The cyclooxygenase-2 product prostaglandin E₂ modulates cardiac contractile function in adult rat ventricular cardiomyocytes

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Accepted 3 September 2003

Abstract

Prostaglandin E₂ (PGE₂), a product of the cyclooxygenase-2 pathway, has been shown to increase cardiac output and modulate cardiac contractile function. However, whether the cardiac contractile response of PGE₂ is due to its action on single ventricular myocytes has not been elucidated. To assess the mechanical effect of PGE₂ at the cellular level, adult rat ventricular myocytes were isolated and stimulated to contract at 0.5 Hz. Mechanical and intracellular Ca²⁺ properties were evaluated using an IonOptix Myocam® analog-to-digital optical detection system. Contractile and intracellular Ca²⁺ properties were evaluated as peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR₉₀), maximal velocity of shortening or relengthening (±dL/dt) and Ca²⁺-induced intracellular Ca²⁺ fluorescence release (CICR), baseline intracellular Ca²⁺ levels and intracellular Ca²⁺ decay rate (τ). PGE₂ (10⁻⁸ to 10⁻³ M) elicited an augmentation in PS but had no effect on TPS, TR₉₀, ±dL/dt, CICR and τ. High concentration of PGE₂ (10⁻⁵ M or higher) reduced the baseline intracellular Ca²⁺ levels. These data indicate that the myocardial contractile response of PGE₂ may be due to its direct cardiac contractile action at the single ventricular myocyte level, probably through a mechanism independent of intracellular Ca²⁺ release.

Keywords: Prostaglandin E₂; Ventricular myocytes; Shortening; Relengthening; Intracellular Ca²⁺

1. Introduction

Cyclooxygenase (COX) catalyzes the oxidation and metabolism of arachidonic acid, leading to the formation of prostaglandin H₂ (PGH₂) and subsequently other prostaglandins such as PGE₂, PGF₂α, PGD₂, and thromboxane A₂ [1,2]. Two COX isoforms, namely COX-1 and COX-2, have been found in mammalian cells with COX-1 being constitutively expressed and COX-2 being normally absent and inducible by various stimuli such as inflammatory cytokines [1,2]. Both prostaglandins and arachidonic acid are believed to participate in multiple physiological as well as pathophysiological responses such as reproductive, immunological, endocrine, tumorigenesis and cardiovascular regulation [1–4]. Arachidonic acid, the precursor for eicosanoid formation, is released in response to receptor activation and pathological stimuli such as myocardial ischemia. It has been demonstrated that release of arachidonic acid in response to receptor activation or pathological stimuli elicits a positive cardiac inotropic response probably through modulation of membrane K⁺ and Ca²⁺ channel activity [5,6]. Although the precise mechanism(s) behind the positive cardiac inotropic actions of arachidonic acid and inflammatory cytokines (such as tumor necrosis factor-α) is largely undefined, it may be speculated that the COX product PGE₂ plays a major role over other prostaglandins in mediating the cardiac function induced by arachidonic acid and inflammatory cytokines [7]. It was shown that induction of COX-2 by interleukin-1β results in the preferential production of PGE₂ in neonatal ventricular myocytes [8]. This is consistent with the observation that the capacity of PGE₂ production is significantly greater than other prostaglandins in many cell types possibly due to a combined effect of COX-2 and PGE₂ synthase [1,7]. PGE₂ has been shown to play a role in protein synthesis and growth in cardiac myocytes [7]. In vivo administration of PGE₂ significantly improves the cardiac contractility and relaxation rate along with reduced heart rate [9]. However, in vivo action of PGE₂ on cardiac electromechanical function may be affected by non-myocyte...
factors such as interstitial connective tissue, fibroblast or nerve terminals. For example, increased ventricular stiffness may reflect a greater amount of interstitial fibrosis and a shift in collagen content rather than a direct effect on the mechanical properties of myocytes themselves. To determine whether the PGE2-induced augmentation on intracellular Ca2+ release in response to electrical stimulation (0.5 Hz) was examined. Unlike its effect on cell shortening, PGE2 (10^{-8} to 10^{-4} M) elicited little effect on CICR and intracellular Ca2+ decay rate (τ). How-

2. Materials and methods

2.1. Isolation of adult rat ventricular myocytes

The experimental procedures described here were approved by the institutional animal use and care committee of the University of North Dakota (Grand Forks, ND, USA). Ventricular myocytes were isolated from adult male Sprague–Dawley rats (200–225 g) as described [10]. Briefly, hearts were rapidly removed and perfused (at 37 °C) with oxygenated (5% CO2–95% O2) Krebs-Henseleit bicarbonate (KHB) buffer (118 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, 10 mM N-[2-hydro-ethyl]-piperazine-N’-[2-ethanesulfonic acid] (HEPES), 11.1 mM glucose, pH 7.4). Hearts were subsequently perfused with a nominally Ca2+-free KHB buffer to remove remnant enzyme and extracellular Ca2+ was added incrementally back to 1.25 mM.

2.2. Myocyte shortening and relengthening

Mechanical properties of ventricular myocytes were assessed by an IonOptix Myocam® detection system (IonOptix Incorporation, Milton, MA, USA). Cells were placed in a chamber mounted on the stage of an inverted Olympus IX-70 microscope and superfused (at 37 °C) with a buffer containing: 131 mM NaCl, 4.7 mM KCl, 1.2 mM CaCl2, 1 mM MgCl2, 10 mM glucose, 10 mM HEPES, at pH 7.4. The cells were field stimulated at a frequency of 0.5 Hz. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-PS (TPS) and time-to-90% relengthening (TR90), maximal velocities of shortening (+dL/dt) and relengthening (+dL/dt) [10].

2.3. Intracellular Ca2+ fluorescence measurement

Myocytes were loaded with fura-2/AM (0.5 μM) for 10 min and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (IonOptix Incorporation) as described [10]. Myocytes were plated on glass cover slips on an Olympus IX-70 inverted microscope and imaged through a Fluor 40× oil objective. Cells were exposed to light emitted by a 75 W lamp and passed through either a 360 or 380 nm filter (bandwidths were ±15 nm), while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm after first illuminating cells at 360 nm for 0.5 s, then at 380 nm for the duration of the recording protocol (333 Hz sampling rate). The 360 nm excitation scan was repeated at the end of the protocol. Qualitative changes in intracellular Ca2+ levels were inferred from the ratio of the fluorescence intensity at two wavelengths (360/380 nm) and were used to determine Ca2+-induced Ca2+ release (change of fura-2 fluorescent intensity, CICR). Intracellular Ca2+ removal was evaluated as the rate of fluorescence decay. To test the effect of PGE2 on intracellular Ca2+ properties, fura-2 fluorescent intensity was recorded before and 5 min after PGE2 administration.

2.4. Data analysis

Data were presented as mean ± S.E.M. Statistical significance (P < 0.05) for each variable was estimated by analysis of variance (ANOVA).

3. Results

3.1. Effect of PGE2 on myocyte shortening (PS)

The average cell length was 117 ± 3 μm (n = 37) in this study. Acute exposure of PGE2 did not affect resting myocyte cell length over the range of concentrations tested. At the end of a 5 min exposure, PGE2 (10^{-8} to 10^{-3} M) elicited a concentration-dependent increase of PS, with a maximal augmentation of 33.2%. The threshold of effect was between 10^{-8} and 10^{-7} M (Fig. 1). PGE2-induced increase on PS was maximal within 4 min of exposure, remained stable for over 20 min and was reversible upon washout (data not shown). PGE2 had no effect on TPS, TR90 and ±dL/dt (Table 1).

3.2. Effect of PGE2 on intracellular Ca2+ transients

To determine whether the PGE2-induced augmentation on PS was due to elevated availability of intracellular Ca2+, the effect of PGE2 on intracellular Ca2+ release in response to electrical stimulation (0.5 Hz) was examined. Unlike its effect on cell shortening, PGE2 (10^{-8} to 10^{-4} M) elicited little effect on CICR and intracellular Ca2+ decay rate (τ). How-
Table 1

Effect of PGE2 on duration and maximal velocity of myocyte shortening and relengthening in adult rat ventricular myocytes

<table>
<thead>
<tr>
<th></th>
<th>TPS (ms)</th>
<th>TR90 (ms)</th>
<th>(\frac{dL/dt}{\mu m/s}^{1})</th>
<th>(\frac{dL/dt}{\mu m/s}^{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>140 ± 5</td>
<td>317 ± 24</td>
<td>91.3 ± 9.7</td>
<td>−75.8 ± 10.9</td>
</tr>
<tr>
<td>PGE2 (10^-8 M)</td>
<td>128 ± 6</td>
<td>252 ± 31</td>
<td>113.8 ± 18.9</td>
<td>−95.5 ± 20.6</td>
</tr>
<tr>
<td>PGE2 (10^-7 M)</td>
<td>146 ± 5</td>
<td>300 ± 22</td>
<td>101.0 ± 11.2</td>
<td>−85.5 ± 11.2</td>
</tr>
<tr>
<td>PGE2 (10^-6 M)</td>
<td>157 ± 5</td>
<td>338 ± 23</td>
<td>102.1 ± 11.1</td>
<td>−82.6 ± 10.2</td>
</tr>
<tr>
<td>PGE2 (10^-5 M)</td>
<td>147 ± 5</td>
<td>291 ± 19</td>
<td>99.0 ± 10.1</td>
<td>−79.2 ± 9.6</td>
</tr>
<tr>
<td>PGE2 (10^-4 M)</td>
<td>148 ± 6</td>
<td>300 ± 21</td>
<td>99.9 ± 10.4</td>
<td>−80.0 ± 10.4</td>
</tr>
<tr>
<td>PGE2 (10^-3 M)</td>
<td>145 ± 6</td>
<td>310 ± 25</td>
<td>89.9 ± 9.7</td>
<td>−71.8 ± 9.9</td>
</tr>
</tbody>
</table>

TPS: time-to-peak shortening; TR90: time-to-90% relengthening; \(\frac{dL/dt}{\mu m/s}\) maximal velocities of shortening and relengthening. Data represent mean ± S.E.M., n = 33-37 cells per group.

![Fig. 1](image1.png)

Fig. 1. Concentration-dependent response of PGE2 (10^-8 to 10^-3 M) on peak cell shortening. Data are presented as percent change of PS from the baseline value (5.12 ± 0.50% of resting cell length). Data represent mean ± S.E.M., n = 33-37 cells per group. * P < 0.05 vs. control (without PGE2).

However, PGE2 significantly reduced the baseline intracellular Ca^{2+} at the concentrations of 10^-3 and 10^-4 M (Fig. 2 and Table 2). A large portion of the fura-2-loaded ventricular myocytes failed to survive the entire PGE2 concentration response range shown in Fig. 1 (10^-6 to 10^-3 M) and stopped beating prior to reaching the highest concentration of PGE2 (10^-3 M). This observation may suggest potential interaction between fura-2 and PGE2. Data from 10^-3 M of PGE2 was thus not available due to this cell variability problem.

![Fig. 2](image2.png)

Fig. 2. Effect of PGE2 (10^-6 to 10^-3 M) on intracellular Ca^{2+} induced Ca^{2+} release in adult rat ventricular myocytes. CICR is presented as the difference of peak (electrically stimulated) and basal fura-2 fluorescent intensity (360/380 nm ratio). A control fluorescent CICR value was obtained 5 min prior to PGE2 addition and is denoted as the "0" concentration of PGE2. Data represent mean ± S.E.M., n = 12–14 cells per group.

Taken together, the lack of stimulatory effect of PGE2 on CICR suggests that an increase in intracellular free Ca^{2+} is unlikely to be responsible for the PGE2-induced stimulatory action on myocyte shortening.

4. Discussion

Our study provided evidence that the major COX product PGE2 directly enhances cardiac contraction in isolated ventricular myocytes, suggesting that the myocardial response of PGE2 may be attributed, at least in part, to its action in individual myocytes. The PGE2-evoked positive cardiac contractile response in isolated ventricular myocytes was not associated with alteration in the duration or maximal velocity of contraction and relaxation. The intracellular Ca^{2+} transient recording revealed that the electrically-stimulated Ca^{2+}-induced Ca^{2+} release and intracellular Ca^{2+} clearing (τ) were not affected by PGE2 with the exception that baseline intracellular Ca^{2+} level was reduced by higher concentrations of PGE2. The discrepancy between myocyte...
The PGE₂-elicited positive cardiac contractile response is also consistent with the PGE₂-elicited protection enhanced cardiac contractility in isolated ventricular myocytes. Secondly, although the direct action of PGE₂ on ion channels, actin, myosin heavy chain, sarcoplasmic Ca²⁺ release, and phospholipase C[14–16] activates these kinases or enzymes or protein kinases such as protein kinase C and phospholipase C[14–16]. Activation of these kinases or enzymes may directly affect the actin–myosin phosphorylation state although direct evidence is still lacking for PGE₂. Secondly, although the direct action of PGE₂ on cardiac contractile elements such as membrane ion channels, actin, myosin heavy chain, sarcoplasmic Ca²⁺ release and Ca²⁺-regulatory proteins or exchanger has not been elucidated in the hearts, PGE₂ has been demonstrated to effectively inhibit certain types of K⁺ channel and stimulate Ca²⁺ influx in various cell types such as vascular smooth muscle cells and osteoblasts[15,17]. Modulation of these membrane ion channels may directly modulate myocardial contractile function and may also be responsible for reduced intracellular Ca²⁺ levels at higher concentrations of PGE₂. Thirdly, PGE₂ and β-adrenergic activation may synergistically stimulate glycolysis and glucose oxidation in the isolated working rat heart and therefore potentiate rather than decrease β-adrenergic stimulation of glucose metabolism and promote cardiac contractile function[18].

Last but certainly not the least, PGE₂ has been shown to protect free radical-induced cardiac dysfunction revealing its positive cardiac response via a mechanism(s) independent of intracellular Ca²⁺ rise such as altered myofilament Ca²⁺ sensitivity. PGE₂ has been shown to activate multiple enzymes or protein kinases such as protein kinase C and phospholipase C[14–16]. Activation of these kinases or enzymes may directly affect the actin–myosin phosphorylation state although direct evidence is still lacking for PGE₂. Secondly, although the direct action of PGE₂ on cardiac contractile elements such as membrane ion channels, actin, myosin heavy chain, sarcoplasmic Ca²⁺ release and Ca²⁺-regulatory proteins or exchanger has not been elucidated in the hearts, PGE₂ has been demonstrated to effectively inhibit certain types of K⁺ channel and stimulate Ca²⁺ influx in various cell types such as vascular smooth muscle cells and osteoblasts[15,17]. Modulation of these membrane ion channels may directly modulate myocardial contractile function and may also be responsible for reduced intracellular Ca²⁺ levels at higher concentrations of PGE₂. Thirdly, PGE₂ and β-adrenergic activation may synergistically stimulate glycolysis and glucose oxidation in the isolated working rat heart and therefore potentiate rather than decrease β-adrenergic stimulation of glucose metabolism and promote cardiac contractile function[18]. In conclusion, our study demonstrates a direct cardiac potentiating response of PGE₂ at the ventricular myocyte level, possibly through an intracellular Ca²⁺-independent mechanism(s). The precise nature of cardiac contractile effects of PGE₂ under both physiological and pathophysiological conditions (e.g., myocardial inflammation, myocardial infarction and ischemia–reperfusion injury) is still far from being clear. Future studies should focus on the action of PGE₂ on cardiac oxidation and antioxidant defense, receptor/G-protein-coupled signal transduction and certain membrane elements of excitation-contraction coupling such as ion channels. These investigations are essential to the better understanding of the cellular effects and pharmacological profiles for PGE₂ in the heart so that optimal management of cardiac function may be achieved.

Acknowledgements

This work was supported in part by Max Baer Heart Fund and NASA Grant # NCC5-582 to JR. We sincerely thank Dr. James Haselton for serving as the work-study faculty mentor for A.LK at University of North Dakota.

References


