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Importance of ryanodine receptors in effects of cyclic GMP is reduced in thyroxine-induced cardiac hypertrophy

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Abstract

We tested the hypothesis that the negative functional effects of cyclic GMP were mediated by ryanodine receptors, and that these effects would be reduced in thyroxine (thyroxine, 0.5 mg/kg/day, 16 days)-induced hypertrophic myocytes. Using rabbit ventricular myocytes from control ($n=9$) and thyroxine ($n=9$) hearts, percent cell shortening (%) and maximum rate of contraction and relaxation were determined using a video edge detector at baseline and after 10^{-6}, 10^{-5} M 8-bromo-cyclic GMP. Dantrolene 10^{-6} M, ryanodine receptor inhibitor, was added alone or after 8-Br-cGMP treatment. Changes in cytosolic Ca^{2+} concentration were assessed in fura-2-loaded control and thyroxine myocytes. 8-Br-cGMP caused a significant decrease in percent shortening, from 5.3±0.9% to 3.9±0.6% at 10^{-5} M in control, and 3.4±0.3% to 2.6±0.4% in thyroxine myocytes. Dantrolene significantly decreased percent shortening from 4.5±0.8% to 3.7±0.1% in control and from 3.7±0.1% to 2.8±0.3% in thyroxine myocytes. In 8-Br-cGMP treated control myocytes, dantrolene did not significantly change myocyte contractility, which suggested that cyclic GMP acted on ryanodine receptors. However, in 8-Br-cGMP treated thyroxine myocytes, dantrolene further reduced myocyte contractility implying that the interaction of cyclic GMP and ryanodine receptors appeared to be interrupted in thyroxine myocytes. Maximum rate of contraction data were consistent with the percent cell shortening data and Ca^{2+} transients changed similarly to myocyte contractility. We conclude that effects of cyclic GMP on myocytes contractility were partially mediated though interaction with ryanodine receptors and the subsequent decrease in cytosolic calcium levels. This interaction was reduced in thyroxine hypertrophic myocytes.
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Keywords: Thyrotoxicosis; Cardiac myocyte shortening; Ca^{2+} transients; Ryanodine receptor; Cyclic GMP

1. Introduction

The second messenger cyclic GMP, a signaling molecule common to nitric oxide and natriuretic peptides, plays an important role in myocardial function. It has been shown that cyclic GMP can reduce myocardial metabolism, inotropy and function (Kuhn, 2004; Kempf and Wollert, 2004). The negative functional effects of cyclic GMP are mainly mediated through the cyclic GMP-dependent protein kinase and this can reduce intracellular Ca^{2+} by activation of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) (Lau et al., 1998; Zhang et al., 2002). It is not clear whether cyclic GMP also affects calcium release or sensitivity in its negative functional role in cardiac myocytes. The cyclic GMP signaling pathway may also be mediated by cyclic GMP protein kinase-independent interaction with other molecules in the cell, such as cyclic GMP-gated cation channels and certain phosphodiesterases (Shah and Maccarthy, 2000; Vila-Petroff et al., 1999).

Ryanodine receptors are intracellular calcium release channels that control the levels of intracellular Ca^{2+} by releasing Ca^{2+} from intracellular calcium-stores in the sarcoplasmic reticulum (Bers, 2004). It was reported that Ca^{2+} release in muscle homogenates was reduced by nitric oxide (Meissner,

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2004). Increasing the intracellular cyclic GMP level with nitric oxide may alter ryanodine receptor activity (Eu et al., 1999). Cyclic GMP-mediated signaling in regulation of ryanodine receptors may involve phosphorylation of ryanodine receptors by cyclic GMP-dependent protein kinase or other factors (Suko et al., 1993; Willmott et al., 2000). The effect of cyclic GMP on ryanodine receptors in cardiac myocytes has not been extensively elucidated.

Hyperthyroidism causes many direct and indirect changes in myocardial function (Fadel et al., 2000). Thyroxine activates a number of signaling pathways and can produce cardiac hypertrophy and dysfunction. Cardiac hypertrophy results from a combination of direct effects of thyroxine on the heart, such as increased protein synthesis, decreased duration of cardiac action potential and functional refractory period, and indirect hemodynamic changes (Polikar et al., 1993; Hu et al., 2003). An increased number of β-adrenoceptors, alterations in muscarinic responses, and cyclic GMP-mediated changes in cyclic GMP signaling have been reported after T4 (Hu et al., 2003; Weiss et al., 1995; Engel et al., 2001). Overexpression of functional ryanodine receptors was also reported in thyroxine-induced cardiac hypertrophy in rabbit myocardium. This relative abundance of ryanodine receptors is considered to be responsible, in part, for the changes in sarcoplasmic reticulum Ca^{2+} release, cytosolic Ca^{2+} transients, and cardiac systolic function associated with thyroid hormone-induced cardiac hypertrophy (Jiang et al., 2000; Wu et al., 2002).

Previous studies from our laboratory have demonstrated altered responses to cyclic GMP in cardiac myocytes from hypertrophied rabbit hearts (Yan et al., 2003). Cyclic GMP protein kinase activity is also reduced in thyroxine-induced hypertrophic cardiac myocytes (Engel et al., 2001; Yan et al., 2003). We examined the role of ryanodine receptors in cyclic GMP-mediated signaling and how cardiac hypertrophy altered this response. We tested the hypothesis that the negative functional effects of cyclic GMP were partially mediated by ryanodine receptors and that these effects would be reduced in thyroxine-induced hypertrophic myocytes. We examine this issue in control and thyrotoxic New Zealand white rabbit myocytes by using dantrolene, an inhibitor of intracellular Ca^{2+} release through ryanodine receptor channels in sarcoplasmic reticulum, before and after cyclic GMP administration.

2. Methods and materials

The investigation was conducted in accordance with the Guide for the Care of Laboratory Animals (DHHS Publication 85-23, revised 1996) and was approved by the Institutional Animal Care and Use Committee.

2.1. Hypertrophic heart model

New Zealand white rabbits ($n=9$) were injected with 0.5 mg/kg/day of thyroxine subcutaneously for 16 days. These animals were weighed on days 1, 8, and 16. The animals in the control group ($n=9$) received no injections, but were weighed prior to the experiment.

2.2. Ventricular myocyte dissociation

Ventricular myocytes were isolated from hearts of New Zealand white rabbits ($n=18$, 1.5–2.5 kg) as previously described (Zhang et al., 2002; Yan et al., 2003). Briefly, the rabbits were anesthetized with sodium pentobarbital (35 mg/kg) followed by the administration of heparin (10 unit/g body weight) intravenously using the circumflex ear vein. The heart was immediately removed after an overdose of pentobarbital (60 mg/kg) and retrograde perfused through the aorta with minimal essential medium (MEM, Sigma) supplemented with 10 mM taurine, 2 mM L-glutamic acid and 20 mM HEPES, pH 7.2. After 5 min of perfusion with MEM, the heart was perfused with MEM containing 0.1% type II collagenase (Worthington) for 16 min. All perfusion media were maintained at 37 °C and equilibrated with water-saturated oxygen.

After collagenase perfusion, the heart was removed from the perfusion apparatus and the ventricle was cut into 8–10 pieces. The tissue suspension was further treated with MEM containing 0.1% collagenase and 0.5% bovine serum albumin (BSA), fraction V (Sigma) at 37 °C and gently swirled at 2 cycles/s for 5 min. A slurry containing isolated myocytes was decanted from tissue suspension. The isolated cells were washed three times in MEM containing 0.5% BSA and centrifuged at low speed (34 *g*) to completely remove the collagenase and subcellular debris. Incubation of the remaining tissue with collagenase was repeated at least two more times. Myocyte viability was assessed by maintenance of a rod-shaped morphology and was between 50–70%. Yields were typically $10–14\times10^6$ rod-shaped cells/heart.

2.3. Myocyte functional measurements

Individual ventricular myocytes were studied for contractile function. Cells were suspended in 2 ml of 2 mM Ca^{2+} MEM solution containing 0.5% BSA maintained at 37 °C in a chamber that was fitted onto the stage of an inverted light microscope (Nikon Eclipse TS100). Two platinum wires were inserted into two parallel sides of the chamber and were used to pace the myocytes by electric field stimulation (1 Hz, 5 ms duration, voltage 10% above threshold, and polarity altered with each pulse). Unloaded shortening of selected cardiac myocytes was measured on-line by using a video edge-detector system and fluorescence system (IonOptix, Milton, MA), which detected the change of position of both edges of the cell. Data were collected continuously. The output of the video edge detector was fed into a television monitor and a desktop computer, which then analyzed the data. Cells used to determine the functional parameters were healthy and could react to different reagents throughout the course of the experiment. Cell contraction measurements were obtained on random cells in each preparation and each cell was required to complete its protocol. Untreated cells

continued to contract at a constant level over the time course of the experiment.

2.4. Intracellular [Ca^{2+}] measurements

For the measurement of intracellular Ca^{2+} concentration, the fluorescent Ca^{2+} indicator, fura-2, was used. Ventricular myocytes were incubated with 2 μM fura-2 acetoxymethyl ester (fura-2 AM, Molecular Probes, Eugene, OR) in MEM solution containing 0.5% BSA and 1 mM $CaCl_2$ at room temperature for 1 h. Unincorporated fura-2 AM was removed by washing myocytes twice in fura-2 AM-free MEM solution.

The fura-loaded cells were suspended in 2 ml of 2 mM Ca^{2+} MEM solution containing 0.5% BSA maintained at 37 °C in a chamber that was fitted onto the stage of an inverted microscope. Ventricular myocytes were paced by electric field stimulation at 1 Hz, 5 ms duration. A dual excitation spectrofluorometer was used to record fluorescence emissions (480–520 nm) elicited from exciting wavelengths at 360 and 380 nm and measurements recorded with the IonOptix Fluorescence System (IonOptix, Milton, MA). Qualitative changes in $[Ca^{2+}]_i$ were determined from the ratio of the fluorescence intensity at 360/380 nm wavelengths.

2.5. Experimental protocol

Ventricular myocytes were used in the following protocol for functional measurements. In all groups, myocytes in appropriate concentration were suspended in a chamber with 2 ml of MEM containing 2 mM Ca^{2+} and 0.5% BSA. After a 10-min stabilization period paced with electrical field stimulation, baseline contraction data for an individual myocyte were recorded. At 5-min intervals, reagents were added to the medium and allowed to diffuse to the cell during which time cell contractility was measured. Dantrolene, a specific inhibitor for ryanodine receptors, was added at 10^{-6} M (dose selected after preliminary studies). In a separate experiment, 8-bromo-cGMP (10^{-6} and 10^{-5} M) was given to myocytes prior to Dantrolene treatment. A minimum of 10 consecutive contractions was used for each data point. For each protocol at least 3 cells in each animal were repeatedly measured. Functional measurements obtained include resting cell length, absolute cell shortening, maximal rate of shortening, maximal rate of cell relaxation, and calculated percentage of cell shortening. For Ca^{2+} measurement, each experimental protocol was performed in at least three myocytes from each animal and three animals were used for each group.

2.6. Statistics

Results are expressed as means ± S.E.M. A repeated measure analysis of variance (ANOVA) was used to compare variables measured under the experimental and control conditions. Duncan's post hoc procedure was used to compare differences between baseline and the various treatments. A Student *T*-test was used to compare heart to body weight ratios and cell length between control and thyroxine animals and to compare the intracellular calcium transients. In all cases, a value of $P<0.05$ was accepted as significant.

3. Results

In the control rabbits, the heart weight-to-body weight ratio was 2.7±0.4 g/kg ($n=9$) and cell length was 114±8 μm. In thyroxine-induced cardiac hypertrophic rabbit, the heart weight-to-body weight ratio was 3.6±0.3 g/kg and cell length was 131±4.5 μm ($n=9$), which were statistically higher than that of control rabbits. There were no statistically significant differences in functional parameters under baseline conditions between control and thyroxine myocytes including percent shortening, maximum rates of shortening and relaxation and timing parameters.

3.1. Dantrolene decreased cell contractility in both control and hypertrophic myocytes

In both groups, dantrolene at 10^{-6} M significantly decreased percent shortening from 4.5±0.8% to 3.0±0.5% in control myocytes and 3.7±0.1% to 2.8±0.3% in thyroxine-induced hypertrophic myocytes (Fig. 1). Maximum rate of shortening (R_{max}) was also decreased in dantrolene treated myocytes, from 66.4±8.3 to 48.8±9.2 μm/s in control and 89.9±11.5 to 70.6±17.7 μm/s in hypertrophic cells (Fig. 1).

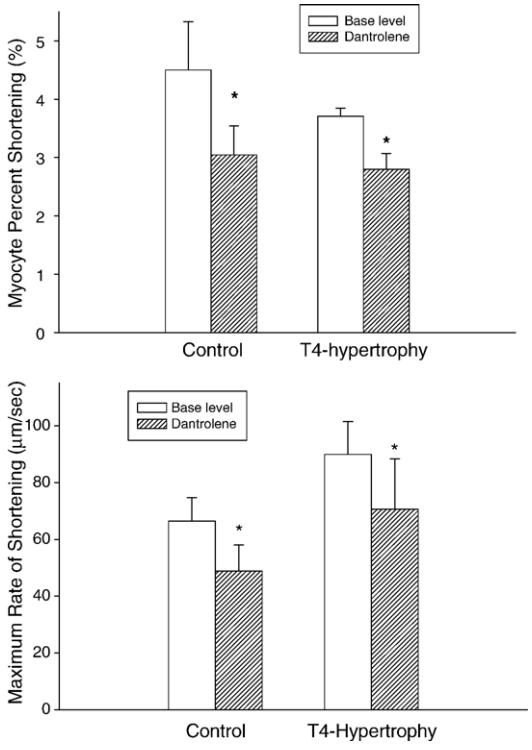

Fig. 1. Data from control and thyroxine hypertrophic ventricular myocytes for percentage shortening before and after cells were treated with dantrolene 10^{-6} M (top). Similar data from control and thyroxine hypertrophic ventricular myocytes for maximum rate of shortening before and after cells were treated with dantrolene 10^{-6} M (bottom). [a]Significantly different from baseline.

Table 1
Effects of 8-Br-cGMP (10^{-5} M) and dantrolene (10^{-6} M) on maximum rate of relaxation (dF, μm/s), time to peak contraction and time to 50% relaxation (50% *R*) in control and thyroxine-induced hypertrophic myocytes

	dF (μm/s)		Time to peak (s)		50% *R* (s)	
	Control	Hypertrophy	Control	Hypertrophy	Control	Hypertrophy
Base level	69.5±5.6	60.6±6.4	0.248±0.025	0.240±0.030	0.327±0.044	0.276±0.007
Dantrolene	57.6±10.9	49.22±8.4	0.319±0.06	0.308±0.089	0.336±0.006	0.286±0.001
Base level	82.2±17.5	73.7±10.5	0.291±0.052	0.226±0.010	0.367±0.062	0.281±0.015
cGMP	61.3±14.1	53.6±9.1	0.272±0.061	0.249±0.015	0.344±0.073	0.308±0.018
Dantrolene	51.6±10.4	37.9±7.8 [a, b]	0.285±0.062	0.248±0.020	0.366±0.080	0.324±0.032

[a] Significantly different from base level.
[b] Significantly different from cGMP.

Maximum rate or relaxation did not change significantly with dantrolene, Table 1.

3.2. Cyclic GMP decreased cell contractility in both control and hypertrophic myocytes

In both groups, 8-Br-cGMP caused a significant decrease in myocyte contractility with percent shortening decreasing from 5.3±0.9% at baseline to 3.9±0.6% in the presence of 10^{-5} M 8-Br-cGMP in control, and 3.4±0.3% to 2.8±0.4% in hypertrophic myocytes (Fig. 2). Maximum rate of shortening decreased from 78.0±11.9 at baseline to 56.2±10.5 μm/s in presence of 10^{-5} M 8-Br-cGMP in control, and 94.7±7.5 to 69.3±11.2 μm/s in hypertrophic myocytes (Fig. 2). Neither 8-Br-cGMP nor dantrolene changed maximum rate of relaxation of control myocytes (Table 1).

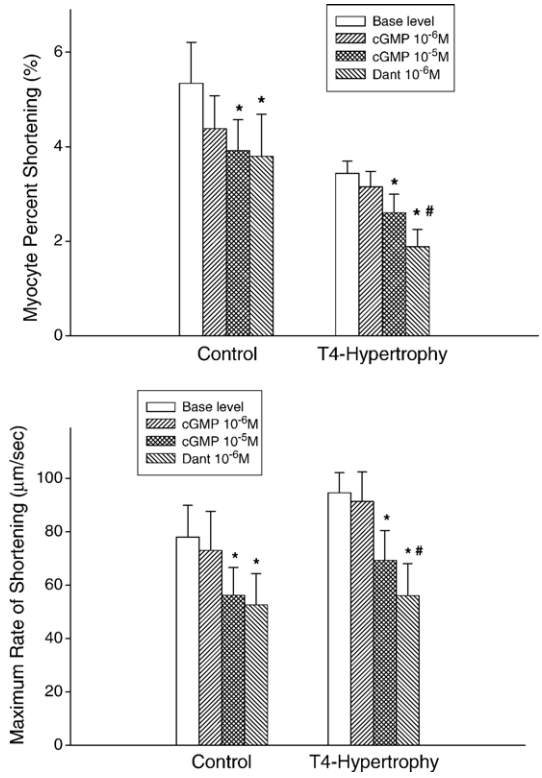

Fig. 2. Data from control and thyroxine hypertrophic ventricular myocytes for percentage shortening before and after cells were treated with 8-Br-cGMP at 10^{-6}, 10^{-5} M followed by dantrolene 10^{-6} M (top). Similar data from control and thyroxine hypertrophic ventricular myocytes for maximum rate of shortening before and after cells were treated with 8-Br-cGMP at 10^{-6}, 10^{-5} M followed by dantrolene 10^{-6} M (bottom). [a]Significantly different from baseline. [b]Significantly different from myocytes treated with 8-Br-cGMP 10^{-5} M alone.

3.3. Dantrolene further reduced cell contractility in cyclic GMP treated hypertrophic myocytes but not control myocyte

In 8-Br-cGMP treated control myocytes, addition of dantrolene did not significantly change myocyte contractility with percent shortening changing from 3.9±0.6% to 3.8±0.9% (Fig. 2) and maximum rate of shortening from 56.2±10.5 to 52.6±11.8 μm/s (Fig. 2). However, in 8-Br-cGMP treated hypertrophic myocytes, dantrolene further reduced myocyte contractility with percent shortening decreasing from 2.8±0.4% to 1.9±0.3% and maximum rate of shortening from 69.3±11.2 to 56.0±12.0 μm/s (Fig. 2). Addition of dantrolene in the presence of cyclic GMP also significantly reduced maximum rate of relaxation in thyroxine-hypertrophy myocytes (Table 1).

Time to peak contraction (TTP) and time to 50% relaxation (50% *R*) of control and thyroxine-induced hypertrophic myocytes were not significantly altered by 8-Br-cGMP and/or dantrolene (Table 1).

3.4. Intracellular Ca^{2+} transient responded similarly to myocyte contractility upon addition of 8-Br-GMP and/or dantrolene

To test whether the effects of cyclic GMP and/or dantrolene on myocyte contractility were correlated with changes in intracellular Ca^{2+} concentration, Ca^{2+} transients were measured in control and hypertrophied myocytes. As shown in Table 2,

Table 2
Effects of dantrolene alone and dantrolene after 8-Br-cGMP on percentage peak height of fluorescence intensity in control (n=3 rabbits) and hypertrophied ventricular myocytes (n=3 rabbits)

% Peak height	Control		T4-hypertrophy	
Baseline	12.0±2.5	11.7±1.8	10.0±1.8	11.4±2.8
8-Br-cGMP 10^{-5} M		10.4±2.0 [a]		8.5±2.6 [a]
Dantrolene 10^{-6} M	10.2±2.4 [a]	9.7±1.9 [a]	7.5±1.9 [a]	6.4±2.4 [a, b]

[a] Significantly different from baseline.
[b] Significantly different from myocytes treated with 8-Br-cGMP.

dantrolene at 10^{-6} M significantly decreased the amplitude of Ca^{2+} transients in both control and hypertrophic myocytes. In both control and thyroxine ventricular myocytes, 8-Br-cGMP significant reduced the Ca^{2+} transients as measured by the change in the percentage change in the peak height of fluorescence intensity. In the 8-Br-cGMP 10^{-5} M treated control myocytes, dantrolene had little effect in changing the Ca^{2+} transient peaks. However, in hypertrophic myocytes, dantrolene further reduced the percentage peak height of fluorescence intensity (Table 2).

4. Discussion

In this study, we found that inhibition of the Ca^{2+} release channel decreased cell contractility and intracellular calcium transients in both control and hypertrophic ventricular myocytes. Similarly, addition of cyclic GMP reduced contractility and intracellular calcium transients. One major finding of this study was that in control myocytes addition of the ryanodine receptor inhibitor dantroline in presence of cyclic GMP did not further reduce myocyte function and calcium transients. This indicated that the effects of cyclic GMP were at least partially mediated by negative regulation of the calcium release channels. The other major finding was that in hypertrophic myocytes, addition of dantrolene after cyclic GMP further decreased myocyte contractility and calcium flux. This suggested that the interaction between cyclic GMP and ryanodine receptors was reduced after thyroxine-induced cardiac hypertrophy.

Cyclic GMP, produced in response to nitric oxide and natriuretic peptides, is an important negative regulator of myocardial function. Elevation of cyclic GMP decreases myocyte contraction, oxygen consumption and can regulate gene expression (Kempf and Wollert, 2004; Zhang et al., 2002; Pilz and Casteel, 2003). The negative functional effects of cyclic GMP are mediated, in part, through the cyclic GMP-dependent protein kinase (Zhang et al., 2002; Shah and Maccarthy, 2000; Kaye et al., 1999). Alternatively, the effects of cyclic GMP may also be mediated by interaction with other molecules in the cell, such as cyclic GMP-gated cation channels and certain cyclic AMP phosphodiesterases (Shah and Maccarthy, 2000; Vila-Petroff et al., 1999). Cyclic GMP may also speed calcium reuptake (Zhang et al., 2002). These interactions can lead to a reduction in calcium flux or sensitivity of the myocytes and may involve reduced release from the sarcoplasmic reticulum calcium release channels (Bers, 2004).

Ryanodine receptors are calcium release channels that control the level of intracellular Ca^{2+} by releasing Ca^{2+} from the sarcoplasmic reticulum which is required for excitation–contraction coupling in skeletal and cardiac muscle (Bers, 2004). Ryanodine receptors are homotetramers and form macromolecular complexes with a number of other proteins that modulate channel activity (Bers, 2004). These channels can be regulated through covalent modifications such as oxidation, nitrosylation, and phosphorylation (Meissner, 2004). Mutations in the cardiac isoform (RyR2) can lead to catecholaminergic polymorphic ventricular tachycardia and other cardiac arrhythmias. In failing human hearts, hyperphosphorylation of RyR2 by the cyclic AMP protein kinase results in defective channel function (Wehrens and Marks, 2003; Marx et al., 2000). The role of cyclic GMP in the control of ryanodine receptors in cardiac myocytes is not clear.

Nitric oxide can increase or decrease cardiac myocyte contractility and ryanodine receptor activity depending on the level of nitric oxide and this can lead to cyclic GMP-dependent or -independent signaling(Shah and Maccarthy, 2000; Ziolo et al., 2001). The effects of nitric oxide on ryanodine receptors and calcium transients are also dependent on level of adrenergic activation (Ziolo et al., 2001). The effect of nitric oxide on ryanodine receptor activity during muscle activation is thought to be mediated mainly by nitrosylation (cyclic GMP independent) in which nitric oxide poly-S-nitrosylation reversibly activates ryanodine receptors and leads to Ca^{2+} release from the sarcoplasmic reticulum (Eu et al., 1999; Stamler and Meissner, 2001). It was proposed that nitric oxide-induced increases in the level of cyclic GMP in myocytes may alter ryanodine receptor activity, although studies in this area are very limited (Eu et al., 1999). It was reported that cyclic GMP-mediated signaling in regulation of ryanodine receptors may involve phosphorylation of ryanodine receptors by the cyclic GMP-dependent protein kinase (Suko et al., 1993; Willmott et al., 2000). In the current study, we found that both dantrolene-induced blockade of calcium release and the addition of cyclic GMP led to reduced myocyte function and Ca^{2+} transients. In control myocytes, addition of dantrolene after cyclic GMP did not significantly change myocyte contractility or calcium transients. This suggests that both dantrolene and cyclic GMP affect calcium release, although they may affect differing parts of this release pathway. It has also been reported that cyclic GMP reduces calcium release from ryanodine receptors in tracheal smooth muscle (Kannan et al., 1997), although smooth and cardiac muscle differ in many respects. This provides evidence to support the idea that the effects of cyclic GMP on ventricular myocytes were partially mediated through interaction with ryanodine receptors.

Thyroid hormone causes many direct and indirect changes in the heart and cardiovascular system (Fadel et al., 2000; Danzi and Klein, 2004). Hyperthyroidism significantly increases in basal metabolic rate, heart rate, blood pressure, myocardial oxygen consumption, and coronary blood flow in thyrotoxic animals (Hu et al., 2003; Straznicka et al., 1998). It causes transient increases in protein synthesis in cardiac myocytes as well as changes in the electrical properties of the cells (Danzi and Klein, 2004). Hyperthyroidism leads to significant cardiac hypertrophy. Our model of hyperthyroidism, thyroxine 0.5 mg/kg/day, has been shown to cause significant cardiac hypertrophy and increased cardiac metabolism within 16 days in this and previous studies (Weiss et al., 1995; Yan et al., 2003).

In the current study, we observed significant cardiac hypertrophy, but no significant changes in baseline ventricular

myocyte functional or timing parameters. This was accompanied by a dantrolene-induced functional decrements and decrements in the cytoplasm calcium transients that were very similar to that observed in the control myocytes. This suggests that the calcium release channels were not significantly affected by 16 days of thyroxine administration. This contrasts with reduced ryanodine receptor activity in dogs with pressure overload hypertrophy (Hittinger et al., 1999). There may be enhanced ryanodine receptor activity after thyroxine (Jiang et al., 2000; Wu et al., 2002). This difference might be related to the very high metabolic demand found during thyrotoxicosis (Weiss et al., 1995).

Nitric oxide and natriuretic peptides, both produced in the heart, are believed to be endogenous inhibitors of maladaptive hypertrophic signaling and inhibit cardiac hypertrophy via cyclic GMP (Kempf and Wollert, 2004). Increases in myocardial cyclic GMP levels have been reported in some pressure-induced cardiac hypertrophy models (Huang et al., 1999; Roitstein et al., 1994), but not others (Rabindranauth et al., 1997). Basal cyclic GMP levels were not reported altered in thyroxine-induced hypertrophic hearts (Weiss et al., 1995). In the current study, we found similar reductions in function and calcium transients with 8-Br-cGMP in the thyroxine and control myocytes. Although 8-Br-cGMP exerted negative functional effects in thyroxine hypertrophic myocytes, cyclic GMP-dependent protein kinase activity was reported by our laboratory to be reduced in thyrotoxic myocytes (Engel et al., 2001; Yan et al., 2003). This may help to explain why dantrolene administration after 8-Br-cGMP further reduced function and calcium transients in the thyroxine myocytes, while this was not observed in controls. The cyclic GMP-mediated signaling in regulation of ryanodine receptors may involve phosphorylation of ryanodine receptors by the cyclic GMP-dependent protein kinase (Suko et al., 1993; Willmott et al., 2000), which is altered in hypertrophy. Other studies showed that cyclic GMP-dependent protein kinase activity is required for the antihypertrophic effects of nitric oxide and atrial natriuretic peptide (Fiedler and Wollert, 2004; Wollert et al., 2003). Further work is necessary to determine what other factors help to replace the interaction between cyclic GMP and ryanodine receptors in thyroxine myocytes. This could be related to L-type calcium channels or the sarcoplasmic reticulum Ca^{2+}-ATPase (Zhang et al., 2002; Shah and Maccarthy, 2000).

In pathological condition, cardiac myocytes tend to adapt to the new working conditions by altering signal transduction pathways and regulating gene expression. Both blockade of ryanodine receptors and addition of cyclic GMP reduced function in control myocytes. In this study, we found that the negative effects of cyclic GMP on myocyte contractility were partially mediated though interaction with sarcoplasmic reticulum calcium release channels and the subsequent decrease in cytosolic calcium transients. In thyrotoxic ventricular myocytes both agents still reduced function and calcium transients. However, the interaction between cyclic GMP and ryanodine receptors was reduced in thyroxine-induced hypertrophic myocytes.

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