Effects of Triiodothyronine on Retention of β -Adrenergic Responsiveness of Voltage-Gated Transmembrane Calcium Current during Culture of Ventricular Myocytes from Neonatal Rabbits

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ABSTRACT

To study the effects of triiodothyronine (T_3) on responsiveness of L-type calcium currents to β -adrenergic stimulation in neonatal hearts, ventricular myocytes were isolated from neonatal rabbits and cultured in medium containing 10% fetal bovine serum to which T_3 had been added to achieve either hypothyroid, euthyroid, or hyperthyroid conditions, as assessed by measurement of free T_3 concentrations. During a 24-h culture period, the striated rod-shaped myocardial cells progressively assumed a stellate shape with reduced surface area; however, the rate constants for diffusion of Na^+ from a microelectrode pipette into the cells remained unchanged. Voltage-dependent characteristics of L-type calcium currents as assessed by whole-cell voltage clamp studies were also unchanged after culture with various concentrations of free T_3 . By contrast, the stimulation of voltage-gated transmembrane calcium current from baseline by isoproterenol was reduced ($p < 0.05$) in hypothyroid cells ($15 \pm 8\%$; $n = 14$) compared with either euthyroid ($86 \pm 15\%$; $n = 18$), hyperthyroid ($54 \pm 16\%$; $n = 12$) or freshly isolated ($50 \pm 12\%$; $n = 10$)

myocytes. The differences in β -adrenergic responsiveness of voltage-gated transmembrane calcium current to isoproterenol between euthyroid, hyperthyroid, and freshly isolated cells were not significant ($p > 0.05$). These results indicate that retention of β -adrenergic responsiveness of voltage-gated transmembrane calcium current in neonatal cardiac myocytes depends on physiologic amounts of active thyroid hormone. Our culture method for neonatal cardiac myocytes will be useful for studying physiologic modulation of β -adrenergic responsiveness. (*Pediatr Res* 37: 277–282, 1995)

Abbreviations

G protein, guanine nucleotide-binding coupling protein
HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid
 I_{Ca} , voltage-gated transmembrane calcium current
 T_3 , triiodothyronine
 T_4 , thyroxine

Inadequate or excessive amounts of thyroid hormone affect both the peripheral vasculature and the ventricular myocardium *in vivo* (1). Direct effects of thyroid hormone on heart muscle include increasing the rate and force of contraction and decreasing diastolic relaxation time (2). These effects, achieved via nuclear actions of the active form of the thyroid hormone T_3 are mediated by T_3 binding to specific nuclear receptors, which function as transcription factors to alter transcription of T_3 -responsive genes (3). Treatment of euthyroid animals with supplemental T_3 alters cellular concentrations of mRNA-

encoding myosin isoforms (4), β -adrenergic receptors (5), Ca^{2+} -dependent ATPase (6), and G proteins (7).

In addition, several *in vivo* and *in vitro* studies have demonstrated alterations in membrane Ca^{2+} channel physiology induced by T_3 (8–11). Using whole-cell voltage clamp studies of freshly isolated ventricular myocytes from hypothyroid, euthyroid, and hyperthyroid adult guinea pigs, Binah *et al.* (10) demonstrated increased inward calcium currents, increased outward potassium currents, and shortened action potential after *in vivo* administration of thyroid hormone. Treatment of cultured chick ventricular myocytes with T_3 increased L-type Ca^{2+} channel density, increased transmembrane calcium flux, and increased intracellular Ca^{2+} concentration during subsequent treatment with isoproterenol (11). Those studies suggest that the direct effects of T_3 on the myocardium result, at least in part, from changes in calcium channel density and β -adrenergic responsiveness.

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Although diminished β -adrenergic responsiveness is a pathologic marker of heart failure, cause-and-effect relations between heart failure and decreased β -adrenergic responsiveness have not been established. To address this question, we sought to develop an *in vitro* model for investigating the effects of thyroid hormone on retention of β -adrenergic responsiveness of I_{Ca} in cultured cardiomyocytes from euthyroid neonatal rabbits.

METHODS

Isolation and culture of cardiomyocytes. Ventricular myocytes were isolated as previously described (12) with some modifications. Newborn rabbits (New Zealand White, 2–3 d old) were administered intraperitoneal heparin (1000 U) and pentobarbital (50 mg) before rapid excision of the heart. Hearts were rinsed in Tyrode's solution (136 mM NaCl, 5.4 mM KCl, 0.33 mM NaH_2PO_4 , 1 mM MgCl_2 , 10 mM Na-HEPES, pH 7.3, 4 mM mannitol, 0.6 mM thiamine, 10 mM glucose, and 2 mM pyruvic acid) containing 3.5 mM CaCl_2 and mounted on a sterile Langendorf perfusion apparatus. All perfusion solutions were sterilized by filtering through a 2- μm filter, and then oxygenated (100% O_2) and maintained at 37°C before use. The coronary arteries were perfused via the aorta with calcium-free Tyrode's solution at 37°C using a peristaltic pump (2.5 mL/min) for 3 min, followed by perfusion at the same rate with Tyrode's solution containing collagenase (1 mg/mL, type 1, Sigma Chemical Co., St. Louis, MO) and protease (0.076 mg/mL, type XIV, Sigma) until the heart was flaccid (7–12 min). The enzyme-containing solution was then flushed from the heart for 3 min with Tyrode's solution containing 0.1 mM CaCl_2 . The atria and connective tissue were removed, and the ventricles were teased apart with forceps. Individual ventricular myocytes were dispersed by gentle shaking in Tyrode's solution containing 0.1 mM CaCl_2 and filtered through sterile nylon mesh (~500 μm pore size; Fisher Scientific, Pittsburgh, PA) to remove residual connective tissue. The myocytes were then separated from smaller connective tissue cells and debris by sedimentation ($1 \times g$ twice for 90 min). After the second sedimentation, the cell pellet was resuspended in culture medium [minimum essential media with Earle's salts and 25 mM Na-HEPES, pH 7.4, 2 mM glutamine, 500 U/mL penicillin G, 500 μg /mL streptomycin, and 10% (vol/vol) fetal bovine serum (Sigma)]. The cells were plated in 75- cm^2 flasks for 4–6 h at 37°C to allow attachment of fibroblasts to the plastic flasks. The nonadherent ventricular myocytes were then transferred to 25- cm^2 tissue culture flasks containing fragments of glass coverslips that had been pretreated for 4–6 h with culture media containing 10% fetal bovine serum to promote attachment of myocytes. Cells were plated at a density of $2\text{--}3 \times 10^6$ cells per 25- cm^2 flask with medium, to which appropriate concentrations of T_3 (Sigma) had been added. The cells were maintained at 37°C in a humidified 95% air-5% CO_2 atmosphere.

Free T_3 assay. Culture medium was collected from each culture flask at the time the cultured cells were used for voltage clamp studies and stored at -20°C until the free T_3 assay was performed. Free T_3 was measured in each sample using a

competitive binding assay (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA). The interassay coefficient of variation for this assay was approximately 5%, and limits of detection were 0.3 and 155 pM.

Current measurements. Transmembrane currents were measured using the whole-cell voltage clamp technique, similar to that described by Hamill *et al.* (13). All voltage clamp studies of cultured cells were performed between 22 and 28 h after plating of the cells into culture flasks. Cells chosen for whole-cell voltage clamp studies were adherent to the glass coverslip, quiescent, and lacked apparent contact with adjacent cells. To perform voltage clamp studies, a glass coverslip with adherent myocytes was removed from the culture flask, placed in a 0.5 mL well on the stage of an inverted microscope (Zeiss, Thornwood, NY), and rinsed with Tyrode's solution containing 0.1 mM CaCl_2 . Transmembrane currents were measured during voltage clamp steps using an EPC-7 patch-clamp amplifier (Adams/List, Great Neck, NY). Currents were filtered at 1 kHz using an eight-pole Bessel filter (Frequency Devices, Haverhill, MA), digitized using the Axolab 1100 data-acquisition system, and analyzed using pCLAMP software (Axon Instruments Inc., Foster City, CA).

Capacitance measurements were made as previously described by Hunyh *et al.* (12) immediately after establishing a whole-cell seal.

Cellular dialysis. The rate of dialysis of the cell with internal pipette solution was determined using the method described by Wetzel *et al.* (14). After the formation of a stable seal, 64 sequential voltage clamps from -120 to 0 mV were performed at 4-s intervals. Because the internal solution (60 mM NaCl, 46 mM CsCl, 20 mM Cs-HEPES, pH 7.1, 1 mM MgCl_2 , 1 mM CaCl_2 , 14 mM EGTA, and 5.5 mM Mg-ATP) and external solution (60 mM NaCl, 75 mM CsCl, 10 mM Cs-HEPES, pH 7.3, 1 mM MgCl_2 , 1 mM CaCl_2 , 0.1 mM CdCl_2 , and 10 mM glucose) contained equal concentrations of Na^+ , the transmembrane Na^+ current at 0 mV decreased to zero as Na^+ ions diffused from the microelectrode into the cell to progressively reduce the transmembrane concentration gradient of Na^+ . Transmembrane currents carried by potassium and calcium ions were blocked by cesium and cadmium ions, respectively. The time constant for diffusion of Na^+ into the cell was determined using nonlinear least-squares regression (BMDP software, University of California Los Angeles).

Measurement of transmembrane calcium currents. For these experiments, the internal solution contained 96 mM CsCl, 20 mM Cs-HEPES, pH 7.1, 14 mM EGTA, 1 mM CaCl_2 , 6 mM MgCl_2 , 10 mM tetraethylammonium-Cl, and 5 mM ATP, and the external solution contained 135 mM CsCl, 1.8 mM Cs-HEPES, pH 7.3, 1.8 mM CaCl_2 , 1 mM MgCl_2 , 10 mM glucose, and 0.01 mM tetrodotoxin. Cesium ions and tetrodotoxin were included to block currents carried by potassium and sodium ions, respectively. The relation between peak I_{Ca} and depolarization potential was determined by performing voltage steps from an initial holding potential of -80 mV to a prepulse potential of -40 mV for 50 ms followed by a 100-ms voltage step to test potentials between -30 and 40 mV in 10-mV increments at 5-s intervals. Steady state activation and inactivation curves for I_{Ca} were constructed as described by Wetzel

et al. (15). Steady state activation and inactivation curves were fitted to the Boltzmann equation using nonlinear least-squares regression.

The response of I_{Ca} to isoproterenol was determined by performing a series of 18 voltage clamps from a holding potential of -80 mV to a prepulse potential of -40 mV for 50 ms, followed by a 100-ms voltage step to 0 mV at 20-s intervals. Transmembrane calcium currents were recorded during each voltage step. After the second voltage clamp, $10 \mu\text{M}$ isoproterenol was added. Basal transmembrane calcium current was the average of the peak inward current measured during the two voltage clamp steps preceding addition of isoproterenol. The maximal isoproterenol-stimulated I_{Ca} was the largest peak transmembrane current during the 16 voltage steps performed after the solution change. Peak I_{Ca} achieved a new steady state within 5 min after addition of isoproterenol. The response to isoproterenol was expressed as the percent increase in maximal peak I_{Ca} with isoproterenol compared with maximal peak I_{Ca} before addition of isoproterenol. All electrophysiologic studies were performed at 23°C .

Statistical analyses. Results are expressed as mean $\pm$ SEM (number of measurements), unless indicated otherwise. Statistical significance was determined using the Student-Newman-Keuls multiple range test. Differences were considered significant at $p < 0.05$. For comparison of the percent increase in I_{Ca} after addition of isoproterenol, a logarithmic transformation of the data was performed before applying the Student-Newman-Keuls multiple-range test.

RESULTS

Free T₃ concentrations during myocyte culture. The mean free T₃ concentration in plasma obtained from neonatal rabbits (1–3 d old) was 2.0 ± 0.2 pM ($n = 13$). Free T₃ concentration in culture media containing 10% fetal bovine serum to which no T₃ had been added was 0.77 ± 0.28 pM ($n = 12$). This culture medium was thus considered to provide hypothyroid culture conditions. With addition of 10 pmol/L T₃ to this hypothyroid culture medium, the free T₃ concentration increased to 3.5 ± 1.2 pM ($n = 6$), and this culture medium was considered to provide euthyroid conditions during cell culture. Finally, addition of 10 nmol/L T₃ yielded a free T₃ concentration beyond the range of the assay (>154 pM; $n = 12$). Because of the dynamic state of T₃ binding to serum proteins, better quantification of free T₃ concentration in the media to which 10 nM T₃ had been added was not possible. This medium was thought to provide hyperthyroid culture conditions.

Cellular morphology. Freshly isolated ventricular cells displayed a striated rod shape and had an eccentrically placed nucleus under phase-contrast microscopy ($\times 450$). Approximately 4 h after initiation of culture, a progressive loss of striations and loss of rod shape commenced, and by 12 h of culture, the cells were spherical in shape with no visible striations. After 24 h of culture, the cells had developed pseudopod-like attachments to the plate and had assumed a stellate shape; no striated rod-shaped cells remained, and most

Figure 1. Photomicrographs of cardiomyocytes. Ventricular myocytes from neonatal rabbits immediately after isolation (A), and after culture in euthyroid medium for 4 h (B) 12 h (C) and 24 h (D) as described in "Methods."

cells were attached to the plastic flasks or glass coverslips (Fig. 1).

Cellular surface area. As the capacitance of biologic membranes is relatively constant ($1 \mu\text{F}/\text{cm}^2$) (16), capacitance was used as a measure of cellular surface area. Mean capacitance of the freshly isolated myocytes was 18.0 ± 1.2 pF ($n = 14$) and was 14.0 ± 2.4 pF, 10.7 ± 1.4 pF, and 10.8 ± 1.9 pF for hypothyroid ($n = 23$), euthyroid ($n = 14$), and hyperthyroid ($n = 14$) cells, respectively. The cells cultured under euthyroid and hyperthyroid conditions were thus slightly smaller than the freshly isolated cells ($p < 0.01$), but cultured cells did not differ in size regardless of the free T₃ concentration present in the culture medium.

Whole-cell voltage clamp characteristics of myocytes. To determine whether the altered cell morphology that developed during short-term culture was associated with changes in the electrophysiologic characteristics of the ventricular myocytes, we measured rate constants for dialysis of the cell with internal pipette solution. The rate of loss of the transmembrane Na⁺ gradient during diffusion of the Na⁺ ion from the pipette solution was determined for freshly isolated and cultured myocytes. Both cultured cells and freshly isolated cells demonstrated exponential decay curves. The time constants for loss of the transmembrane potential were not different among cells cultured in euthyroid (30.7 ± 7 s; $n = 7$), hypothyroid (35.6 ± 5 s; $n = 12$), or hyperthyroid (33.0 ± 3.6 s; $n = 5$) conditions and freshly isolated cells (37.3 ± 6.0 s; $n = 7$). In all groups, loss of the transmembrane Na⁺ gradient was 80% complete within 4 min. These results indicate that changes in the cell morphology that occur in these cells do not drastically affect the ability to control the intracellular ionic environment of ventricular myocytes using the whole-cell voltage clamp technique.

Transmembrane calcium currents. The relation between maximal I_{Ca} and transmembrane voltage in cultured myocytes was similar to that for freshly isolated cells (Fig. 2). Maximal I_{Ca} in all cells occurred at transmembrane potentials between -10 and 0 mV. Steady state activation and inactivation curves for I_{Ca} (Fig. 3) displayed no significant difference between cell

Figure 2. I_{Ca} recordings from ventricular myocytes. Representative I_{Ca} standardized to cell surface area for a freshly isolated myocyte (A) and myocytes cultured for 24 h with euthyroid conditions (B), hypothyroid conditions (C), and hyperthyroid conditions (D). In each panel I_{Ca} was measured during a 400-ms depolarization to 0 mV before (I) and during (II) exposure of the cell to 10 μ M (-)-isoproterenol.

Figure 3. Plot of peak I_{Ca} vs. applied transmembrane voltage in ventricular myocytes. Panels indicate results for freshly isolated myocytes (A, $n = 18$) and myocytes cultured for 24 h with euthyroid conditions (B, $n = 18$), with hyperthyroid conditions (C, $n = 12$), and with hypothyroid conditions (D, $n = 10$).

groups. Specifically, the membrane potential producing half-maximal activation of I_{Ca} , half-maximal inactivation of I_{Ca} , and the dependence of I_{Ca} activation on transmembrane voltage, as determined from the fitted Boltzmann equation, were similar for all the experimental groups (Table 1 and Fig. 4). The dependence of I_{Ca} inactivation on transmembrane voltage was slightly higher in freshly isolated cells (-7.2 ± 0.3 mV)

Table 1. Calculated kinetic constants for steady state activation and inactivation of I_{Ca}

Treatment	K_A	V_{hA} (mV)	K_I	V_{hI} (mV)
Freshly isolated cells ($n = 7$)	5.8 ± 0.3	-23 ± 3	$-7.2 \pm 0.3^*$	-40 ± 2
Euthyroid culture ($n = 10$)	6.0 ± 0.4	-17 ± 1	-5.7 ± 0.4	-34 ± 2
Hypothyroid culture ($n = 14$)	6.5 ± 0.2	-20 ± 1	-5.1 ± 0.1	-36 ± 1
Hyperthyroid culture ($n = 14$)	6.0 ± 0.4	-19 ± 2	-5.4 ± 0.2	-33 ± 2

Values for K_A , K_I , V_{hA} , and V_{hI} were determined by fitting the data shown in Figure 4 to the Boltzmann equation ($F_x = [1 + e^{(V-V_h)/K}]^{-1}$). K_A and K_I are exponential coefficients from the Boltzmann equation for activation and inactivation constants, respectively. V_{hA} and V_{hI} are transmembrane potentials at half-maximal activation and half-maximal inactivation, respectively.

* Significant difference ($p < 0.05$) compared with other experimental groups.

Figure 4. Plots of percent maximal I_{Ca} vs. transmembrane voltage. Calculations were performed as described by Wetzel *et al.* (15). Filled circles indicate the proportion of calcium channels that are closed after depolarization from each transmembrane voltage to 0 mV. Open circles indicate the proportion of calcium channels that are open after depolarization from -80 mV to each transmembrane voltage. Data were obtained from freshly isolated cells (A, $n = 7$) and from myocytes cultured for 24 h with euthyroid conditions (B, $n = 10$), hyperthyroid conditions (C, $n = 14$), and hypothyroid conditions (D, $n = 14$).

compared with cultured cell groups (-5.1 ± 0.1 , -5.4 ± 0.2 , and -5.7 ± 0.4 mV for cells cultured under hypothyroid, hyperthyroid, and euthyroid conditions, respectively). These results demonstrate that kinetics for the transmembrane calcium current are not significantly altered in ventricular myocytes during culture and are unaffected by alterations in free T_3 concentrations.

Response to isoproterenol. The response of I_{Ca} to isoproterenol was significantly greater in freshly isolated cells ($50 \pm 12\%$; $n = 10$) and in cells cultured under euthyroid ($86 \pm 15\%$;

$n = 18$), or hyperthyroid ($54 \pm 16\%$; $n = 12$) conditions than in cells cultured under hypothyroid conditions ($15 \pm 8\%$; $n = 14$). Differences in the degree of stimulation of I_{Ca} by isoproterenol between freshly isolated myocytes and cells cultured under either euthyroid or hyperthyroid conditions were not significant. These results indicate that maintenance of the responsiveness of I_{Ca} in cultured ventricular myocytes to β -adrenergic stimulation requires physiologic concentrations of T₃.

DISCUSSION

We have developed a method for *in vitro* culture of ventricular cardiomyocytes from neonatal rabbits and demonstrated that physiologic concentrations of free T₃ maintain responsiveness of myocardial I_{Ca} to β -adrenergic stimulation during short-term culture. Neonatal ventricular myocytes cultured in hypothyroid conditions have a diminished response to β -adrenergic stimulation. These data suggest that providing physiologic concentrations of free T₃ during short-term culture is important for preserving normal calcium channel sensitivity to β -adrenergic agonists.

T₃ concentrations during cell culture. T₄, the primary circulating thyroid hormone in mammals, is the prohormone for T₃, which has four to 10 times greater affinity for thyroid hormone receptors and is the most physiologically important thyroid hormone (17). The conversion of T₄ to T₃ is catalyzed by the enzyme iodothyronine 5'-deiodinase, an enzyme that occurs in most tissues. However, the heart uses T₃ that is derived largely from the circulation rather than producing T₃ itself (17). Therefore, to circumvent any problems related to limited conversion of the prohormone T₄ to T₃ by cardiomyocytes, we used T₃ in our studies. As greater than 99% of T₃ in the circulation is bound to plasma-binding proteins and biologically unavailable (17), only free unbound concentrations of T₃ were assessed in our studies. The free concentration of T₃ (0.77 ± 0.28 pM) in minimum essential media with added 10% fetal bovine serum was significantly lower than the free T₃ concentration measured in plasma from neonatal rabbits (2.0 ± 0.2 pM), as well as lower than the concentrations reported for human infants at birth (2.0 – 3.5 pM) (18, 19) or at 2–8 d of age (5.7 – 7.1 pM) (18–20). Thus culture medium unsupplemented with T₃ provides a hypothyroid environment for *in vitro* culture of the ventricular myocytes. By contrast, the free T₃ concentration present in our euthyroid medium is slightly greater than the concentration of T₃ measured in the plasma of neonatal rabbits, which may have been artificially lowered by the presence of heparin (data on the effects of heparin on free T₃ assay provided by Diagnostic Products Corp.).

The effects of added T₃ on response of I_{Ca} to isoproterenol. In contrast to the findings in several previous studies (10, 11), we did not find an effect of supraphysiologic T₃ levels on L-type calcium current density (Table 2) or on the response of I_{Ca} to β -adrenergic stimulation. Kim *et al.* (11) concluded that 10 nM T₃ added to serum-free culture medium increases L-type calcium channel number and activity in spontaneously contracting monolayers of chick ventricular myocytes as assessed by dihydropyridine binding and nifedipine-sensitive ⁴⁵Ca²⁺ uptake, but they did not compare the cultured cells with

Table 2. I_{Ca} in cardiomyocytes before and after treatment with isoproterenol

Treatment	Peak I_{Ca} before isoproterenol (pA/pF)	Peak I_{Ca} with isoproterenol (pA/pF)	Increase in I_{Ca} with isoproterenol (%)
Freshly isolated cells ($n = 10$)	-6.1 ± 1.3	-8.2 ± 1.2	50 ± 12
Euthyroid culture ($n = 18$)	-4.4 ± 0.6	-6.9 ± 0.7	86 ± 15
Hypothyroid culture ($n = 14$)	-6.5 ± 0.9	-7.2 ± 0.9	$15 \pm 8^*$
Hyperthyroid culture ($n = 12$)	-5.9 ± 0.7	-8.7 ± 1.2	54 ± 16

Peak I_{Ca} was measured before and during exposure of cells to 10 μ M (–)-isoproterenol, and the percent increase of I_{Ca} was calculated for each cell.

* Significant difference ($p < 0.05$) compared with other experimental groups.

freshly isolated cells. By contrast, we measured calcium channel activity directly, without other sources of calcium uptake, *e.g.* sodium-calcium exchange, before and after culture. By using quiescent myocytes, we eliminated the effects of T₃ on the strength and rate of contraction, which may secondarily affect calcium uptake. In addition, comparison of contracting and noncontracting ventricular myocytes has revealed differences in cellular morphology (21), levels of the nonstimulatory, noninhibitory guanine nucleotide binding protein designated G_o α (22), and synthetic rates of proteins (23). Some differences may thus occur between noncontracting and contracting ventricular myocytes during treatment with T₃. Alternatively, endothelial cells present during culture may secrete growth factors that alter gene expression in the cultured cardiac myocytes.

The mechanism by which responsiveness of I_{Ca} to β -adrenergic stimulation is preserved under euthyroid culture conditions may be related to maintenance of normal expression of one or more components of the cellular signaling cascade that couples cardiac β -adrenergic receptors to L-type calcium channels. The major components of this signaling pathway are the β -adrenergic receptors, G protein that stimulates adenylyl cyclase, adenylyl cyclase, and protein kinase A. In addition, changes in other cellular components may indirectly affect this signaling pathway, *e.g.* muscarinic receptors and G proteins that inhibit adenylyl cyclase. Cardiac levels of a number of these proteins are affected by thyroid hormone status. Specifically, expression of β -adrenergic receptor is increased by thyroid hormone (24), and the promoter region of the human gene encoding the β_1 -adrenergic receptor contains a consensus thyroid hormone response element and possibly a second thyroid hormone response element (25). Conversely, added thyroid hormone seems to increase the degradation of the cardiac muscarinic receptor in neonatal rat cardiomyocytes (26). Although the expression of the α -subunit of G protein that stimulates adenylyl cyclase in heart does not seem to be modulated by thyroid hormone, cardiac levels of G proteins that inhibit adenylyl cyclase are modulated by thyroid hormone treatment (7). Finally, altered cellular levels of β -subunits of G proteins can modify β -adrenergic regulation of I_{Ca} in cardiac myocytes from neonatal rabbits (Chen F, Van Dop C, Wetzel

GT, Lee RH, Friedman WF, Klitzner TS, unpublished observations). In addition, activity of L-type calcium channels in the chick heart is increased by thyroid hormone (11). Thyroid hormone thus modulates expression of numerous proteins involved in mediating cellular responsiveness to β -adrenergic stimulation. Our finding of the requirement for euthyroid conditions during culture of neonatal cardiomyocytes to maintain cellular responsiveness of I_{Ca} to β -adrenergic agonists fits well with these data. Based on these published data, we speculate that thyroid hormone maintains β -adrenergic responsiveness in rabbit ventricular myocytes during primary culture by maintaining the expression of several proteins involved in modulating the β -adrenergic signaling pathway.

In summary, our results demonstrate that neonatal ventricular myocytes retain basic electrophysiologic properties in short-term culture as assessed by the whole-cell voltage clamp technique. In addition, β -adrenergic responsiveness of transmembrane L-type calcium channels is preserved in the presence physiologic levels of T_3 . We propose that this *in vitro* model of the neonatal myocardium is appropriate for evaluating the β -adrenergic signaling cascade in a controlled environment amenable to experimental manipulation.

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