 is highly dependent on the P_i concentration present in the Ca^{2+} uptake medium (Fig. 3B). Similar results were obtained with T_4, or with oxalate (5–20 mM) instead of P_i (data not shown).

The effect of T_3 on ATPase activity, measured in the absence and in the presence of P_i, is shown in Fig. 4. T_3 inhibited the ATPase activity of SR vesicles more strongly in the presence of P_i.

The binding of T_4 to SR vesicles and purified ATPase

The binding of thyroxine to the purified (Ca^{2+}+ Mg^{2+})-ATPase and SR vesicles was measured using [^{125}I]T_4 and the equilibrium dialysis technique. Fig. 5 shows that [^{125}I]T_4 binds both to the purified ATPase and to the SR vesicles. At 2.5 μM T_4, 12 nmol T_4 were bound per mg of purified protein and about 9 nmol T_4 per mg SR proteins. Assuming a molecular weight of 110000 for the (Ca^{2+}+ Mg^{2+})-ATPase [27] and that the enzyme constituted about 55% of the total SR protein, a molar ratio of about 1.2 and 1.4 T_4 bound per purified ATPase and membrane bound ATPase, respectively, are calculated from the data of Fig. 5. The T_4 concentration required for half saturation of the binding sites was about 0.8 μM for both the purified and membrane bound ATPases. Thus, the inhibitory effect of T_4 on the Ca^{2+}-ATPase and the coupled Ca^{2+} transport probably results from its interaction with the (Ca^{2+}+ Mg^{2+})-ATPase. However, the purified ATPase still contained bound proteolipid; consequently, we measured the binding of T_4 to the proteolipid released by heat from the purified ATPase [28]. About 1 mol of T_4 bound per mol of proteolipid was obtained by assuming a molecular weight of 12000 for the proteolipid (data not shown). We also measured the binding of T_4

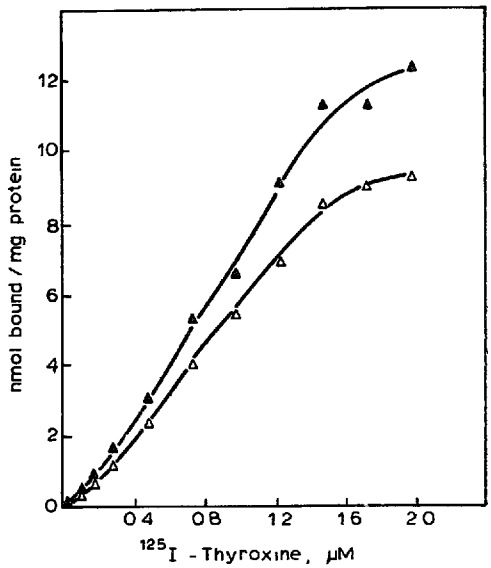

Fig. 5. [^{125}I]T_4 binding to SR membranes and to purified (Ca^{2+} + Mg^{2+})-ATPase. The binding of [^{125}I]T_4 to SR membranes (△) or purified ATPase (▲) was measured by the equilibrium dialysis procedure [25] except that the dialysis was performed at 22°C for 16 h. The incubation mixture contained, in a total volume of 1 ml, 20 mM Tris-HCl (pH 7.5), 100 mM KCl and the indicated concentration of [^{125}I]T_4 (containing 0.02 μCi/ml) and was dialyzed against 20 ml of 20 mM Tris-HCl (pH 7.5) and 100 mM KCl.

to proteolipid-depleted ATPase [28] and found that T_4 also binds to this ATPase (data not shown).

To determine the target protein(s) of T_4, the L-[^{125}I]thyroxine binding to SR proteins was also assessed by means of gel overlay technique. Fig. 6 shows the L-[^{125}I]thyroxine binding activity of several SR proteins.

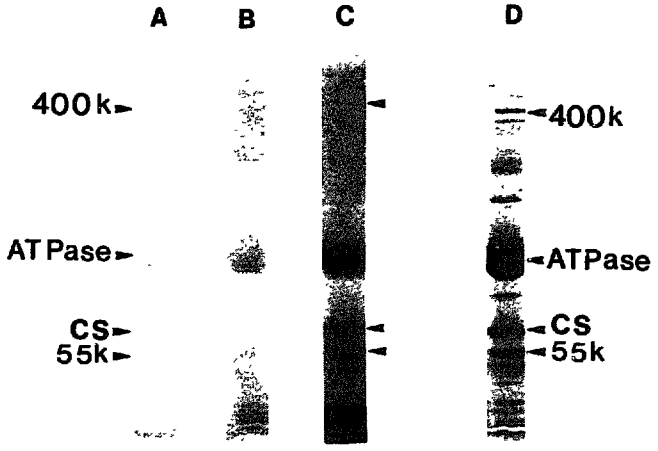

Fig. 6. [^{125}I]Thyroxine gel overlay of SR proteins. SR membranes (75 μg of protein) were subjected to SDS-polyacrylamide gel electrophoresis (4–13% acrylamide) according to Laemmli [39]. Thereafter, the binding of [^{125}I]thyroxine to SR proteins, using the gel overlay method, was determined as described previously [33]. Lanes A, B and C are autoradiograms of gels incubated with: 0.05, 0.1 and 0.2 μCi/ml of [^{125}I]thyroxine, respectively and lane D is a Coomassie-stained gel of lane C. ATPase, (Ca^{2+} + Mg^{2+})-ATPase; CS, calsequestrin. Molecular weight standards were myosin, 200000; β-galactosidase, 116000; phosphorylase *b*, 92500; bovine serum albumin, 66200 and ovalbumin 45000 (Bio-Rad).

T_4 binds to the $(Ca^{2+}+Mg^{2+})$-ATPase, calsequestrin and to the 400 kDa and 55 kDa proteins. No significant labelling of other proteins was observed when the [^{125}I]thyroxine concentration was increased 4-fold.

Discussion

The data presented here show that the thyroid hormones inhibit both Ca^{2+} accumulation and Ca^{2+}-ATPase activities of the SR vesicles. Modification of T_4 or T_3 in the amino acid portion of the molecules affects their inhibitory action only slightly, whereas modification of the ring portion of the molecules resulted in T_4 and T_3 analogs which were unable to inhibit Ca^{2+} accumulation (Table I). These results are consistent with the physiological activity of the various T_3 and T_4 analogs.

In an attempt to reveal the mechanism by which T_4 or T_3 generates the inhibition of Ca^{2+} accumulation, the effect of these hormones on several partial reactions of the Ca^{2+}-transport cycle was studied. We found that they had no significant effect on the rate of the ADP–ATP exchange, the steady-state level of phosphoenzyme formed from ATP or the ATP and Ca^{2+} binding to the ATPase. In contrast, T_4 and T_3 strongly inhibited the ATP–P_i exchange and E-P formation from P_i; two reactions in which P_i is a substrate. Since the ATP–P_i exchange is coupled to Ca^{2+} exchange between the pools of Ca^{2+} located separately in the vesicles and in the assay medium [26], and because T_4 and T_3 inhibit Ca^{2+} accumulation, its inhibitory effect on the ATP–P_i exchange is expected. These observations together with the findings that the ATPase activity of the purified $(Ca^{2+}+Mg^{2+})$-ATPase and of the membrane-bound enzyme (measured in the presence of A23187) are not significantly affected by the thyroid hormones suggest that the inhibition of Ca^{2+} accumulation by T_3 and T_4 is not due to their direct effect on the activity of the $(Ca^{2+}+Mg^{2+})$-ATPase.

The findings that the inhibition of both Ca^{2+} accumulation and the coupled ATP hydrolysis by the hormones is dependent on the presence of P_i in the assay medium (Figs. 3 and 4) and that the hormones inhibit the partial reactions of Ca^{2+} transport when P_i is a substrate, such as the ATP–P_i exchange and E-P formation from P_i, may suggest that thyroid hormones inhibit the transport of P_i that is coupled to Ca^{2+} transport and, hence, that they inhibit the P_i-stimulated, ATP-dependent Ca^{2+} accumulation.

It has been shown that oxalate and P_i are co-transported at a 1:1 ratio with Ca^{2+} [29,30]. Furthermore, Carley and Racker [31] showed that the mechanism of P_i transport is not a passive movement in response to a Ca^{2+} gradient but, rather, that it is catalyzed by a specific protein which is inactivated during the purification of the $(Ca^{2+}+Mg^{2+})$-ATPase. We suggest that the interaction of T_3 or T_4 with the P_i transporter is the main cause of the inhibition of both Ca^{2+} transport and the coupled ATP hydrolysis.

Recently, Fliegel et al. [32], using a photoactive derivative of T_3, have demonstrated the presence of thyroid hormone binding protein within the lumen of the SR. The binding of T_4 to protein other than its receptor has also been demonstrated recently [33]. It has been shown that T_4 binds to myosin light chain kinase and thereby inhibited its activity ($I_{50} = 8\ \mu M$). Thus, the question is whether the effect of T_3 or T_4 on Ca^{2+} transport is due to their interaction with the receptor or, as suggested above, they act by direct interaction with other protein(s) involved in Ca^{2+} transport such as the Ca^{2+}-ATPase or the P_i transporter. Our [^{125}I]thyroxine gel overlay results (Fig. 6) indicate that [^{125}I]T_4 binds directly to the Ca^{2+}-ATPase and to other proteins such as calsequestrin, the 55-kDa and the 400 kDa (ryanodine receptor) proteins. It should be mentioned that the SR 55 kDa protein was recently identified as the multifunctional thyroid hormone binding protein [34]. These results may suggest that one of these proteins is the anion transporter of SR membranes, which binds T_3 or T_4 at the μM range whereas the binding affinity of their receptor is at the nM range.

The observation that T_3 or T_4 decreased Ca^{2+} accumulation by the SR suggests that the hormones might increase the myoplasmic free Ca^{2+} concentration. Indeed, it has been shown that there is an increased intracellular Ca^{2+} level in skeletal muscle treated by T_4 [7,8]. This might explain, in part, the alteration of the contraction-relaxation cycle observed in skeletal and cardiac muscle in hyperthyroidism [35]. Furthermore, increased protein degradation has been observed when there was an excess of thyroid hormone [10,36]. In addition, it has been shown that increased intracellular Ca^{2+} levels appear to favor muscle catabolism [37]. It is tempting, therefore, to speculate that the increased rate of protein degradation in muscle treated with thyroid hormone is a result of elevated intracellular Ca^{2+} concentration.

Further studies on the interaction of the thyroid hormones or their analogs with the P_i transport system of the SR membranes could provide the identification and characterization of the anion transporter.

Acknowledgements

This research was supported in part by the Fund for Basic Research administered by the Israeli Academic of Science and Humanities. We thank Prof. D.H. MacLennan for the hospitality extended to us and for enabling us to perform part of these experiments in his laboratory and Mrs. R. Shami for her expert technical assistance.

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