Thyroid hormone-induced overexpression of functional ryanodine receptors in the rabbit heart

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Thyroid hormone-induced overexpression of functional ryanodine receptors in the rabbit heart. Am J Physiol Heart Circ Physiol 278: H1429–H1438, 2000.—Modifications in the Ca\(^{2+}\) uptake and -release functions of the sarcoplasmic reticulum (SR) may be a major component of the mechanisms underlying thyroid state-dependent alterations in heart rate, myocardial contractility, and metabolism. We investigated the influence of hyperthyroid state on the expression and functional properties of the ryanodine receptor (RyR), a major protein in the junctional SR (J SR), which mediates Ca\(^{2+}\) release to trigger muscle contraction. Experiments were performed using homogenates and J SR vesicles derived from ventricular myocardium of euthyroid and hyperthyroid rabbits. Hyperthyroidism, with attendant cardiac hypertrophy, was induced by the injection of L-thyroxine (200 µg/kg body wt) daily for 7 days. Western blotting analysis using cardiac RyR-specific antibody revealed a significant increase (>50%) in the relative amount of RyR in the hyperthyroid compared with euthyroid rabbits. Ca\(^{2+}\)-dependent, high-affinity \(^{3}H\) ryanodine binding was also significantly greater (>40%) in J SR from hyperthyroid rabbits. The Ca\(^{2+}\) sensitivity of \(^{3}H\) ryanodine binding and the dissociation constant for \(^{3}H\) ryanodine did not differ significantly between euthyroid and hyperthyroid hearts. Measurement of Ca\(^{2+}\)-release rates from passively Ca\(^{2+}\)-preloaded J SR vesicles and assessment of the effect of RyR-Ca\(^{2+}\)-release channel (CRC) blockade on active Ca\(^{2+}\)-uptake rates revealed significantly enhanced (>2-fold) CRC activity in the hyperthyroid, compared with euthyroid, J SR. These results demonstrate overexpression of functional RyR in thyroid hormone-induced cardiac hypertrophy. Relative abundance of RyR may be responsible, in part, for the changes in SR Ca\(^{2+}\) release, cytosolic Ca\(^{2+}\) transient, and cardiac systolic function associated with thyroid hormone-induced cardiac hypertrophy.

HEART IS a major target for thyroid hormone action, and changes in thyroid status lead to striking alterations in cardiac contractile function and energy metabolism (9, 43). Increases in thyroid hormone levels have been found to enhance myocardial contractility, the speed of systolic contraction and diastolic relaxation, cardiac output, and heart rate (12, 28, 33). On the other hand, decreases in these parameters were observed in hypothyroid state. Evidence from a number of studies suggests that the mechanisms underlying these changes include direct transcriptional regulation of cardiac genes by thyroid hormone, which in turn impacts on myocyte Ca\(^{2+}\) cycling at the level of the myofilaments and the sarcoplasmic reticulum (SR) (9, 34). Thyroid hormone-responsive elements have been identified in the promoter region of cardiac α-myosin heavy chain (α-MHC) and sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2) genes, and thyroid state-dependent alterations in the transcriptional activity of these genes have been documented (1, 4, 9, 14, 18, 31, 44). A shift from the β-myosin heavy chain (β-MHC) (slow) to α-MHC (fast) has been demonstrated during transition from hypothyroid to hyperthyroid state. Changes in MHC isoforms correlated with alterations in myosin ATPase activity and actomyosin cross-bridge cycle rate (23). Thyroid hormone-induced increases in the levels of myocardial SERCA2 mRNA and protein have been documented (1, 18, 21, 22, 35). It has also been reported that thyroid hormone-mediated changes in SERCA2 protein levels were inversely related to alterations in the levels of SERCA2 inhibitor protein phospholamban (20, 22). On the basis of these observations, it has been suggested that thyroid hormone-mediated changes in the relative ratio of phospholamban to Ca\(^{2+}\)-ATPase regulate the Ca\(^{2+}\)-uptake rates by SR and the relaxation properties of the myocardium (20, 22).

In addition to the Ca\(^{2+}\)-cycling properties of the myofilaments and the rate of Ca\(^{2+}\) sequestration by the SR, the speed of Ca\(^{2+}\) delivery to the myofilaments and the intensity of Ca\(^{2+}\) signal are important determinants of cardiac systolic function, strength of contraction, and relaxation duration. However, relatively less is known about the influence of thyroid hormone on the cellular events associated with the rise in cytoplasmic Ca\(^{2+}\) on myocyte excitation. In the cardiomyocyte, the major mechanism for excitation-induced elevation of intracellular Ca\(^{2+}\) involves Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR (3). Recent evidence suggests that sarco(endo)plasmic reticulum Ca\(^{2+}\)-channels are closely associated with a cluster of SR Ca\(^{2+}\)-release channels (CRC), called ryanodine receptors (RyR), in the diadic junctions forming discrete Ca\(^{2+}\)-release units (41). According to the "cluster bomb" model (39), voltage activation of Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channel serves as the local Ca\(^{2+}\) signal to activate the coupled RyR, causing further increase in local Ca\(^{2+}\) and cross-activation of other RyR.
within the release unit. These local Ca\(^{2+}\)-release events have been visualized directly as "Ca\(^{2+}\) sparks" (6), and the recruitment of these events, as a function of Ca\(^{2+}\)-channel activation, underlies the whole cell Ca\(^{2+}\)-transient induced by transsarcolemmal Ca\(^{2+}\) influx (5, 26). Evidence from a study using ferret ventricular muscle indicates that, in the hypothyroid state, decreased peak tension during isometric contraction is associated with a Ca\(^{2+}\)-transient of decreased amplitude and prolonged duration, whereas opposite changes in the time course of Ca\(^{2+}\)-transient and associated isometric tension occur in the hyperthyroid state (28). Thyroid hormone-induced increase in Ca\(^{2+}\)-influx across the sarcolemma, apparently due to an increase in the number of L-type Ca\(^{2+}\) channels, has been reported in cultured ventricular myocytes (19). The steady-state level of mRNA encoding the RyR-CRC has been shown to be increased in the hyperthyroid and decreased in the hypothyroid rabbit heart (1). It is not known whether the thyroid state-dependent changes in mRNA levels are accompanied by parallel changes in the cardiac tissue levels of RyR protein and RyR function. The present study was undertaken to determine the influence of thyroid hormone on RyR protein expression and the functional properties of the RyR in the rabbit heart.

**METHODS**

**Animals**

Twelve-week-old New Zealand White male rabbits were obtained from a local breeder and were maintained on ordinary rabbit chow in the Health Sciences Center animal care facility of this institution. Hyperthyroidism was induced by injecting L-thyroxine (T\(_4\)) intramuscularly at 200 \(\mu g/\)kg body wt daily for 7 days (1). Age-matched untreated rabbits were used as controls ( euthyroid group). The care of animals and the protocols used were in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Ethics Committee of the University of Western Ontario.

**Chemicals**

Reagents for electrophoresis were obtained from Bio-Rad Laboratories (Mississauga, Ontario, Canada). \(^{45}\)CaCl\(_2\) and \(^{3}H\)ryanodine were from NEN (Mississauga, Ontario, Canada). Monodonal antibody against cardiac RyR isoform (RyR2) was purchased from Affinity BioReagents (Golden, CO). Polyclonal antibody against the skeletal muscle RyR isoform (RyR1) was a generous gift from Drs. V. Sorentino and A. Conti (San Raffaele Scientific Institute, Milan, Italy). All other chemicals were from Sigma Chemical (St. Louis, MO) or BDH Chemicals (Toronto, Ontario, Canada).

**Preparation of Muscle Homogenates**

In addition to SR membranes, cardiac muscle homogenates from euthyroid and hyperthyroid rabbits were used in some experiments. The homogenates were prepared by homogenizing the ventricular tissue in 10 vol (based on tissue weight) of 10 mM Tris-maleate-100 mM KCl buffer (pH 6.8) using a Polytron homogenizer (three 15-s bursts with 30-s interval between bursts; speed setting 5.5). The homogenates were filtered through four layers of cheese cloth and used for experiments.

**SDS-PAGE and Immunoblotting of RyR**

The protein composition of Sr and LSR vesicles isolated from euthyroid and hyperthyroid hearts was analyzed by SDS-PAGE using 4–18% gradient gels as described previously (16). Western immunoblotting procedure was used to localize and quantify RyR in SR membrane vesicles and cardiac muscle homogenates. For this, samples of homogenate and SR vesicles (25 \(\mu\)g protein/lane in each case) were first subjected to SDS-PAGE on 6% homogenous or 4–18% gradient gels. The protein bands were visualized using the enhanced chemiluminescence detection system from Amersham. The images of the protein bands were optimized, captured, and analyzed by ImageMaster VDS gel documentation system (Pharmacia BDH Chemicals (Toronto, Ontario, Canada)). Other chemicals were from Sigma Chemical (St. Louis, MO) or BDH Chemicals (Toronto, Ontario, Canada). A. Conti (San Raffaele Scientific Institute, Milan, Italy). All other chemicals were from Sigma Chemical (St. Louis, MO) or BDH Chemicals (Toronto, Ontario, Canada).
Biotech, San Francisco, CA). The Western blotting detection system was determined to be linear with respect to the amount of SR/homogenate protein in the range 10–40 µg using this camera-densitometry system.

Measurement of High-Affinity 
[^3H]Ryanodine Binding

High-affinity ryanodine binding was measured as described by Timmerman et al. (42) with minor modifications. Briefly, J SR vesicles (25 µg) were incubated at 37°C for 60 min in a buffered medium (total volume 100 µl) containing 150 mM KCl, 200 mM HEPES (adjusted to pH 7 with KOH), 0.25–50 nM[^3H]ryanodine (~57,000 counts·min⁻¹·pmol⁻¹), 0.2 mM EGTA, and variable amounts of CaCl₂ to obtain free [Ca²⁺] ranging from 0.05 to 12.94 µM as calculated by the computer program of Fabiato (10). The binding reaction was terminated by filtration through 0.22-µm GS Millipore filters using a Millipore filtration technique as described previously (38). Passive 45Ca²⁺ efflux from passively 45Ca²⁺-loaded JSR vesicles as described previously (38). Passive 45Ca²⁺ loading was performed by incubating JSR vesicles at 4°C for 1 h in medium A [50 mM Tris-maleate (pH 6.8), 120 mM KCl, 5 mM MgCl₂, and 2 mM potassium oxalate; total volume 150 µl] or for 16 h in medium B [50 mM Tris-maleate (pH 6.8), 120 mM KCl, 5 mM MgCl₂, and 5 mM 45CaCl₂; total volume 150 µl]. To initiate Ca²⁺ release, aliquots of 45Ca²⁺-loaded vesicles were diluted 40-fold into a Ca²⁺-release medium [50 mM Tris-maleate (pH 6.8) containing (in mM) 120 KCl, 0.1 CaCl₂, and 1 mM EGTA] that was preincubated for 5 min at 37°C. Subsequently, aliquots of the incubation mixture were filtered through Millipore filters at 15-s intervals for a period of 5 min. The filters were washed with 3 ml of ice-cold 10 mM Tris-maleate buffer (pH 6.8) containing 10 mM MgCl₂ and 10 µM ruthenium red and dried at 60°C, and the 45Ca²⁺ radioactivity was determined by liquid scintillation counting.

The rate of Ca²⁺ uptake was calculated from the linear regression line derived from the uptake determined at the three time points. The Ca²⁺-uptake rates were also measured under conditions of CRC blockade by including 25 µM ruthenium red (45) or 625 µM ryanodine (8) in the incubation medium.

Measurement of Blood Hormone Levels

Blood samples were collected by cardiac puncture at the time when the rabbits were killed, and the samples were then centrifuged. The serum samples were treated with polyethylene glycol to precipitate any endogenous antibodies (24), and the hormones were assayed by a fully automated chemiluminescent immunoassay analyzer (Chiron ACS-180).

Data Analysis

Results are presented as means ± SE. Statistical significance was evaluated by the Student’s t-test; P < 0.05 was taken as the level of significance.

RESULTS

Establishment of Hyperthyroid State and Cardiac Hypertrophy

Ventricular weight normalized for body weight was increased significantly in the thyroid hormone-treated rabbits (hyperthyroid group) compared with the untreated control (euthyroid group) animals (Fig. 1A).
This difference in the ratio of ventricular weight to body weight was due to both an increase in ventricular weight (euthyroid: 5.43 ± 0.11 g, n = 10; hyperthyroid: 6.08 ± 0.08 g, n = 10) and a decrease in body weight (euthyroid: 3.06 ± 0.05 kg, n = 10; hyperthyroid: 2.70 ± 0.04 kg, n = 10) in the hyperthyroid group. The blood levels of T3 and triiodothyronine (T3) were significantly elevated (Fig. 1, B and C), whereas thyroid-stimulating hormone levels were significantly decreased (Fig. 1D) in the hyperthyroid compared with euthyroid. These data demonstrate that the thyroid hormone treatment protocol used in this study resulted in the development of hyperthyroid state with attendant cardiac hypertrophy in the rabbit.

Effects of Thyroid Hormone on RyR Protein Expression

The relative levels of RyR protein in euthyroid and hyperthyroid rabbit hearts were determined by quantitative immunoblotting using a monoclonal antibody specific for the cardiac isoform, RyR2. Experiments using unfractionated cardiac muscle homogenates revealed significantly higher expression levels (~43% increase) of RyR2 in the hyperthyroid compared with the euthyroid (Fig. 2) heart. In additional experiments, cardiac muscle homogenates were fractionated to yield membrane vesicles enriched in JSR and LSR, and Western blotting analysis of RyR2 was performed using these membrane vesicles. As shown in Fig. 3, the relative amount of RyR2 was markedly higher (~2-fold) in JSR vesicles from the hyperthyroid heart, compared with those from the euthyroid heart. LSR vesicles from both euthyroid and hyperthyroid hearts had much lower levels of RyR2 than JSR vesicles. No significant difference in the relative amount of RyR2 was evident between LSR vesicles from hyperthyroid and euthyroid hearts. The protein profile of JSR and LSR vesicles isolated from hyperthyroid hearts was essentially similar to the protein profile of corresponding membrane vesicle preparations from euthyroid hearts (Fig. 4). Therefore, differences in the relative purity of the membrane vesicles isolated from the hyperthyroid versus euthyroid hearts does not contribute to the observed increase in expression levels of RyR2 in the hyperthyroid JSR. Densitometric analysis of Coomassie blue-stained RyR2 band in SDS-PAGE gels showed a significantly higher content of RyR protein in JSR vesicles derived from the hyperthyroid compared with euthyroid hearts (Fig. 5). Also evident was a significant increase (~75%) in the Ca2+-ATPase content of both JSR and LSR vesicles derived from the hyperthyroid compared with euthyroid hearts (Fig. 4).

Although the heart normally expresses only the RyR2 isoform, it was of interest to examine whether...
other RyR isoforms are also expressed in the hyperthyroid state. Western immunoblotting analysis using a polyclonal antibody specific for the RyR1 isoform showed no evidence of RyR1 expression in cardiac muscle of hyperthyroid or euthyroid rabbits; under similar experimental conditions, RyR1 isoform was readily detected in rabbit fast-twitch skeletal muscle, which normally expresses this isoform (results not shown).

**Functional Properties of RyR in Euthyroid and Hyperthyroid Hearts**

Having established thyroid hormone-induced overexpression of RyR in the rabbit heart, the functional properties of these receptors in euthyroid versus hyperthyroid hearts were compared using various criteria as described below.

**Ca\(^{2+}\)**-dependent, high-affinity \[^{3}H\]ryanodine binding. The plant alkaloid ryanodine binds preferentially to SR CRC that are open, and changes in \[^{3}H\]ryanodine binding are thought to reflect changes in gating properties of the ryanodine receptors (7, 30). Figure 6A shows the specific \[^{3}H\]ryanodine binding to JSR vesicles derived from euthyroid and hyperthyroid hearts as a function of free \(\text{Ca}^{2+}\) concentration. For these experiments, the binding assay medium contained 40 nM \[^{3}H\]ryanodine, which permitted saturation of high-affinity binding sites (see below). In both euthyroid and hyperthyroid groups, specific \[^{3}H\]ryanodine binding had a threshold for detection at 0.1 µM free \(\text{Ca}^{2+}\) and increased to a maximal value at 1.3 µM free \(\text{Ca}^{2+}\). At a given subsaturating (0.38 µM) or saturating (3.25 µM) free \(\text{Ca}^{2+}\) in the assay, the levels of specific \[^{3}H\]ryanodine binding were significantly higher (~50% increase) in the hyperthyroid, compared with the euthyroid, group (Fig. 6, B and C). In additional experiments, specific \[^{3}H\]ryanodine binding to JSR vesicles from euthyroid and hyperthyroid hearts was determined at...
varying concentrations of the radioligand in the presence of a saturating concentration of free Ca\(^{2+}\) (3.25 µM). Results from a typical experiment are shown in Fig. 7. At the range of \([3H]\)ryanodine concentrations used (0.25–50 nM), specific binding was saturable in both groups (Fig. 7A). Nonspecific binding was <15% under these assay conditions. Scatchard plots of the data indicated a single binding site (Fig. 7B). Average values for maximum binding sites (B\(_{\text{max}}\)) and the dissociation constant (K\(_d\)) for \([3H]\)ryanodine derived from experiments using six separate JSR preparations each from euthyroid and hyperthyroid hearts are summarized in Table 1. It can be seen that the value for B\(_{\text{max}}\) was significantly greater for hyperthyroid, compared with euthyroid, hearts; the K\(_d\) for \([3H]\)ryanodine did not differ between the two groups.

Ca\(^{2+}\) release from Ca\(^{2+}\)-preloaded JSR vesicles. To assess the Ca\(^{2+}\)-release function of the ryanodine receptors, JSR vesicles isolated from euthyroid and hyperthyroid hearts were subjected to passive Ca\(^{2+}\) loading, and the rate of Ca\(^{2+}\) release from the Ca\(^{2+}\)-preloaded vesicles was measured (38). For these experiments, the Ca\(^{2+}\) loading of JSR vesicles was performed for 1 h in the presence of oxalate in the incubation medium, or for 16 h in the absence of oxalate in the incubation medium. As shown in Fig. 8, A and C, irrespective of the Ca\(^{2+}\) loading conditions used, the rate of Ca\(^{2+}\) release was significantly greater in the hyperthyroid group compared with the euthyroid group. However, during passive Ca\(^{2+}\) loading (in the absence or presence of oxalate), JSR vesicles from the hyperthyroid hearts...
accumulated a greater amount of Ca$^{2+}$ than did JSR vesicles from the euthyroid hearts. Therefore, the Ca$^{2+}$ release rates expressed as a percentage of the initial Ca$^{2+}$ load did not differ appreciably between the euthyroid and hyperthyroid groups (Fig. 8, B and D).

Assessment of RyR function using CRC blockers. Difference in the rates of ATP-energized Ca$^{2+}$ uptake measured in the presence and absence of CRC blockers is a commonly used parameter to assess RyR function in isolated SR vesicles (8). The results presented in Fig. 9 compare the rates of ATP-driven Ca$^{2+}$ uptake in JSR vesicles from euthyroid and hyperthyroid hearts in the absence and presence of CRC blockers. At concentrations known to block Ca$^{2+}$ release (8, 45), ruthenium red (25 µM) and ryanodine (625 µM) both stimulated the rates of Ca$^{2+}$ uptake in JSR vesicles of euthyroid and hyperthyroid hearts (Fig. 9, A and B). JSR vesicles from hyperthyroid hearts exhibited significantly higher rates of Ca$^{2+}$ uptake compared with those from euthyroid hearts both in the absence and presence of CRC blockers. The rate of Ca$^{2+}$ release, defined as the difference in the rate of Ca$^{2+}$ uptake observed in the absence and presence of CRC blockade, was significantly greater (2- to 5-fold) in the hyperthyroid compared with the euthyroid group (Fig. 9C).

DISCUSSION

The results presented here demonstrate that induction of hyperthyroid state, with attendant cardiac hypertrophy, is accompanied by overexpression of RyR protein in the rabbit myocardium. The elevated expres-
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Assessment of the effect of RyR-CRC blockade on active Ca2+-uptake rates also revealed significantly higher CRC activity in the hyperthyroid, compared with euthyroid, JSR (Fig. 9C). Previous studies have demonstrated abbreviation of cytosolic Ca2+-transient (Ca2+<sup>+</sup>) in hyperthyroid, compared with euthyroid heart, which is due to an increase in both the rate of rise and rate of decline in Ca2+<sup>+</sup> (2, 29). Because the rising phase of Ca2+<sup>+</sup>-transient predominantly reflects the release of Ca2+ from the SR (3), the thyroid hormone-induced overexpression of RyR and associated enhancement in Ca2+-channel activity reported here provide a mechanistic basis for the faster rate of rise of the Ca2+<sup>+</sup> transient in the hyperthyroid heart, at the molecular level. According to the current concept of excitation-contraction coupling, one or a small number of L-type Ca2+-channels in the sarcolemma and a cluster of directly proximal RyR in the adjacent JSR serve as discrete Ca2+-release units producing spatially localized transient elevations of Ca2+<sup>+</sup> (<sup>2</sup>+sparks) on myocyte excitation (5, 6, 26, 39, 41). In this scheme, the functional efficacy of RyR would be expected to depend on their spatial proximity to the L-type channels as well as the maintenance of optimal stoichiometry of coupling between the two molecular components of the Ca2+-release unit. Our results show that high-affinity [3H]ryanodine binding in J SR vesicles from euthyroid and hyperthyroid hearts was Ca2+-dependent and saturable in the physiological range of cytosolic free Ca2+. B<sub>max</sub> was significantly greater in the hyperthyroid compared with euthyroid group, which is in accordance with the data from Western blotting experiments demonstrating upregulation of RyR protein expression. The dissociation constants for [3H]ryanodine and the Ca2+-sensitivity of [3H]ryanodine binding did not differ significantly between euthyroid and hyperthyroid hearts. These findings imply that the gating properties of RyR and the affinity of their Ca2+-activation sites remain unaltered in the hyperthyroid heart. Measurement of the rates of Ca2+ release from passively Ca2+-preloaded J SR vesicles demonstrated significantly higher rates of release in the case of hyperthyroid compared with euthyroid group, which could be attributed to the relatively higher density of RyR in the hyperthyroid J SR. However, in these experiments, the initial Ca2+-load was invariably greater in J SR vesicles from the hyperthyroid compared with euthyroid group, and the Ca2+-release rates (expressed as a percentage of initial Ca2+-load) did not differ significantly between the two groups. Previous studies have demonstrated that the rate of Ca2+-release from the SR is also dependent on intraluminal Ca2+-load (13, 37). Thus the SR Ca2+-load dependence of Ca2+-release function is retained in the hyperthyroid heart. The higher level of Ca2+-accumulation in hyperthyroid JSR during passive Ca2+-loading reflects an enhanced Ca2+-storage capacity of the membranes in the hyperthyroid state and is presumably due to larger intravesicular volume and/or an increased level of the Ca2+-storage protein, calsequestrin. Cardiac mRNA level of calsequestrin, however, was found not to be influenced by thyroid state in the rabbit (1).

We used various criteria to compare the functional properties of RyR in euthyroid and hyperthyroid hearts, and these included specific high-affinity [3H]ryanodine binding, unidirectional Ca2+-release, and ATP-driven Ca2+-uptake in the presence and absence of CRC blockers. At low nanomolar concentrations ryanodine opens the RyR-CRC or locks it in the open state, whereas at high micromolar concentrations it promotes the closed conformation of the channel (7, 30). Therefore, high-affinity [3H]ryanodine binding, which is dependent on free Ca2+-concentration (7, 30), can be correlated with the functional state of the RyR. Our results show that high-affinity [3H]ryanodine binding in J SR vesicles from euthyroid and hyperthyroid hearts was Ca2+-dependent and saturable in the physiological range of cytosolic free Ca2+. B<sub>max</sub> was significantly greater in the hyperthyroid compared with euthyroid group, which is in accordance with the data from Western blotting experiments demonstrating upregulation of RyR protein expression. The dissociation constants for [3H]ryanodine and the Ca2+-sensitivity of [3H]ryanodine binding did not differ significantly between euthyroid and hyperthyroid hearts. These findings imply that the gating properties of RyR and the affinity of their Ca2+-activation sites remain unaltered in the hyperthyroid heart. Measurement of the rates of Ca2+-release from passively Ca2+-preloaded J SR vesicles demonstrated significantly higher rates of release in the case of hyperthyroid compared with euthyroid group, which could be attributed to the relatively higher density of RyR in the hyperthyroid J SR. However, in these experiments, the initial Ca2+-load was invariably greater in J SR vesicles from the hyperthyroid compared with euthyroid group, and the Ca2+-release rates (expressed as a percentage of initial Ca2+-load) did not differ significantly between the two groups. Previous studies have demonstrated that the rate of Ca2+-release from the SR is also dependent on intraluminal Ca2+-load (13, 37). Thus the SR Ca2+-load dependence of Ca2+-release function is retained in the hyperthyroid heart. The higher level of Ca2+-accumulation in hyperthyroid JSR during passive Ca2+-loading reflects an enhanced Ca2+-storage capacity of the membranes in the hyperthyroid state and is presumably due to larger intravesicular volume and/or an increased level of the Ca2+-storage protein, calsequestrin. Cardiac mRNA level of calsequestrin, however, was found not to be influenced by thyroid state in the rabbit (1).
channels ([3H]PN200-110 binding sites), which was also associated with augmented transsarcolemmal Ca2+ influx (19).

Our results also showed significantly higher rates of ATP-energized Ca2+ uptake by J SR vesicles from the hyperthyroid, compared with those from the euthyroid heart (Fig. 9, A and B). This observation is in conformity with previous studies demonstrating enhanced Ca2+-uptake activity of cardiac SR from hyperthyroid animals (25, 40) and cardiomyocytes cultured in the presence of thyroid hormone (20). This enhancement in SR Ca2+-sequestering activity likely results from the thyroid hormone-induced upregulation of SERCA2 and downregulation of phospholamban (20–22) and contributes to a faster rate of decline of Ca2+ (2, 29) and acceleration of diastolic relaxation (12, 28, 33) in the hyperthyroid heart.

In conclusion, this study demonstrates overexpression of RyR protein in the hyperthyroid rabbit heart. The relative abundance of RyR may be responsible, in part, for the changes in SR Ca2+ release, Ca2+ transient, and cardiac systolic function associated with thyroid hormone-induced cardiac hypertrophy.

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