Prostaglandin E2 Reduces Cardiac Contractility via EP3 Receptor

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Background—Prostaglandin E2 (PGE₂) EP receptors EP3 and EP4 signal via decreased and increased cAMP production, respectively. Previously, we reported that cardiomyocyte-specific EP4 knockout mice develop dilated cardiomyopathy with reduced ejection fraction. Thus, we hypothesized that PGE₂ increases contractility via EP4 but decreases contractility via EP3.

Methods and Results—The effects of PGE₂ and the EP1/EP3 agonist sulprostone on contractility were examined in the mouse Langendorff preparation and in adult mouse cardiomyocytes. Isolated hearts of adult male C57Bl/6 mice were perfused with PGE₂ (10⁻⁶ M) or sulprostone (10⁻⁶ M) and compared with vehicle. Both PGE₂ and sulprostone decreased +dp/dt (P<0.01) and left ventricular developed pressure (P<0.001) with reversal by an EP3 antagonist. In contrast, the EP4 agonist had the opposite effect. Adult mouse cardiomyocytes contractility was also reduced after treatment with either PGE₂ or sulprostone for 10 minutes. We then examined the acute effects of PGE₂, sulprostone, and the EP4 agonist on expression of phosphorylated phospholamban and sarcoendoplasmic reticulum Ca²⁺-ATPase 2a in adult mouse cardiomyocytes using Western blot. Treatment with either PGE₂ or sulprostone decreased expression of phosphorylated phospholamban corrected to total phospholamban, whereas treatment with the EP4 agonist had the opposite effect. Sarcoendoplasmic reticulum Ca²⁺-ATPase 2a expression was unaffected. Finally, we examined the effect of these compounds in vivo using pressure–volume loops. Both PGE₂ and sulprostone decreased +dp/dt, whereas the EP4 agonist increased +dp/dt.

Conclusions—Contractility is reduced via the EP3 receptor but increased via EP4. These effects may be mediated through changes in phospholamban phosphorylation and has relevance to detrimental effects of inflammation. (Circ Heart Fail. 2016;9:e003291. DOI: 10.1161/CIRCHEARTFAILURE.116.003291.)

Key Words: cardiomyopathy, dilated | heart contractility | inflammation | phospholamban | prostaglandin E2

Original Article

Prostaglandin E2 (PGE₂) elicits biological effects through 4 distinct receptor subtypes termed EP1, EP2, EP3, and EP4 that couple to different second messenger systems. Whereas activation of EP2 and EP4 increases cAMP, activation of EP1 increases intracellular calcium and activation of EP3 decreases cAMP. Thus, the effect of PGE₂ is dependent on the profile of EP receptors expressed in different tissues and cell types.

Our laboratory recently reported that male mice lacking the EP4 receptor subtype only in cardiac myocytes (EP4 knockout) have reduced cardiac function with age and develop a phenotype of dilated cardiomyopathy. However, these in vivo studies could not discern whether the EP4 knockout mice have intrinsic defects in myocyte contractility or whether decreased cardiac function results from whole system abnormalities in the sympathetic nervous system that regulates both the speed and force of contraction or whether these mice have conduction defects. Moreover, although we reported that EP3 mRNA is not increased in the heart of EP4 knockout mice to compensate for lack of EP4, we now suggest that the absence of EP4 allows the effects of EP3 stimulation to proceed unopposed. There are few studies that have reported on the influence of PGE₂ in isolated myocyte contractility and the EP receptor(s) involved. Wang et al showed that cardiomyocyte-specific deletion of cyclooxygenase-2 increased ventricular tachycardia in response to electric stimulation of the heart and infusion of PGE₂, was reported to depress premature ventricular beats in humans, consistent with its ability to reduce ischemia-induced arrhythmias in animal models. There is only 1 published article describing the effect of PGE₂ on isolated rat myocytes in vitro, and it found that PGE₂ increased contractility without effects on intracellular calcium. However, these few reports do not allude to potential mechanisms by which PGE₂ increases the rate or force of contractions, including which PGE₂ receptor subtype is involved.

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Phospholamban is a crucial component of excitation–
contraction coupling. The phosphorylation of phospholamban
(p-PLN) physically separates it from sarcoendoplasmic reticu-
ulum Ca²⁺-ATPase 2a (SERCA2a) and, therefore, removes its
inhibitory effect, making for increased SERCA activity and
triggering the release of large amounts of Ca²⁺ from the sarco-
plasmic reticulum to increase contractility. Therefore, we tested
the ability of PGE, and various EP agonists to alter contraction
and examined the hypothesis that PGE, acting through its EP4
receptor can increase contraction via increased p-PLN, whereas
PGE, acting through its EP3 receptor has the opposite effect.
This hypothesis was tested in isolated mouse ventricular myo-
cytes, in the ex vivo mouse working heart Langendorff prepara-
tion and acutely in vivo using pressure–volume (PV) loops.

Methods

Animal Use

The wild-type and EP4 knockout mice used in this study were
bred and genotyped at Henry Ford Hospital and have been previ-
ously described. C57Bl/6 mice used for the myocardial infarction
(MI) studies and contractility studies were from Jackson laborato-
ries. The mouse model of MI using permanent ligation of the left
anteriordescending coronary artery was previously described by
us as was the isolation of adult mouse ventricular cardiomyocytes
(AVM). All studies involving the use of animals were approved by
the Institutional Review Committee at Henry Ford Hospital, in ac-
cordance with federal guidelines.

Chemicals

The EP1/EP3 agonist (sulprostone), EP4 agonist (CAY 10598),
and the EP3 agonist (L798106) were obtained from Cayman Chemical
(Ann Arbor, MI). All drugs were dissolved in 100% ethanol. All other
chemicals were obtained from Sigma Aldrich.

Mouse Langendorff Studies

Mice were anesthetized with isoflurane and injected with 250 U hepa-
rin. Hearts were rapidly excised and placed in ice-cold Krebs solution
(mmol/L: NaCl 118.5, KCl 4.7, MgSO4 2.46, KH₂PO₄ 1.21, glucose
12, CaCl₂ 1.7, Na pyruvate 2, NaHCO₃ 25, pH 7.4, bubbled in 95%
O₂/5% CO₂). The aortas were cannulated and retrogradely perfused
constantly at 3.5 mL/min at 37°C. Hearts were allowed to beat spon-
taneously. Coronary perfusion pressure was constantly measured
using an in-line pressure transducer (MLT0380/D; ADInstruments,
Australia). After 10-minute equilibration, chemicals or vehicle was
added to the buffer and perfusion was continued for 30 minutes. Left
ventricular (LV) end-diastolic pressure, LV end-systolic pressure, and
heart rate were monitored and recorded continuously using PowerLab
system (ADInstruments). LV developed pressure (LVDP) was calcu-
lated by subtracting LV end-diastolic pressure from LV end-systolic
pressure. Hearts were excluded from further study if they exhibited
≥1 of the following exclusion criteria: LV end-diastolic pressure>20
mm Hg, LVDP <60 mm Hg, heart rate <300 bpm or arrhythmias.

Isolation of Adult Cardiomyocytes for Contractility
Studies

Isolation of cardiomyocytes from 16- to 21-week-old C57Bl/6 male
mice was performed using modifications of the method described by
O’Connell et al and has previously been described by us. 2,3-butanedi-
one monoxime (10 mmol/L) was omitted as it is a known inhibitor
of contractility. Freshly isolated AVM prepared in Tyrode solution
were loaded with 1 µmol/L Fura-2 AM (Molecular Probes, Eugene,
OR) for 5 minutes at room temperature, washed, and rested for 15
minutes. After cells were loaded and rested, cardiomyocytes were di-
vided into aliquots and treated with either vehicle or PGE, (10⁻⁴ M).
In another set of experiments, cells were divided and treated with
either vehicle or 10⁻⁴ M sulprostone for 10 minutes and washed. An
aliquot of cells was added to the chamber, and cells were allowed to
attach for 2 minutes and then superfused with Tyrode solution at 37°C
and electrically stimulated at 3 Hz using a biphasic pulse. Contraction
amplitude and intracellular calcium transients were recorded online
using a dual-excitation spectrophotometer and video edge detection
system (IonOptix), and a minimum of 50 transients were analyzed
for each cell. As indicators of contractility, peak shortening and the
speed of contraction and relaxation were measured. To ensure that
the response to the various agonists was not because of reduced cell
viability, in some experiments, the cells treated with test agents were
assessed first and then the cells treated with vehicle were assessed.

Cell Culture and Western Blot

Phospholamban and p-PLN protein expression was measured by
Western blot in AVM treated with PGE, CAY 10598, and sulprostone
for various times. For the cell culture studies to investigate the effect
of PGE, and EP agonists on phospholamban and p-PLN expression,
we used primary cultures of AVM from 18- to 20-week-old C57Bl/6
male mice. Cells were plated, and after 1 hour the media was changed
to serum-minus media. After an additional hour, the cells were treated
and cell lysates were harvested at appropriate times.

Western blot analysis was performed under reducing conditions
using 20 µg of total protein. After electrophoresis, proteins were trans-
ferred overnight to a polyvinylidene fluoride membrane. Membranes
were blocked for 1 hour in 5% milk (wt/vol in TBS-Tween) and in-
cubated overnight (4°C) with a 1:1000 dilution of phospholamban or a
p-PLN antibody that recognizes phosphorylation at Ser⁵⁷/Thr⁷⁷ (Cell
Signaling, Danvers, MA). After washing with TBS-tween, membranes
were incubated with a horseradish peroxidase-conjugated donkey anti-
rabbit secondary antibody for 1 hour at room temperature at a dilution
of 1:2000. After further washing, they were developed using a Super
Signal West Pico chemiluminescent substrate (Pierce, Rockford, IL).

PV Loops

PV loops were performed in male 18- to 20-week-old C57Bl/6 mice
using a closed chest method with Nembutal (sodium pentobarbital)
anesthesia. Briefly, mice were surgically ventilated and the PV cath-
er (Transonic Scisense Inc., London, Ontario, Canada) was inserted
into the LV via the right carotid artery using the surgical procedure de-
scribed by Pucher et al. The left jugular vein was also cannulated for
drug administration. After stabilization of the signal for 10 minutes,
baseline PV loops were obtained at steady state. PGE, EP4 agonist,
or sulprostone was then infused at a rate of 30 µg/min per kilogram
body weight, and data were collected for over a period of 30 minutes.
In other experiments, a vehicle (0.1%) ethanol was infused to ensure
that the vehicle had no effect over the experimental period. All data
were collected and analyzed using iWOX 408 Labscribe v3 software.

Statistical Analysis

All statistics were performed by a statistician in the Department of
Public Health Sciences of Henry Ford Hospital using the statistical
package SAS version 9.4. For the contractility data, statistics are re-
ported as means±SEM with n representing the number of cells. For
all other data, n represents the number of experiments or the number
of mice used. Groups were compared with Student t test except where
normality was not present, and a 2-sample Wilcoxon test was used.
For the PV loop data, a paired t test was used to compare parameters
before and after administration of experimental drugs. P<0.05 was
considered as evidence of a statistically significant difference for ex-
perimental data with the P values being 2 sided.

Results

Effect of PGE2 and Sulprostone on AVM Contractility

The top panel of Figure 1A shows mean data of transients
from cells treated with vehicle and cells treated with PGE,.
The bottom panel of the same figure shows mean transients from cells treated with vehicle and cells treated with sulprostone. Treatment with PGE₂ (10⁻⁶ M) for 10 minutes reduced contractility as measured by peak height (6.84±0.7 μm for vehicle versus 3.85±0.3 μm for PGE₂; \( P < 0.001 \)), departure velocity (−250.0±25.2 μm/s for vehicle versus −142.2±11.2 μm/s for PGE₂; \( P < 0.001 \)), and return velocity (147.9±19.3 μm/s for vehicle versus 65.8±8.6 μm/s for PGE₂; \( P < 0.001 \)).

Under basal conditions, treatment of AVM for 10 minutes with 10⁻⁶ M sulprostone also reduced contractility as measured by peak height (7.41±0.4 μm for vehicle versus 4.44±0.43 μm for sulprostone; \( P < 0.001 \)), departure velocity (−281.3±15.7 μm/s for vehicle versus −194.6±19.0 μm/s for sulprostone; \( P < 0.001 \)), and return velocity (178.8±12.8 μm/s for vehicle versus 120.4±15.3 μm/s for sulprostone; \( P = 0.008 \)). The mean data for the effects of PGE₂ and sulprostone are presented in Figure 1B.

With regard to changes in intracellular calcium, treatment with 10⁻⁶ M PGE₂ increased \( \text{sin exp tau} \) (the exponential decay time constant for calcium) from a value of 0.082±0.003 in vehicle-treated cells to 0.094±0.004 in PGE₂-treated cells, \( P = 0.022 \). Treatment with sulprostone did not significantly increase this parameter. These results indicate a slower return to baseline calcium levels in cells treated with PGE₂.
Effect of PGE₂, Sulprostone and EP4 Agonist (CAY 10598) on Contractility of the Isolated Heart

Isolated hearts of 18- to 20-week-old male C57Bl/6 mice were mounted on the Langendorff apparatus, equilibrated for 10 minutes, and then perfused with PGE₂ (10⁻⁶ M) or sulprostone (10⁻⁶ M) for 30 minutes. Values at the end of equilibration were set to 100%. Compared with vehicle, PGE₂ decreased +dp/dt (77.8±3% for PGE₂ versus 96.7±3% for vehicle; \( P = 0.004 \)) and LVDP (77.2±2% versus 96.8±3%; \( P < 0.001 \)). Sulprostone decreased +dp/dt (75.9±2% versus 96.7±3%; \( P < 0.001 \)), −dp/dt (72.2±1% versus 85.7±1%; \( P = 0.01 \)) and LVDP (70.9±1% versus 96.8±3%; \( P < 0.001 \)).

The effects of both PGE₂ and sulprostone were reversed by the EP3 antagonist, L789106 (10⁻⁶ M). In contrast to the effect of sulprostone and PGE₂, perfusion of the EP4 agonist into isolated working hearts increased their contractility. Compared with vehicle, the EP4 agonist, CAY 10598 (10⁻⁶ M) increased +dp/dt (75.9±2% versus 96.7±3%; \( P < 0.001 \)), −dp/dt (72.2±1% versus 85.7±1%; \( P = 0.01 \)) and LVDP (70.9±1% versus 96.8±3%; \( P < 0.001 \)).

To confirm specificity of the EP4 agonist, we then performed experiments to determine the effect of the EP4 agonist in working hearts from EP4 knockout mice in which the EP4 receptor is deleted only in cardiac myocytes. As anticipated, the EP4 agonist had no effect on contractility of hearts obtained from these knockout animals (Figure 2).

Effect of PGE₂, Sulprostone, and EP4 Agonist In Vivo

The acute in vivo effects of the above compounds were determined using PV loops in a closed chest approach. Similar to the results observed in the isolated adult myocytes and Langendorff preparation, both PGE₂ and sulprostone decreased contractility when compared with baseline measurements. In contrast, acute treatment with the EP4 agonist significantly improved contractility as measured by increased +dp/dt and heart rate. These results are presented in the Table and Figure 3.

Effect of PGE₂, Sulprostone, and EP4 Agonist (CAY 10598) on p-PLN and SERCA2a Expression

Treatment of AVM for 15 minutes with either PGE₂ or sulprostone decreased the expression of p-PLN corrected to total phospholamban, by 67% and 43%. SERCA2a expression was unaffected (data not shown), which was anticipated in this short experimental time frame. In contrast, treatment with the EP4 agonist increased p-PLN corrected to total phospholamban by 3.7±0.6-fold, \( P = 0.005 \), n=3 separate preparations. To further explore the role for the EP receptors in p-PLN, we examined AVM from 13- to 15-week-old male EP4 knockout mice. In these mice, treatment with PGE₂, for 15 minutes reduced p-PLN/total phospholamban by an average of 81%, a value that appeared greater than the 67% reduction observed in C57Bl/6 mice. Pretreatment

![Figure 2. Cardiac function was determined using the Langendorff preparation. L798106 is an EP3 inhibitor. A and B, Prostaglandin E2 (PGE₂) and sulprostone (Sulp) reduced percent left ventricular (LV) +dp/dt via EP3. C, EP4 agonist (CAY 10598) increased percent LV +dp/dt. D, EP4 agonist (CAY 10598) had no effect on cardiac function in EP4 knockout (KO) hearts. n=4 to 6 per group. Statistical significance: ** \( P < 0.01 \), *** \( P < 0.001 \).](attachment:figure2.png)
with the EP3 antagonist in AVM from EP4 knockout mice prevented the ability of PGE$_2$ to reduce p-PLN/total phospholamban (Figure 4B).

**EP3 and EP4 Are Increased in the MI Heart**

To determine whether the expression of EP3 and EP4 is altered in the failing heart, we performed real-time reverse transcription
polymerase chain reaction on LV samples from hearts obtained from C57Bl/6 mice that were subjected to permanent ligation of the LAD for 2 weeks. All samples were obtained from the border-remote zone and not the infarcted section. Our data show that EP3 is increased 3.37±0.8-fold in the MI heart compared with sham-operated controls (P=0.016), and EP4 mRNA is also increased in those same hearts although to a lesser extent (2.12±0.35-fold; P=0.007).

Discussion
This study provides direct evidence that exogenous PGE 
reduces contractility of the in vivo heart, the isolated working heart, and single adult ventricular myocytes via its EP3 receptor subtype, whereas stimulation of the EP4 receptor has opposite results. These effects may be mediated by alterations in the p-PLN, a protein that negatively regulates SERCA activity.

There have been many studies reporting the effect of PGE 
on contractility, but the results have been conflicting and do not elucidate the contribution of the various receptor subtypes. Using the PGE-perfused mouse isolated heart, Liu et al reported that PGE 
attenuates the adrenergic-induced cardiac contractile response in animal hearts, whereas studies by Pecha et al did not support a role for PGE 
in regulating catecholamine-induced inotropy. Whether these differences relate to the different preparations used (isolated heart versus atrial and ventricular trabeculae) is not known. Moreover, Klein et al used a system similar to the one used in our studies to observe that PGE 
augmented peak shortening in adult rat cardiomyocytes independent of changes in calcium, whereas concentrations >10^{-5} M reduced intracellular calcium. Church et al also reported that PGE 
(10^{-5} M) increases the contraction frequency of neonatal rat cardiomyocytes, but only spontaneous beating was measured. Although our recent data conflict with those described above, our results across a spectrum of preparations ranging from isolated cardiomyocytes through the Langendorff preparation and in vivo using

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**Figure 4.** A, Effect of prostaglandin E2 (PGE 
(10^{-6} M), sulprostone (Sulp; 10^{-6} M) and EP4 agonist, CAY 10598 (10^{-6} M) on p-PLN (phosphorylated phospholamban) expression in isolated myocytes obtained from C57Bl/6 mice. Top, Representative Western blots of phosphorylated and total PLN (T-PLN) and bottom is bar graph showing mean data after 15-min treatment.

B, Effect of PGE 
(10^{-6} M) on p-PLN expression in isolated myocytes obtained from 13- to 15-week-old EP4 knockout (KO) mice. Experiments were performed in the absence or presence of the EP3 inhibitor (EP3i), L798106 (10^{-6} M). Top, Representative Western blots of p-PLN and T-PLN and bottom is bar graph showing mean data after 15-min treatment.
PV loops are consistent and suggest that the main effect of PGE$_2$ via EP3 is to reduce contractility whereas it augments contractility via EP4. Our data are thus consistent with the reduced contractility noted in our previous studies using the cardiomyocyte-specific EP4 knockout mouse.

Clinical reports have also implicated the EP1/EP3 receptor agonist, sulprostone, in heart failure or cardiomyopathy. Vital et al$^{11}$ reported a case of a peripartum heart failure, whereas another case showed that sulprostone caused coronary spasm, bradycardia, and subsequent asystole.$^{12}$ In EP3-overexpressing mice, LV ejection fraction was severely decreased in transgenic hearts whereas the relative LV mass was significantly increased.$^{13}$ Using isolated rat atria, Wolkowicz et al$^{14}$ also reported that an EP1 agonist increased contractile force that was sensitive to Rho kinase inhibitors. In a Langendorff preparation, both endogenous PGE$_2$ and an exogenous EP4 agonist were shown to protect the heart from ischemia-reperfusion injury via EP4.$^{15}$ These latter results are consistent with our findings that the EP4 agonist improves cardiac function in the same working heart preparation.

It is well established that PGE$_2$ signals through 4 receptors (EP1, EP2, EP3, and EP4) that signal via different mechanisms. In the mouse LV, expression of EP3 and EP4 mRNAs is higher than that of EP1 and EP2. Indeed, Xiao et al$^{15}$ were unable to detect EP1 in the mouse heart using quantitative PCR, whereas other literature supports its presence.$^{16,17}$ The results in our present study provide direct evidence that exogenous PGE$_2$ can reduce contractility of both the isolated heart and isolated myocytes acutely via its EP3 receptor. We were rather surprised to note that the effect of PGE$_2$, mirrored that of the EP1/EP3 selective agonist sulprostone, despite the fact that both the isolated heart and the isolated cardiac myocyte express EP4 receptors abundantly as measured by real-time reverse transcription polymerase chain reaction. Although the reason for this finding is not clear, one could speculate that either EP4 but not EP3 is rapidly internalized after agonist stimulation$^{18-20}$ or that the receptors are compartmentalized differently.$^{21}$ However, we have data (not shown) indicating that within a 1-hour time frame, AVM are able to increase cAMP production in response to repeated doses of either PGE$_2$ or the EP4 agonist, suggesting that the former may not be correct in cardiac myocytes. Further experiments are needed to examine these possibilities.

Because both cyclooxygenase-2 and microsomal PGE synthase-1 are upregulated in the infarcted heart to increase production of PGE$_2$, we were interested to examine whether the expression of EP3 and EP4 was also affected. Although our experiments were limited to studies of mRNA levels because of difficulties with the commercially available EP3 and EP4 antibodies, our data show that both EP3 and EP4 mRNAs are upregulated after MI although this is seemingly greater for EP3. We thus speculate that the altered balance between EP3 and EP4 in heart failure contributes to the diminished contractility observed in pathological conditions characterized by increased PGE$_2$.

Almost a decade ago, Schutte et al$^{22}$ reported that the administration of PGE$_2$ to healthy sheep improved cardiac contractility and relaxation while decreasing heart rate. In contrast, however, similar administration to sheep in congestive heart failure reduced cardiac function with increased preload. These results lead the authors to speculate that PGE$_2$ might not be a suitable agent for treatment of congestive heart failure because of the worsening effect it had on the cardiodynamics of the failing heart. Our results suggest that administration of a selective EP4 agonist might be a more promising option. Indeed, the first clinical report was very recently published showing that an EP4 agonist had a lucistropic and vasodilator effect in healthy volunteers.$^{23}$ This was followed by a concurrent publication showing that acute infusion of an EP4 agonist to normal anesthetized dogs increased ejection fraction and +dP/dt but decreased end systolic pressure.$^{24}$ Our results add to evidence for a protective effect of EP4 but provide additional mechanistic insight.

Ca$^{2+}$ is known to be important in myocyte contraction and relaxation. Selective stimulation of EP2 or EP4 receptors attenuates histamine-evoked Ca(2+) signals potentially demonstrating that PGE$_2$ via these receptors can regulate heart contractility by alterations in intracellular calcium. Our data with isolated myocytes show that treatment with PGE$_2$ slows calcium reuptake. Surprisingly, we could not detect such an effect using sulprostone, but whether this relates to our inability to detect smaller changes with this drug is unknown. Phospholamban is a phosphoprotein in cardiac sarcoplasmic reticulum that is a reversible regulator of the Ca(2+) (+)-ATPase (SERCA2a) activity and cardiac contractility. Dephosphorylated phospholamban inhibits SERCA2a and phospholamban phosphorylation, at either Ser$^{16}$ by protein kinase A or Thr$^{17}$ by Ca(2+) (+)-calmodulin-dependent protein kinase, reverses this inhibition. Through this mechanism, phospholamban is a key modulator of sarcoplasmic reticulum Ca(2+) (+) uptake, Ca(2+) (+) load, contractility, and relaxation.$^2$ Previously, our gene array data on LV from EP4 knockout mice showed reduced phospholamban in knockout mice with the reduction correlating with ejection fraction (data submitted to the GEO database-NCBI). However, these data were obtained from older mice in various stages of heart failure and could not discern phosphorylation status. In support of our data, Liu et al$^8$ reported that PGE$_2$ inhibits adrenergic-induced p-PLN and the contractile response in animal hearts. However, they did not observe any effect under basal conditions and they implicated the EP4 receptor, whereas our results showing that PGE$_2$ reduces p-PLN in isolated myocytes suggests that this is an EP3-mediated event, consistent with the Langendorff data.

In conclusion, our data clearly show that PGE$_2$, has an acute and direct effect on cardiac contractility; a positive inotropic effect mediated by its EP4 receptor and a negative inotropic effect mediated by its EP3 receptor. These effects were consistent in experiments ranging from isolated myocyte contractility studies through those in the intact animal. Our results have importance in situations where PGE$_2$ is elevated such as various inflammatory conditions and thus suggest a new deleterious relationship between inflammation and cardiac function that is mediated via the PGE$_2$ EP3 receptor subtype.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Our laboratory previously reported that cardiomyocyte-specific EP4 knockout mice develop dilated cardiomyopathy with reduced ejection fraction. We thus hypothesized that prostaglandin E2 (PGE2) increases contractility via EP4 but decreases contractility via EP3. Our present study examine the influence of PGE2 and its receptor subtypes (EP1–4) on cardiac contractility using isolated mouse cardiomyocytes, the Langendorff heart preparation, and in vivo using pressure–volume loops in the anesthetized mouse. Our studies show that PGE2 has opposing effects on contractility dependent on which receptor subtype is activated. In general, contractility was reduced via the EP3 receptor but increased via EP4. In the working heart, both PGE2 and the EP1/EP3 agonist sulprostone decreased left ventricular developed pressure via EP3, whereas the EP4 agonist had the opposite effect. Single myocyte contractility was also reduced after treatment with either PGE2, or sulprostone. The negative inotropic effects of PGE2, and the EP3 agonist seemed to be mediated by decreased phosphorylation of phospholamban without effects on SERCA2a expression. The in vitro hemodynamic effects of PGE2, and sulprostone were mimicked acutely in vivo using pressure–volume loops. Both PGE2, and sulprostone decreased +dp/dt, whereas the EP4 agonist increased +dp/dt. Our results may have potential clinical significance as we also observed increased EP3 expression in mice subject to myocardial infarction. If these results translate to the patient population, they suggest that blockade of the EP3 receptor could ameliorate worsening cardiac function observed in inflammatory conditions characterized by increased PGE2. Furthermore, they suggest a potentially protective role for EP4 agonists.
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