Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 481 (2003) 25–31
www.elsevier.com/locate/ejphar

Negative functional effects of cyclic GMP are altered by cyclic AMP phosphodiesterases in rabbit cardiac myocytes

Harvey R. Weiss[a,*], Michael J. Lazar[b], Kusum Punjabi[a], James Tse[c], Peter M. Scholz[b]

[a] *Heart and Brain Circulation Laboratory, Department of Physiology and Biophysics, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854-5635, USA*
[b] *Heart and Brain Circulation Laboratory, Department of Surgery, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635, USA*
[c] *Heart and Brain Circulation Laboratory, Department of Anesthesia, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635, USA*

Received 17 April 2003; received in revised form 26 August 2003; accepted 3 September 2003

Abstract

In this study, we tested the hypothesis that the negative functional effects of cyclic GMP on cardiac myocytes would be affected by the actions of cyclic GMP on cyclic AMP phosphodiesterases. Ventricular myocytes from eight rabbits were used to determine the functional and cyclic AMP changes caused by 10^{-7}, 10^{-6}, 10^{-5} M 8-Bromo-cGMP alone and after the administration of 10^{-6} M milrinone (cyclic GMP-inhibited cyclic AMP phosphodiesterase inhibitor) or 10^{-6} M *erythro*-9-(2-Hydroxy-3-3-nonyl)adenine (EHNA, cyclic GMP-stimulated cyclic AMP phosphodiesterase inhibitor). 8-Br-cGMP dose-dependently reduced %shortening by 35 ± 4% of baseline at 10^{-5} M. This effect was significantly blunted by EHNA at all doses. The maximum rate of shortening was reduced by 31 ± 3% by 10^{-5} M 8-Br-cGMP. This effect of 8-Br-cGMP was significantly enhanced (42 ± 4%) in the milrinone group. A similar pattern was observed in the maximum rate of relaxation data. Cyclic AMP levels were significantly increased from a baseline level of 4.0 ± 0.8 pmol/10^5 myocytes by milrinone (+60%), EHNA (+61%) and 8-Br-cGMP (+47%). The combination of EHNA plus 8-Br-cGMP increased cyclic AMP levels significantly more that the combination of milrinone plus 8-Br-cGMP. Exogenous cyclic GMP reduces myocyte function, while raising cyclic AMP possibly through cyclic GMP-inhibited cyclic AMP phosphodiesterase effects. Blocking cyclic GMP-inhibited cyclic AMP phosphodiesterase enhances the functional effects cyclic GMP, while blocking cyclic GMP-stimulated cyclic AMP phosphodiesterase reduced these effects. The study demonstrated a functional interaction between cyclic GMP and cyclic AMP related to the cyclic GMP affected cyclic AMP phosphodiesterases.

Keywords: Cardiac myocyte; Cyclic GMP; Cyclic AMP; Cyclic GMP affected cyclic AMP phosphodiesterases; (Rabbit)

1. Introduction

The second messenger guanosine 3′, 5′-cyclic monophosphate (cyclic GMP) has negative functional and metabolic effects on both the myocardium and isolated cardiac myocytes (Murad, 1994; Shah and MacCarthy, 2000; Shah et al., 1994). Many studies have shown that cyclic GMP antagonizes the positive metabolic and functional effects of catecholamines and cyclic AMP (Corwell et al., 1994; Mery et al., 1993; Shah and MacCarthy, 2000). The mechanisms of action of cyclic GMP include phosphorylation of proteins by cyclic GMP-dependent protein kinase and effects on cyclic GMP-stimulated or cyclic GMP-inhibited cyclic AMP phosphodiesterases, which change the level of cyclic AMP (Corwell et al., 1994; Mery et al., 1993). There may also be an interaction between these second messengers at the level of their protein kinases (Yan et al., 2000; Shah and MacCarthy, 2000). Several studies have shown that protein phosphorylation is a major pathway for the effects of cyclic GMP, and that blocking of this pathway will significantly diminish the effects of cyclic GMP (Coppenolle et al., 1997; Sumiim and Sperelakis, 1995; Wang et al., 2000). The cyclic AMP-dependent protein kinase plays a key role in the mediation of the effects of cyclic AMP on cardiac myocytes, by phosphorylating proteins including those involved in Ca^{2+} transport and in the control of the contractile apparatus (Wang et al.,

* Corresponding author. Tel.: +1-732-235-4626; fax: +1-732-235-5038.
E-mail address: hweiss@umdnj.edu (H.R. Weiss).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.09.006

2000; Hove-Madsen et al., 1996; Sumiim and Sperelakis, 1995).

Since cyclic GMP levels can affect the level of cyclic AMP in the heart, this can cause significant interaction and effects on function (Naim et al., 1998). In fact, several groups have reported positive functional effects of nitric oxide and cyclic GMP in cardiac myocytes that have been, in part, ascribed to increases in cyclic AMP (Hirota et al., 2001; Kojda and Kottenberg, 1999). There is a large superfamily of cyclic nucleotide phosphodiesterases that degrade cyclic AMP and cyclic GMP (Francis et al., 2001; Steinberg and Brunton, 2001). The activity of at least two cyclic AMP phosphodiesterases are affected by cyclic GMP, the cyclic GMP-stimulated cyclic AMP phosphodiesterase and the cyclic GMP-inhibited cyclic AMP phosphodiesterase. Depending on the relative activity of the phosphodiesterases, cyclic GMP can either raise or lower the level of cyclic AMP (Naim et al., 1998; Kojda and Kottenberg, 1999; Gustafsson and Brunton, 2002; Weiss et al., 1999). The roles of these various phosphodiesterase isozymes have not been well established, but it has been suggested that they may be important in regulating L-type Ca^{2+} current (Mery et al., 1993; Ahmmed et al., 2001; Kajimoto et al., 1997; Vandecasteele et al., 2001).

We tested the hypothesis that the negative functional effects of cyclic GMP on cardiac myocytes would be affected by the action of cyclic GMP on cyclic AMP phosphodiesterases. This hypothesis was tested in isolated ventricular myocytes from New Zealand white rabbits. The effects of increasing doses of a cell permeable form of the second messenger cyclic GMP (8-Bromo-cGMP) on myocyte function were determined. These effects were also determined after blockade of the cyclic GMP-inhibited cyclic AMP phosphodiesterase with milrinone and the cyclic GMP-stimulated cyclic AMP phosphodiesterase with *erythro*-9-(2-Hydroxy-3-3-nonyl)adenine (EHNA). We determined that cyclic GMP increased the level of cyclic AMP and that the negative functional effects of cyclic GMP depended, in part, on the activity of cyclic GMP on cyclic GMP-inhibited and cyclic GMP-stimulated cyclic AMP phosphodiesterases.

2. Methods

New Zealand white rabbits ($n = 10$), weighing 2–3 kg, were used in this study. Experiments were performed on ventricular myocytes isolated from the hearts of these rabbits. All experiments were conducted in accordance with Guide for the Care of Laboratory Animals (DHHS Publication No. 85-23, revised 1996) and were approved by our Institutional Animal Care and Use Committee.

2.1. Cell dissociation

Freshly isolated ventricular myocytes were prepared by a standard method as described previously (Yan et al., 2000; Straznicka et al., 1999; Zhang et al., 2002). The rabbits were anesthetized and the heart was rapidly removed after an overdose of pentobarbital (100 mg/kg). Retrograde aortic perfusion of the heart was immediately begun at 70 mm Hg constant pressure with HEPES buffered minimal essential medium (MEM). This low-Ca^{2+} MEM solution had an osmolality of 296 mOsm, and the free Ca^{2+} activity was 2–5 μM. After 5 min of perfusion with low-Ca^{2+} MEM, the heart was perfused at 50 mm Hg with a 60-ml volume of low-Ca^{2+} MEM supplemented with 0.1% collagenase (Worthington type II). After 20 min of collagenase perfusion with recirculation, the heart was removed from the perfusion apparatus and cut into 8–10 pieces in MEM containing 1 mM $CaCl_2$ and 0.5% bovine serum albumin. This Ca^{2+}-MEM was supplemented with 0.1% collagenase. The tissue suspension was gently swirled by a wrist action shaker for 5 min. A slurry containing isolated heart cells was decanted from the tissue suspension. The isolated cells were washed three times. The combined, washed cells were then maintained at room temperature. The viability of the myocytes was about 55–70%. Yields were typically $10–14 \times 10^8$ rod-shaped cells per heart. Cells previously isolated in this fashion have been shown to have an intact cell coat as well as a functional sarcolemma and normal permeability barriers to extracellular ions, ADP and succinate.

2.2. Cell shortening measurement

Isolated cardiac myocytes were put into a open chamber (37 °C) on the stage of an inverted microscope (Zeiss Axiovert 125) with 2.0 mM Ca^{2+} MEM solution. The volume of the chamber was 3.5 ml. At least 5 min was allowed to let the myocytes equilibrate. Myocytes were paced with extracellular field electrical stimulation (1–4 Hz, 5-ms duration, voltage at 10% above threshold, and the polarity alternated each pulse) by two platinum wires inserted into the center of the myocyte suspension. Unloaded cell shortening was measured on-line using a video-edge detector (Myotrack system, Crystal Biotech, Model VED-114) and a camera, which detected the change of the position of both edges of the cell. The output of the video-edge detector was fed into both a television monitor and a desktop computer. We determined percent shortening as (100∗(maximum length − minimum length)/maximum length) and maximal rate of shortening from the maximal first derivative of shortening. A 5-min stabilization was allowed after which contraction data for the myocytes were recorded from a minimum of 10 consecutive contractions. Observations were performed individually on at least four cells per heart. Cell viability at the conclusion of the experiment was assessed by maintenance of rod-shaped morphology and by responsiveness to electrical pacing.

2.3. Cyclic AMP measurements

In order to determine cyclic AMP levels, myocytes were warmed to 0 °C and homogenized in ethanol using a

Table 1
Effects of 8-Br-cGMP alone and with milrinone or EHNA on percent shortening (%), maximum rate of shortening (μm/s) and maximum rate of relaxation

	%Shortening				Maximum rate of shortening			Maximum rate of relaxation	
	CON	MIL	EHNA	CON	MIL	EHNA	CON	MIL	EHNA
Baseline	6.0 ± 0.3	4.7 ± 0.5	5.3 ± 0.6	83.9 ± 5.1	74.4 ± 5.0	78.6 ± 8.2	84.5 ± 5.4	73.0 ± 4.9	76.8 ± 8.5
Treatment		4.9 ± 0.6	4.7 ± 0.5		78.7 ± 8.1	73.8 ± 8.7		74.1 ± 7.3	71.4 ± 8.6
10^{-7} M 8-Br-cGMP	4.8 ± 0.5	3.8 ± 0.5[a]	4.3 ± 0.5	68.7 ± 5.7[b]	64.2 ± 6.9[a]	69.6 ± 7.4	68.2 ± 5.1[b]	63.9 ± 6.6	68.0 ± 7.3
10^{-6} M 8-Br-cGMP	4.4 ± 0.4[b]	3.5 ± 0.5[a]	3.9 ± 0.5	65.2 ± 5.6[b]	57.2 ± 5.7[a]	60.4 ± 5.7	63.9 ± 5.3[b]	57.4 ± 6.3[a]	59.3 ± 5.5[b]
10^{-5} M 8-Br-cGMP	4.2 ± 0.4[b]	2.8 ± 0.2[a,b,c]	3.7 ± 0.5	64.3 ± 5.8[b]	49.5 ± 2.9[a,b,c]	58.9 ± 5.6[a,b]	63.5 ± 5.6[b]	52.2 ± 3.7[a,b,c]	57.7 ± 5.7[a,b]

Values are presented as mean ± S.E.M., $N = 10$.
[a] Significantly different from treatment.
[b] Significantly different from baseline.
[c] Significantly different from CON.

Brinkmann Polytron in an ice bath. The homogenate was centrifuged at 30,000 × *g* for 15 min in a Sorvall RC-5B centrifuge. The supernate was recovered. The pellet was resuspended in 1 ml of 2:1 ethanol–water and centrifuged as before. The combined supernatants were evaporated to dryness in a 60 °C bath under a stream of nitrogen gas. The final residue was dissolved in 1.5 ml of assay buffer (0.05 M sodium acetate, pH 5.8, containing sodium azide). Cyclic AMP levels were determined by radioimmunoassay using a scintillation proximity assay (Amersham). This assay measures the competitive binding of ^{125}I-labeled cyclic AMP specific antibody. After construction of the standard curve, cyclic AMP levels were determined directly from the counts in pmol/10^5 myocytes. The levels were corrected for overlap of 8-Br-cGMP onto cyclic AMP.

2.4. Experimental protocol

The following protocol was used for the cell shortening and cyclic AMP measurements. Myocytes were placed in a chamber with 2 mM Ca^{2+}-MEM during electrical field stimulation (1 Hz, 5 ms, and voltage 10% above threshold) and were allowed to stabilize for 10 min. A 5-min recording was made for baseline measurements. Cell shortening data were then measured after the addition of 8-Bromo-guanosine 3′, 5′-cyclic monophosphate (8-Br-cGMP) 10^{-7}, 10^{-6}, and 10^{-5} M. After baseline measurements, cell shortening data were also obtained after the addition of milrinone (10^{-6} M), a cyclic GMP-inhibited cyclic AMP phosphodiesterase inhibitor, or *erythro*-9-(2-Hydroxy-3-3-nonyl)adenine (EHNA, 10^{-6} M), a cyclic GMP-stimulated cyclic AMP phosphodiesterase inhibitor, followed by 8-Br-cGMP 10^{-7}, 10^{-6}, and 10^{-5} M, in a dose-related manner. At least 5 min was allowed to obtain steady state after the addition of each reagent before measurements were taken. Identically treated groups of cells from each protocol were also frozen and later used to measure cyclic AMP levels. There groups use to analyze cyclic AMP were control, 8-Br-cGMP 10^{-5} M, milrinone 10^{-6} M, EHNA 10^{-6} M and their combination.

2.5. Statistics

All results are expressed as means ± S.E.M. A repeated measure analysis of variance (ANOVA) was used to compare variables measured in the experimental and control conditions. Duncan's multiple range test was used to compare the differences post hoc. This analysis was used to determine differences between groups and treatments for both cardiac myocyte function and cyclic AMP levels. In all cases, $P < 0.05$ was accepted as significant.

3. Results

3.1. Functional data

We report five parameters of myocyte function, percent shortening (%), maximal rate of shortening (μm/s), maximal rate of relaxation (μm/s), time to peak contraction (s) and 90% relaxation time (s) in electrically stimulated contracting ventricular cells. The baseline values for these parameters

Table 2
Effects of 8-Br-cGMP alone and with milrinone or EHNA on time to peak shortening (s) and time to 90% relaxation (s)

	Time to peak shortening			Time to 90% relaxation		
	CON	MIL	EHNA	CON	MIL	EHNA
Baseline	0.38 ± 0.01	0.40 ± 0.03	0.31 ± 0.02	0.36 ± 0.02	0.31 ± 0.02	0.31 ± 0.01
Treatment		0.40 ± 0.02	0.33 ± 0.01		0.33 ± 0.01	0.31 ± 0.02
10^{-7} M 8-Br-cGMP	0.41 ± 0.02	0.44 ± 0.03	0.37 ± 0.02	0.37 ± 0.02	0.35 ± 0.01	0.33 ± 0.02
10^{-6} M 8-Br-cGMP	0.40 ± 0.02	0.45 ± 0.02	0.35 ± 0.02	0.39 ± 0.01	0.35 ± 0.02	0.35 ± 0.02
10^{-5} M 8-Br-cGMP	0.44 ± 0.01[a]	0.45 ± 0.02	0.35 ± 0.03	0.38 ± 0.02	0.35 ± 0.03	0.34 ± 0.03

Values are presented as mean ± S.E.M., $N = 10$.
[a] Significantly different from baseline.

are presented in Tables 1 and 2. These initial values were similar in the control, milrinone and EHNA groups.

Increasing doses of 8-Br-cGMP led to a dose-dependent decrease in percent shortening, which was observed in all three groups, Table 1. Fig. 1 shows the decrement in percent shortening caused by 8-Br-cGMP as a percent of the starting value. Neither milrinone nor EHNA significantly affected percent shortening. However, EHNA significantly reduced the decrement in percent shortening caused by 8-Br-cGMP at all three doses. Milrinone did not significantly alter the response to 8-Br-cGMP in terms of percent shortening. However, it did significantly reduce the minimal value for percent shortening observed with 10^{-5} M 8-Br-cGMP compared to the control group, Table 1.

There was a dose-dependent decrease in maximal rate of shortening caused by increasing doses of 8-Br-cGMP, which was observed in all three groups, Table 1. Fig. 2 shows the decrement in maximal rate of shortening caused by 8-Br-cGMP as a percent of the starting value. The maximum rate of shortening was not significantly altered by administration of milrinone or EHNA. EHNA significantly reduced the decrement in maximal rate of shortening caused by 10^{-7} and 10^{-5} M 8-Br-cGMP. Milrinone significantly increased the maximal rate of shortening response to 10^{-5} M 8-Br-cGMP. It also significantly reduced the minimal value for maximal rate of shortening observed with 10^{-5} M 8-Br-cGMP compared to the control group, Table 1.

Increasing doses of 8-Br-cGMP led to a dose-dependent decrease in maximal rate of relaxation in all three groups, Table 1. Fig. 3 shows the decrement in maximal rate of relaxation caused by 8-Br-cGMP as a percent of the initial value. Neither milrinone nor EHNA significantly affected the maximum rate of relaxation. EHNA significantly reduced the decrement in maximal rate of relaxation caused by 10^{-7} and 10^{-5} M 8-Br-cGMP. Milrinone significantly increased the response to 10^{-5} M 8-Br-cGMP in terms of maximal rate of relaxation. Compared to the control group, milrinone also significantly reduced the maximal rate of relaxation observed with 10^{-5} M 8-Br-cGMP.

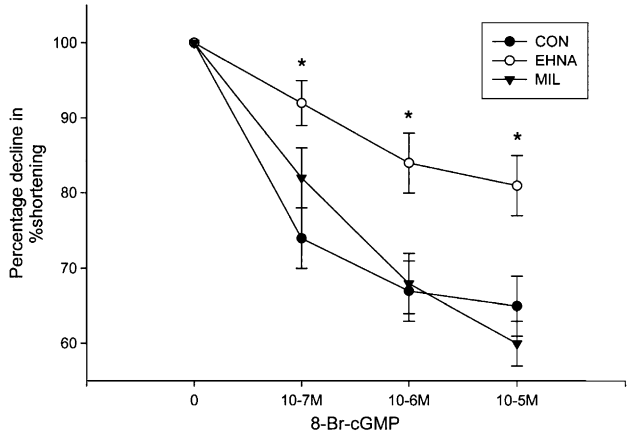

Fig. 1. The percentage decline in percent shortening caused by increasing doses of 8-Br-cGMP in the control group and the groups treated with milrinone and EHNA is shown ($N=10$). All values are significantly below the initial value. *Significantly different from control group value.

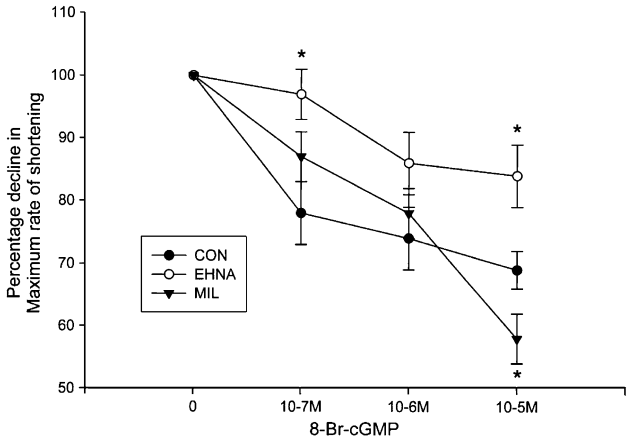

Fig. 2. The percentage decline in maximum rate of shortening caused by increasing doses of 8-Br-cGMP in the control group and the groups treated with milrinone and EHNA is shown ($N=10$). All values are significantly below the initial value. *Significantly different from control group value.

The administration of 8-Br-cGMP led to an increase in the time to peak contraction that was statistically significant at 10^{-5} M in the control group, Table 2. Neither milrinone nor EHNA significantly affected the time to peak contraction. The time to 90% relaxation was not significantly altered in any of the three treatment groups by 8-Br-cGMP, milrinone or EHNA.

3.2. Cyclic AMP data

Compared to the basal value, the level of cyclic AMP was significantly increased by the two cyclic AMP phosphodiesterase inhibitors, milrinone (+60%) and EHNA (+61%), Fig. 4. Myocyte cyclic AMP levels were also significantly increased by 10^{-5} M 8-Br-cGMP (+47%)

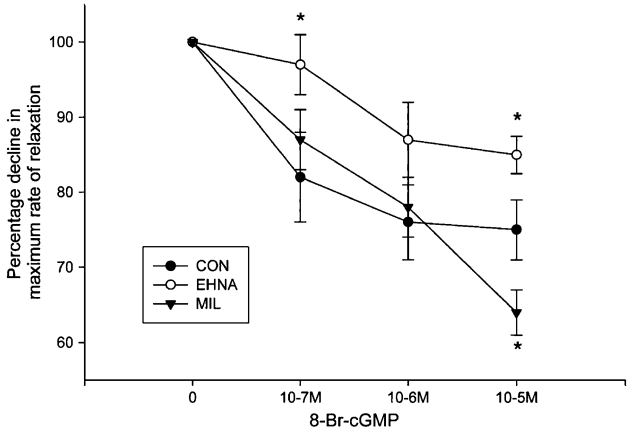

Fig. 3. The percentage decline in maximum rate of relaxation caused by increasing doses of 8-Br-cGMP in the control group and the groups treated with milrinone and EHNA is shown ($N=10$). All values except 10^{-7} M 8-Br-cGMP in the presence of EHNA are significantly below the initial value. *Significantly different from control group value.

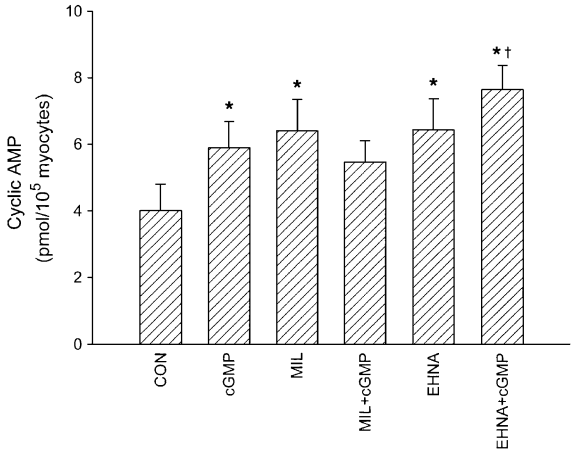

Fig. 4. Cyclic AMP levels in ventricular myocytes at baseline or treated with 10^{-5} M 8-Br-cGMP, milrinone, EHNA or their combination are shown ($N=8$). *Significantly different from the control group value. †Significantly different from the milrinone plus 8-Br-cGMP group value.

alone. The level of cyclic AMP in the milrinone plus 8-Br-cGMP group was not significantly increased over the control group. The cyclic AMP level in ventricular myocytes of the EHNA plus 8-Br-cGMP group was significantly greater than the control group as well as the milrinone plus 8-Br-cGMP group.

4. Discussion

In this study, we demonstrated that increasing the level of cyclic GMP in ventricular myocytes led to a reduction in function, despite an increase in the inotropic second messenger cyclic AMP. The major finding of this study was that the negative functional effects of cyclic GMP were enhanced by blocking the cyclic GMP-inhibited cyclic AMP phosphodiesterase and this reduced the rise in cyclic AMP and that the negative functional effects of cyclic GMP were blunted by blockade of the cyclic GMP-stimulated cyclic AMP phosphodiesterase. Cyclic GMP caused a greater rise in cyclic AMP after cyclic GMP-stimulated AMP phosphodiesterase blockade compared to blockade of cyclic GMP-inhibited cyclic AMP phosphodiesterase. The effects of cyclic GMP on the level of cyclic AMP appear to depend on the relative activity of cyclic GMP-stimulated cyclic AMP phosphodiesterase and cyclic GMP-inhibited cyclic AMP phosphodiesterase. At baseline, the actions of cyclic GMP-inhibited cyclic AMP phosphodiesterase seem to predominate but this does not prevent the negative functional effect of cyclic GMP on ventricular myocytes.

The use of isolated rabbit ventricular myocytes in this study reduced concerns arising from the use of heart tissues containing heterogeneous cell types. The yields of healthy myocytes were high (60–80% viability). By using isolated myocytes, we established that the effects seen on cyclic AMP and cell function were accounted for entirely by ventricular myocytes. Measurement errors in our cyclic AMP data due to damaged cells should be small, since only about 20% of the cells in our preparation were rounded and non-contracting. Cell contraction measurements were obtained on at least four random cells in each preparation, and each cell was required to complete its protocol. Untreated cells continued to contract with a constant shortening over the time course of the experiment. Milrinone is a selective cyclic GMP-inhibited cyclic AMP phosphodiesterase inhibitor. EHNA is the only available selective cyclic GMP-stimulated cyclic AMP phosphodiesterase inhibitor (Gustafsson and Brunton, 2002). It does affect adenosine levels, but this effect should not be important in isolated well-oxygenated myocytes. We found the doses of EHNA and milrinone to be effective phosphodiesterase inhibitors in myocytes in preliminary experiments and previous studies (Weiss et al., 1999), although others have used higher doses of milrinone (Cone et al., 1999).

Many studies have reported cyclic GMP to have negative metabolic and functional effects on intact hearts and isolated myocytes, and it has also been demonstrated to be antagonistic to cyclic AMP (Shah and MacCarthy, 2000; Murad, 1994; Shah et al., 1994; Corwell et al., 1994; Mery et al., 1993). The mechanisms of action include inhibition of L-type Ca^{2+} channels, protein phosphorylation by cyclic GMP-dependent protein kinases, and effects on cyclic GMP-stimulated and cyclic GMP-inhibited cyclic AMP phosphodiesterases (Coppenolle et al., 1997; Hove-Madsen et al., 1996; Kajimoto et al., 1997; Mery et al., 1993; Sumiim and Sperelakis, 1995; Wang et al., 2000). The second messenger cyclic GMP can also affect the Ca^{2+} calcium reuptake mechanism in ventricular myocytes (Zhang et al., 2002). It had been suggested that the effects of cyclic GMP on the inhibition of transmembrane Ca^{2+} current were mediated by the activation of a cyclic GMP-dependent protein kinase that phosphorylated a channel or regulatory protein (Wang et al., 2000; Sumiim and Sperelakis, 1995; Coppenolle et al., 1997). In the current study, we found a significant dose-dependent negative functional effect of cyclic GMP in rabbit ventricular myocytes. This may be related to action of the cyclic GMP protein kinase (Straznicka et al., 1999; Sumiim and Sperelakis, 1995; Wang et al., 2000; Yan et al., 2000).

There have also been reports of additional effects of cyclic GMP mediated by the hydrolysis or activation of cyclic AMP via a cyclic GMP-dependent cyclic AMP phosphodiesterases (Hirota et al., 2001; Kajimoto et al., 1997; Mery et al., 1993; Naim et al., 1998). The cyclic GMP induced changes in cyclic AMP can affect myocyte function (Dittrich et al., 2001; Hirota et al., 2001; Kojda and Kottenberg, 1999; Vandecasteele et al., 2001). In the current study, the negative functional effects of cyclic GMP were accompanied by increase in the level of cyclic AMP in the ventricular myocytes. This may be related to the predominance of cyclic GMP-inhibited cyclic AMP phosphodiesterase activity in heart (Shahid and Nicholson, 1990). Thus, it is unlikely that

actions of the cyclic GMP affected cyclic AMP phosphodiesterases were the cause of these negative functional effects.

On the basis of their particular primary protein sequence, cyclic AMP and cyclic GMP phosphodiesterases have been classified into 11 families (Francis et al., 2001), many of which are found in the heart. These cyclic AMP phosphodiesterase isozymes have been shown to be of major importance as targets for pharmacological interventions in the heart (Levy and Bailey, 2000; Hirota et al., 2001). Two of these myocardial cyclic AMP phosphodiesterases are affected by cyclic GMP. A cyclic GMP-stimulated phosphodiesterase and cyclic GMP-inhibited phosphodiesterase hydrolyze cyclic AMP. Recent in vivo and in vitro experimental evidence shows that cyclic GMP-stimulated and cyclic GMP-inhibited cyclic AMP phosphodiesterases may mediate effects on the force of contraction in mammalian cardiac tissue (Kajimoto et al., 1997; Naim et al., 1998; Weiss et al., 1999). Changes in cyclic GMP levels can affect cyclic AMP levels and also myocardial function (Dittrich et al., 2001; Kajimoto et al., 1997; Naim et al., 1998; Vandecasteele et al., 2001).

There is evidence for a greater activity of cyclic GMP-inhibited compared to cyclic GMP-stimulated cyclic AMP phosphodiesterase in the heart (Shahid and Nicholson, 1990). Clinically useful cyclic AMP phosphodiesterase inhibitors for cardiac problems are usually cyclic GMP-inhibited cyclic AMP phosphodiesterase inhibitors (Levy and Bailey, 2000; Movsesian, 1999). We found that the addition of cyclic GMP to control ventricular myocytes increased the level of cyclic AMP in the heart. This would be consistent with greater cyclic GMP-inhibited cyclic AMP phosphodiesterase activity. However, this increase in cyclic AMP was not sufficient to counter the negative functional effects of the exogenous cyclic GMP. Others have reported positive inotropic effects of nitric oxide and cyclic GMP related, in part, to their action on cyclic AMP phosphodiesterases (Hirota et al., 2001; Kojda and Kottenberg, 1999). In the current study, we also found that inhibition of either cyclic GMP-inhibited or cyclic GMP-stimulated cyclic AMP phosphodiesterase did not cause functional changes in the heart even though cyclic AMP levels increased. This may be related to the low endogenous production of cyclic AMP in isolated cardiac myocytes without basal sympathetic stimulation.

When cyclic GMP-stimulated cyclic AMP phosphodiesterase activity was inhibited with EHNA, cyclic AMP levels were increased and the negative function effects of cyclic GMP were blunted. This may be directly related to the inhibitory effect of cyclic GMP on cyclic GMP-inhibited cyclic AMP phosphodiesterase. When cyclic GMP-inhibited cyclic AMP phosphodiesterase activity was inhibited with milrinone and cyclic GMP was added, the cyclic AMP level did not significantly rise and the negative functional effects of cyclic GMP were increased compared to cyclic GMP alone. This may be related to the stimulatory effect of cyclic GMP on cyclic GMP-stimulated cyclic AMP phosphodiesterase. This study demonstrated a strong interaction between cyclic GMP and cyclic GMP-stimulated and cyclic GMP-inhibited cyclic AMP phosphodiesterases. These effects of cyclic GMP on cyclic AMP levels through effects on cyclic GMP affected cyclic AMP phosphodiesterases have been demonstrated in myocytes and other tissue types (Gustafsson and Brunton, 2002; Naim et al., 1998; Weiss et al., 1999). This may help to explain some of the reports of positive inotropic effects of nitric oxide and cyclic GMP (Hirota et al., 2001; Kojda and Kottenberg, 1999). Even though cyclic GMP-inhibited cyclic AMP phosphodiesterase activity appears to be more important than cyclic GMP-stimulated cyclic AMP phosphodiesterase activity, cyclic GMP-stimulated cyclic AMP phosphodiesterase modulates and limits the effects of cyclic GMP on cyclic AMP. This appears to aid the negative functional effects of the second messenger cyclic GMP on ventricular myocytes.

In summary, we found that increased cyclic GMP led to a reduction in function of ventricular myocytes, despite an increase in the inotropic second messenger cyclic AMP. These negative functional effects of cyclic GMP were enhanced by blocking the cyclic GMP-inhibited cyclic AMP phosphodiesterase and this reduced the rise in cyclic AMP. The negative functional effects of cyclic GMP were blunted by blockade of the cyclic GMP-stimulated cyclic AMP phosphodiesterase. Cyclic GMP caused a greater rise in cyclic AMP after cyclic GMP-stimulated cyclic AMP phosphodiesterase blockade compared to blockade of cyclic GMP-inhibited cyclic AMP phosphodiesterase. The effects of cyclic GMP on the level of cyclic AMP appear to depend on the relative activity of cyclic GMP-stimulated and cyclic GMP-inhibited cyclic AMP phosphodiesterase and this affects myocyte function.

Acknowledgements

This study was supported, in part, by U.S.P.H.S. grant HL 40320 and a grant-in-aid from the American Heart Association, Heritage Affiliate.

References

Ahmmed, G.U., Xu, Y., Dong, P.H., Zhang, Z., Eiserich, J., Chiamvimonvat, N., 2001. Nitric oxide modulates cardiac Na^+ channel via protein kinase A and protein kinase G. Circ. Res. 89, 1005–1013.

Cone, J., Wang, S., Tandon, N., Fong, M., Sun, B., Sakurai, K., Yoshitake, M., Kambayashi, J., Liu, Y., 1999. Comparison of the effects of cilostazol and milrinone on intracellular cAMP levels and cellular function in platelets and cardiac cells. J. Cardiovasc. Pharmacol. 34, 497–504.

Coppenolle, F.V., Ahidouch, A., Guilbault, P., 1997. Regulation of endogenous Ca^{2+} channels by cyclic AMP and cyclic GMP-dependent protein kinase in Pleurodeles oocytes. Mol. Cell. Biochem. 168, 155–161.

Corwell, T.L., Arnold, E., Boerth, N.J., 1994. Inhibition of smooth muscle growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. Am. J. Physiol. 267, C1405–C1413.

Dittrich, M., Jurevicius, J., Georget, M., 2001. Local response of L-type Ca^{2+} current to nitric oxide in frog ventricular myocytes. J. Physiol. (Lond.) 534, 109–121.

Francis, S.H., Turko, I.V., Corbin, J.D., 2001. Cyclic nucleotide phosphodiesterases: relating structure and function. Prog. Nucleic Acid Res. Mol. Biol. 65, 1–52.

Gustafsson, A.B., Brunton, L.L., 2002. Attenuation of cAMP accumulation in adult rat cardiac fibroblasts by IL-1beta and NO: role of cGMP-stimulated PDE2. Am. J. Physiol. 283, C463–C471.

Hirota, Y., Ishida, H., Genka, C., 2001. Physiological concentration of nitric oxide induces positive inotropic effects through cGMP pathway in isolated rat ventricular myocytes. Jpn. J. Physiol. 51, 455–461.

Hove-Madsen, L., Mery, P.F., Jurevicius, J., 1996. Regulation of myocardial calcium channels by cyclic AMP metabolism. Basic Res. Cardiol. 91 (Suppl. 2), 1–8.

Kajimoto, K., Hagiwara, N., Kasanuki, H., 1997. Contribution of phosphodiesterase isozymes to the regulation of the L-type calcium current in human cardiac myocytes. Br. J. Pharmacol. 121, 1549–1556.

Kojda, G., Kottenberg, K., 1999. Regulation of basal myocardial function by NO. Cardiovasc. Res. 41, 514–523.

Levy, J.H., Bailey, J.M., 2000. Phosphodiesterase inhibitors: the inotropes of choice for the new millennium? J. Cardiothorac. Vasc. Anesth. 14, 365–366.

Mery, P.F., Pavoine, C., Belhassen, L., 1993. Nitric oxide regulates cardiac Ca^{2+} current. Involvement of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. J. Biol. Chem. 268, 26286–26295.

Movsesian, M.A., 1999. Beta-adrenergic receptor agonists and cyclic nucleotide phosphodiesterase inhibitors: shifting the focus from inotropy to cyclic adenosine monophosphate. J. Am. Coll. Cardiol. 34, 318–324.

Murad, F., 1994. The nitric oxide-cGMP signal transduction system for intracellular and intercellular communication. Recent Prog. Horm. Res. 49, 239–248.

Naim, K.L., Rabindranauth, P., Weiss, H.R., Tse, J., 1998. Positive inotropy due to lowering cyclic GMP is also mediated by increases in cyclic AMP in control and hypertrophic hearts. Can. J. Physiol. Pharm. 76, 605–712.

Shah, A.M., MacCarthy, P.A., 2000. Paracrine and autocrine effects of nitric oxide on myocardial function. Pharmacol. Ther. 86, 49–86.

Shah, A.M., Spurgeon, H.A., Sollott, J., 1994. 8-Bromo-cGMP reduces the myofilament response to Ca^{2+} in intact cardiac myocytes. Circ. Res. 74, 970–978.

Shahid, M., Nicholson, C.D., 1990. Comparison of cyclic nucleotide phosphodiesterase isoenzymes in rat and rabbit ventricular myocardium: positive inotropic and phosphodiesterase inhibitory effects of Org 30029, milrinone and rolipram. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 695–698.

Steinberg, S.F., Brunton, L.L., 2001. Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. Annu. Rev. Pharmacol. Toxicol. 41, 751–773.

Straznicka, M., Gong, G., Yan, L., 1999. Cyclic GMP protein kinase mediates negative metabolic and functional effects of cyclic GMP in control and hypertrophied rabbit cardiac myocytes. J. Cardiovasc. Pharmacol. 34, 229–236.

Sumiim, K., Sperelakis, N., 1995. cGMP-dependent protein kinase regulation of the L-type Ca^{2+} current in rat ventricular myocytes. Circ. Res. 77, 803–812.

Vandecasteele, G., Verde, I., Rucker-Martin, C., 2001. Cyclic GMP regulation the in L-type Ca^{2+} channel in human atrial myocytes. J. Physiol. (Lond.) 533, 329–340.

Wang, Y., Wagner, M.B., Joyner, R.W., 2000. cGMP-dependent protein kinase mediates stimulation of L-type calcium current by cGMP in rabbit atrial cells. Cardiovasc. Res. 48, 310–322.

Weiss, H.R., Gong, G.X., Straznicka, M., 1999. Cyclic GMP and cyclic AMP induced changes in control and hypertrophic cardiac myocyte function interact through cyclic GMP affected cyclic AMP phosphodiesterases. Can. J. Physiol. Pharm. 77, 229–235.

Yan, L., Lee, H., Huang, M.W., 2000. Opposing functional effects of cyclic GMP and cyclic AMP may act through protein phosphorylation in rabbit cardiac myocytes. J. Auton. Pharm. 20, 111–121.

Zhang, Q., Yan, L., Weiss, H.R., 2002. Cyclic GMP induced reduction in cardiac myocyte function is partially mediated by activation of the sarcoplasmic reticulum Ca^{2+}-ATPase. Pharmacology 64, 106–112.