Microsomal Prostaglandin E\textsubscript{2} Synthase-1 Deletion Leads to Adverse Left Ventricular Remodeling After Myocardial Infarction

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Background—Pharmacological inhibition of cyclooxygenase-2 increases the risk of myocardial infarction (MI) and stroke. Microsomal prostaglandin (PG) E\textsubscript{2} synthase-1 (mPGES-1), encoded by the \textit{Ptges} gene, functions downstream from cyclooxygenase-2 in the inducible PGE\textsubscript{2} biosynthetic pathway. We caused acute MI in \textit{Ptges\textsuperscript{+/+}} and \textit{Ptges\textsuperscript{−/−}} mice to define the role of mPGES-1 in cardiac ischemic injury.

Methods and Results—Twenty-eight days after MI, \textit{Ptges\textsuperscript{−/−}} mice develop more left ventricular (LV) dilation, have worse LV systolic and diastolic function, and have higher LV end-diastolic pressure than \textit{Ptges\textsuperscript{+/+}} mice but have similar pulmonary wet-to-dry weight ratios, cardiac mass, infarct size, and mortality. The length-to-width ratio of individual cardiomyocytes is significantly greater in \textit{Ptges\textsuperscript{−/−}} than \textit{Ptges\textsuperscript{+/+}} mice after MI, a finding consistent with eccentric cardiomyocyte hypertrophy in \textit{Ptges\textsuperscript{−/−}} mice. Expression of atrial natriuretic peptide, brain natriuretic peptide, and α- and β-myosin heavy chain, markers of ventricular hypertrophy, is higher in the LV of \textit{Ptges\textsuperscript{−/−}} than \textit{Ptges\textsuperscript{+/+}} mice after MI. \textit{Ptges\textsuperscript{+/−}} mice express cyclooxygenase-2 and mPGES-1 protein in inflammatory cells adjacent to the infarct after MI but do not express these proteins in cardiomyocytes. \textit{Ptges\textsuperscript{+/−}} mice express cyclooxygenase-2 in inflammatory cells adjacent to the infarct and do not express mPGES-1 in any cells in the heart. Levels of PGE\textsubscript{2} but not PGD\textsubscript{2}, thromboxane A\textsubscript{2}, PGI\textsubscript{2}, or PGF\textsubscript{2α} are higher in the infarct and LV remote from the infarct after MI in \textit{Ptges\textsuperscript{+/+}} than \textit{Ptges\textsuperscript{−/−}} mice.

Conclusions—In \textit{Ptges\textsuperscript{+/−}} mice, mPGES-1 in inflammatory cells catalyzes PGE\textsubscript{2} biosynthesis in the LV after MI. Deletion of mPGES-1 leads to eccentric cardiac myocyte hypertrophy, LV dilation, and impaired LV contractile function after acute MI. (\textit{Circulation}. 2008;117:1701-1710.)

Key Words: hypertrophy • inflammation • myocardial infarction • prostaglandins • remodeling

Prostaglandins (PGs) are biologically active lipid mediators that are synthesized by the sequential action of phospholipase A\textsubscript{2}, cyclooxygenase (COX), and PG synthase enzymes. Many aspects of cardiac physiology, including heart rate, coronary blood flow, coronary microvascular permeability, and left ventricular (LV) contractility, are regulated by PGs.\textsuperscript{1–3} PGE\textsubscript{2}, the principal PG generated by ventricular cardiomyocytes,\textsuperscript{4} may be synthesized by microsomal PGE\textsubscript{2} synthase-1 (mPGES-1), an inducible enzyme,\textsuperscript{5,6} or by mPGES-2 or cytosolic PGE\textsubscript{2} synthase, which are constitutively expressed.

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Studies with mice harboring a targeted deletion of \textit{Ptges} (\textit{Ptges\textsuperscript{−/−}} mice) have shown that elimination of mPGES-1 expression decreases pain, fever, and inflammation in vivo.\textsuperscript{7–9} From these observations, pharmacological inhibition of mPGES-1 has been proposed as an alternative to inhibition of...
COX-2 in the management of patients with pain and inflammatory diseases. Targeted deletion of Ptgesc also attenuates brain ischemia-reperfusion injury and reduces plaque burden in fat-fed Ptgesc+/low-density lipoprotein receptor-deficient (LDLR−/−) mice but has no effect on thrombomodulin biosynthesis, clot formation, or blood pressure. In contrast, inhibition of the PGE2 receptor EP4 attenuates cardiomyocyte hypertrophy in vitro, and deletion of EP4 increases infarct size and compromises LV contractile function after 1 hour of coronary occlusion and 1 day of reperfusion in vivo. Therefore, although deletion of mPGES-1 has potentially beneficial effects in multiple disease processes, deletion of mPGES-1 and attenuated PGE2 biosynthesis may have deleterious effects on the cardiac response to myocardial infarction (MI).

LV remodeling takes place after MI and is characterized by a state of volume overload. This maladaptive state is associated with an increase in LV wall stress that leads to eccentric cardiomyocyte hypertrophy with the addition of sarcomeres in series and longitudinal cell growth, infarct zone thinning, and LV dilation. PGE2 induces hypertrophy of ventricular cardiomyocytes in vitro, and mPGES-1 catalyzes the majority of PGE2 biosynthesis by cardiomyocytes and inflammatory cells. In this study, we evaluated the effect of targeted deletion of mPGES-1 on cardiomyocyte hypertrophy and LV remodeling after MI. We found that mice lacking mPGES-1 generate less PGE2 in the LV and develop eccentric cardiomyocyte hypertrophy, LV dilation, impaired LV systolic and diastolic function, and elevated LV end-diastolic pressure (LVEDP) after MI compared with wild-type littersmates. In contrast, Ptgesc+/+ and Ptgesc−/− mice have similar pulmonary wet-to-dry weight ratios, cardiac mass, infarct size, and mortality after MI.

Methods
Reagents were from Sigma Chemical Co (St Louis, Mo) unless otherwise stated. The methodology used for real-time quantitative polymerase chain reaction (PCR) and immunoblotting studies has been described. Housing and experimental procedures were approved by the Animal Care Committee of the University Health Network and were in accordance with the Guide for the Care and Use of Laboratory Animals Research Statutes, Ontario (1980).

Ptgesc Gene–Targeted Mice
Construction of the mPGES-1–deficient mouse line (Ptgesc−/−, DBA/11acl background) was carried out as described. PCR-based genotyping of tail DNA extracts, immunoblotting, and assays of mPGES-1 activity from multiple-organ lysates confirmed the absence of mPGES-1 in Ptgesc−/− mice. Wild-type littersmates were used as controls for studies with Ptgesc−/− mice; all mice were generated by breeding heterozygous mice. The genetic status of mice (Ptgesc+/+ or Ptgesc−/−) remained unknown to investigators carrying out surgical procedures, echocardiography, acquisition of pressure-volume loops, and morphometric assessments.

MI Model
Eight- to 12-week–old male Ptgesc−/− mice or their wild-type littersmates were sedated with ketamine (50 mg/kg) and xylazine (5 mg/kg), intubated, ventilated, and maintained with 2% isoflurane. Through a left thoracotomy, the left coronary artery was ligated at a proximal location under the left atrial appendage, thereby simulating acute coronary artery thrombosis. Ischemia was confirmed by the appearance of hypokinesis and pallor distal to the occlusion and by ST elevation on ECG.

Invasive Hemodynamic Assessment
Under isoflurane anesthesia, a micromanometer and conductance 1.4F catheter (SPR-839, Millar Instruments, Houston, Tex) was introduced into the LV through the right carotid artery. After stabilization, signals were recorded continuously at a sampling rate of 1000/s with a pressure-volume conductance system coupled to a Powerlab/4SP analog-to-digital converter (AD Instruments, Mountain View, Calif). All pressure-volume loops were analyzed with a cardiac pressure-volume analysis program (PVAN 3.3, Millar Instruments).

Echocardiographic Assessment of LV Function
Mice were anesthetized with ketamine (25 mg/kg) and xylazine (2.5 mg/kg). Isoflurane was not used for these studies. Recordings were performed with a Sequoia C256 Ultrasound System (Siemens Medical, Mountain View, Calif) with a 13-MHz linear-array transducer (15L8). M-mode and 2-dimensional images were obtained in the parasternal short axis at the level of the papillary muscles. For each measurement, 3 consecutive cardiac cycles were recorded and averaged by a single experienced examiner. M-mode imaging was recorded at a sweep speed of 200 mm/s for offline measurement of LV end-diastolic and LV end-diastolic dimensions, and the endocardium was traced by covering the innermost edge of the endocardial surface. The LV end-diastolic area was determined as the largest cavity size and the LV end-diastolic area as the smallest cavity size during the cardiac cycle.

Volumetry, Morphometric Analysis, and Collagen Content
At baseline and 28 days after MI, hearts were arrested in diastole, perfusion fixed with 10% formalin at an intraventricular pressure of 20 mm Hg in situ, explanted, weighed, cut into 1-mm transverse sections, and photographed for morphometric measurements. LV chamber volume, LV diameter, LV surface area, and scar area were measured by planimetry with image analysis software (Scion Image, National Institutes of Health Software, Bethesda, Md). The percentage of infarcted LV was calculated by dividing the surface area of the scar by the total surface area of the LV transverse sections. After staining with picrosirius red, total collagen content in the interventricular septum, remote from the zone of infarction, was determined by laser scanning confocal microscopy.

Statistical Analysis
Data are presented as mean±SEM. Analyses of data recorded at 1 time point were performed by 2-tailed unpaired Student t tests that assumed unequal variance. Analyses of data recorded at several time points for 1 group were performed by 1-way ANOVA to evaluate the effect of time; if significant, Bonferroni correction for multiple comparisons was applied when post hoc analysis between different time points was carried out. Analyses of data recorded at several time points for 2 groups (Ptgesc−/− mice, Ptgesc−/− mice) were performed by 2-way ANOVA (to evaluate the effect of group, time, and group–time interactions); if significant, Bonferroni correction for multiple comparisons was applied when post hoc analysis between different time points or between different groups at the same time point was carried out. Survival after coronary ligation was assessed by a log-rank test. A value of P<0.05 was accepted as statistically significant. In total, 119 Ptgesc−/− mice and 129 Ptgesc−/− mice were used in this study.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Online-Only Data Supplement
We describe the methodology used for the measurement of PG levels in the expanded Methods and Results sections in the online-only Data Supplement. The ratio of phosphorylated to total AKT glyco-
gen synthase kinase (GSK), jun N-terminal kinase-1 (JNK1), protein kinase C (PKC), or PKCα and the expression of Nab1 in cardiomyocytes from the LV remote from the infarct is presented in online-only Data Supplement Figure I. Immunohistochemical analysis of COX-2 and mPGES-1 in the LV remote from the infarct is presented in online-only Data Supplement Figure II. PG levels and the expression of PG biosynthetic enzymes in the LV remote from the infarct and in the infarct and peri-infarct tissue are presented in online-only Data Supplement Figures III and IV, respectively. The in vivo 2-dimensional echocardiographic assessment of cardiac structure and function of hearts from Ptges+/− and Ptges−/− mice before and 7 and 28 days after coronary artery ligation is presented in online-only Data Supplement Table I.

Results
Ptges−/− Mice Develop LV Dilation and Impaired LV Contractile Function After MI
We did not identify any differences in cardiac mass, dimensions, or function (Figure 1, the Table, and online-only Data Supplement Table I) or cardiomyocyte morphology (Figure 2A through 2G) between noninjured adult Ptges+/− and Ptges−/− mice. To evaluate the effect of Ptges gene deletion on the cardiac response to acute MI, we subjected 8- to 12-week-old Ptges+/− and Ptges−/− mice to left anterior descending coronary artery ligation. Survival of Ptges+/− and Ptges−/− mice after MI was similar (Figure 1A). The decreases in LV fractional shortening (Figure 1B) observed in Ptges−/− mice 7 days after coronary ligation were comparable and were consistent with the development of a significant MI in these mice. Between 7 and 28 days after MI, fractional shortening (Figure 1B) did not change in Ptges+/− mice but decreased significantly in Ptges−/− mice. Twenty-eight days after MI, fractional shortening (Figure 1B) and dP/dtmax (Figure 1C), load-dependent indexes of LV systolic function, and preload-adjusted maximal power (the Table) were significantly worse in Ptges−/− mice than Ptges+/− mice, dP/dtmin (Figure 1D) and the time constant of isovolumetric LV relaxation (τ; Figure 1E), measures of LV diastolic function, also were significantly worse in Ptges−/− than Ptges+/− mice 28 days after MI. LV volume was 54%
Eccentric Hypertrophy After MI

similar in these mice 3, 7, and 28 days after coronary ligation but the wet-to-dry weight ratio of pulmonary tissue was lower in the Ptges+/− mice than in the Ptges+/+ mice. More ANP, BNP, and -myosin heavy chain (MHC) are fetal cardiac genes that are re-expressed when cardiomyocytes hypertrophy. Cardiomyocytes in the LV remote from the zone of infarction undergo hypertrophy in both Ptges+/+ and Ptges−/− mice, as shown by immunostaining with anti-MHC (Figure 2K) in Ptges−/− mice (Figure 1M), but the wet-to-dry weight ratio of pulmonary tissue was similar in these mice 3, 7, and 28 days after coronary ligation (Figure 1N).

Cardiomyocytes in Ptges−/− Mice Undergo Eccentric Hypertrophy After MI

We found that cardiomyocytes in the LV remote from the zone of infarction underwent hypertrophy in both Ptges+/+ and Ptges−/− mice after MI, but the extent of hypertrophy was attenuated in Ptges−/− mice (Figure 2A and 2B). To study the geometry of individual cardiomyocytes, we enzymatically digested hearts before and 28 days after MI and identified rod-shaped cardiomyocytes by immunostaining with anti-troponin I (Figure 2C). The increase in cardiomyocyte surface area after MI was significantly greater in Ptges+/+ than Ptges−/− mice (Figure 2D). The increases in length of cardiomyocytes from Ptges+/+ and Ptges−/− mice after MI were similar (Figure 2E). In contrast, the width of cardiomyocytes from Ptges+/+ mice did not change after MI, whereas the width of cardiomyocytes from Ptges−/− mice decreased (Figure 2F), thereby resulting in a significantly greater length-to-width ratio of cardiomyocytes from Ptges−/− than Ptges+/+ mice 28 days after MI (Figure 2G). These observations confirm that cardiomyocytes in Ptges−/− mice underwent growth by eccentric hypertrophy after MI.

Expression of Molecular Markers of Cardiomyocyte Hypertrophy in Ptges+/+ and Ptges−/− Mice After MI

The genes encoding atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and - and -myosin heavy chain (MHC) are fetal cardiac genes that are re-expressed when cardiomyocytes hypertrophy. Cardiomyocytes in the LV remote from the zone of infarction expressed significantly more ANP, BNP, -MHC, and -MHC mRNA (Figure 2H through 2K) in Ptges−/− than Ptges+/+ mice 3 and 7 days after coronary ligation. Twenty-eight days after coronary ligation, -MHC mRNA levels were higher in the LV of Ptges+/+ than Ptges−/− mice, whereas -MHC mRNA levels remained higher in Ptges−/− than Ptges+/+ mice.

Table. Morphometric and Invasive Hemodynamic Analyses of Hearts From Ptges+/+ and Ptges−/− Mice Before and 28 Days After Left Coronary Artery Ligation

<table>
<thead>
<tr>
<th>Before LCA Ligation</th>
<th>28 Days After LCA Ligation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ptges+/+ (n=9)</td>
</tr>
<tr>
<td>Body mass, g</td>
<td>20.73±1.06</td>
</tr>
<tr>
<td>Cardiac mass, g</td>
<td>131±6.2</td>
</tr>
<tr>
<td>LV diameter, cm</td>
<td>0.32±0.033</td>
</tr>
<tr>
<td>Septum diameter, cm</td>
<td>0.13±0.023</td>
</tr>
<tr>
<td>LV volume/body mass</td>
<td>1.08±0.10</td>
</tr>
<tr>
<td>LV diameter/body mass</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>Septum diameter/body mass ×10^3</td>
<td>6.3±0.1</td>
</tr>
<tr>
<td>Cardiac mass/body mass ×10^3</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>358±31</td>
</tr>
<tr>
<td>LV end-systolic pressure, mm Hg</td>
<td>109±7.3</td>
</tr>
<tr>
<td>LV end-systolic volume, μL</td>
<td>*</td>
</tr>
<tr>
<td>LV end-diastolic volume, μL</td>
<td>*</td>
</tr>
<tr>
<td>Stroke volume, μL</td>
<td>*</td>
</tr>
<tr>
<td>Cardiac output, μL/min</td>
<td>*</td>
</tr>
<tr>
<td>Arterial elastance, mm Hg/μL</td>
<td>*</td>
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<tr>
<td>Preload-adjusted maximal power, mW/μL^2</td>
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LCA indicates left coronary artery. Hearts were subjected to hyperkalemic arrest, perfusion fixed at systemic pressure in situ, explanted, fixed an additional 24 hours, and then processed for morphometric analysis. Alternatively, hemodynamics were assessed with a 1.4F catheter advanced from the right carotid artery into the LV.

*Before coronary ligation, significant variability in measurements based on calculated volumes was observed, possibly because of the relatively small size of the LV in mice not subjected to MI compared with the size of the micromanometer and conductance catheter. Therefore, these values are not reported. However, morphometric and 2-dimensional echocardiographic analyses documented similar LV volume, cardiac dimensions, and fractional shortening in Ptges+/+ and Ptges−/− mice before LCA ligation.

†P<0.05, day 0 vs 28, Ptges+/+ or Ptges−/− mice; ‡P<0.05, Ptges+/+ vs Ptges−/− mice at 0 or 28 days after LCA ligation.
mPGES-1 Is Expressed in Inflammatory Cells in the LV After MI

We then evaluated the expression of selected PG biosynthetic enzymes in the heart. In the LV remote from the infarct, COX-2 mRNA increased in Ptges<sup>−/−</sup> and Ptges<sup>+/+</sup> mice, and mPGES-1 mRNA increased transiently in Ptges<sup>−/−</sup> mice (Figure 4A and 4B), but no COX-2 or mPGES-1 protein was identified in this part of the heart up to 28 days after MI (online-only Data Supplement Figure II). In the infarct and peri-infarct tissue, the increase in COX-2 mRNA was greater in Ptges<sup>−/−</sup> than Ptges<sup>+/+</sup> mice, whereas mPGES-1 mRNA increased progressively after MI in Ptges<sup>−/−</sup> mice (Figure 4C and 4D). COX-2 protein was identified in inflammatory cells in and adjacent to the infarct in Ptges<sup>−/−</sup> (Figure 4E) and Ptges<sup>+/+</sup> mice (Figure 4G) 3 and 7 but not 28 days after MI. mPGES-1 protein was also identified in inflammatory cells in and adjacent to the infarct in Ptges<sup>−/−</sup> mice 3 and 7 days and, to a lesser extent, 28 days after MI (Figure 4F). No mPGES-1 protein was identified in the heart of Ptges<sup>−/−</sup> mice (Figure 4H), and no COX-2 or mPGES-1 protein was identified in cardiomyocytes from Ptges<sup>−/−</sup> or Ptges<sup>+/+</sup> mice at any time point after coronary ligation (Figure 4E through 4H). Therefore, in Ptges<sup>−/−</sup> mice, coronary ligation leads to the recruitment of inflammatory cells to the infarct and peri-infarct zones that express COX-2 and mPGES-1 protein.

**Figure 2.** Eccentric cardiomyocyte hypertrophy and altered expression of fetal cardiac genes in mice lacking mPGES-1 after MI. A, Cardiomyocytes remote from the zone of infarction. B, Cardiomyocyte surface area by computerized planimetry. C, Before and 28 days after MI, hearts from Ptges<sup>−/−</sup> and Ptges<sup>+/+</sup> mice were explanted, digested with collagenase, and stained with anti–troponin I. The surface area (D), length (E), width (F), and length-to-width ratio (G) of ≈100 cardiomyocytes from individual hearts were assessed. Levels of ANP (H), BNP (I), β-MHC (J), and α-MHC (K) mRNA in cardiomyocytes remote from the zone of infarction measured by quantitative real-time PCR and normalized to GAPDH mRNA levels. Data represent ≥8 independent experiments for each group. Open bars indicate Ptges<sup>−/−</sup> mice; solid bars, Ptges<sup>+/+</sup> mice. a, P<0.05 vs time 0; b, P<0.05, Ptges<sup>−/−</sup> vs Ptges<sup>+/+</sup> mice at any time point.

Differential Activation of Signaling Cascades That Regulate Cardiomyocyte Hypertrophy in Ptges<sup>−/−</sup> and Ptges<sup>+/+</sup> Mice After MI

Next, we evaluated signaling pathways that have been implicated in the regulation of cardiomyocyte hypertrophy. The ratios of activated (phosphorylated) to total of JNK2, extracellular signal-regulated kinase (ERK)-1, ERK2, GSKα, and PKCδ were significantly higher in cardiomyocytes remote from the zone of infarction in Ptges<sup>−/−</sup> than Ptges<sup>+/+</sup> mice 3 days after MI (Figure 3A through 3G). Calcineurin protein levels in the LV were similar in Ptges<sup>−/−</sup> and Ptges<sup>+/+</sup> mice (Figure 3H), but the levels of calcineurin and MCI1 mRNA, which is regulated by an intragenic cluster of nuclear factor of activated T cells (NFAT) consensus binding sites and has been used as an index of the activation level of the calcineurin-NFAT pathway, were greater in Ptges<sup>−/−</sup> than Ptges<sup>+/+</sup> mice 3 days after MI (Figure 3I and 3J). No differences in the ratio of phosphorylated to total AKT, GSKβ, JNK1, PKCa, or PKCe or in the expression of Nab1, all of which have been implicated in the regulation of cardiomyocyte hypertrophy, were identified between Ptges<sup>−/−</sup> and Ptges<sup>+/+</sup> mice after MI (online-only Data Supplement Figure Ia through If). Thus, mPGES-1 modulates multiple signaling pathways that regulate cardiomyocyte hypertrophy.

mPGES-1 Regulates PGE 2 Biosynthesis in the LV After MI

Ptges<sup>−/+</sup> and Ptges<sup>−/−</sup> mice had similar levels of PGE 2 (Figure 4I), PGD 2, thromboxane B 2 (TxB 2; a TxA 2 metabolite), PGF 2α, and 6k-PGF 1α (a PGI 2 metabolite) in the LV before MI (online-only Data Supplement Figure IIIa through IIId). Three and 7 days after MI, PGE 2 levels were significantly higher in the LV (Figure 4I) and infarct (which includes the peri-infarct zone; Figure 4J) of Ptges<sup>−/−</sup> than Ptges<sup>−/−</sup> mice. Interestingly, levels of PGE 2 in the infarct and peri-infarct zones of Ptges<sup>−/−</sup> mice 3 and 7 days after MI were ∼8-fold higher than the levels of PGE 2 in the LV remote from the infarct. Twenty-eight days after MI, levels of PGE 2 remained above baseline levels in the LV and infarct but were not statistically different between Ptges<sup>−/−</sup> and Ptges<sup>+/+</sup> mice. The levels of PGD 2, TxB 2, PGF 2α, and 6k-PGF 1α and the expression of PGD 2 synthase, TxA 2 synthase, and PGI 2 synthase mRNA in the LV remote from the infarct (online-only Data Supplement Figure IVa through IVd and IVh through IVj) increased after MI, but no differences were found in the levels of these PGs or the expression of their respective terminal synthases between Ptges<sup>−/−</sup> and Ptges<sup>+/+</sup> mice 3, 7, and 28 days after MI. Therefore, coronary ligation leads to a selective increase in mPGES-1–catalyzed PGE 2 biosynthesis in the heart.
Discussion
After acute MI, viable cardiomyocytes remote from the zone of infarction undergo hypertrophy, a critical step in postinfarction LV remodeling. This adaptation is necessary to sustain cardiac function and to prevent heart failure, a leading cause of death after MI. Our findings provide direct evidence that a lack of mPGES-1 leads to eccentric hypertrophy of viable cardiomyocytes, LV dilation, and impaired LV systolic and diastolic function after acute MI. Although Ptges−/− mice also develop higher LVEDP than Ptges+/+ mice 28 days after MI, Ptges−/− mice do not develop pulmonary edema; the wet-to-dry weight ratios of lungs from these mice after MI are similar. This finding and the observation that the percentage of infarcted LV was similar in Ptges+/+ and Ptges−/− mice may explain why no difference was found in the survival of these mice after MI. The failure of Ptges−/− mice to develop pulmonary edema and heart failure after MI may be due to the fact that relatively young mice (8 weeks old) do not develop heart failure even after large (up to 28% of the LV) infarctions.17

The relatively low heart rate of mice undergoing echocardiographic assessment (200 bpm to 300 bpm; online-only Data Supplement Table I) may be a manifestation of the anesthesia used for these studies and is below the resting heart rate of these mice (350 bpm to 450 bpm; the Table). Importantly, the measurements of LV dimensions and LV contractile function obtained by 2 independent methods, morphometric analysis of myocardium perfusion fixed in situ and invasive hemodynamic assessment, are consistent with the echocardiographic data. These findings support the conclusion that the echocardiographic measurements are accurate, the relatively low heart rate observed during echocardiographic assessment notwithstanding.

Inflammatory Cells Are the Likely Source of PGE2 Biosynthesis in the LV After MI
The cell type that produces PGE2 in the heart after MI has not been identified. However, although cardiomyocytes,17,25 cardiac fibroblasts,25 and inflammatory cells18,26 can express mPGES-1 and synthesize PGE2 in vitro, inflammatory cells are the likely source of PGE2 biosynthesis in the LV because inflammatory cells were the only cells in the heart that express mPGES-1 protein after MI (see Figure 4E through 4H). Because the increase in COX-2 and mPGES-1 protein expression after MI is confined to inflammatory cells in the infarct and peri-infarct regions, we postulate that PGE2 produced by inflammatory cells in and adjacent to the infarct diffuses to and regulates the hypertrophy of cardiomyocytes.
remote from the zone of infarction. Eccentric hypertrophy of viable cardiomyocytes in *Ptges*−/− mice remote from the zone of infarction may be due to a relative deficiency of mPGES-1–catalyzed PGE2 biosynthesis because PGE2 levels in this part of the LV are 4-fold lower in *Ptges*−/− mice than *Ptges*+/+ mice after MI and PGE2 induces cardiomyocyte hypertrophy in vitro.4,14

**mPGES-1 Catalyzes PGE2 Biosynthesis in the Heart After MI**
mPGES-1 mRNA expression and protein synthesis increase in the infarct zone of *Ptges*+/+ mice after MI. mPGES-2 and cytosolic PGE2 synthase (cPGES) mRNA also are expressed in the infarct and in the LV remote from the infarct after MI (online-only Data Supplement Figures IIIf, IIIg, IVf, and IVg, respectively). Unexpectedly, cPGES mRNA levels in the infarct and LV remote from the infarct were transiently higher in *Ptges*−/− mice than in *Ptges*+/+ mice after coronary ligation. Although mPGES-1, mPGES-2, and cPGES are all expressed in the LV after MI, the observation that PGE2 levels were 4-fold higher in the LV and infarct of *Ptges*+/+ than of *Ptges*−/− mice 3 and 7 days after coronary ligation provides direct evidence that mPGES-1 catalyzes the majority of PGE2 biosynthesis in the heart up to 7 days after MI. PGE2 levels remain elevated in *Ptges*+/+ mice 28 days after MI, despite the fact that mPGES-1 protein levels in the infarct are low at this time. Therefore, it is possible that cPGES and/or mPGES-2, both with mRNA species that were identified in the LV and infarct after MI, also could contribute to PGE2 biosynthesis 28 days after MI in *Ptges*+/+ (and *Ptges*−/−) mice.

**mPGES-1 Modulates Signaling Cascades That Regulate Cardiomyocyte Hypertrophy After MI**

Multiple signaling molecules have been implicated in the pathophysiology of eccentric myocardial hypertrophy. Thus, the absence of telomerase,27 Kruppel-like factor 15,28 the transcription factor GATA4,29 or focal adhesion kinase (in the ventricles)30 leads to eccentric cardiomyocyte hypertrophy. Because PGE2 increases GATA-4 binding activity, 31 de-

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**Figure 4.** Attenuated PGE2 biosynthesis in the infarct and LV in *Ptges*−/− mice after MI. COX-2 (A) and mPGES-1 (B) mRNA levels in the LV after MI. COX-2 (C) and mPGES-1 mRNA (D) levels in the infarct after MI. The infarct includes tissue from the infarction and adjacent peri-infarct zones. mRNA was measured by quantitative real-time PCR, and results are normalized to GAPDH mRNA levels. Immunohistochemical analysis of the heart for COX-2 (E) and mPGES-1 (F) in *Ptges*+/+ mice and COX-2 (G) and mPGES-1 (H) in *Ptges*−/− mice 0, 3, 7, and 28 days after MI. Images are representative of ≥3 independent experiments at each time point shown. PGE2 levels in the LV (I) and infarct (J) after MI. Data represent ≥9 independent experiments for each group. Open bars indicate *Ptges*+/+ mice; solid bars, *Ptges*−/− mice. a, *P* < 0.05 vs time 0; b, *P* < 0.05, *Ptges*−/− vs *Ptges*+/+ mice at any time point.
increased PGE\(_2\) levels in the LV of Ptges\(^{-/-}\) mice could attenuate GATA4 activation and promote eccentric cardiomyocyte hypertrophy after MI. Activation of the mitogen-activated protein kinase kinase 5 (MEK5)-ERK5 signaling cascade also leads to eccentric hypertrophy of rat neonatal ventricular cardiomyocytes, and transgenic expression of MEK5 results in eccentric cardiomyocyte hypertrophy in the absence of apoptosis or fibrosis,\(^{33}\) a finding reminiscent of the eccentric cardiomyocyte hypertrophy and lack of fibrosis or apoptosis observed in Ptges\(^{-/-}\) mice after MI. The observation that the activity of multiple signaling molecules, including JNK2, ERK1, ERK2, GSK\(\alpha\), and PKC\(\alpha\), and the activity of the calcineurin-NFAT pathway are increased in cardiomyocytes in Ptges\(^{-/-}\) compared with Ptges\(^{+/+}\) mice suggests that multiple signaling cascades may contribute to the eccentric cardiac myocyte hypertrophy observed in Ptges\(^{-/-}\) mice after MI. Because Jnk2\(^{-/-}\) mice and transgenic mice expressing dominant-negative JNK1 and JNK2 exhibit enhanced myocardial growth 3 days after transverse aortic constriction,\(^{33}\) JNK2 activation may attenuate cardiomyocyte hypertrophy in Ptges\(^{-/-}\) mice after MI.

mPGES-1 Attenuates Prohypertrophic Fetal Cardiac Gene Expression After MI

Expression of the genes encoding \(\alpha\)-MHC, \(\beta\)-MHC, ANP, and BNP is increased when ventricular myocytes hypertrophy.\(^{20}\) \(\alpha\)-MHC, which is upregulated in the heart after birth, has high ATPase activity, whereas \(\beta\)-MHC has low ATPase activity. Three days after MI, \(\beta\)-MHC expression increases 5-fold after MI in Ptges\(^{-/-}\) mice but does not change in Ptges\(^{+/+}\) mice, whereas \(\alpha\)-MHC expression does not change in Ptges\(^{-/-}\) mice but decreases 50% in Ptges\(^{+/+}\) mice. The relative increase in \(\beta\)-MHC compared with \(\alpha\)-MHC expression in the LV of Ptges\(^{-/-}\) mice after MI, a molecular signature of pathological cardiac remodeling, may lead to a reduction in myofilibrillar ATPase activity, reduced shortening velocity of cardiac myofibers, and eventual contractile dysfunction.\(^{24}\) Because alterations in fetal gene re-expression occur before the onset of eccentric cardiomyocyte hypertrophy,\(^{20}\) the increased ratio of \(\beta\)-MHC to \(\alpha\)-MHC expression may be predictive of subsequent LV dysfunction in Ptges\(^{-/-}\) mice after MI. Expression of 2 other markers of ventricular hypertrophy, the natriuretic peptides ANP and BNP, was increased PGE\(_2\) levels in the LV of Ptges\(^{-/-}\) mice could attenuate GATA4 activation and promote eccentric cardiomyocyte hypertrophy after MI. Activation of the mitogen-activated protein kinase kinase 5 (MEK5)-ERK5 signaling cascade also leads to eccentric hypertrophy of rat neonatal ventricular cardiomyocytes, and transgenic expression of MEK5 results in eccentric cardiomyocyte hypertrophy in the absence of apoptosis or fibrosis,\(^{33}\) a finding reminiscent of the eccentric cardiomyocyte hypertrophy and lack of fibrosis or apoptosis observed in Ptges\(^{-/-}\) mice after MI. The observation that the activity of multiple signaling molecules, including JNK2, ERK1, ERK2, GSK\(\alpha\), and PKC\(\alpha\), and the activity of the calcineurin-NFAT pathway are increased in cardiomyocytes in Ptges\(^{-/-}\) compared with Ptges\(^{+/+}\) mice suggests that multiple signaling cascades may contribute to the eccentric cardiac myocyte hypertrophy observed in Ptges\(^{-/-}\) mice after MI. Because Jnk2\(^{-/-}\) mice and transgenic mice expressing dominant-negative JNK1 and JNK2 exhibit enhanced myocardial growth 3 days after transverse aortic constriction,\(^{33}\) JNK2 activation may attenuate cardiomyocyte hypertrophy in Ptges\(^{-/-}\) mice after MI.

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Possible Effect of Pharmacological Inhibition of mPGES-1 on Postinfarction LV Remodeling

The increased risk of MI and stroke\(^{41,42}\) and increased mortality after MI\(^{43}\) in patients taking selective COX-2 inhibitors are proposed to be due to an imbalance of prothrombotic eicosanoids (increased TxA\(_2\)) and antiplatelet aggregation (decreased PGI\(_2\)).\(^{44}\) We noted that targeted deletion of the gene encoding mPGES-1 does not alter the levels of TxA\(_2\) or PGI\(_2\) in the heart after MI. Therefore, pharmacological inhibition of mPGES-1 may not be associated with the perturbations in TxA\(_2\) and PGI\(_2\) metabolism that increase the risk of arterial thrombosis in patients taking COX-2 inhibitors but may lead to eccentric cardiomyocyte hypertrophy and compromise LV function and LV remodeling after acute MI. This hypothesis should be interpreted with caution because the effect of individual gene deletions on cardiac physiology in mice may not be observed in patients taking pharmacological inhibitors of the corresponding gene product. For example, the diffuse cardiac fibrosis noted in 50% of mice lacking COX-2\(^{-/-}\) has not been identified in patients taking selective COX-2 inhibitors. In addition, COX-2 inhibition was shown to be cardioprotective in mice\(^{46,47}\) but led to adverse LV remodeling and LV rupture in a porcine MI model.\(^{48}\) These observations underscore the need for caution in extrapolating findings in mice to humans without studies in larger animals first.

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Disclosures

Dr Audoly has stock ownership or options in Pfizer Inc and has received fees (or fees are pending) for patents, licenses, or licensing. Dr Jakobsson has received consulting fees from several companies and has patents related to COX and COX inhibitors. Drs Rubin and Lindsay have received consultancy fees from several companies and have patents related to COX-2 inhibitors. Drs Liu and Jakobsson have research grants from several research foundations and have patents related to COX inhibitors. Dr Geisslinger has a patent pending related to COX-2 inhibitors.
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**CLINICAL PERSPECTIVE**

Pharmacological inhibition of microsomal prostaglandin E2 synthase-1 (mPGES-1) has been proposed as an alternative to inhibition of cyclooxygenase-2 in the management of patients with pain and inflammatory diseases. Here, we examined the role of prostaglandin E_{2} (PGE_{2}) in postinfarction cardiac remodeling by taking advantage of homozygote mPGES-1 knockout mice, which have low basal and induced PGE_{2} synthesis. Contrary to our original hypothesis, we found that deletion of mPGES-1 did not affect the size of the infarct after coronary ligation. However, the mPGES-1 knockout animals had worse left ventricular systolic and left ventricular diastolic function, more ventricular dilation, and markedly attenuated cardiomyocyte hypertrophy in the region remote from the infarction compared with wild-type mice. Coupled with the observation that the bulk of PGE_{2} biosynthesis in the infarct was carried out by inflammatory cells, these findings suggest that diffusion of PGE_{2} from the infarct and peri-infarct regions influences the hypertrophy of cardiomyocytes remote from the infarction. These data imply that hypertrophy in the region remote from the infarct is not regulated purely by mechanical forces but also by inflammatory mediators such as PGE_{2}. The potential clinical importance of these observations is significant because the millions of patients who previously took cyclooxygenase-2 inhibitors are potential candidates to take pharmacological inhibitors of mPGES-1, which are currently in development. Our findings emphasize the importance of carefully evaluating cardiac function in patients at risk for myocardial infarction who are treated with agents that selectively block PGE_{2} biosynthesis, which have been proposed to have less cardiovascular toxicity than inhibitors of cyclooxygenase-2.