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Negative functional effects of cGMP mediated by cGMP protein kinase are reduced in T_4 cardiac myocytes

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Abstract

We tested the hypothesis that in isolated rabbit cardiac myocytes, the negative functional effects of cyclic GMP are partly mediated by cyclic GMP-dependent protein kinase activity, and that these effects are altered in thyroxine (T_4, 0.5 mg/kg/day for 16 days)-induced hypertrophic myocytes. Using isolated ventricular myocytes from control ($N = 8$) and T_4 ($N = 8$) hypertrophic hearts, data for percent cell shortening (%) and maximum rate of contraction ($\mu m/s$) were collected using a video edge detector at baseline, after the addition of 10^{-6} M 8-bromo-cyclic GMP (8-Br-cGMP), 10^{-5} M 8-Br-cGMP, and 10^{-6} M KT5823 (10-methoxy-10-methoxycarbonyl-9, 10, 11, 12-tetrahydro-9, 12-epoxy-(1*H*)-diinidolo [1, 2, 3, f–g: 3′, 2′, 1′-k-j]-pyrrolidino-[3,4-i] [1,6]-benzodiazocin-2-methyl-1-one, cyclic GMP protein kinase inhibitor). Protein phosphorylation was determined autoradiographically after gel electrophoresis. In both control and T_4 myocytes, 8-Br-cGMP caused a significant decrease in percent shortening ($5.56 \pm 0.49\%$ to $3.02 \pm 0.47\%$ in control and $4.34 \pm 0.33\%$ to $3.13 \pm 0.17\%$ in T_4 myocytes) and maximal rate of contraction 57.35 ± 6.05 to 36.82 ± 3.17 $\mu m/s$ in control and 58.49 ± 3.28 to 42.88 ± 2.29 $\mu m/s$ in T_4 myocytes). KT5823 significantly increased percent shortening to $3.77 \pm 0.28\%$ and rate to 48.68 ± 4.71 $\mu m/s$ after 8-Br-cGMP only in control myocytes. In T_4 myocytes, the changes in percent shortening and rate after KT5823 were not significant. Protein phosphorylation was increased by 8-Br-cGMP in control and to a lesser extent in T_4 myocytes, but the increment was reduced by KT-5823 in control only. These data demonstrated that cyclic GMP had negative functional effects partially mediated by cyclic GMP protein kinase in control myocytes. Cyclic GMP also exerted negative functional effects in thyroxine-induced hypertrophic myocytes, but cyclic GMP protein kinase activity was not an important regulator of these effects in T_4 ventricular myocytes.

Keywords: Nitric oxide (NO); Second messenger; Myocyte; Hypertrophy; (Rabbit)

1. Introduction

The second messenger guanosine 3′,5′-cyclic monophosphate (cyclic GMP) has been shown to exert negative metabolic and functional effects on cardiac myocytes (Lohmann et al., 1991; Paulus et al., 1994; Shah et al., 1994). Increased levels of cyclic GMP have been found to result in reduction of local myocardial metabolism and force development both in vivo and in vitro in a variety of species, including humans (Lohmann et al., 1991; Paulus et al., 1994; Sperelakis et al., 1994). In the rabbit heart, previous work has demonstrated that changes in cyclic GMP levels lead to inverse changes in myocardial oxygen consumption (Weiss and Tse, 1995; Weiss et al., 1994). Previous work has also shown that an increase in cyclic GMP concentration resulted in a concentration-dependent reduction in myocyte contractility (Brady et al., 1993; Sperelakis et al., 1994; Sudgen and Bogoyevitch, 1995). The action of cyclic GMP may be mediated through protein phosphorylation, cyclic GMP-stimulated or -inhibited cyclic AMP phosphodiesterases and direct or indirect inhibition of L-type calcium channels leading to reductions in cytosolic calcium (Hartzell, 1989; Lohmann et al., 1991; Mery et al., 1991; Lincoln et al., 1994; Shah et al., 1994; Sperelakis et al., 1994; Yan et al., 1998). Several studies have shown that protein phosphorylation is a major pathway for the effects of cyclic GMP, and that blocking

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this pathway will significantly diminish the effects of cyclic GMP (Mery et al., 1991; Sperelakis et al., 1994; Wahler and Dollinger, 1995). It has also been suggested that the regulation of L-type calcium channels by cyclic GMP is mediated by cyclic GMP protein kinase (Mery et al., 1991; Sperelakis et al., 1994; Wahler and Dollinger, 1995).

Hyperthyroidism causes many direct and indirect changes in myocardial function (Dillman, 1990; Klein, 1990). Some of these changes, such as increased basal metabolic rate and oxygen consumption, are due to the direct effects of thyroid hormone on various tissues (Oppenheimer et al., 1987). Others, such as cardiac hypertrophy, result 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 (Crie et al., 1983; ; Siehl et al., 1985; Klein, 1988; Klein and Hong, 1986). There are many reports of an increased number of β-adrenoceptor receptors in the hearts of thyrotoxic animals and humans (Tse et al., 1980; Eliades and Weiss, 1989; Liggett et al., 1989; Crozatier et al., 1991). There may also be alterations in muscarinic responses with thyroxine (Weiss and Tse, 1995). In addition, previous work has shown that the relationship between cyclic GMP and myocardial oxygen consumption is altered in thyroxine-induced cardiac hypertrophy in vivo (Weiss et al., 1994, 1995). It is not clear what part of the altered relationship between cyclic GMP and myocardial function is directly related to effects of thyroxine on cardiac myocytes.

In this study, we tested the hypothesis that in isolated rabbit cardiac myocytes, the negative functional affects of cyclic GMP are partially mediated by cyclic GMP-dependent protein kinase activity, and that these effects are altered in thyroxine-induced hypertrophic myocytes. We used the cyclic GMP analogue 8-Bromo-cyclic GMP and a selective inhibitor of cyclic GMP protein kinase, KT5823, to test this hypothesis. Ventricular myocytes from control and thyroxine-treated rabbit hearts were used in this study. We found that cyclic GMP exerts negative functional effects in both control and hypertrophic myocytes, but that the importance of the cyclic GMP protein kinase significantly decreased after thyroxine.

2. Materials and methods

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Institutional Animal Care and Use Committee. Eight New Zealand white rabbits were injected with 0.5 mg/kg/day of thyroxine intramuscularly for 16 days. These animals were weighed on days 1, 8, and 16. The animals in the control group received no injections, but were weighed prior to the experiment.

2.1. Cell dissociation

New Zealand white rabbits (weighing 2–3 kg, $N = 8$ thyroxine, $N = 8$ control) were anesthetized with sodium pentobarbital (35 mg/kg) followed by the administration of heparin (10 units/g body weight) intravenously using the circumflex ear vein. The heart was rapidly removed and weighed after an overdose of pentobarbital (60 mg/kg). Retrograde aortic perfusion of the heart was immediately begun at 70 mmHg constant pressure with *N*-2-hydroxyethylpiperazine-*N*′-2-ethane-sulfonic acid (HEPES, pH = 7.5) buffered with minimal essential medium (MEM). This perfusion medium contained (in mM): NaCl 117, KCl 5.7, $NaHCO_3$ 11, NaH_2PO_4 1.5, $MgCl_2$ 1.7, HEPES 21.1, glucose 11.7, amino acids and vitamins. We added 2 mM L-glutamine, 10 mM taurine, and the pH was adjusted to 7.2 with NaOH. This low Ca^{2+} MEM solution had an osmolarity of 296 mosM and a free Ca^{2+} activity of 2–5 μM. After 5 min of perfusion with low Ca^{2+} MEM, the heart was perfused at 50 mmHg with 60 ml of low Ca^{2+} MEM supplemented with 0.1% collagenase (Worthington type II). All perfusion media were maintained at 37°C and equilibrated with a water saturated gas mixture (85% O_2, 10% N_2, 5% CO_2). After 20 min of recirculation of the collagenase perfusate, the heart was removed from the perfusion apparatus and cut into smaller pieces in MEM containing 1 mM $CaCl_2$ and 0.5% bovine serum albumin (fraction V, Sigma, St. Louis, MO). This Ca^{2+}-MEM was supplemented with 0.1% collagenase. The tissue suspension was gently swirled in 50 ml centrifuged tubes at 37°C by a wrist action shaker (2 cps) for 5 min. A slurry containing isolated myocytes was decanted from the tissue suspension. The isolated cells were washed three times followed by low-speed centrifugation (34 g) to completely remove the collagenase and subcellular debris and then resuspended in MEM. Incubation of the remaining tissue with collagenase was repeated at least two more times. Myocyte viability in the MEM suspension was between 70% and 80%. Yields were typically $10–14 \times 10^6$ rod-shaped cells/heart.

2.2. Myocyte shortening measurements

Isolated cardiac ventricular myocytes were put into a chamber (37°C) on the stage of an inverted microscope (Zeiss Axiovert 125) in 2 mM Ca^{2+}-MEM solution. The volume of the chamber was 3.5 ml. Two platinum wires inserted into the center of the myocyte chamber were used to pace the myocytes by electric field stimulation (1 Hz, 5-ms duration, voltage at 10% above threshold, alternating polarity after each pulse). Unloaded cell shortening was

measured on-line using a Myotrack system (Data Sciences International, St. Paul, MN), including a camera and a video edge detector that detected the change of the position of both edges of the cell. The data were collected continuously at a rate of 240 data points/s. The output of the video edge detector was fed into both a television monitor and a desktop computer, which was used to analyze the data. An example of this data is shown in Fig. 1. After a 5-min stabilization period, contraction data for the individual ventricular myocyte were recorded for a minimum of 10 consecutive contractions. We used at least three myocytes per experiment. We then determined percent shortening (%), maximal velocity of shortening (μm/s), time to peak (s), and 90% relaxation (s).

2.3. In vitro phosphorylation reactions and phosphoprotein analysis

In vitro phosphorylation reactions and phosphoprotein analysis were performed five times. All reactions were carried out in microfuge tubes at room temperature. Myocytes from healthy adult New Zealand White rabbits, harvested, as described, were homogenized (Brinkmann Polytron homogenizer: 15 s at 20,000 rpm) in buffer (5 mM Tris–HCl, pH 7.4, 1 mM $MgCl_2$, 0.25 M sucrose) and centrifuged at 15,500 rpm for 20 min at 4°C. The supernatant was aliquoted and used as the myocyte extract for all phosphorylation reactions. An activator, 8-bromo-cyclic GMP (only the higher dose), and inhibitor, KT 5823 (specific cyclic GMP-dependent protein kinase inhibitor), were added to 10 μl of extract (0.5 mg total protein/ml). Ten minutes were allowed for each reactant to equilibrate. After equilibration, each reaction was cooled on ice. Gamma-^{33}P-ATP (1 μl) at 10 μCi/μl was added to initiate the reaction. The reaction was terminated 15 min later by adding a volume of Bio Rad reducing sample buffer equal to the entire reaction volume. The samples were heated at 95°C for 5 min and electrophoresed using miniature 12% SDS polyacrylamide slab gels. The gels were then stained with Coomassie Brilliant Blue and dried overnight using a Promega Gel Drying Kit and exposed to X-ray film at −20°C for 24 h. The exposed X-ray film demonstrated phosphate labeled proteins, which were then sized by comparison to molecular weight standard markers.

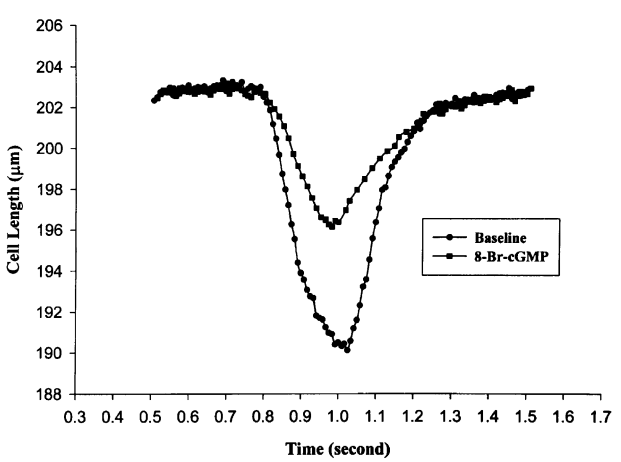

Fig. 1. An example of cell shortening data collected from a thyroxine treated ventricular myocyte. Shortening is shown at baseline (circle) and after 10^{-5} M 8-Br-cGMP (square). Note the reduced shortening after 8-Br-cGMP.

2.4. Experimental protocol

The following protocol was used for the myocyte functional measurements. Myocytes were suspended in the chamber with 2 mM Ca^{2+}-MEM at an appropriate concentration and the cells were allowed to stabilize for 10–15 min. The ventricular myocytes were paced with electrical field stimulation. A 5-min interval was allowed between the addition of reagents during which cell contractility was measured. In the control group, 8-bromo-cyclic GMP (8-Br-cGMP) 10^{-6} M was added, followed by 8-Br-cGMP 10^{-5} M, followed by the cyclic GMP protein kinase inhibitor KT 5823 10^{-6} M (10-methoxy-10-methoxycarbonyl-9, 10, 11, 12-tetrahydro-9, 12-epoxy-(1*H*)-diindolo [1, 2, 3, f–g: 3′, 2′, 1′-k-j]-pyrrolidino-[3,4-i] [1,6]-benzodiazocin-2-methyl-1-one). In the thyroxine-induced hypertrophy group, 8-Br-cGMP 10^{-6} M was added, followed by 8-Br-cGMP 10^{-5} M, and KT 5823 10^{-6} M. For the protein phosphorylation assay, we used only the higher dose of 8-Br-cGMP with and without KT2823.

2.5. Statistics

Results are expressed as mean ± S.E.M. A repeated measure analysis of variance was used to compare variables measured in the baseline and experimental periods for both the thyroxine and control myocytes. Duncan's multiple range test was used to compare differences post hoc. This analysis was used to determine differences between groups and treatments for myocyte function. Regression analysis was performed on the effects of 8-Br-cyclic GMP on percent shortening and maximum rate of shortening in control and T_4 myocytes. In all cases, a $P < 0.05$ was accepted as significant.

3. Results

The heart weight to body weight ratio of the thyroxine-induced cardiac hypertrophic rabbits was statistically greater than controls (5.57 ± 0.25 vs. 4.22 ± 0.19 g/kg, $P = 0.001$). Length and width measurements of 50 myocytes from each heart were made. Thyroxine-induced

hypertrophic myocytes were both significantly longer (156.3 ± 1.5 vs. 135.7 ± 0.4 μm, $P = 0.0001$) and wider (32.5 ± 0.1 vs. 25.5 ± 0.3 μm, $P = 0.0001$) than control ventricular myocytes.

The percent cell shortening data for ventricular myocytes from control and thyroxine-induced hypertrophic ($N = 8$) hearts are depicted in Fig. 2. There were no significant functional differences between control and thyroxine-induced hypertrophic myocytes under baseline conditions. Percent cell shortening decreased significantly from baseline values after the cyclic GMP analogue was added in both groups. The maximum percentage decrease was significantly greater in control ($46 \pm 3\%$) vs. thyroxine ($26 \pm 5\%$). Percent shortening decreased further with the addition of the higher dose of 8-Br-cGMP. The line of best fit was percent shortening $= -8.7 \times 10^4$ (Dose) + 4 for control and -1.6×10^5 (Dose) + 5 for thyroxine-treated myocytes. In the control myocytes, the addition of KT5823 caused a significant increase in percent cell shortening compared to 10^{-5} M 8-Br-cGMP. However, KT5823 did not significantly affect percent shortening in the thyroxine-induced hypertrophic myocytes.

The maximal velocity of shortening data for control and thyroxine-induced hypertrophic myocytes are presented in Fig. 3. There were no significant functional differences between control and thyroxine-induced hypertrophic myocytes. In the control myocytes, the maximal velocity of shortening decreased significantly after the addition of the higher dose of 8-Br-cGMP. In the thyroxine-induced hypertrophic myocytes, a significant reduction in maximal velocity of shortening occurred after both doses of 8-Br-cGMP. The maximum percentage decrease was not different in control ($35 \pm 3\%$) vs. thyroxine ($25 \pm 5\%$). The line

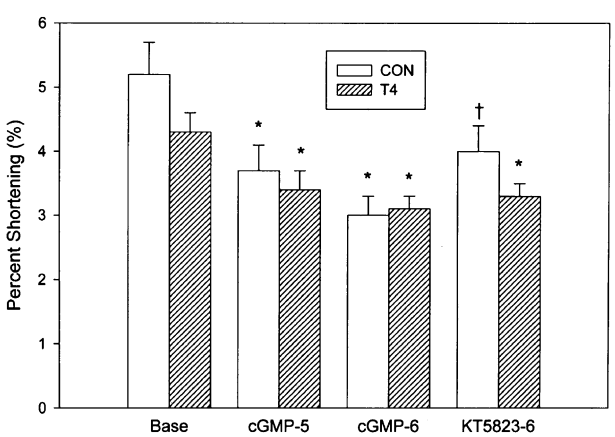

Fig. 2. Percent cell shortening of isolated ventricular myocytes from control and thyroxine induced hypertrophic ($N = 8$) rabbit hearts are shown. Values are presented under basal (BASE) conditions, after the addition of two doses of 8-Br-cGMP followed by the addition of the cyclic GMP-dependent protein kinase inhibitor KT5823. Note that 8-Br-cGMP reduced percent cell shortening of the myocytes and that KT5823 partially restored it. *, Significantly different from BASE. †, Significantly different from 8-Br-cGMP 10^{-5} M.

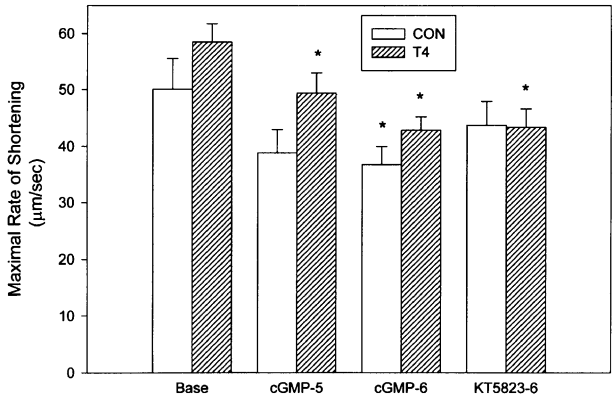

Fig. 3. The maximal velocity of shortening of isolated ventricular myocytes from control and thyroxine induced hypertrophic rabbit hearts are shown. Values are presented under basal (BASE) conditions, after the addition of two doses of 8-Br-cGMP followed by the addition of the cyclic GMP-dependent protein kinase inhibitor KT5823. Note that 8-Br-cGMP reduced the maximal velocity of shortening of the myocytes and that KT5823 partially restored it. *, Significantly different from BASE.

of best fit was percent shortening $= 1.2 \times 10^6$ (Dose) + 55 for control and 8.9×10^5 (Dose) + 45 for thyroxine-treated myocytes. In the control myocytes, maximal velocity of shortening returned toward baseline levels after the addition of the cyclic GMP protein kinase inhibitor, whereas maximal velocity of shortening in the thyroxine-induced hypertrophic myocytes was still significantly slower than baseline after KT5823 was added.

There were no significant differences in the time to peak shortening caused by 8-Br-cGMP or KT5823 in control (0.33 ± 0.03 (s) baseline, 0.38 ± 0.03 8-Br-cGMP 10^{-6} M, 0.38 ± 0.02 8-Br-cGMP 10^{-5} M, 0.35 ± 0.03 KT5823) or T_4 (0.28 ± 0.02, 0.34 ± 0.03, 0.33 ± 0.02, 0.35 ± 0.02) myocytes. Similarly, the time to 90% relaxation was not altered by treatment in either the control (0.29 ± 0.02, 0.28 ± 0.02, 0.26 ± 0.01, 0.27 ± 0.02) or T_4 (0.28 ± 0.01, 0.28 ± 0.01, 0.28 ± 0.02, 0.27 ± 0.01) myocytes. No significant differences were found between these parameters in comparisons between control and T_4 myocytes.

In examination of protein phosphorylation in the control myocytes, the addition of 8-Br-cGMP enhanced the labeling of five specific protein bands at molecular weight of 97, 53, 31, 22 kDa and a double band at about 47 kDa, (Fig. 4, Panel B). When we added KT 5823 after 8-Br-cyclic GMP, all the bands were lightened. KT 5823 alone had no effect on basal phosphorylation (Fig. 4, Panel A). In thyroxine-induced hypertrophic rabbit myocytes, the effect of these doses of 8-Br-cGMP on protein phosphorylation were greatly reduced. The strongest effect appeared at 31 kDa. KT 5823 had no effect on this pattern of protein phosphorylation in the thyroxine-treated myocytes. The addition of the protein kinase inhibitor alone exerted no effect on the protein phosphorylation pattern of unstimu-

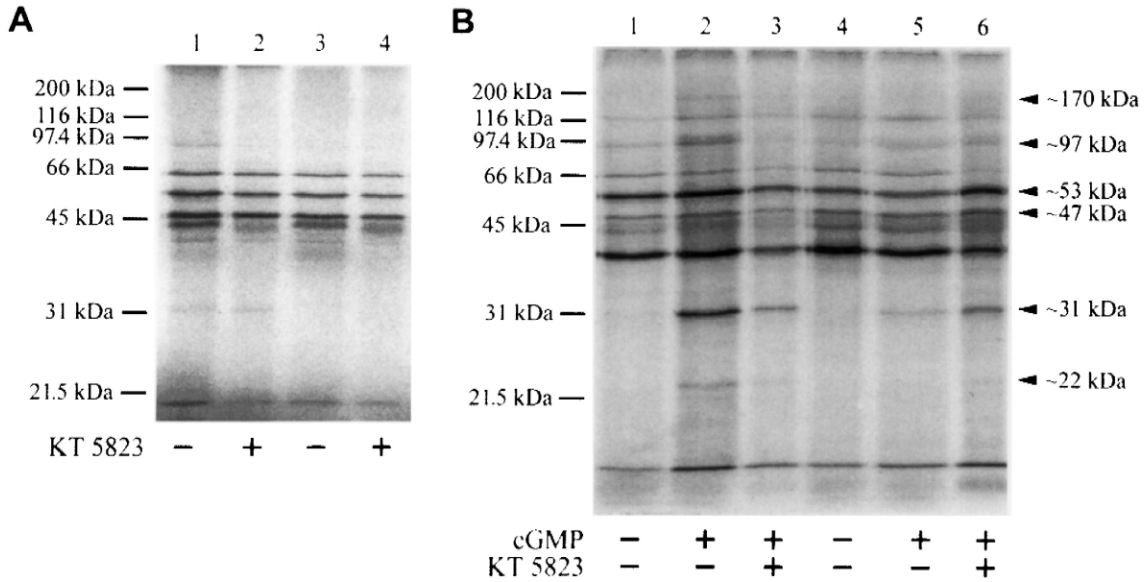

Fig. 4. The effects of 8-Br-cGMP and KT5823 on protein phosphorylation of both control and thyroxine-induced hypertrophic rabbit ventricular myocytes. In panel A, basal phosphorylation in control (lane 1) and thyroxine treated myocytes (lane 3) are shown. The effect of KT5823 on basal phosphorylation is shown in control (lane 2) and thyroxine (lane 4) myocytes. Note the lack of effect of KT5823 alone. In panel B, basal phosphorylation is shown in lanes 1 (control) and 4 (thyroxine). The effects of 8-Br-cGMP on both groups of myocytes are shown in lanes 2 (control) and 5 (thyroxine). The effects of addition of both KT5823 and 8-Br-cGMP are shown in lanes 3 (control) and 6 (thyroxine), respectively. Molecular weight standards are shown at the left. Note that in the control group, 8-Br-cGMP increased phosphorylation of five protein bands at 97, 53, 47, 31 and 22 kDa and KT5823 reduced the labeling (labeled at the right). The effects of 8-Br-cGMP were reduced in the thyroxine treated myocytes and KT5823 had no effects.

lated control or thyroxine-treated myocyte extracts. Similar results were obtained in two control and three thyroxine-treated animals.

4. Discussion

One major finding of this study was that cyclic GMP dose dependently decreased percent cell shortening and maximal velocity of shortening in both control and thyroxine-induced hypertrophic myocytes to a similar extent. We found that the addition of a cyclic GMP protein kinase inhibitor partially reversed the negative functional effects of cyclic GMP in control myocytes. The other major finding was that the cyclic GMP protein kinase was not an important mediator of these negative functional effects of cyclic GMP in thyroxine-induced hypertrophic myocytes. This difference was demonstrated by the increase in percent cell shortening and maximal rate of shortening in control myocytes after the addition of a selective inhibitor of cyclic GMP protein kinase and the lack of effect of this inhibitor in thyroxine-induced hypertrophic myocytes. Further, cyclic GMP increased protein phosphorylation more in control than thyroxine-treated myocytes and KT5823 reduced this effect in control myocytes only. This suggests that there is a shift in the mechanisms through which cyclic GMP exerts its negative functional effects in thyroxine-treated myocytes.

The yield of healthy ventricular myocytes was high (70–80% viability) from both control and hypertrophic rabbits. The viability of the myocytes at the end of each experiment was confirmed by rechecking the percentage of rod-shaped myocytes and assessing their morphology. By using isolated myocytes, we established that the effects seen on percent cell shortening and maximal velocity of shortening were accounted for entirely by myocytes. This could be disputed in intact heart preparations, with heterogeneous cell types. 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. A cyclic GMP analogue, 8-bromo-cyclic guanosine monophosphate, was used to increase the levels of cyclic GMP within the ventricular myocyte. In previous studies, this chemical analogue has shown a greater affinity for cyclic GMP-dependent protein kinase than cyclic GMP, but a lesser affinity for the cyclic GMP affected phosphodiesterases (Lohmann et al., 1991). We used KT 5823 in order to inhibit the cyclic GMP protein kinase. This agent is a selective inhibitor for this protein kinase (Haikala et al., 1997; Straznicka et al., 1999; Vila-Petroff et al., 1999).

The cellular effects of cyclic GMP have been studied in various tissue types, including the myocardium (Lohmann et al., 1991; Murad, 1994; Shah et al., 1994; Sperelakis et al., 1994; Weiss et al., 1994). Our results demonstrated

that cyclic GMP reduced percent cell shortening and maximal velocity of shortening in control myocytes. This study addressed the issue of the importance of cyclic GMP protein kinase in the negative functional effects of cyclic GMP on ventricular myocytes.

Cyclic GMP protein kinase is a dimer of two identical 75 kDa subunits and can be activated in vitro by cyclic GMP at concentrations below 1 μM (Landgarf and Hofmann, 1989). Cyclic GMP protein kinases are serine/threonine kinases that exist in two general classes in vertebrate cells: type I and type II. Type-II kinases have only been detected in intestinal epithelial cells, whereas type-I cyclic GMP protein kinases are widely distributed and have been isolated from soluble extracts from various tissues, including myocardium (Lincoln and Keely, 1980; Sumii and Sperelakis, 1995; Tohse et al., 1995). Several mechanisms for cyclic GMP have been determined, but the predominant mechanism of action of cyclic GMP in isolated myocytes is felt to be via cyclic GMP protein kinase (Sperelakis et al., 1994). Although the exact mechanism by which cyclic GMP kinase acts is not yet understood, previous studies have shown that one effect is the reduction of intracellular Ca^{2+} (Mery et al., 1991; Francis and Corbin, 1994; Tohse et al., 1995). Recent studies have also demonstrated that the regulation of L-type calcium channels by cyclic GMP in different species was partially mediated by cyclic GMP-dependent protein kinase although this may not be the major mechanism of its action (Mery et al., 1991; Francis and Corbin, 1994; Sperelakis et al., 1994; Sudgen and Bogoyevitch, 1995; Sumii and Sperelakis, 1995; Tohse et al., 1995; Wahler and Dollinger, 1995; Van der Zypp and Majewski, 1998). In preliminary studies, KT 5823 had no effect on cell function or protein phosphorylation when added alone (Straznicka et al., 1999). In the present study, inhibition of cyclic GMP protein kinase reversed the negative functional effects of cyclic GMP in control myocytes and also lightened the bands that had been darkened by 8-Br-cGMP. This indicated that cyclic GMP protein kinase phosphorylation was a major mediator of cyclic GMP action in isolated control myocytes under our experimental conditions.

Hyperthyroidism is associated with an elevated cardiac output, cardiac hypertrophy, and mild hypertension (Chilian et al., 1985; Gerdes et al., 1997). There are also reports of enhanced myocardial protein synthesis with hyperthyroidism (Siehl et al., 1985; Oppenheimer et al., 1987). This model of hyperthyroidism, 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 (Eliades and Weiss, 1989; Liggett et al., 1989; Murad, 1994; Weiss et al., 1994, 1995). An increased number of β-adrenergic receptors has been described in the hearts of thyrotoxic animals and humans (Eliades and Weiss, 1989; Liggett et al., 1989; Tse et al., 1980; Crozatier et al., 1991). There may also be alterations in muscarinic responses with thyroxine (Weiss and Tse, 1995). In addition, the relationship between cyclic GMP and myocardial oxygen consumption is altered in thyroxine-induced cardiac hypertrophy in vivo (Weiss et al., 1994, 1995).

In vivo experiments have demonstrated that the effects of cyclic GMP were enhanced in thyroxine-induced hypertrophy, but basal levels of cyclic GMP were not altered (Weiss et al., 1995). Cyclic GMP levels may increase during some forms of heart failure, (Michel et al., 1990; Jakob et al., 1995) although there may not be alterations in the production of cyclic GMP in the heart after thyroxine-induced hypertrophy (Tse et al., 1980). Our in vitro data indicated that cyclic GMP did exert negative functional effects in isolated thyroxine-induced hypertrophic myocytes, but that the action of cyclic GMP was somewhat less than to control myocytes. The differences between in vitro and in vivo responses to cyclic GMP after thyroxine treatment may be the result of differences in the extracellular environment, including different levels of catecholamines, thyroid hormones, etc. It also indicated that the enhanced negative metabolic response to thyroxine in vivo (Weiss et al., 1995) was not directly related to differences in the ventricular myocytes.

We found a negative functional effect of cyclic GMP in the control and thyroxine-treated hypertrophic myocytes. There was, however, no longer a significant functional effect of the cyclic GMP protein kinase inhibitor in the hypertrophic myocytes. There are conflicting reports in the literature regarding the amount of cyclic GMP protein kinase present in thyroxine-induced hypertrophy. One author has found decreased levels of cyclic GMP-dependent protein kinase in thyroxine-induced hypertrophy in the rat heart (Tse et al., 1980). However, Ecker et al. (1989) found that cyclic GMP protein kinase levels were not decreased in thyroxine-induced hypertrophic rats, but that cyclic GMP-dependent protein kinase levels were decreased in pressure-induced hypertrophy. The findings of this study indicated that cyclic GMP protein kinase was not an important mediator of cyclic GMP's negative functional effects in thyroxine-induced hypertrophy, since inhibiting cyclic GMP protein kinase did not lead to a reversal of cyclic GMP's effects on percent cell shortening and maximal velocity of shortening in isolated thyroxine-induced hypertrophic myocytes. This is also supported by the protein phosphorylation data. In thyroxine-induced hypertrophic myocytes, we found that 8-Br-cGMP caused the phosphorylation of only one of the five proteins, which had been phosphorylated in control animals. In addition, KT5823 did not have significant effects on protein phosphorylation in the thyroxine-treated myocytes. These data indicate a shift in the mechanism through which cyclic GMP exerts its negative functional influence after thyroxine-induced cardiac hypertrophy. This could indicate an increase in the relative importance of cyclic GMP affected cyclic AMP phosphodiesterases or effects on L-type Ca^{2+} channels after thyroxine.

In summary, we found that cyclic GMP caused a similar decrease in percent cell shortening and maximal velocity of shortening in isolated cardiac myocytes from both control and thyroxine-induced hypertrophic hearts. Inhibition of the cyclic GMP protein kinase partially restored function to the control, but not hypertrophic myocytes. In addition, protein phosphorylation was enhanced by cyclic GMP and reduced by a cyclic GMP protein inhibitor in control, but not hypertrophic myocytes. This indicated that the cyclic GMP protein kinase was not an important mediator of the functional effects of cyclic GMP in thyroxine-induced hypertrophy. This suggested that there was an alteration in the mechanism by which cyclic GMP regulates function in ventricular myocytes in thyroxine-induced cardiac hypertrophy.

Acknowledgements

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