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Thyroid Hormone-Induced Alterations of Ryanodine and Dihydropyridine Receptor Protein Expression in Rat Heart

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Thyroid hormone-induced cellular dysfunctions may be associated with changes in the intracellular Ca^{2+} concentration. The ryanodine receptor, a Ca^{2+} release channel of the SR, is responsible for the rapid release of Ca^{2+} that activates cardiac muscle contraction. In the excitation-contaction coupling cascade, activation of ryanodine receptors is initiated by the activity of sarcolemmal Ca^{2+} channels, the dihydropyridine receptors. 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. In the present study, quantitative changes of ryanodine receptors and the dihydropyridine receptors, and the functional consequences of these changes in various thyroid states were investigated. In hyperthyroid hearts, [^{3}H]ryanodine binding and ryanodine receptor mRNA levels were increased, but protein levels of ryanodine were not changed significantly. However, the above parameters were markedly decreased in hypothyroid hearts. In case of dihydropyridine receptor, there were a significant increase in the mRNA and protein levels, and [^{3}H]nitrendipine binding, whereas no changes were observed in these parameters of hypothyroid hearts. Our findings indicate that hyperthyroidism is associated with increases in ryanodine receptor and dihydropyridine receptor expression levels, which is well correlated with the ryanodine and dihydropyridine binding. Whereas opposite changes occur in ryanodine receptor of the hypothyroid hearts.

Key Words: Heart, Thyroid hormone, Ryanodine receptor, Dihydropyridine receptor

INTRODUCTION

Thyroid hormone-induced cardiac hypertrophy is extensively studied to investigate mechanisms altering cardiac function. Cardiac hypertrophy after thyroxine administration is associated with an increased rate of tension development and an enhanced velocity of fiber shorting. This contrasts with a depression of cardiac contractile velocity associated with hypothyroidism. An increased velocity of contraction was caused by a shift of myosin heavy chain isoforms (Mercadier et al, 1981; Nadel-Ginard & Mahdavi, 1994; Haddard et al, 1998) and increased speed of diastolic relaxation. An increased rate of relaxation is thought to be resulted from changes in the velocity of cytoplasmic Ca^{2+} sequestration by the sarcoplasmic reticulum (SR) (Alpert et al, 1987; Arai et al, 1991).

SR is an important determinant in the regulation of intracellular Ca^{2+} fluxes, which contribute to myocardial contractility (Luo et al, 1994). During the excitation of cardiac muscle, extracellular Ca^{2+} entry is believed to be primarily mediated by the dihydropyridine receptor (DHPR), which functions in tissue as a voltage-dependent L-type Ca^{2+} channel localized in the T-tubule membranes and is the sensor of their depolarization (Marty et al, 1995). The influx of extracellular Ca^{2+} triggers the release of SR Ca^{2+} through the activation of SR Ca^{2+} release channel (ryanodine receptor), which is called as Ca^{2+}-induced Ca^{2+} release. For cardiac muscle relaxation, SR Ca^{2+}-

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ATPase mediates the uptake of Ca^{2+} from the cytosol, which is controlled by phospholamban, the regulator of the SR Ca^{2+}-ATPase. The effects of thyroid hormone on the gene expression of these Ca^{2+}-cycling proteins in small animals are well documented. Previously, we have shown that experimental cardiac hypertrophy produced by thyroid hormone administration results in increased rates of Ca^{2+} uptake, which are associated with coordinate increases in expression of Ca^{2+}-ATPase mRNA and protein level (Kim et al, 1999).

Aims of the present study was to examine the mechanisms altering SR function in hyperthyroid and hypothyroid rat hearts, with the following objective: 1) to determine the effect of thyroid hormone on the expression of mRNA encoding SR ryanodine receptor that are responsible for calcium release in rat hearts, 2) to determine the corelation between quantitative changes in ryanodine receptor levels and the functional consequences of these changes in various thyroid states and, 3) to determine whether thyroid hormone affects the functional state of Ca^{2+} channel, dihydropyridine receptor and its mRNA and protein expression levels.

METHODS

Animal models

Male Sprague-Dawley (SD) rats weighing 250 g (8 weeks after birth) were randomly divided into three groups (euthyroid, hyperthyroid, and hypothyroid). 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 & Clausen, 1984). No spontaneous deaths were observed during the experimental period.

Preparation of heavy sarcoplasmic reticulum vesicles

Heavy sarcoplasmic reticulum (HSR) vesicles were prepared from the ventricular muscle of rat heart as described for rabbit muscle (Valdivia et al, 1992). Briefly, rat heart was cut into small pieces and ground by a food procssor in a solution containing; 0.1 M NaCl, 5 mM Tris-Maleate, pH 6.8. The homogenate was centrifuged for 30 min at 5,500×g and the supernatant was centrifuged again for 30 min at 12,000×g. The pellet was resuspended and incubated in a high-K buffer containing 0.1 M KCl, 5 mM Tris-MES, pH 6.8. The suspension was centrifuged for 60 min at 130,000×g. The pellet containing vesicles was resuspended in 10% sucrose solution and subjected to a discontinuous sucrose gradient centrifugation for 5 h at 100,000×g. Microsomal fractions were recovered from the interfaces of 20, 30, 35, 40% of sucrose layers and each fraction was centrifuged for 90 min at 100,000×g. The centrifuged subfractions was resuspended in a solution containing 0.3 M sucrose, 0.1 M KCl, 5 mM Na-PIPES, pH 6.8, frozen in liquid nitrogen, and stored at −80°C before use. The concentration of protein was determined by the Lowry method (1951).

Ca^{2+} release experiment

SR $^{45}Ca^{2+}$ release were measured by a modified method of Ghosh et al (1988). Briefly, $^{45}Ca^{2+}$ uptake was performed in an uptake medium containing 1.8 μM $^{45}CaCl_2$ (with 2.16 Ci/mmol $^{45}Ca^{2+}$), 50 μM $CaCl_2$, 120 mM KCl, 30 mM hepes (pH 7.4), 1 mM $MgCl_2$, and 5 mM KCN. The uptake was initiated by the addition of 1 mM ATP. After the completion of uptake, HSR vesicles were transferred onto a filter paper (Whatman GF/B) and washed three times with a washing solution containing 120 mM KCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$, and 30 mM HEPES (pH 7.4). The radiactivity remained in the HSR vesicles was determined by a liquid scintillation spectrometer. SR $^{45}Ca^{2+}$ release was commenced by adding release-inducing reagents after 10 min of $^{45}Ca^{2+}$ uptake.

Measurement of [^{3}H]ryanodine equilibrium binding

[^{3}H]ryanodine binding was carried out for 90 min at 37°C in 0.1 ml of 0.2 M KCl, 1 mM Na_2EGTA, 0.995 mM $CaCl_2$, 10 mM Na-PIPES, pH 7.2. Free Ca^{2+} concentration was 10 μM, which was calculated with a computer program using affinity constants of Fabiato (1988). [^{3}H]ryanodine (60 mCi/mmol) was purchased from Du Pont-New England Nuclear and was diluted directly in the incubation medium to achieve concentrations in the saturable range of 1~30 nM. During incubation, cardiac SR (0.3~0.5 mg/ml) were the last reagent to be added to the me-

dium. Samples were filtered on whatman GF/B glass fiber filters, using a Brandel (Gaithersburg, MD) cell harvester, and washed twice with 5 ml of distilled water. The nonspecific binding was measured in the presence of 10 μM unlabeled ryanodine and was subtracted from each sample. In order to establish whether [^{3}H]ryanodine binding was affected by proteolytic degradation that may have occurred during the binding assay, control incubations were carried out in the presence or absence of the protease inhibitors pepstatin A (1 μM), iodoacetamide (1 mM), phenylmethylsulfonyl fluoride (0.1 mM), leupeptin (1 μM), and benzamidine (1 mM). Addition of the protease inhibitor mixture did not result in a significant change in the site density or in the affiniity of [^{3}H]ryanodine binding. Radioactivity was measured by scintillation counting.

[^{3}H]nitrendipine binding assay

Binding of [^{3}H]nitrendipine to membrane fractions was monitored according to a method reported earlier (Finkel et al, 1987). Membrane preparations (0.08~0.1 mg protein/tube) were incubated with 0.035~5 nM [^{3}H]nitrendipine, unless otherwise indicated in the text, in the absence or presence of 2.5 μM unlabeled nitrendipine, a concentration sufficient to inhibit more than 95% of the specific [^{3}H]nitrendipine binding. Assays were terminated after 1 h at room temperature by filtration (GF/C filters, Whatman, Clifton, New Jersey). Filters were washed twice with 5 ml cold Tris-HCl buffer. The radioactivity of the filters was counted in a scintillation counter at an efficiency of 39~41%. The nonspecific [^{3}H]nitrendipine binding (in the presence of nitrendipine) was subtracted from the total binding (in the absence of nitrendipine) to obtain the specific binding of [^{3}H]nitrendipine.

cDNA expression in E. coli

The pCRII (vector inserted 389 bp ryanodine receptor cDNA, Pharmacia Biotec.) contructs or the pGEX (vector inserted 378 bp dihydropyridine receptor cDNA, Pharmacia Biotec.) contructs in *E. coli* DH5 α cells (Pharmacia Biotec.) were grown in 5~10 ml Luria Bertani (LB) medium, supplemented with 100 g of ampicillin/ml, to an A_{600}=1.0~1.5 at 37°C, then induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside and incubated 12~16 h at 20°C with constant shaking. The cells were harvested by centrifugateion (10,000×g, 5 min) and the pellet was used as the DNA purification source. The cardiac-specific ryanodine receptor cDNA and dihydropyridine receptor cDNA were a generous gift from Dr. Do Han Kim in Kwangju Institute for Science and Technology.

Ryanodine receptor and dihydropyridine receptor cDNA isolation

DNA was isolated using DNA purification system procedure described by Promega. The pellet harvested by centrifugation was resuspended in 250 μl cell resuspension solution and mixed by inverting the tube 4 times after added 250 μl cell lysis solution. 10 μl alkaline protease solution added in the suspension, then added 350 μl neutralization solution and centrifuged at 14,000×g for 10 min. The supernatant was transferred to the Minipreps spin column (Promega) by decanting and centrifuged for 5~10 min at 14,000 g. The resulting supernatant was transferred to the same spin colum and centrifuged at 14,000×g for 1 min. The plasmid DNA was eluted by adding of 100 μl nuclease-free water to the spin colum and centrifuged at 14,000×g for 1min. After eluting the DNA was digested at 37°C for 1 h with EcoRI (for ryanodine receptor cDNA), and BamH and Xho (for dihydropyridine receptor cDNA), the resulting fragments were detected by 1.2% agarose gel electrophoresis.

Oligonucleotides

A synthetic 60-mer oligonucleotides were used in the hybridization, which is specific 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 [γ-^{32}P]ATP by using T4 polynucleotide kinase and unincorporated nucleotides were removed by C18 Sep-Pak cartridge (Millipore).

RNA isolation and dot blot analysis of ryanodine receptor

Total cellular RNA of hearts with different thyroid status was isolated by guanidine isothiocyanate method (Chomczynski & Sacchi, 1987). For dot blots, RNA samples were denatured by heating at 60°C for 5 min, cooled on ice, diluted with 6 vol of 4% formaldehyde in 20 SSC. 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 the 389 bp cardiac-specific ryanodine receptor cDNA probe or the 378 bp dihydropyridine receptor cDNA probe, and then 18S rRNA oligonucleotide. 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 ryanodine receptor and dihydropyridine receptor probe 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 ryanodine receptor cDNA 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 ryanodine receptor mRNAs, signals with ryanodine receptor probes were normalized to those with the 18S rRNA probe in the same membranes. Statistical significance was determined by unpaired Student's t-test.

Quantitative immunoblots

The cardiac-specific ryanodine receptor and dihydropyridine receptor monoclonal antibodies (RyR-Ab) were purchased from Affinity Bioreagents Inc. (Golden, USA). The relative ratios of ryanodine receptor and dihydropyridine receptor in cardiac SR from hypothyroid, euthyroid, and hyperthyroid hearts were estimated by quantitative immunoblotting. Cardiac SR proteins separated by SDS-polyacrylamide gel electrophoresis (4% for ryanodine receptor and 6% for dihydropyridine receptor) 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 a monoclonal antibody (1 : 1,000 dilution) specific for ryanodine receptor or dihydropyridine receptor, and incubated with a secondary antibody (goat anti-mouse-HRP conjugate) from Amersham International (Little Chalfont, UK) at 1 : 2,000 for 2 h. 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 ImageQuant software, with data reported as integrated density units. The density associated with ryanodine receptor and dihydropyridine receptor was linear in the range of 10 ~ 50 μg cardiac SR protein loaded onto the gel lanes.

RESULTS

Effects of various thyroid states on SR Ca^{2+} release and [^{3}H]ryanodine binding

Hyperthyroidism was associated with increases in sarcoplasmic reticulum (SR) Ca^{2+}-ATPase and decreases in phospholamban levels whereas opposite changes in these proteins occur in hypothyroidism, which influence SR Ca^{2+} function, and thus cardiac function (Kim et al, 1999). Thus it was of special interest to determine whether thyroid hormone also influence SR Ca^{2+} release, i.e. cardiac contraction parameter. We analyzed the Ca^{2+} release of cardiac heavy SR vesicles from euthyroid, hyperthyroid, and hypothyroid rats (Fig. 1). The Ca^{2+} release from the cardiac heavy SR vesicles, which is a major factor in determining contraction rate, was increased in hyperthyroid rats but was not decreased in hypothyroid rats when compared with euthyroidism. [^{3}H] ryanodine binding can be used to monitor changes in the functional state of the Ca^{2+} release channel

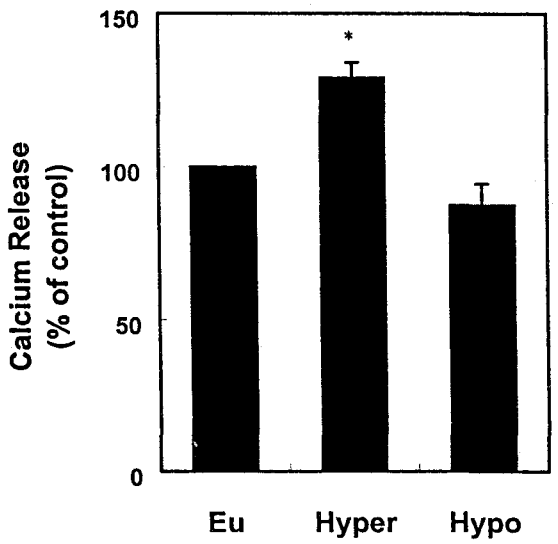

Fig. 1. Ca^{2+} Release of cardiac heavy sarcoplasmic reticulum vesicles from euthyroid, hyperthyroid and hypothyroid rats. Values are mean±S.E. of six different hearts, each assayed in triplicate. Statistical significant difference ($p<0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). EU, euthyroid; HYPER, hyperthyroid; HYPO, hypothyroid

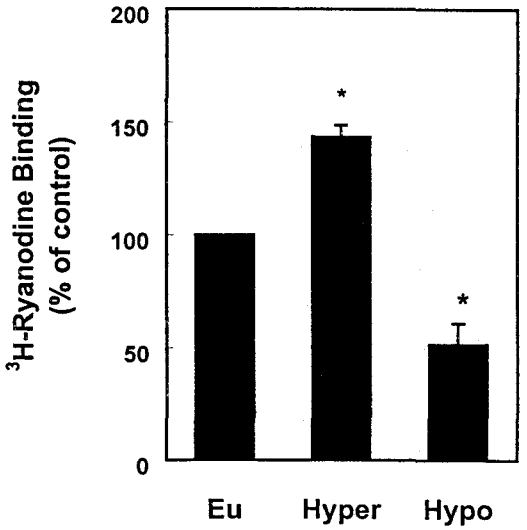

Fig. 2. [^{3}H]ryanodine binding of cardiac heavy sarcoplasmic reticulum vesicles from euthyroid, hyperthyroid and hypothyroid rats. [^{3}H]ryanodine binding was carried out for 90 min at 37°C in 0.1 ml of 0.2 mM Na_2EGTA, 0.995 mM $CaCl_2$, 10 mM Na-PIPES, pH 7.2. The calculated free Ca^{2+} was 10 μM. [^{3}H]ryanodine (60 mCi/mmol) (Du Pont-New England Nuclear) was diluted directly in the incubation medium to achieve concentrations in the saturable range of 1~30 nM. During incubation, cardiac (0.3~0.5 mg/ ml) microsomes were the last reagent to be added to the medium. Values are mean±S.E. of six different hearts, each assayed in triplicate. Statistical significant differences (P 0.05) to euthyroid group were estimated by unpaired Student's t-test and denoted by asterisk (*). EU, euthyroid; HYPER, hyperthyroid; HYPO, hypothyroid

(Hawkes et al, 1992; Meissner and el-Hashem, 1992). We studied specific [^{3}H]ryanodine binding to preparations of cardiac heavy SR vesicles from euthyroid, hyperthyroid and hypothyroid rats to determine whether changes in the functional state of ryanodine receptor is reflected in various thyroid conditions (Fig. 2). [^{3}H]ryanodine binding was increased in hyperthyroid rats, and decreased in hypothyroid rats. These results show that ryanodine receptor was also influenced by various thyroid states.

Effects of various thyroid states on expression levels of ryanodine receptor and dihydropyridine receptor

Because thyroid hormone is known to alter the rate of systolic contraction, which is in part controlled by the activities of ryanodine receptor and dihydropyridine receptor (Amidi et al, 1968; Grossman et al, 1971), we examined the expression of these genes and proteins of rat heart in various thyroid states. We analyzed the expression level of mRNA for cardiac ryanodine receptor using an RNA dot blot method (Fig. 3). The cardiac ryanodine receptor mRNA level was increased in hyperthyroid rats and was decreased in hypothyroid rats. Similar analysis on protein expression levels of ryanodine receptor showed that cardiac ryanodine receptor protein was also decreased in hypothyroidism. In hyperthyroid hearts, there was an increase in the expression levels of ryanodine receptor, but the increase was not statistically significant (Fig. 4).

The expression level of the mRNA and the protein for dihydropyridine receptor localized in the T-tubule membranes was also examined in these heart preparations (Fig. 5, 6). In hyperthyroidism, the dihydropyridine receptor mRNA and protein levels were increased as similar to an increased level of ryanodine receptor (Fig. 4), but in hypothyroidism, the dihydropyridine receptor mRNA and protein levels were not decreased as noted for ryanodine receptor (Fig. 5, 6). These relative changes in the expression pattern of ryanodine receptor were similar to those observed in Ca^{2+} release from SR.

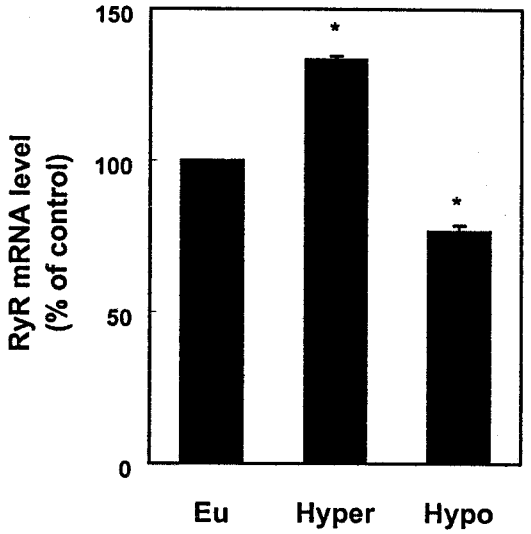

Fig. 3. RNA dot blot analysis of ryanodine receptor from euthyroid, hyperthyroid and hypothyroid rat hearts. Levels of ryanodine receptor mRNA corrected by 18S rRNA level in the same membrane were normalized to the mean of euthyroid group. Values are mean±S.E. of six different hearts, each assayed in triplicate. Statistical significant difference ($p<0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). RyR, ryanodine receptor; EU, euthyroid; HYPER, hyperthyroid; HYPO, hypothyroid

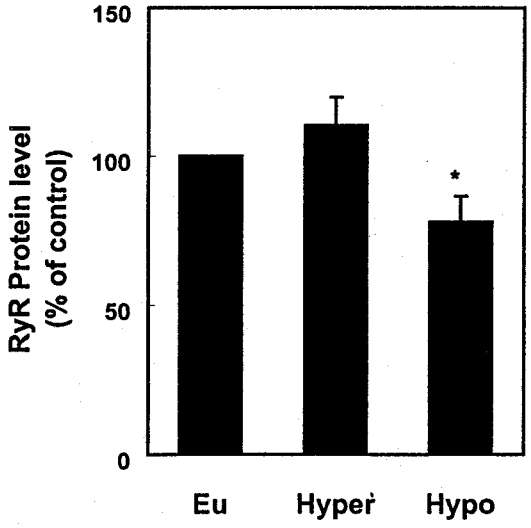

Fig. 4. Western blot analysis of ryanodine receptor in euthyroid, hyperthyroid, and hypothyroid rat hearts. Immunoblots were reacted with 1 : 1,000 dilution of ryanodine receptor monoclonal antibody. Values are mean± S.E. of six different hearts, each assayed in triplicate. Statistical significant difference ($p<0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). EU, euthyroid; HYPER, hyperthyroid; HYPO, hypothyroid

Effects of various thyroid states on [^{3}H]nitrendipine binding

In order to examine the regulatory role of thyroid hormone on sarcolemmal Ca^{2+}-channels, dihydropyridine receptor, the effects of hyperthyroidism and hypothyroidism on rat heart performance and sarcolemmal Ca^{2+} handling were studied. Studies of [^{3}H]nitrendipine binding with cardiac sarcolemmal membrane revealed an increase in hyperthyroid hearts, and no changes in hypothyroid hearts when compared with euthyroidism (Fig. 7). These results were similiar to the expression level of dihydropyridine receptor mRNA and protein (Fig. 5, 6).

DISCUSSION

Principal findings of the present study in the hyperthyroid and hypothyroid rat hearts are that the functional changes were associated with alterations in the expression of ryanodine receptor and dihydropyridine receptor which contributed to the cardiac con-

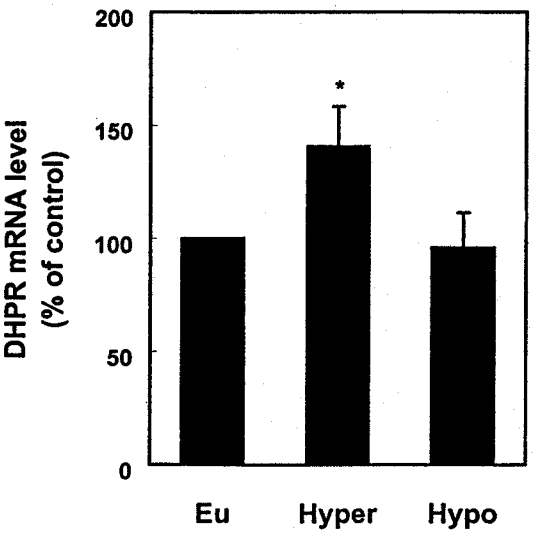

Fig. 5. RNA dot blot analysis of dihydropyridine receptor from euthyroid, hyperthyroid and hypothyroid rat hearts. Levels of ryanodine receptor mRNA corrected by 18S rRNA level in the same membrane were normalized to the mean of euthyroid group. Values are mean±S.E. of six different hearts, each assayed in triplicate. Statistical significant difference ($p<0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). DHPR, dihydropyridine receptor; EU, euthyroid; HYPER, hyperthyroid; HYPO, hypothyroid

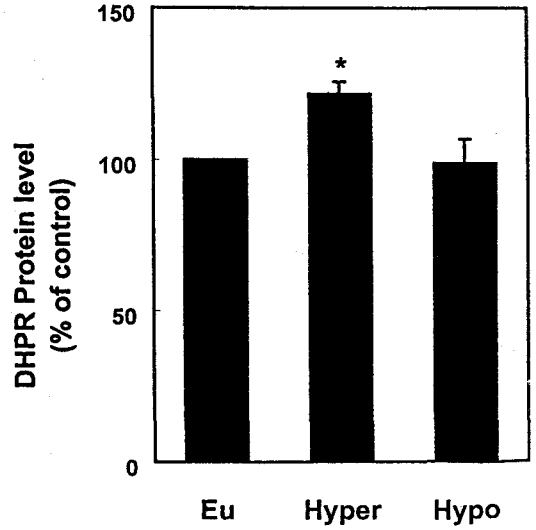

Fig. 6. Western blot analysis of dihydropyridine receptor in euthyroid, hyperthyroid and hypothyroid rat hearts. Immunoblots were reacted with 1 : 1,000 dilution of dihydropyridine receptor monoclonal antibody. Values are mean±SE of six different hearts, each assayed in triplicate. Statistical significant difference ($p<0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). DHPR, dihydropyridine receptor; EU, euthyroid; HYPER, hyperthyroid; HYPO, hypothyroid

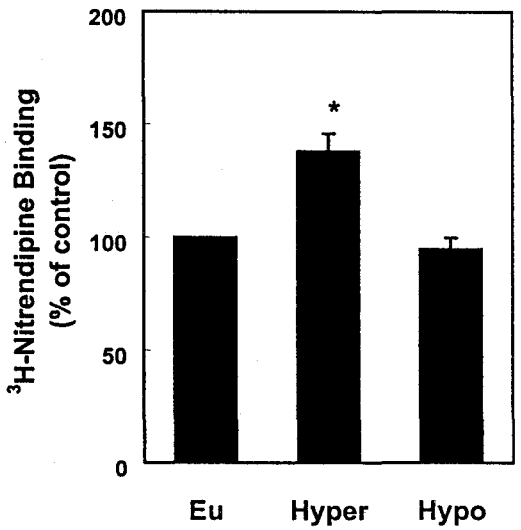

Fig. 7. [^{3}H]nitrendipine binding of cardiac heavy sarcoplasmic reticulum vesicles from euthyroid, hyperthyroid and hypothyroid rats. Values are mean±S.E. of three different hearts, each assayed in triplicate. Statistical significant difference ($p<0.05$) to euthyroid group was estimated by unpaired Student's t-test and denoted by asterisk (*). EU, euthyroid; HYPER, hyperthyroid; HYPO, hypothyroid

traction. Previously we have demonstrated that the expression of the mRNA and protein levels of SR Ca^{2+}-ATPase was upregulated in hyperthyroid hearts and downregulated in hypothyroid hearts. However, examinations of the phospholamban mRNA and protein levels in the same hearts revealed an opposited trend of changes compared with the SR Ca^{2+}-ATPase (Kim et al, 1999). This indicates that the SR Ca^{2+} uptake and the myocardial relaxation rate in hypothyroidism were depressed as a consequence of downregulation of Ca^{2+}-ATPase and overexpression of its inhibitor, phospholamban.

In addition to relaxant parameters, in the present study we have demonstrated that the expression levels of the mRNA coding for the cardiac ryanodine receptor are upregulated in hyperthyroidism and downregulated in hypothyroidism (Fig. 3). Furthermore, we found that thyroid hormone-induced changes in the protein level for SR ryanodine receptor is increased in the hyperthyroid and decreased in the hypothyroid rat hearts (Fig. 4). In case of dihydropyridine receptor, mRNA and protein expression levels were also increased in hyperthyroid rats, however, there was no changes of mRNA and protein expression levels in the hypothyroid rats.

The functional significance of these changes in ryanodine receptor and dihydropyridine receptor levels in hyperthyroidism and hypothyroidism was reflected in basal contractile parameters (Morkin et al, 1983). In hyperthyroid hearts the ryanodine receptor and dihydropyridine receptor resulted in enhanced release of Ca^{2+} from the SR and the stimulation of myocardial contraction rates, but hypothyroidism was associated with a opposite changes. These data indicate that in chronic hyperthyroid and hypothyroid status, regulation of Ca^{2+} release is accomplished primarily by increasing or decreasing the number of functional protein molecules for the Ca^{2+} release channel. More important, these alterations in Ca^{2+} release are coordinately regulated.

In addition to expression levels for ryanodine receptor and dihydropyridine receptor, we also observed functional state of the Ca^{2+} channel as monitored by [^{3}H]ryanodine binding to ryanodine receptor and [^{3}H]nitrendipine binding to dihydropyridine receptor in various thyroid conditions. [^{3}H]ryanodine binding was increased in hyperthyroid hearts, and decreased in hypothyroid hearts, which is correlated

with changes in the expression levels of ryanodine receptor mRNA and protein in hyperthyroidism and hypothyroidism, respectively. This result may be suggest that the specific affinity of [^{3}H]ryanodine to the Ca^{2+} release channel was significantly increased in cardiac SR of rats with hyperthyroidism in association to the increased changes in the Ca^{2+} channel density. It remains to be confirmed whether an electrical response to ryanodine is occurring in agreement with this observation at [^{3}H]ryanodine binding assay. Our data on [^{3}H]nitrendipine binding to Ca^{2+} channel, dihydropyridine receptor, in cardiac sarcolemmal membranes of rats with hyperthyroidism showed increased activities, but examination of the binding in the hypothyroidism revealed no changes compared with euthyroidism.

In summary, our data indicate that thyroid hormones increased the expression levels in the mRNA and protein of ryanodine receptor and of dihydropyridine receptor. Whereas opposite changes in the expression of ryanodine receptor mRNA and protein occur in hypothyroidism. The increased expression of the Ca^{2+} release channel and dihydropyridine receptor could lead to an increased Ca^{2+} release and Ca^{2+} influx, and thus cardiac contraction in hyperthyroidism.

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