Reduction in interaction between cGMP and cAMP in dog ventricular myocytes with hypertrophic failure

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Zhang, Qihang, Michael Lazar, Bruno Molino, Roberto Rodriguez, Tomer Davidov, Jun Su, James Tse, Harvey R. Weiss, and Peter M. Scholz. Reduction in interaction between cGMP and cAMP in dog ventricular myocytes with hypertrophic failure. Am J Physiol Heart Circ Physiol 289: H1251–H1257, 2005.—Baseline function and signal transduction are depressed in hearts with hypertrophic failure. We tested the hypothesis that the effects of cGMP and its interaction with cAMP would be reduced in cardiac myocytes from hypertrophic failing hearts. Ventricular myocytes were isolated from control dogs, dogs with aortic valve stenosis hypertrophy, and dogs with pacing hypertrophic failure. Myocyte function was measured using a video edge detector. Cell contraction data were obtained at baseline, with cAMP; with cGMP; with cGMP and cAMP; and with cGMP and cAMP plus an inhibitor of the cGMP-stimulated and cGMP-inhibited cAMP phosphodiesterases. We used erythro-9-(2-hydroxy-3-onyl)adenine (EHNA; a cAMP phosphodiesterase [PDE2] inhibitor) plus 8-bromo-cGMP, or milrinone (a PDE3 inhibitor) plus 8-bromo-cGMP. Baseline percent shortening and maximal rates of shortening (Rmax) and relaxation were slightly reduced in hypertrophied and relaxed myocytes were slightly reduced in hypertrophic myocytes and were significantly lower in failing myocytes (Rmax for control dogs, 95.3 ± 17.3 %; hypertrophy dogs, 88.2 ± 5.5 %; failure dogs, 53.2 ± 6.4 μm/s); 8-Bromo-cGMP dose dependently reduced myocyte function in all groups. However, EHNA (10−4 M) and milrinone (10−6 M) significantly reduced the negative effects of cGMP on cell contractility in control and hypertrophy but not in failing myocytes, significantly reduced the negative effects of cGMP on cell contractility in control and hypertrophy but not in failing myocytes, significantly reduced the negative effects of cGMP on cell contractility in control and hypertrophy but not in failing myocytes. Both combinations of EHNA-cGMP and milrinone-cGMP significantly increased intracellular cAMP in control, hypertrophic, and failing myocytes. These data indicated that the cGMP signaling pathway was preserved in hypertrophic failing cardiac myocytes. However, the interaction of cGMP with the cAMP signaling pathway was impaired in these failing myocytes.

cardiac; guanosine 5'-cyclic monophosphate; adenosine 5'-cyclic monophosphate; phosphodiesterase; hypertrophy; heart; canine

The second-messenger cGMP can reduce myocardial metabolism, inotropy, and function (21, 22, 35). The negative functional effects of cGMP in cardiac myocytes are mediated through cGMP-gated ion channels, cGMP-dependent protein kinases, and cGMP-regulated phosphodiesterases (16, 23, 28, 32). Previous studies from our laboratory (13, 31, 35) have shown that elevation in cGMP decreases myocyte oxygen consumption and myocyte contraction in rabbit and canine hearts and cardiac ventricular myocytes. These negative functional effects of cGMP can be altered by cAMP. There are reports of both enhancement and inhibition (3, 5, 9, 11, 26, 30, 31). The intracellular concentration of cAMP is regulated at the level of synthesis through adenylyl cyclase as well as the level of degradation through several cAMP phosphodiesterases, which break down cAMP into 5'-AMP. The activity of two forms of these cAMP phosphodiesterases, cGMP-stimulated cAMP phosphodiesterase (PDE2) and cGMP-inhibited cAMP phosphodiesterase (PDE3) is regulated by the level of intracellular cGMP (5, 13, 30). In the myocardium, a small increase in cGMP levels predominantly inhibits PDE3 (11). This would function to increase myocardial cAMP levels, which may limit the negative functional effects of cGMP.

Cardiac hypertrophy develops as an adaptive and compensatory mechanism in response to chronic increase in hemodynamic pressure overload (6, 14). This tends to maintain cardiac function, although the cAMP signal transduction system may be depressed (13, 20, 27, 34). If the stress continues, there can be a transition to heart failure. Baseline myocardial function and signal transduction through both cGMP and cAMP are markedly depressed in hearts with hypertrophic failure (1, 2, 8, 17, 20, 25, 27, 29). Failing hearts have been shown to be associated with decreased myocardial function and downregulation of the β-adrenergic system in human and animal models (2, 25, 29). In dogs, basal cAMP levels and adenylyl cyclase activity were significantly lower in failing heart than in control or hypertrophic hearts (1, 7). Basal cGMP levels were significantly elevated in hypertrophic and failing hearts. Basal guanyl cyclase activity was also significantly elevated in failing hearts compared with control and hypertrophic hearts (8). How these changes in cGMP affect myocyte function in failing hearts is not known. Furthermore, changes in the interaction between cGMP and cAMP have not been reported in hypertrophic cardiac failure.

We tested the hypothesis that the functional effects of cGMP and its interaction with cAMP would be reduced in ventricular myocytes from hypertrophic failing hearts. This was tested through the use of a cell-permeable form of cGMP and inhibitors of the cGMP-stimulated and cGMP-inhibited cAMP phosphodiesterases. We used erythro-9-(2-hydroxy-3-onyl)adenine (EHNA), which is a cAMP phosphodiesterase (PDE2) inhibitor, or milrinone, which is a PDE3 inhibitor, to increase cAMP levels by preventing its degradation. The hypothesis was examined in ventricular myocytes isolated from control dog hearts, hearts with left ventricular hypertrophy induced by aortic valve stenosis, and rapid ventricular pacing.
induced failing hearts with left ventricular hypertrophy. We thought that the transition from hypertrophy would affect the responses to cAMP and cGMP. We found that cGMP had similar negative functional effects in all three groups of ventricular myocytes, but the interaction with cAMP was reduced with cardiac failure.

**METHODS**

The investigation was conducted in accordance with the **Guide for the Care and Use of Laboratory Animals** (NIH Publication No. 85-23, Revised 1996) and was approved by our Institutional Animal Care and Use Committee.

**Preparation of experimental model.** Left ventricular hypertrophy was produced in 6- to 8-week-old puppies (4-6 kg body wt) by creation of valvular aortic stenosis (8, 19). The animals were anesthetized with intravenous pentobarbital sodium (30 mg/kg) and were intubated and maintained on 0.5-1.0% halothane and a 50% nitrous oxide-50% oxygen mixture. Direct arterial blood pressure measurement (using percutaneous arterial catheters) and continuous ECGs were recorded. The heart was exposed using a right thoracotomy. The noncoronary aortic sinus of Valsalva was plicated with a single horizontal mattress stitch using a nonabsorbable suture reinforced with Teflon-felt pledgets. All animals had a palpable thrill in the ascending aorta after plication. The pericardium was repaired, and the thoracotomy was closed over a temporary chest tube.

After ∼5 mo, congestive heart failure was induced in six left ventricular hypertrophic dogs after implantation of a rapid pacemaker. Animals were anesthetized with intravenous pentobarbital sodium and were intubated and maintained on halothane and a mixture of nitrous oxide and oxygen. Direct arterial blood pressure measurements and continuous ECGs were recorded. Pacemaker leads were secured to the epicardium with nonabsorbable sutures. Leads were brought through the incision to a supracapsular pocket and connected to a multiprogrammable implantable pacemaker (Medtronic; Minneapolis, MN). The pericardium was repaired, and the thoracotomy was closed over a temporary chest tube. The pacemaker was initially programmed at a rate of 220 beats/min and was adjusted to 240 beats/min over 72 h. Cardiac failure occurred by 5 wk after pacemaker implantation. Symptomatology of cardiac failure included ascites, lassitude, difficulty in breathing, and poor eating. Previous studies had demonstrated significantly reduced cardiac function at this time (7, 8). Approximately 6 mo after aortic valve plication and 5 wk after pacemaker implantation, we studied the six left ventricular hypertrophic and six pacing-induced failure dogs and compared them with six control age-matched dogs.

**Ventricular myocyte dissociation.** Animals were anesthetized with pentobarbital sodium (30 mg/kg), intubated, and ventilated with a volume ventilator. The heart was excised for cell dissociation. Left ventricular myocytes were prepared as previously described (31, 35). Briefly, the circumflex area was removed from the heart and immediately submerged in ice-cold minimal essential medium (MEM; Sigma) supplemented with 10 mM taurine, 2 mM l-glutamic acid, and 20 mM HEPES, pH 7.2. While submerged, the circumflex area was quickly cannulated and retrograde perfusion of the circumflex artery using Langendorff apparatus was begun at 90 mmHg constant pressure at 37°C. After 5 min of perfusion with MEM, the heart was perfused with MEM that contained 0.1% type II collagenase (Worthington) for 25 min. All perfusion media were maintained at 37°C and equilibrated with water-saturated oxygen. After collagenase perfusion, the circumflex area was removed from the perfusion apparatus and cut into small pieces. The tissue suspension was further treated with MEM that contained 0.1% collagenase and 0.5% bovine serum albumin (BSA, fraction V; Sigma) at 37°C and gently stirred at 2 cycles/s for 5-10 min. A slurry that contained isolated myocytes was decanted from tissue suspension. The isolated cells were washed three times in MEM that contained 0.5% BSA and were 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 functional measurements.** Individual ventricular myocytes were studied for function. Cells were suspended in 2 ml of 2 mM Ca²⁺ MEM solution that contained 0.5% BSA maintained at 37°C in a chamber that was fitted onto the stage of an inverted light microscope (Zeiss Axiovert 125; Carl Zeiss). 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 online using a video edge-detector system (model VED-114; Crystal Biotech; Patton Biomedical) and a video camera that 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 were then used to analyze the data. Cells used to determine the functional parameters were healthy and could react to agents that alter cAMP or cGMP levels throughout the course of the experiment. Cell contraction measurements were obtained from three or four 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.

**Experimental protocol.** Ventricular myocytes from normal (n = 6), hypertrophy (n = 6), and failing (n = 6) canine hearts were used in the protocol for cell functional measurements. In all groups, myocytes in appropriate concentration were suspended in a chamber with 2 ml of MEM that contained 2 mM Ca²⁺ and 0.5% BSA. After a 10-min stabilization period during which the cells were 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 cell contractility was measured. In all groups, 8-bromo-cGMP was added at concentrations of 10⁻⁷, 10⁻⁶, and 10⁻⁵ M. Data were also obtained at baseline, with 10⁻⁷ M EHNA plus 8-bromo-cGMP (10⁻⁷, 10⁻⁶, and 10⁻⁵ M), or with 10⁻⁶ M milrinone plus 8-bromo-cGMP (10⁻⁷, 10⁻⁶, and 10⁻⁵ M). A minimum of 10 consecutive contractions was used for each data point. For each of the three protocols, at least three or four cells in each animal were repeatedly measured. Measurements obtained included resting cell length, absolute cell shortening, maximal rate of shortening, rate of cell relaxation, and calculated percentage of cell shortening.

**Determination of cAMP level.** Myocytes from control, hypertrophic, and failing hearts were collected and incubated under electric stimulation and were gently swirled at 2 cycles/s at 37°C for 5 min in the absence or presence of 10⁻⁵ M 8-bromo-cGMP, 10⁻⁶ EHNA, 10⁻⁶ M milrinone, a combination of 10⁻⁵ M 8-bromo-cGMP and 10⁻⁶ EHNA, or 10⁻⁵ M 8-bromo-cGMP with 10⁻⁶ M milrinone. At the end of the incubation, myocyte samples were centrifuged, and pellets were frozen in liquid nitrogen and immediately stored at −80°C.

cAMP levels were determined using a competitive binding assay (Amer sham; Arlington Heights, IL) measuring the displacement of [³²P]cAMP from a binding protein. Samples (200 μl) were placed in scintillation vials with 10 ml of scintillation fluid and were counted in a scintillation counter. The level of cAMP was corrected for the interference caused by 8-bromo-cGMP. After a standard curve was constructed, cAMP levels were determined directly from the counts by a linear regression to obtain values in picomoles per 10⁵ myocytes. cAMP values were also calculated on a per unit volume basis from cell length and weight determinations by assuming that the myocytes were cylinders.

**Statistics.** Results are expressed as means ± SE. A repeated-measure ANOVA was used to compare variables measured at baseline and with the various agents employed in the three experimental groups.
groups. Duncan’s post hoc test was used to compare differences between the various treatments and groups. In all cases, a value of \( P < 0.05 \) was accepted as significant.

RESULTS

Body and heart weights of all control, hypertrophic, and hypertrophic failure dogs were determined to evaluate the degree of cardiac hypertrophy. Although body weights of canines from all groups were similar (control, 23.4 ± 1.3; hypertrophy, 21.6 ± 1.2; failure, 23.1 ± 0.3 kg), heart weights were increased in hypertrophic and failing heart dogs (control, 181 ± 7; hypertrophy, 246 ± 26; failure, 245 ± 13 g). The heart weight-to-body weight ratio in hypertrophy (11.40 ± 0.97 g/kg) and failure (10.61 ± 0.61 g/kg) dogs was higher than that of control canines (7.82 ± 0.45 g/kg).

Baseline myocyte function in control, hypertrophic, and failing myocytes. Baseline ventricular myocyte maximal rate of shortening and relaxation and percent shortening were slightly reduced in hypertrophic myocytes with no statistical difference compared with controls. In contrast, baseline contractility in the failing myocytes was much lower than that of control myocytes (Table 1). All reported parameters were reduced in the failing myocytes. Neither EHNA nor milrinone alone altered myocyte function in the control, hypertrophic, or hypertrophic failure groups (Table 1).

Effects of cGMP on myocyte function: interaction with milrinone and EHNA. In control as well as in hypertrophic and failing myocytes, cGMP reduced myocyte contractility. These data are presented as percent decrements from initial values for maximal rate of shortening, maximal rate of relaxation, and percent shortening (Figs. 1, 2, and 3). When 8-bromo-cGMP at \( 10^{-7}, 10^{-6}, \) or \( 10^{-5} \) M concentration was added to control ventricular myocytes, percent shortening, maximum rate of shortening, and relaxation were all decreased in a concentration-dependent manner. When 8-bromo-cGMP at \( 10^{-5} \) M concentration was added to control myocytes, percent shortening was decreased 50%. Percent shortening of hypertrophic myocytes was further reduced by 8-bromo-cGMP treatment.

Table 1. Effects of cAMP phosphodiesterase inhibitors EHNA and milrinone on cell contractility of control, hypertrophy, and failure ventricular myocytes

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>Percent Shortening, %</th>
<th>Maximum Rate of Shortening, ( \mu \text{m/s} )</th>
<th>Maximum Rate of Relaxation, ( \mu \text{m/s} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.97±0.34</td>
<td>79.1±11.0</td>
<td>78.7±11.5</td>
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<td>EHNA</td>
<td>4.02±0.31</td>
<td>66.5±9.1</td>
<td>66.4±9.8</td>
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<tr>
<td>Baseline</td>
<td>4.98±0.63</td>
<td>75.8±6.7</td>
<td>75.2±6.2</td>
</tr>
<tr>
<td>Milrinone</td>
<td>4.72±0.80</td>
<td>68.9±10.1</td>
<td>69.3±9.7</td>
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<tr>
<td>Hypertrophy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.55±0.25</td>
<td>77.0±1.8</td>
<td>77.0±2.7</td>
</tr>
<tr>
<td>EHNA</td>
<td>3.55±0.21</td>
<td>59.0±3.1</td>
<td>61.3±5.0</td>
</tr>
<tr>
<td>Baseline</td>
<td>4.37±0.39</td>
<td>69.6±3.0</td>
<td>67.6±2.6</td>
</tr>
<tr>
<td>Milrinone</td>
<td>4.55±0.37</td>
<td>70.9±6.8</td>
<td>73.1±7.1</td>
</tr>
<tr>
<td>Failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.96±0.63*</td>
<td>54.5±5.3*†</td>
<td>56.4±5.6*†</td>
</tr>
<tr>
<td>EHNA</td>
<td>2.8±0.49*</td>
<td>39.4±4.3*†</td>
<td>43.0±4.7*†</td>
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<tr>
<td>Baseline</td>
<td>3.71±0.43</td>
<td>48.4±3.8*†</td>
<td>48.1±3.3*†</td>
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<tr>
<td>Milrinone</td>
<td>3.15±0.50</td>
<td>41.8±4.4*†</td>
<td>44.6±5.2*†</td>
</tr>
</tbody>
</table>

Values are means ± SE. EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine. *\( P < 0.05 \) vs. control; †\( P < 0.05 \) vs. hypertrophy.

Fig. 1. Decrement in maximum rate of shortening (as percentage of initial value) of control, hypertrophy, and failure myocytes obtained using a video edge detector before and after cells were treated with 8-bromo-cGMP at concentrations of \( 10^{-7}, 10^{-6}, \) and \( 10^{-5} \) M. Effects of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) and milrinone (MIL) on 8-bromo-cGMP-reduced myocyte maximum rate of shortening were compared with myocytes treated with 8-bromo-cGMP alone (CON). \*\( P < 0.05 \), significantly different from baseline (Base); \#\( P < 0.05 \), significantly different from myocytes treated with 8-bromo-cGMP alone.
Decrement in maximum rate of relaxation (as percentage of initial value) of control, hypertrophy, and failure myocytes obtained using a video edge detector before and after cells were treated with 8-bromo-cGMP at concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M. Effects of EHNA and milrinone on 8-bromo-cGMP-reduced myocyte maximum rate of relaxation were compared with myocytes treated with 8-bromo-cGMP alone. *P < 0.05, significantly different from baseline; #P < 0.05, significantly different from myocytes treated with 8-bromo-cGMP alone.

Decrement in percentage of shortening (as percentage of initial value) of control, hypertrophy, and failure myocytes obtained using a video edge detector before and after cells treated with 8-bromo-cGMP at concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M. Effects of EHNA and milrinone on 8-bromo-cGMP-reduced myocyte percentage of shortening were compared with myocytes treated with 8-bromo-cGMP alone. *P < 0.05, significantly different from baseline; #P < 0.05, significantly different from myocytes treated with 8-bromo-cGMP alone.
myocytes and failing myocytes decreased 40 and 50%, respectively, in the presence of 8-bromo-cGMP at $10^{-5}$ M concentration despite the lower initial baseline value compared with controls. Similarly, the maximal rates of shortening and relaxation were also decreased in control, hypertrophic, and failing myocytes by 8-bromo-cGMP.

Addition of EHNA at $10^{-6}$ M or milrinone at $10^{-6}$ M concentration to control ventricular myocytes reduced the negative effects of 8-bromo-cGMP on cell contractility. Percent shortening of control myocytes was 77% of the baseline when treated with both EHNA and 8-bromo-cGMP at $10^{-5}$ M and 81% when treated with both milrinone and 8-bromo-cGMP at $10^{-5}$ M, whereas it was 50% when treated with 8-bromo-cGMP alone (Fig. 1). Similarly, in control myocytes, 8-bromo-cGMP at $10^{-6}$ and $10^{-5}$ M concentrations no longer lowered the maximum rates of shortening and relaxation in the presence of EHNA or milrinone (Figs. 2 and 3). In hypertrophic myocytes, EHNA and milrinone reduced the negative effects of 8-bromo-cGMP at $10^{-5}$ M concentration on myocyte contractility to a lesser degree. Percent shortening of hypertrophic myocytes was 70% when both EHNA and 8-bromo-cGMP were present compared with baseline and 73% when both 8-bromo-cGMP and milrinone were present, whereas it was 60% when treated with 8-bromo-cGMP alone. Neither EHNA nor milrinone reversed the effects of 8-bromo-cGMP on myocyte percent shortening, maximum rate of shortening, or relaxation despite the lower initial baseline value compared with controls.

**Effects of 8-bromo-cGMP, EHNA, and milrinone on intracellular cAMP levels.** The intracellular cAMP levels of ventricular myocytes from control, hypertrophic, and failing canine hearts were measured while the cells were electrically stimulated. When exogenous 8-bromo-cGMP ($10^{-5}$ M) was added to cells, the cAMP level was increased in control, hypertrophic, and failing myocytes (Fig. 4). When EHNA alone was added to myocytes, the intracellular cAMP level did not change in any of the three groups. When milrinone alone was added to myocytes, the cAMP level was only increased in control myocytes and did not change in hypertrophic and failing myocytes. Similarly, these inhibitors did not change myocyte contractility. When a combination of EHNA and 8-bromo-cGMP or milrinone and 8-bromo-cGMP was added to the myocytes, the cAMP level was increased in all of these conditions in control, hypertrophic, and failing myocytes. The differences between baseline values and the various treatments were the same when cAMP values were examined on a per unit volume basis (Fig. 5). Baseline values were not different between groups, although cells treated with EHNA or milrinone had lower values in the failing myocytes compared with controls.

**DISCUSSION**

One major finding of this study was that cGMP dose dependently reduced ventricular myocyte contractility in control, hypertrophic, and failing hearts, which suggests that the cGMP signaling pathway was preserved in hypertrophic and hypertrophic failing cardiac myocytes. This was despite a previously reported reduction in the effects of this pathway in vivo during hypertrophic failure (8). We also found that specific cGMP-affected cAMP phosphodiesterase inhibitors (EHNA and milrinone) reduced the negative functional effects of cGMP in control and hypertrophic myocytes but not in failing myocytes. This indicates that the cGMP-regulated cAMP phosphodiesterases can have a major impact on the negative effects of cGMP on myocyte function. In all groups, the combination of phosphodiesterase inhibitors and cGMP led to a significant increase in cAMP. Thus in cardiac failure, the inability of cAMP to prevent the negative effects of cGMP must be related to a downstream component in the cAMP signaling system.

Nitric oxide and its second messenger cGMP usually have negative metabolic and functional effects on cardiac myocytes as reported in many but not all studies (11, 31). This signal transduction system causes reductions in local myocardial shortening, force development, and oxygen consumption (10, 11, 21). Nitric oxide activates soluble guanylate cyclase in myocytes and produces cGMP molecules. The effects of cGMP operate through direct inhibition of L-type Ca$^{2+}$ channels, cGMP-dependent protein kinase, and cGMP-regulated cAMP phosphodiesterases (12, 23, 31). In this study, we showed that ventricular myocytes from control, hypertrophic, and failing canine heart all responded to cGMP in a similar...
dose-dependent manner. Myocyte percent shortening, maximum rate of shortening, and relaxation were decreased in the presence of cGMP in all groups.

The cAMP phosphodiesterases play an important role in cAMP degradation. cGMP-regulated cAMP phosphodiesterases serve as a bridge between the cAMP and cGMP signaling pathways and are a possible feedback mechanism for cGMP-regulated cardiac function. Phosphodiesterase inhibitors have been extensively used in clinical, academic, and pharmaceutical research (4, 15). In this study, we showed that milrinone, an inhibitor for PDE3, increased intracellular cAMP in control myocytes, whereas EHNA, an inhibitor of PDE2, did not cause a statistically significant increase in cAMP. This could be due to a lesser amount of PDE2 compared with PDE3 that exists in canine cardiac myocytes (22). It could also be related to dosing or the low intrinsic production of cAMP in isolated myocytes due to a lack of sympathetic activity. In vivo studies have shown that milrinone can increase cAMP levels as well as heart function (13, 15). However, in this study, milrinone only increased intracellular cAMP levels and did not increase myocyte contractility. This could be explained by a low basal level of cAMP due to a lack of stimulation by the sympathetic nervous system in isolated ventricular myocytes. Neither EHNA nor milrinone alone significantly altered intracellular cAMP levels in hypertrophic or failing myocytes.

cGMP affects cAMP degradation through effects on the cGMP-regulated cAMP phosphodiesterases. Because cGMP stimulates PDE2 and inhibits PDE3, a combination of cGMP and EHNA should result in inhibition of both PDE2 and PDE3, and a combination of cGMP and milrinone should result in inhibition of PDE3 and stimulation of PDE2. This was observed in isolated rabbit myocytes (30). In the present study, we found that both combinations significantly increased intracellular cAMP levels in all groups. These combinations reduced the negative effects of cGMP on control myocytes, which suggests that cGMP interacts with cAMP through effects on the cGMP-regulated cAMP phosphodiesterases.

Hypertrophy is often an important precursor to heart failure (6). Two major factors, mechanical stress and neural/hormone levels, stimulate intracellular signaling pathways, alter gene expression, and result in increases in cardiac myocyte size, extracellular matrices, and heart mass. The molecular composition of failing heart is altered in various aspects including contractile proteins, Ca$^{2+}$ cycling, signal transduction pathways, metabolism, endocrine function, and extracellular matrix (17). Experimental animal models have been developed to investigate heart hypertrophy and failure (18, 34). Most pacing models lead to dilated myocardial failure without hypertrophy. Previous studies have demonstrated that in aortic valve stenosis-induced left ventricular hypertrophy in dogs, baseline metabolism and cardiac function were similar to control values (13, 19). Basal ventricular myocyte function was unchanged in hypertrophied myocytes compared with control myocytes. However, myocardial and myocyte function and metabolism were diminished in the transition from hypertrophy to cardiac failure (8, 13). Our model demonstrates a combination of both cardiac hypertrophy and symptoms of failure. In the present study, we found a significant decrease in basal myocyte function in this hypertrophic failure group.

We found that the negative functional effects of cGMP on ventricular myocyte function were preserved in both hypertrophic and hypertrophic failing myocytes. This was true for all reported parameters. This suggests the possibility of using the cGMP signal transduction system for treatment of human cardiac failure as has been previously suggested (32, 33). The myocardial response to inhibition of guanylyl cyclase by methylene blue on in vivo regional myocardial work and oxygen consumption was preserved in hypertrophic hearts but was markedly diminished in failing hearts (8). By using isolated ventricular myocytes and stimulating rather than inhibiting, we found that the individual hypertrophic and hypertrophic failing myocytes in vitro still preserved the ability to respond to increases in cGMP. This difference could be related to the differences in the endogenous level of cGMP. Nitric oxide, which stimulates cGMP production, can act as a negative regulator of cardiomyocyte hypertrophy (32). Mechanisms for this inhibitory effect of nitric oxide/cGMP on cardiomyocyte hypertrophy are not well understood. Gene transfer of cGMP-dependent protein kinase I enhanced the antihypertrophic effects of nitric oxide as shown by the enhanced inhibitory effects of the nitric oxide donor S-nitroso-N-acetyl-l-arginine and cGMP on α-1-adrenergic cardiomyocyte hypertrophy (33). This preserved negative functional effect of cGMP might be protective against high sympathetic activity in failure.

Although some studies have shown a decreased response to catecholamines and a decreased β-adrenoceptor number in hypertrophied left ventricle (13, 20, 25, 29), other studies suggested maintenance of responsiveness to β-adrenoceptor stimulation in the hypertrophied model (13, 20, 27). We found that hypertrophic myocytes were similar to control myocytes with minimally reduced contractility and a similar lack of myocyte responses to cAMP phosphodiesterase inhibitors. Baseline myocyte contractility in failing myocytes was significantly lower than that of control myocytes. We did not observe significantly reduced baseline cAMP levels in these failing myocytes as was reported in vivo (7, 25). This could be related to the method of data normalization or the lack of sympathetic stimulation in vitro. It has been reported that the effects of milrinone were reduced in a conscious pacing-induced failure model (18), although we observed little effect of milrinone alone in any group. In addition, in our failing myocytes, the ability of phosphodiesterase inhibitors to interfere with the cGMP pathway through the cAMP pathway was lost. We found in canine ventricular myocytes that the phosphodiesterase inhibitors reduced the negative functional effects of cGMP and were accompanied by an increase in intracellular cAMP. However, this cross-talk or effect of cAMP in overcoming the negative functional effects of cGMP was diminished in hypertrophic heart failure. The interaction between cAMP and cGMP in the control of myocyte function was lost in hypertrophic failing myocytes, although both combinations increased cAMP levels in these myocytes from failing hearts. These changes in the effects and interactions of the cAMP phosphodiesterases in failing myocytes may help to explain their limited success in treating heart failure in humans. This suggests that some downstream components, e.g., cAMP protein kinase, various phosphorylated proteins, or other parts of this pathway, were altered in the hypertrophic failing myocytes. This appeared to prevent the phosphodiesterase inhibitors from blocking the negative functional effects of cGMP in these myocytes.
In summary, these data indicate that cGMP reduces cell contractility in control, hypertrophic, and failing ventricular myocytes and suggest that the cGMP signaling pathway was preserved in hypertrophic failing cardiac myocytes. cGMP-regulated cAMP phosphodiesterase inhibition reversed the negative functional effects of cGMP on myocyte function in control ventricular myocytes and also led to a significant increase in cAMP when the inhibitors were administered simultaneously. In hypertrophic myocytes, both the functional and cAMP effects of cGMP and the cAMP phosphodiesterase inhibitors were preserved. However, in the hypertrophic failing myocytes, the negative functional effects of cGMP were preserved, but the interaction of cAMP with the cGMP signaling pathway was impaired. This was despite a significant increase in cAMP when the inhibitors were combined with cGMP. This suggests an important alteration in signal transduction in these failing myocytes.

GRANTS

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