Korean J Physiol Pharmacol
Vol 3: 223−230, April, 1999

Thyroid Hormone-Induced Alterations of Ca^{2+}-ATPase and Phospholamban Protein Expression in Cardiac Sarcoplasmic Reticulum

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Alterations of cardiovascular function associated with various thyroid states have been studied. In hyperthyroidism left ventricular contractility and relaxation velocity were increased, whereas these parameters were decreased in hypothyroidism. The mechanisms for these changes have been suggested to include alterations in the expression and/or activity levels of various proteins; α-myosin heavy chain, β-myosin heavy chain, β-receptors, the guanine nucleotide-binding regulatory protein, and the sarcolemmal Ca^{2+}-ATPase. All these cellular alterations may be associated with changes in the intracellular Ca^{2+} concentration. The most important regulator of intracellular Ca^{2+} concentration is the sarcoplasmic reticulum (SR), which serves as a Ca^{2+} sink during relaxation and as a Ca^{2+} source during contraction. The Ca^{2+}-ATPase and phospholamban are the most important proteins in the SR membrane for muscle relaxation. The dephosphorylated phospholamban inhibits the SR Ca^{2+}-ATPase through a direct interaction, and phosphorylation of phospholamban relieves the inhibition. In the present study, quantitative changes of Ca^{2+}-ATPase and phospholamban expression and the functional consequences of these changes in various thyroid states were investigated. The effects of thyroid hormones on (1) SR Ca^{2+} uptake, (2) phosphorylation levels of phospholamban, (3) SR Ca^{2+}-ATPase and phospholamban protein levels, (4) phospholamban mRNA levels were examined. Our findings indicate that hyperthyroidism is associated with increases in Ca^{2+}-ATPase and decreases in phospholamban levels whereas opposite changes in these proteins occur in hypothyroidism.

Key Words: Phospholamban, SR Ca^{2+}-ATPase, Heart, Thyroid hormone

INTRODUCTION

Thyroid hormone influences cardiac function by direct and indirect actions; changes in the cardiovascular system are prominent clinical consequences in thyroid dysfunctional states. In hyperthyroidism, there is tachycardia, increased stroke volume, increased cardiac index, cardiac hypertrophy, and decreased peripheral vascular resistance. On the other hand, in hypothyroidism, there is bradycardia, decreased cardiac index, pericardial effusion, increased peripheral vascular resistance, and elevations of mean arterial pressure (For a review, see Braverman et al, 1994). Thyroid hormones play a direct role in regulating myocardial gene expression. Thyroid hormones also indirectly influence cardiac function. The sensitivity of the cardiac myocyte to catecholamines is enhanced in hyperthyroidism and depressed in hypothyroidism, possibly due to changes in the expression of myocardial β-adrenergic receptors.

The cardiovascular manifestations of thyroid hormone have been studied extensively. Thyroid hormones have profound effects on cardiac function. Increased velocity of contraction was caused by a shift of myosin heavy chain isoforms (Haddad et al, 1998; Mercadier et al, 1981; Nadel-Ginard, 1989). Increased speed of diastolic relaxation which is thought to be resulted from changes in the velocity of cytoplasmic Ca^{2+} sequestration by the sarco-

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plasmic reticulum (SR) (Alpert et al, 1987; Arai et al, 1991). The SR is the most important regulator of Ca^{2+} homeostasis that serves as a sink for Ca^{2+} ions during relaxation and as a Ca^{2+} source during contraction. Important proteins in the SR membrane are the ryanodine receptor (which is involved in the release of Ca^{2+} from the SR), the Ca^{2+}-ATPase (which mediates the uptake of Ca^{2+} from the cytosol during muscle relaxation), and phospholamban (the regulator of the SR Ca^{2+}-ATPase). In particular, Ca^{2+}-ATPase is known to be inhibited by phospholamban in its dephosphorylated state through a decrease in the affinity of the enzyme for Ca^{2+} and phosphorylation of phospholamban relieves the inhibition (Edes & Kranias, 1988; Kim et al, 1990).

The effects of thyroid hormone on the gene expression of these Ca^{2+}-cycling proteins in small animals are well documented. Experimental cardiac hypertrophy produced by thyroid hormone administration results in increased rates of tension development and decline, which are associated with coordinate increases in the expression of ryanodine receptor and Ca^{2+}-ATPase mRNA. However, the effects of thyroid hormone on phospholamban are more controversial (Nagai et al, 1989; Arai et al, 1991; Kimura et al, 1994). In small animals treated with thyroid hormone, steady state phospholamban mRNA expression decreases, however, PCR-quantified phospholamban mRNA from serial myocardial biopsies doubled after when patients with hypothyroidism and heart failure were treated with thyroid hormone (Landenson et al, 1992).

Accordingly, the aim of the present study was to examine the mechanisms altering SR function in hypothyroid and hyperthyroid rat hearts. More specifically, this study aims: 1) to determine whether the expression of SR proteins controlling calcium uptake is regulated in a coordinate manner during the various thyroid states; 2) to determine quantitative changes in phospholamban and SR Ca^{2+}-ATPase levels and the functional consequences of these changes in various thyroid state; and 3) to determine the effect of thyroid hormone on the expression of phospholamban mRNA.

METHODS

Animal models

Male Sprague-Dawley (SD) rats weighing 250 g (8 weeks after birth) were randomly divided into three groups (hypothyroid, euthyroid, and hyperthyroid). The euthyroid and hyperthyroid animals were fed a standard laboratory rat chow. The animals in the hyperthyroid group received a daily subcutaneous injection of L-triiodothyronine (T_3, 400 μg/kg body wt) for 14 days. To induce hypothyroidism, animals were maintained on an iodine-deficient diet with 2% (wt/vol) $KClO_4$ in their drinking water for 6 weeks (Van Hardeveld and Clausen, 1984). No spontaneous deaths were observed during the experimental period.

Preparation of cardiac sarcoplasmic reticulum vesicles

The membrane fraction enriched with sarcoplasmic reticulum (SR) from rat heart was isolated according to the method described previously (Feher & LeBolt, 1990), with slight modification. After removal of atrium, visible fat, and connective tissues, ventricular tissue was cut into small pieces and homogenized (15 sec $\times$ 4 with 15 sec intervals) in a buffer containing 10 mM imidazole, pH 7.0, 1 M KCl and 10 mM sodium metabisulfite, using a Virtis homogenizer at a speed setting of 7. The homogenate was centrifuged at 4,000 $\times$ g for 20 min, and the supernatant was centrifuged again at 11,000 $\times$ g for 25 min. The supernatant was centrifuged at 40,000 $\times$ g for 25 min and this supernatant was centrifuged again at 100,000 $\times$ g for 30 min. The resultant pellet was suspended in 10 mM imidazole, pH 7.0, 0.6 M KCl, and 0.3 M sucrose and then centrifuged at 100,000 $\times$ g for 45 min. The final pellet was suspended in 10 mM imidazole, pH 7.0, 0.1 M KCl, and 0.3 M sucrose, frozen in liquid nitrogen, and stored at -80°C before use. Protein concentration was measured by the method of Lowry et al (1951) and the Ca^{2+} transport assay was completed within 2 h.

Ca^{2+}-ATPase activity

The activity of Ca^{2+}-ATPase of the cardiac SR vesicles was measured by the method of Niggli et al (1979). Briefly, the activity was monitored in a physiological solution containing 120 mM KCl, 30 mM Hepes, pH 7.4, 0.5 mM $MgCl_2$, 0.5 mM Na-ATP, 0.4 mM NADH, 50 μM $CaCl_2$, 2 mM phosphoenolpyruvate, 1 IU/ml of pyruvate kinase, and 1 IU/ml of lactate dehydrogenase. The concentration of free Ca^{2+} in the solution was adjusted by using Ca-EGTA buffers calculated by a computer program

(Fabiato, 1988). The formation of ADP by the activity of Ca^{2+}-ATPase is quantitatively coupled to the oxidation of NADH in the reaction solution, resulting in an absorbance decrease at 340 nm. Decrease in absorbance was continuously monitored, and the activity was calculated from the slope of the decrease in absorbance.

Determination of calcium uptake

Calcium uptake was determined at 37°C by using $^{45}CaCl_2$ and a modification of the Millipore filtration technique (Martonosi & Feretos, 1964). In the Millipore filtration assay, the rate of Ca^{2+} uptake was determined in a medium containing 50 μg of SR protein per ml, 40 mM histidine-HCl, pH 7.0, 0.1 M KCl, 5 mM $MgCl_2$, 2.5 mM oxalate, 5 mM NaN_3, 0.5 mM EGTA, various amounts of $^{45}CaCl_2$ to yield $10^{-7} \sim 10^{-5}$ M free Ca^{2+}, using Millipore filters with a pore size of 0.45 μm (type GS). Calcium uptake was initiated by the addition of 5 mM ATP. The initial rates of Ca^{2+} uptake were calculated using least squares linear regression analysis of the 20-, 40-, and 60-sec values of Ca^{2+} uptake.

Determination of phosphorylation level of phospholamban

Phosphorylation of cardiac SR (20 μg) was carried out, in 50 mM potassium phosphate, pH 7.0, 10 mM $MgCl_2$, 10 mM NaF, 0.5 mM EGTA, 1 mM DTT, 15 units of catalytic subunit of the cAMP-dependent protein kinase, and 50 μM [γ^{32}P]ATP for 5 min at 30°C. Reactions were terminated by the addition of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis sample buffer (62.5 mM Tris-HCl, pH 6.8, 7% glycerol, 3% SDS, 5% β-mercaptoethanol, and 0.05% bromophenolblue). Gel electrophoresis was performed on SDS-polyacrylamide gels (10~18%) according to the method of Laemmli (1970), and then autoradiograms were obtained from stained gels using Fuji film.

Quantitative immunoblots

The phospholamban monoclonal antibody (PLB-Ab) was purchased from Upstate Biotechnology Inc. (Lake Placid, USA), and the Ca^{2+}-ATPase monoclonal antibody (SERCA-Ab) was purchased from Affinity Bioreagents Inc. (Golden, USA). The relative ratios of Ca^{2+}-ATPase and phospholamban in cardiac SR from hypothyroid, euthyroid, and hyperthyroid hearts were estimated by quantitative immunoblotting. Cardiac SR proteins separated by SDS-polyacrylamide gel electrophoresis (10~18% gradient slab gels) according to the method of Laemmli (1970), were transferred electrophoretically on to polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore, Bedford, USA). The transferred proteins were incubated with blocking solution containing 5% dried milk, and 0.1% Tween 20 in phosphate buffered saline for 1 h at room temperature. After blocking, the transblots were reacted with PLB-Ab (1 : 1000 dilution) or SERCA-Ab (1 : 1000 dilution), and incubated with a secondary antibody (goat anti-mouse-HRP conjugate) from Amersham International (Little Chalfont, UK) at 1 : 1000 for 1 h, respectively. Antibody binding was detected using ECL (enhanced chemiluminescence) horseradish peroxidase developing agents from Amersham International (Little Chalfont, UK), and membranes were exposed to a sheet of autoradiography film. The developed bands were quantitated by the scanned image using Image Quant software, with data reported as integrated density units. The density associated with phospholamban was linear in the range of 10~50 μg cardiac SR protein loaded onto the gel lanes, while the corresponding linear range for the SR Ca^{2+}-ATPase was between 5 and 25 μg cardiac SR protein.

RNA isolation and dot blot analysis

Total cellular RNA of hearts with different thyroid status was isolated by guanidine isothiocyanate method (Chomczynski & Sacchi, 1987). Two fold serial dilutions of the RNAs, starting with 10 μg, were spotted onto Gene Screen Plus membranes using a dot blot manifold (Bio-Rad), prehybridized and hybridized to a specific phospholamban or SR Ca^{2+}-ATPase oligonucleotide, and then 18S rRNA oligonucleotide according to manufacturer's instructions. Prehybridizations were performed in 5×SSPE (1×SSPE contains 150 mM NaCl, 10 mM sodium phosphate, 1 mM EDTA, pH 7.4), 50% deionized formamide, 5×Denhardt's solution, 1% SDS, and 100 μg/ml salmon sperm DNA at 42°C for 3 h. Hybridizations were done in the same solution without DNA at 42°C for 24 h with oligonucleotides specific to phospholamban or SR Ca^{2+}-ATPase at a concentration of 1×10^6 cpm/ml. The filters were washed twice in a 2×SSPE at room temperature,

once in 0.1×SSPE and 1% SDS at 50°C for 30 min, and twice in 0.1×SSPE at room temperature for 15 min, successively. Autoradiography was done with Kodak X-Omat AR film at −70°C for 1 day. To determine the relative RNA levels, the signals in the films were quantitated by transmittance densitometer (BioRad, model GS-670) with comparison of standards which were blotted in another filter and autoradiographed in the same film and their radioactivities were checked by scintillation counter later. After the membranes were striped of phospholamban or SR Ca^{2+}-ATPase probe in 5 mM Tris-HCl, pH 7.5, and 2 mM EDTA, pH 8.0 at 65°C for 2 h, prehybridization and hybridization with a specific probe to 18S rRNA were performed, and autoradiography was done as described above. ^{32}P-labeled probe was added to hybridization solution at a concentration of 4.2×10^6 cpm/ml (1.3 pmol/ml) with cold oligonucleotide at 5 times the concentration of ^{32}P-labeled probe.

For the calculation of relative amounts of phospholamban or SR Ca^{2+}-ATPase mRNAs, signals with phospholamban or SR Ca^{2+}-ATPase probes were normalized to those with the 18S rRNA probe in the same membranes. Statistical significance was determined by unpaired Student's t-test.

Oligonucleotides

Three kinds of synthetic 60-mer oligonucleotides were used in the hybridization; one is specific for phospholamban (5'-TGA CGC GCT TGC TGG GGC ATT TCA ATA GTC GAG GCT CTC CTG ATA GCC GAG CGA GTA AGG 3'), one for Ca^{2+}-ATPase (5' AGG TGT GTT GCT AAC AAC GCA GAT GCA CGC ACC CGA ACA CCC TTA TAT TTC TGC AAA TGG 3'), and one for 18S rRNA (5' GTA TCT GAT CGT CTT CGA ACC TCC GAC TTT CGT TCT TGA TTA ATG AAA ACA TTC TTG GCA 3'). The oligonucleotides were end-labeled with [$\gamma^{32}P$]ATP by using T4 polynucleotide kinase and unincorporated nucleotides were removed by C18 Sep-Pak cartridge (Millipore).

RESULTS

Effects of various thyroid states on weight parameters and SR Ca^{2+}-ATPase

In comparison with euthyroid hearts, the hyperthyroid ones exhibited significant increases in the ratio of heart weight to body weight and SR Ca^{2+}-ATPase activities (Table 1). On the other hand, hypothyroidism resulted in lower ratios of heart weight to body weight and SR Ca^{2+}-ATPase activities when compared with euthyroidism.

Table 1. Effect of different thyroid states on Heart/Body weight and sarcoplasmic reticulum Ca^{2+}-ATPase

	HYPO	EU	HYPER
Heart/Body weight (g/kg)	2.4±0.4*	3.1±0.5	4.0±0.6*
SR calcium ATPase (nmol/mg/min)	150±22*	230±34	381±37*

The animals in the hyperthyroid group received a daily subcutaneous injection of L-triiodothyronine (T_3, 400 μg/kg body wt) for 14 days. To induce hypothyroidism, animals were maintained on an iodine-deficient diet with 2% (wt/vol) $KClO_4$ in their drinking fluid for 6 weeks. Values shown represent the means±SD of at least 5 different hearts. Statistically significant difference ($p<0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). SR, sarcoplasmic reticulum; HYPO, hypothyroid; EU, euthyroid; HYPER, hyperthyroid

Initial rates of ATP-dependent oxalate-facilitated Ca^{2+} uptake were obtained in cardiac SR vesicles from different thyroid states assayed at various Ca^{2+} concentrations (Fig. 1). Since Ca^{2+} uptake into the SR is a major factor in determining relaxation rate, we measured this parameter in heart SR isolated from hypothyroid, euthyroid and hyperthyroid rats. The maximum rates of Ca^{2+} uptake showed a significant increase in hyperthyroidism when compared with euthyroidism, but hypothyroidism was associated with an opposite trend (Fig. 1). These relative changes in SR Ca^{2+} uptake rates were similar to those observed in SR Ca^{2+}-ATPase activity in isolated SR membranes (Table 1).

Effects of various thyroid states on phosphorylation of phospholamban

To determine whether the changes of the cardiac SR function in various thyroid states are associated with the level of phospholamban phosphorylation, phosphorylation of phospholamban by the catalytic

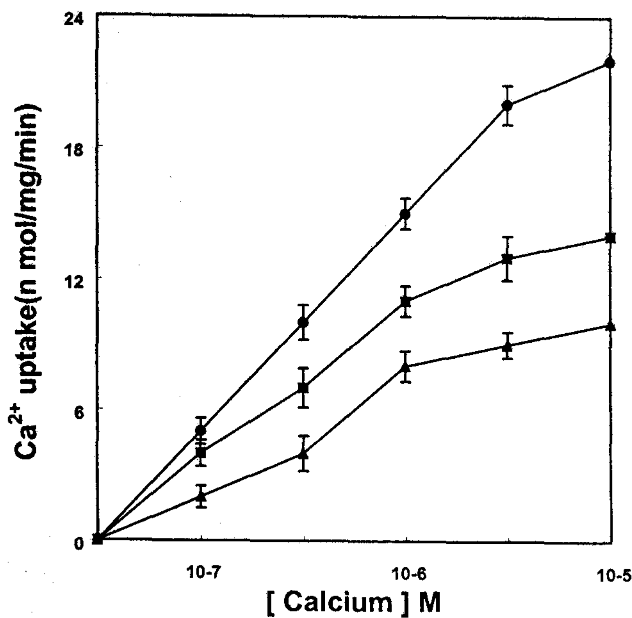

Fig. 1. Calcium dependence curve of sarcoplasmic reticulum Ca^{2+} uptake from hearts with different thyroid status. The initial rates of sarcoplasmic reticulum Ca^{2+} uptake from hypothyroid (▲), euthyroid (■), and hyperthyroid (●) hearts were measured at various free Ca^{2+} concentrations. Each value represents the mean ± SD of six different hearts, each assayed in triplicate.

subunit of the cAMP-dependent protein kinase and [γ^{32}P]ATP was studied. Levels of phospholamban phosphorylation were 148±21, 100±19, and 45±10% in the hypothyroid, euthyroid, and hyperthyroid hearts, respectively, when the values found in euthyroid animals were taken as 100 percent (Table 2).

Effects of various thyroid states on protein expression levels of phospholamban and SR Ca^{2+}-ATPase

To examine whether the observed changes in the SR Ca^{2+} uptake reflected the altered expression of the SR Ca^{2+}-ATPase and phospholamban in hypothyroid and hyperthyroid rat hearts, the relative levels of these proteins were determined by quantitative immunoblotting (Table 3). Administration of thyroid hormone (T_3) to hearts for 14 days induced a marked increase in the expression levels of the SR Ca^{2+}-ATPase protein compared with euthyroid hearts. On the other hand, in hypothyroid animals the expression of the SR Ca^{2+}-ATPase was depressed. Examination of the phospholamban levels in the same hearts revealed an opposite trend in the expression of this protein compared with the SR Ca^{2+}-ATPase. There was a significant decrease of the phospholamban levels in the hyperthyroid hearts, while there was a marked increase in the hypothyroid hearts compared with euthyroid hearts. The relative ratio of phospholamban to Ca^{2+}-ATPase as an index of SR function was observed to increase in hypothyroid rats, whereas it was decreased in hyperthyroid rats compared with euthyroid rats. These changes in the relative levels of

Table 2. Percent changes in the relative phosphorylation of phospholamban in rat hearts

	HYPO	EU	HYPER
PLB	148±21*	100±19	45±10*

Phosphorylation levels of phospholamban were determined by SDS-PAGE and autoradiography. The values found in euthyroid animals were taken as 100 percent and the changes in hypothyroid and hyperthyroid hearts were expressed as percent of euthyroid values. Each value represents the mean ± SD of five different hearts, measured at two protein concentrations, each in quadruplicate. Statistically significant difference ($p < 0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). PLB, phospholamban; HYPO, hypothyroid; EU, euthyroid; HYPER, hyperthyroid

Table 3. Percent changes in the relative protein amounts of phospholamban and Ca^{2+}-ATPase in rat hearts

	HYPO	EU	HYPER
PLB	130±18*	100±20	75±13*
Ca-A	76±14*	100±18	138±12*
PLB/Ca-A	1.71*	1.00	0.54*

Sarcoplasmic reticulum Ca^{2+}-ATPase and phospholamban protein levels were determined by quantitative immunoblotting. The values found in euthyroid animals were taken as 100 percent and the changes in hypothyroid and hyperthyroid hearts were expressed as percent of euthyroid values. Each value represents the mean ± SD of five different hearts, measured at two protein concentrations, each in quadruplicate. Statistically significant difference ($p < 0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). PLB, phospholamban; Ca-A, Ca^{2+}-ATPase; HYPO, hypothyroid; EU, euthyroid; HYPER, hyperthyroid

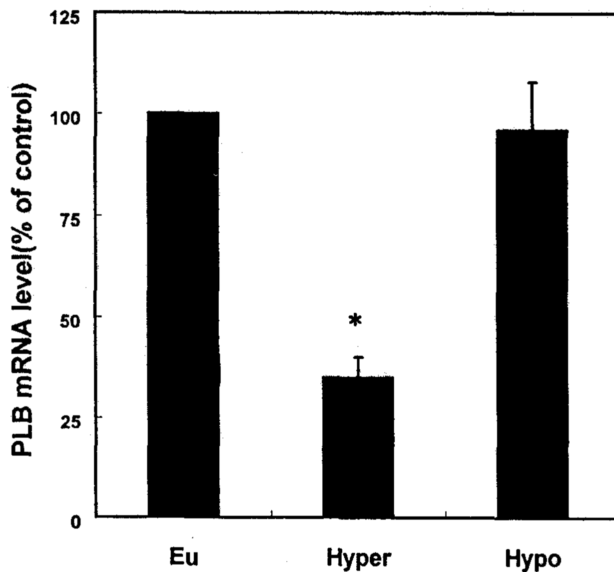

Fig. 2. RNA dot blot analysis of phospholamban level in euthyroid, hypothyroid, and hyperthyroid rats. Levels of phospholamban mRNA corrected by 18S rRNA level in the same membrane were normalized to the mean of euthyroid group. Values are mean ± SD of six different hearts, each assayed in triplicate. Statistically significant difference ($p < 0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). PLB, phospholamban; EU, euthyroid; HYPER, hyperthyroid; HYPO, hypothyroid.

phospholamban and the SR Ca^{2+}-ATPase (Table 3), and in the phosphorylation of phospholamban (Table 2) in hypothyroidism and hyperthyroidism were indicative of the alterations in SR Ca^{2+} uptake properties and in myocardial relaxation rates in these hearts.

Effect of various thyroid states on mRNA levels of phospholamban

Phospholamban mRNA level was decreased significantly in hyperthyroidism as noted for protein. However in hypothyroidism, the relative level of phospholamban mRNA did not change in contrast to an increased expression level of protein (Fig. 2).

DISCUSSION

The principal findings of the present study in the hypothyroid and hyperthyroid rat hearts are that the functional changes of cardiac SR were associated with alterations in the expression of SR Ca^{2+}-ATPase and phospholamban proteins. In the present study, the maximum rates of Ca^{2+} uptake were decreased significantly in hypothyroid rat hearts, but the rates were increased in hyperthyriod rat heart (Fig. 1). These changes in SR Ca^{2+} uptake rates were closely related to those observed in SR Ca^{2+}-ATPase activities of hypothyroid and hyperthyroid rat hearts (Table 1).

Using quantitative immunoblotting, we have demonstrated that the functional changes in the hypothyroid and hyperthyroid rat hearts were associated with the alteration of expression levels of phospholamban and the SR Ca^{2+}-ATPase. Our findings involving the SR Ca^{2+}-ATPase and phospholamban protein levels in hypothyroid and hyperthyroid hearts are consistent with the previous report (Kiss et al, 1994). There were decreases in the SR Ca^{2+}-ATPase protein levels in hypothyroidism and increases in hyperthyroidism. However, examination of the phospholamban protein levels in the same hearts revealed an opposite trend of changes compared with SR Ca^{2+}-ATPase (Table 3). The inverse changes in the expression of the two SR proteins resulted in a significant increase in the relative ratio of phospholamban to SR Ca^{2+}-ATPase in hypothyroid hearts. However, the relative ratio of phospholamban to SR Ca^{2+}-ATPase in hyperthyroid hearts was decreased significantly. These changes in the relative levels of phospholamban and the SR Ca^{2+}-ATPase in hypothyroid and hyperthyroid rat hearts were closely correlated with the functional changes in these hearts. Thus, in hyperthyroid hearts both increased Ca^{2+}-ATPase and decreased phospholamban levels resulted in enhanced SR Ca^{2+} uptake. On the other hand, in hypothyroidism the SR Ca^{2+} uptake was depressed as a consequence of downregulation of Ca^{2+}-ATPase gene expression and overexpression of its inhibitor, phospholamban. These findings suggest that the protein levels of phospholamban in relation to SR Ca^{2+}-ATPase are an important determinant of the SR function.

In the previous study, Arai et al (1991) reported that the mRNA level for phospholamban was decreased in both hyperthyroid and hypothyroid rat hearts. Using RNA dot blot analysis, we found that hyperthyroidism induced a decrease in the phospholamban mRNA level, while hypothyroidism had virtually no effect on phospholamban mRNA levels (Fig. 2), which is in agreement with other study (Nagai et al, 1989). Our data suggest that the

phospholamban mRNA expression does not show a coordinate regulation of the protein levels of the phospholamban. In hyperthyroid hearts, both phospholamban mRNA and protein expression levels decreased in parallel. However, in hypothyroid hearts there was no significant change in the mRNA levels of the phospholamban, while the phospholamban protein level increases. These discrepancies between phospholamban mRNA and protein levels may be explained by a shorter half-life of the message and/or an increased stability of the protein in hypothyroidism. The functional significance of these changes remains unexplained.

Previously, Kiss et al (1994) showed that in the absence of isoproterenol, there were no significant differences in the ^{32}P label associated with phospholamban among the hypothyroid, euthyroid, and hyperthyroid hearts, although the levels of phospholamban were different among these groups. Thus, the lack of differences of phospholamban phosphorylation in these hearts may be due to the changes of cardiac protein kinases and phosphatases by thyroid hormone levels (Kiss et al, 1994). However, we have found that the phosphorylation level of phospholamban was higher in hypothyroid hearts, whereas it was lower in hyperthyroid hearts (Table 2), which reflects the protein levels of phospholamban in these states (Table 3). A close correlation of phospholamban phosphorylation levels with phospholamban protein levels may reflect that alterations in these levels are true in spite of the other opinions.

These data indicate that in chronic hyperthyroid and hypothyroid condition regulation of Ca^{2+} uptake is accomplished primarily by the increase or decrease in the number of functional protein, the Ca^{2+}-ATPase. More important, these alterations in Ca^{2+} uptake are coordinately regulated. In summary, our data indicate that thyroid hormones have an inverse effect on phospholamban and the SR Ca^{2+}-ATPase protein levels. The increased expression of the Ca^{2+}-ATPase leads to an increased Ca^{2+} uptake capacity in hyperthyroidism, whereas opposite changes in the expression of these proteins occur in hypothyroidism.

ACKNOWLEDGENENTS

This work was supported in part by grants from the Research Aid Program for basic Medicine from the Ministry of Education (1996), and the Asan Institute for Life Sciences (1997-002, 1998-002).

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