Augmented Cardiac Hypertrophy in Response to Pressure Overload in Mice Lacking the Prostaglandin I₂ Receptor

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Background—In the heart, the expressions of several types of prostanoid receptors have been reported. However, their roles in cardiac hypertrophy in vivo remain unknown. We intended to clarify the roles of these receptors in pressure overload–induced cardiac hypertrophy using mice lacking each of their receptors.

Methods and Results—We used a model of pressure overload–induced cardiac hypertrophy produced by banding of the transverse aorta in female mice. In wild-type mice subjected to the banding, cardiac hypertrophy developed during the observation period of 8 weeks. In mice lacking the prostaglandin (PG) I₁ receptor (IP⁻/⁻), however, cardiac hypertrophy and cardiomyocyte hypertrophy were significantly greater than in wild-type mice at 2 and 4 weeks but not at 8 weeks, whereas there was no such augmentation in mice lacking the prostanoid receptors other than IP. In addition, cardiac fibrosis observed in wild-type hearts was augmented in IP⁻/⁻ hearts, which persisted for up to 8 weeks. In IP⁻/⁻ hearts, the expression level of mRNA for atrial natriuretic peptide, a representative marker of cardiac hypertrophy, was significantly higher than in wild-type hearts. In vitro, cicaprost, an IP agonist, reduced platelet-derived growth factor–induced proliferation of wild-type noncardiomyocytes, although it could not inhibit cardiomyotrophin–I–induced hypertrophy of cardiomyocytes. Accordingly, cicaprost increased cAMP concentration efficiently in noncardiomyocytes.

Conclusions—IP plays a suppressive role in the development of pressure overload–induced cardiac hypertrophy via the inhibition of both cardiomyocyte hypertrophy and cardiac fibrosis. Both effects have been suggested as originating from the action on noncardiomyocytes rather than cardiomyocytes. (Circulation. 2005;112:84-92.)

Key Words: hypertrophy • myocardium • pressure • prostaglandins • thromboxanes

Cardiac hypertrophy in response to pressure overload is a compensatory mechanism to maintain circulatory homeostasis.¹ When the mechanical overload on the heart is severe and prolonged, however, it is an important risk factor in cardiac morbidity and mortality.² In addition, many patients suffer from this condition because hypertension is a common cardiovascular disease that over time leads to cardiac hypertrophy. Therefore, the mechanisms underlying the development of pressure overload–induced cardiac hypertrophy have been studied extensively. Until now, several extracellular signaling molecules, such as endothelin, angiotensin II, and cardiotoxin-1, have been proposed as mediators promoting pressure overload–induced cardiac hypertrophy.³⁻⁵ In contrast, little is known about these signaling molecules that ameliorate pressure overload–induced cardiac hypertrophy.

Prostanoids, consisting of prostaglandins (PGs) and thromboxane, exert various actions in the cardiovascular system.⁶⁻⁸ PGI₂ is known as a vasodilator and an inhibitor of platelet activation. In contrast, thromboxane A₂ is a potent vasoconstrictor and a platelet stimulator. In the heart, several prostanoids, such as PGE₂ and PGI₂, have been reported to show cardioprotective actions against ischemia/reperfusion injury.⁹⁻¹¹ In regard to cardiac hypertrophy, several reports suggest the participation of prostanoids in its development. These include inhibitory effects of PGI₂ and its analogue on angiotensin II–induced hypertrophy in cultured cardiomyocytes¹² and their inhibitory effects on proliferation and collagen synthesis in cultured cardiac fibroblasts.¹³ In addition, synthesis of PGE₂ and PGI₂ has been reported to be elevated in the hypertrophied and failing heart¹⁴,¹⁵ along with an upregulation of cyclooxygenase (COX)-2.¹⁶ The hypertrophic effect of exogenously administered PGF₂α on cardiomyocytes has also been reported.¹⁷,¹⁸ Furthermore, several types and subtypes of prostanoid receptors have been reported to be expressed in the heart.⁶,⁷ These results suggest
some roles played by these receptors in the development of pressure overload–induced cardiac hypertrophy; their exact roles in vivo, however, remain unknown.

DP, EP, FP, IP, and TP are the receptors for PGD$_2$, PGE$_2$, PGF$_{\alpha}$, PGI$_2$, and thromboxane A$_2$, respectively. In addition, there are 4 subtypes of EP: EP$_1$, EP$_2$, EP$_3$, and EP$_4$. In the present study, we intended to clarify the roles of prostanoid receptors in pressure overload–induced cardiac hypertrophy using mice each lacking one of these receptors.

**Methods**

**Mice**

The generation and maintenance of mice lacking EP$_2$, EP$_3$, EP$_4$, FP, IP, or TP (EP$_2^{-/-}$, EP$_3^{-/-}$, EP$_4^{-/-}$, FP$^{-/-}$, IP$^{-/-}$, and TP$^{-/-}$ mice, respectively) have been reported. All mice, including the wild-type, EP$_2$ mice, have a genetic background of C57BL/6 mice. EP$_4$ experiments in which EP$_4^{-/-}$ mice were used had a mixed genetic background similar to that of EP$_4^{-/-}$ mice. In the present study, we intended to clarify the roles of prostanoid receptors in pressure overload–induced cardiac hypertrophy using mice each lacking one of these receptors.

**Expression of mRNAs for Prostanoid Receptors**

To examine the expression of mRNAs for prostanoid receptors in the heart, we prepared total RNA from the heart using Isogen (Nippon Gene) and performed reverse transcription–polymerase chain reaction (RT-PCR) as reported. A similar procedure was used in the examination of IP mRNA expression in cardiomycocytes and noncardiomycocytes.

**A Model of Pressure Overload–Induced Cardiac Hypertrophy**

Pressure overload was produced by banding of the transverse aorta (transverse aortic constriction procedure) according to the reported method with minor modifications. Briefly, mice anesthetized with ketamine (100 mg/kg IP) and xylazine (5 mg/kg IP) were maintained under a respirator (model 480-7, Shimano Industry). After thoracotomy, the transverse aorta was exposed between the carotid arteries and was constricted by ligation with 7-0 nylon string along with a blunted 24-gauge needle, which needle was then pulled out. As controls, sham-operated mice were produced; they received an identical operation except for the aortic ligation. At indicated times after the banding, wet weight of the heart (HW) and body weight (BW) were measured, and their ratios (HW/BW ratio) were used as indices of cardiac hypertrophy.

To assess the degree of pressure overload, a polyethylene cannula, connected to a pressure transducer, was inserted into the right and left carotid arteries in some of the wild-type and IP$^{-/-}$ mice. Arterial pressure was measured before and at a steady state within 10 minutes after the banding.

Blood pressure and heart rate of conscious mice were measured by the tail-cuff method (BP-98A, Softron) between 9 AM and noon at indicated times after the banding, as reported. The values were obtained by averaging at least 5 measurements. The results showed that blood pressure and heart rate were not significantly different between wild-type and IP$^{-/-}$ mice at 1, 2, 4, or 8 weeks of the banding (data not shown). Similarly, blood pressure and heart rate at 4 weeks of the banding were not significantly different among wild-type, EP$_2^{-/-}$, EP$_3^{-/-}$, FP$^{-/-}$, and TP$^{-/-}$ mice or between F2 wild-type and EP$_2^{-/-}$ mice (Table 1).

**Histological Analysis of the Heart**

At indicated times, hearts were fixed with 10% formalin and embedded in paraffin. Five transverse sections (5-μm thickness)

| Table 1. Effects of Aortic Banding on Heart Rate and Blood Pressure in Mice Lacking Prostanoid Receptors |
|---|---|---|---|
|       | n  | HR, bpm | SBP, mm Hg | DBP, mm Hg |
| WT    |   |        |            |            |
| EP$_2^{-/-}$ | 5 | 14  | 639±11 | 90±2  |
| EP$_3^{-/-}$ | 5 | 14  | 598±19 | 106±2 |
| EP$_4^{-/-}$ | 4 | 14  | 643±21 | 105±3 |
| IP$^{-/-}$ | 8 | 14  | 636±21 | 101±5 |
| TP$^{-/-}$ | 7 | 14  | 646±12 | 100±3 |
| F2 WT |  7 | 14  | 607±30 | 110±4 |

**Role of PGI$_2$, Receptor in Cardiac Hypertrophy**

Heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured by the tail-cuff method in wild-type (WT), EP$_2^{-/-}$, EP$_3^{-/-}$, EP$_4^{-/-}$, FP$^{-/-}$, IP$^{-/-}$, TP$^{-/-}$, and F2 wild-type (F2 WT) mice at 4 weeks of aortic banding. Each value represents mean±SEM.

were prepared from the middle segment of the heart at intervals of 0.3 mm. The sections were stained with hematoxylin and eosin for examination of their gross appearance and were stained with the van Gieson method for measurement of cardiac hypertrophy and cardiac fibrosis. Cardiomycocyte hypertrophy was assessed by measuring cross-sectional area of 100 cardiomycocytes having nearly circular capillary profiles and nuclei in the left ventricle near the endocardial region. Cardiac fibrosis was assessed separately as interstitial and perivascular fibrosis by calculating the ratio of van Gieson–stained area of interstitial or perivascular fibrosis to total area of cardiac tissue in each section. These analyses were performed by digital planimetry with the use of NIH Image computer software.

**Expression of mRNAs for COXs and Atrial Natriuretic Peptide**

We examined whether the cardiac expression of mRNAs for COX-1, a constitutive isoform, and COX-2, an inducible isoform, changes on pressure overload. We also examined cardiac expression of mRNA for atrial natriuretic peptide (ANP), a representative marker of cardiac hypertrophy. Total RNA was prepared from the left ventricles at indicated times and the expression levels of the mRNAs were determined by RT-PCR with the use of the expression level of 18S ribosomal RNA (rRNA) as an internal control. The sequences of the primers used were as follows: COX-1 (sense), 5'-CTGCT-GAGAAAGGGATTACTT3'; COX-1 (antisense), 5'-GTGC-GAC-CCCGCGTTAATGTT3'; COX-2 (sense), 5'-ACACTCATATCAG-GCACCC3'; COX-2 (antisense), 5'-GGACGGTTTCTTTCCCACTCAG3'; ANP (sense), 5'-ATGGGCTCTTCTCCATCACCC3'; ANP (antisense), 5'-TCACACTGGGGCCTCATACTG3'; 18S rRNA (sense), 5'-ATCTCGCGATGACATATGC3'; and 18S rRNA (antisense), 5'-CCAGGTTATCAGTACAGC3'.

**Measurements of 6-Keto-PGF$_{\alpha}$ Contents in the Heart**

To determine whether pressure overload increases PGI$_2$ production in the heart, we measured the tissue contents of 6-keto-PGF$_{\alpha}$, a stable metabolite of PGI$_2$. Tissue samples were prepared from the left ventricle of wild-type mice at indicated times after the aortic banding, and the contents of 6-keto-PGF$_{\alpha}$ were measured with an EIA kit (Cayman Chemical).

**In Vitro Examination of Cardiomycocyte Hypertrophy and Noncardiomycocyte Proliferation**

 Cultures of cardiomycocytes and noncardiomycocytes were performed as reported. In short, cardiac ventricles of fetal mice were minced and then incubated with a buffer containing 0.1% collagenase (Wako) for 60 minutes at 37°C. The cells were filtered through a nylon mesh, suspended in a culture medium (DMEM/F-12 supple-
mented with 100 U/mL penicillin and 100 μg/mL streptomycin) containing 2.5% fetal calf serum, and then preplated onto a dish to separate cardiomyocytes from noncardiomyocytes. After incubation for 30 minutes, unattached cardiomyocytes were harvested and used for an assay. Attached noncardiomyocytes were grown to near confluence and then used for an assay.

Hyertrophy of cardiomyocytes was estimated by [14C]leucine incorporation, and proliferation of noncardiomyocytes was by [3H]thymidine incorporation and cell number. Cardiomyocytes were plated into 24-well culture plates at 10⁵ cells per well. Noncardiomyocytes were plated into 24-well culture plates at 10⁴ cells per well and into 6-well culture plates at 5×10⁶ cells per well for examination of [H]thymidine incorporation and cell number, respectively. After 48 hours of culture, culture medium was changed to a fresh one containing vehicle or cicaprost (10⁻⁵ mol/L, Schering), and indomethacin (10⁻⁴ mol/L, Sigma). In cardiomyocytes, cardiotoxin-1 (10⁻⁶ mol/L, Genzyme/Technne) and [¹⁴C]leucine (0.1 mCi/mL, Amersham) were added to the culture medium, and the cells were cultured for 48 hours. In noncardiomyocytes, platelet-derived growth factor (PDGF) (Peprotec) and fetal calf serum was added at 5 ng/mL cultured for 48 hours. For examination of [³H]thymidine incorporation, the cells were cultured for 24 hours, and then [³H]thymidine (2 mCi/mL, Amersham) was added, and the cells were cultured for an additional 6 hours. For each cell number count, the cells were cultured for 48 hours, harvested by trypsin-EDTA treatment, and counted with a hemocytometer. Amounts of [¹⁴C]leucine and [³H]thymidine incorporated into cardiomyocytes and noncardiomyocytes, respectively, were quantified by liquid scintillation counter.

Measurements of cAMP Accumulation
The cardiomyocytes and noncardiomyocytes were preincubated for 30 minutes at 37°C in the culture medium containing 10⁻⁵ mol/L indomethacin and 1 mmol/L 3-isobutyl-1-methylxanthine (Sigma-Aldrich). Then the cardiomyocytes were stimulated with vehicle, cicaprost (10⁻⁶ or 10⁻⁵ mol/L), or isoproterenol (10⁻⁷ mol/L, Nacala
tesque), and the noncardiomyocytes were stimulated with vehicle or cicaprost (10⁻⁶ or 10⁻⁵ mol/L). After stimulation for 30 minutes at 37°C, the levels of intracellular cAMP (cAMP) were measured as reported. We determined the protein contents of the cells by use of a BCA protein assay kit (Pierce Chemical).

Statistical Analysis
All values are expressed as mean±SEM. Statistical analysis was performed with 1-way (Figure 5 and Tables 1 and 2) or 2-way (Figures 1C, 2B, 3B, and 4 and Table 3) ANOVA followed by Duncan’s test for multiple comparisons. A difference was considered statistically significant at P<0.05 from the 2-tailed test. All data were analyzed with the software program Super ANOVA, version 1.11.

Results
Expression of mRNAs for Prostanoid Receptors in the Heart
We first examined which types and subtypes of prostanoid receptors are expressed in the heart using the RT-PCR method. We found the expression of mRNAs for EP2, EP3, FP, IP, and TP but not for EP1 and DP (Figure 1A).

In Vivo Model of Pressure Overload–Induced Cardiac Hypertrophy
On the basis of the results of RT-PCR analysis, we next examined cardiac hypertrophy using a model of pressure overload–induced cardiac hypertrophy in EP2⁻/⁻, EP3⁻/⁻, EP1⁻/⁻, FP⁻/⁻, IP⁻/⁻, and TP⁻/⁻ mice. In wild-type mice, HW and HW/BW ratio at 4 weeks after aortic banding were significantly higher than that in sham-operated mice, indicat-

<p>| TABLE 2. Effects of Aortic Banding on BW, HW, and BW/HW Ratio in Mice Lacking Prostanoid Receptors |
|---------------------------------|---|---|---|</p>
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW, g</th>
<th>HW, mg</th>
<th>HW/BW, mg/g</th>
</tr>
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<tr>
<td><strong>Sham-operated mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>14</td>
<td>22.5±0.6</td>
<td>104.3±2.6</td>
<td>4.7±0.1</td>
</tr>
<tr>
<td>EP2⁻/⁻</td>
<td>6</td>
<td>21.6±0.8</td>
<td>99.4±3.9</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td>EP3⁻/⁻</td>
<td>5</td>
<td>22.8±0.2</td>
<td>106.0±2.1</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td>FP⁻/⁻</td>
<td>5</td>
<td>21.5±0.5</td>
<td>103.9±2.2</td>
<td>4.8±0.1</td>
</tr>
<tr>
<td>IP⁻/⁻</td>
<td>8</td>
<td>23.7±0.8</td>
<td>113.1±5.7</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td>TP⁻/⁻</td>
<td>5</td>
<td>22.0±0.7</td>
<td>105.9±2.9</td>
<td>4.8±0.1</td>
</tr>
<tr>
<td>F2 WT</td>
<td>7</td>
<td>21.6±0.5</td>
<td>99.9±3.3</td>
<td>4.7±0.2</td>
</tr>
<tr>
<td>EP1⁻/⁻</td>
<td>7</td>
<td>22.9±1.5</td>
<td>110.7±5.0</td>
<td>4.9±0.1</td>
</tr>
<tr>
<td><strong>Aortic-banded mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>8</td>
<td>22.7±0.5</td>
<td>151.6±9.1</td>
<td>6.7±0.5</td>
</tr>
<tr>
<td>EP2⁻/⁻</td>
<td>5</td>
<td>23.1±0.5</td>
<td>147.5±9.1</td>
<td>6.4±0.3</td>
</tr>
<tr>
<td>EP3⁻/⁻</td>
<td>7</td>
<td>25.1±0.6</td>
<td>158.1±9.6</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>FP⁻/⁻</td>
<td>5</td>
<td>21.7±1.0</td>
<td>152.7±11.7</td>
<td>7.1±0.8</td>
</tr>
<tr>
<td>IP⁻/⁻</td>
<td>6</td>
<td>24.2±0.4</td>
<td>195.5±14.1</td>
<td>8.2±0.7†</td>
</tr>
<tr>
<td>TP⁻/⁻</td>
<td>5</td>
<td>24.4±1.0</td>
<td>160.9±8.1</td>
<td>6.6±0.4*</td>
</tr>
<tr>
<td>F2 WT</td>
<td>6</td>
<td>23.8±0.7</td>
<td>150.9±12.4</td>
<td>6.3±0.4</td>
</tr>
<tr>
<td>EP1⁻/⁻</td>
<td>5</td>
<td>24.3±0.9</td>
<td>162.2±12.0</td>
<td>6.7±0.5*</td>
</tr>
</tbody>
</table>

BW and HW were measured in wild-type (WT), EP2⁻/⁻, EP3⁻/⁻, FP⁻/⁻, IP⁻/⁻, TP⁻/⁻, F2 wild-type (F2 WT), and EP1⁻/⁻ mice at 4 weeks of aortic banding or sham operation. Each value represents mean±SEM.

*P<0.05 vs corresponding sham-operated mice; †P<0.05 vs aortic-banded wild-type mice.

ing that the procedure induced cardiac hypertrophy. In IP⁻/⁻ mice, however, HW and HW/BW ratio were significantly greater than those in wild-type mice, suggesting that IP mediated an antihypertrophic effect (Table 2 and Figures 1B and 1C). In contrast, there were no such differences among wild-type, EP2⁻/⁻, EP3⁻/⁻, FP⁻/⁻, TP⁻/⁻, and TP⁻/⁻ mice or between F2 wild-type and EP3⁻/⁻ mice (Table 2). In sham-operated groups, there was no significant difference in HW, BW, and HW/BW ratio among wild-type, EP2⁻/⁻, EP3⁻/⁻, FP⁻/⁻, and TP⁻/⁻ mice or between F2 wild-type and EP3⁻/⁻ mice (Table 2). These results suggest that IP is the only prostanoid receptor able to affect the development of pressure overload–induced cardiac hypertrophy, at least in the present model.

When the time course of the hypertrophy was examined, it was already apparent at 1 week after the banding, reached a maximum level at 2 weeks, and continued at a similar level thereafter (Figure 1C and Table 3). The HW or HW/BW ratio at 1 week was not significantly different between wild-type and IP⁻/⁻ mice. At 2 and 4 weeks, however, both HW and HW/BW ratio in IP⁻/⁻ mice were significantly greater than those in wild-type mice (Figure 1C and Table 3). At 8 weeks after the banding, however, there was no significant difference in the HW/BW ratio between wild-type and IP⁻/⁻ mice, whereas HW was still significantly greater in IP⁻/⁻ mice than in wild-type mice. There was no significant difference in BW after the banding between wild-type and IP⁻/⁻ mice throughout the experiment (Table 3). These results clearly indicate that IP participates in the suppression of pressure overload–induced cardiac hypertrophy.
Before the banding, the systolic pressures in carotid arteries were 71.0±1100.6±8.8 (n=4) and 70.0±1106.4 (n=3) mm Hg in wild-type and IP⁻/⁻ mice, respectively. After the banding, the systolic pressures in right carotid artery increased to 88.5±1108.5 and 87.3±1107.5 mm Hg, respectively, and those in left carotid artery decreased to 65.5±1106.9 and 65.0±1104.9 mm Hg, respectively, resulting in a pressure gradient of 23.0±1101.6 and 22.3±1102.7 mm Hg, respectively. This suggests that a similar degree of pressure overload was given between wild-type and IP⁻/⁻ hearts by the procedure.

Cardiomyocyte Hypertrophy and Cardiac Fibrosis

Cardiac hypertrophy is associated frequently with both cardiomyocyte hypertrophy and cardiac fibrosis, with the latter consisting of both interstitial and perivascular fibrosis. To determine whether the antihypertrophic role of IP is due to inhibition of cardiomyocyte hypertrophy or cardiac fibrosis, we measured the cross-sectional area of cardiomyocytes (Figure 2) and the area of cardiac fibrosis (Figure 3). In wild-type mice, the cross-sectional area of cardiomyocytes and the area of cardiac fibrosis increased significantly after the banding compared with those in sham-operated mice, indicating the development of both cardiomyocyte hypertrophy and cardiac fibrosis.

At 1 week after the aortic banding, the cross-sectional area of cardiomyocytes was not significantly different between wild-type and IP⁻/⁻ hearts (Figure 2B). At 2 and 4 weeks after the banding, however, it was significantly more augmented in IP⁻/⁻ hearts than in wild-type hearts, indicating that IP-mediated signal suppressed the development of cardiomyocyte hypertrophy. At 8 weeks after the banding, the cross-sectional area of cardiomyocytes in IP⁻/⁻ hearts was not significantly different from that in wild-type hearts, suggesting that mechanisms leading to cardiomyocyte hypertrophy caught up with the suppressive effect of IP.

In wild-type hearts, the increase in the area of perivascular fibrosis was already apparent at 1 week after the banding, and it further increased gradually thereafter (Figure 3B, top). In contrast, the increase in the area of interstitial fibrosis was not apparent at 1 week after the banding, reached a maximum level at 2 weeks, and then declined gradually (Figure 3B, bottom). The difference in the appearance times of perivascular and interstitial fibrosis may be derived from the different timing of inflammatory cell infiltration into these 2 areas, and the late-phase decline of interstitial fibrosis may suggest decreased contents of type III collagen during progression of cardiac fibrosis. At 1 week after the banding, both fibrotic areas were not significantly different between wild-type and IP⁻/⁻ hearts (Figure 3B). At 2 and 4 weeks, however, these areas were significantly enlarged in IP⁻/⁻ hearts compared with those in wild-type hearts. In contrast to cardiomyocyte hypertrophy, these fibrotic areas at 8 weeks were still significantly larger in IP⁻/⁻ hearts than in wild-type hearts.
hearts, suggesting a potent antifibrotic role of IP. These results indicate that IP participates in the suppression of both cardiomyocyte hypertrophy and cardiac fibrosis.

There was no significant difference in the cross-sectional area of cardiomyocytes and the area of interstitial and perivascular fibrosis among wild-type, EP2<sup>-/-</sup>, EP<sup>-/-</sup>, FP<sup>+</sup>, and TP<sup>+/+</sup> mice or between F2 wild-type and EP4<sup>+/+</sup> mice at 4 weeks after the banding (see online-only Data Supplement).

**Expression of ANP mRNA in the Heart**

We next examined whether IP deficiency affects the cardiac expression of hypertrophy-related genes, in which we used ANP mRNA as a marker. In wild-type mice, the expression level of ANP mRNA increased significantly at 2 weeks of the banding compared with that in sham-operated mice (Figure 4), indicating that pressure overload induced gene expression along with the development of cardiac hypertrophy. In IP<sup>-/-</sup> hearts, however, expression levels were significantly higher than those in wild-type hearts throughout the experimental period. Moreover, in IP<sup>-/-</sup> hearts, its significant increase was apparent as early as 1 week after the banding. Interestingly, the expression level in sham-operated IP<sup>-/-</sup> mice was slightly but significantly higher than that in sham-operated wild-type mice, suggesting an inhibitory effect of basally produced PGI<sub>2</sub> on ANP mRNA expression. These results suggest that stimulation of IP is able to modulate the expression of hypertrophy-related genes in the heart.

**Expression of COX mRNAs and Production of PGI<sub>2</sub> in the Heart**

To determine whether pressure overload affects the expression of COXs and production of PGI<sub>2</sub>, we measured the expression levels of COX mRNAs and 6-keto-PGF<sub>1α</sub> contents in the left ventricle of wild-type mice. The expression level of mRNA for COX-1 or COX-2 did not change significantly after the banding throughout the experimental period compared with that before the banding (data not shown). In addition, the expression of COX-2 mRNA was barely detectable by the present method, and its level was significantly lower than that of COX-1 mRNA compared with the use of an 18S rRNA as an internal control. Accordingly, there was no significant change in the cardiac level of 6-keto-PGF<sub>1α</sub> throughout the experimental period; these values were 10.4±2.1 (n=4) and 9.3±2.3 (n=5) pg/mg wet weight in aortic-banded and sham-operated groups, respectively, at 1 week of the banding. These results indicate that the pressure overload did not affect COX mRNA expression or PGI<sub>2</sub> production in the heart, whereas significant amounts of PGI<sub>2</sub> were being produced in the heart irrespective of the presence or absence of pressure overload.

**Effects of Cicaprost on Noncardiomyocyte Proliferation and Cardiomyocyte Hypertrophy**

To determine whether pressure overload affects the expression of COXs and production of PGI<sub>2</sub>, we measured the expression levels of COX mRNAs and 6-keto-PGF<sub>1α</sub> contents in the left ventricle of wild-type mice. The expression level of mRNA for COX-1 or COX-2 did not change significantly after the banding throughout the experimental period; these values were 10.4±2.1 (n=4) and 9.3±2.3 (n=5) pg/mg wet weight in aortic-banded and sham-operated groups, respectively, at 1 week of the banding. These results indicate that the pressure overload did not affect COX mRNA expression or PGI<sub>2</sub> production in the heart, whereas significant amounts of PGI<sub>2</sub> were being produced in the heart irrespective of the presence or absence of pressure overload.

There was no significant difference in the cross-sectional area of cardiomyocytes and the area of interstitial and perivascular fibrosis among wild-type, EP<sup>-/-</sup>, EP<sup>-/-</sup>, FP<sup>+</sup>, and TP<sup>+/+</sup> mice or between F2 wild-type and EP4<sup>+/+</sup> mice at 4 weeks after the banding (see online-only Data Supplement).
TABLE 3. Effects of Aortic Banding on BW and HW in Wild-Type and IP−/− Mice

<table>
<thead>
<tr>
<th></th>
<th>Weeks After Aortic Banding</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>BW, g</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WT (SO)</td>
<td>20.8±0.6 (4)</td>
<td>22.7±1.4 (4)</td>
<td>22.5±1.2 (4)</td>
<td>24.1±1.0 (4)</td>
</tr>
<tr>
<td>WT (AB)</td>
<td>20.7±0.6 (10)</td>
<td>21.7±0.4 (9)</td>
<td>22.7±0.4 (9)</td>
<td>23.5±0.7 (6)</td>
</tr>
<tr>
<td>IP−/− (SO)</td>
<td>21.8±1.6 (4)</td>
<td>22.8±1.3 (4)</td>
<td>22.2±0.8 (4)</td>
<td>24.1±0.8 (4)</td>
</tr>
<tr>
<td>IP−/− (AB)</td>
<td>20.7±0.3 (7)</td>
<td>22.7±0.3 (8)</td>
<td>23.6±0.7 (7)</td>
<td>26.1±0.9 (9)</td>
</tr>
<tr>
<td><strong>HW, mg</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>WT (SO)</td>
<td>98.0±1.6 (4)</td>
<td>104.6±5.5 (4)</td>
<td>104.6±5.0 (4)</td>
<td>115.5±3.9 (4)</td>
</tr>
<tr>
<td>WT (AB)</td>
<td>113.9±4.6 (10)</td>
<td>144.2±3.7 (9)*</td>
<td>143.8±7.6 (9)*</td>
<td>151.1±9.3 (6)*</td>
</tr>
<tr>
<td>IP−/− (SO)</td>
<td>107.7±5.4 (4)</td>
<td>108.9±5.5 (4)</td>
<td>99.0±3.4 (4)</td>
<td>109.7±7.0 (4)</td>
</tr>
<tr>
<td>IP−/− (AB)</td>
<td>122.7±6.6 (7)</td>
<td>172.1±6.4 (8)*†</td>
<td>177.2±11.6 (7)*†</td>
<td>181.5±10.4 (9)*†</td>
</tr>
</tbody>
</table>

BW and HW were measured in wild-type (WT) and IP−/− mice at 1, 2, 4, and 8 weeks of aortic banding (AB) or sham operation (SO). Each value represents mean±SEM. Numbers in parentheses represent number of animals.

*P<0.05 vs corresponding sham-operated mice; †P<0.05 vs aortic-banded wild-type mice.

Discussion

In the present study pressure overload produced by the aortic banding caused marked cardiac hypertrophy, consisting of cardiomyocyte hypertrophy and cardiac fibrosis. The degree of cardiac hypertrophy after the banding was significantly greater in IP−/− mice than in wild-type mice and was accompanied by augmentation of both cardiomyocyte hypertrophy and cardiac fibrosis. In addition, the increase in the expression level of ANP mRNA after the banding was significantly augmented in IP−/− hearts compared with wild-type hearts. These findings provide direct evidence that IP-mediated signaling plays a suppressive role in the development of pressure overload–induced cardiac hypertrophy and fibrosis.

Synthesis of PGI2 has been reported to be elevated in the hypotrophied and failing heart along with an upregulation of COX-2. In the present study, however, we found no significant increase in COX mRNA and PGI2 production after the aortic banding. This discrepancy may be derived from the differences in experimental conditions used or diseases studied. Accordingly, COX-2 upregulation in the heart has been reported in patients having congestive heart failure and increased cardiac PGI2 production was transient in dogs subjected to aortic banding. Recently, involvement of proinflammatory cytokines in the pathogenesis of heart failure due to a variety of causes has been suggested. Our procedure of aortic banding was relatively mild in degree and did not induce apparent cardiac failure, suggesting little participation of inflammatory cytokines, a common inducer of COX-2, in the pathogenesis of the present cardiac hypertrophy. Nevertheless, cardiac 6-keto-PGF1α concentrations before and after the aortic banding were grossly estimated to be 30 to 50 nmol/L, a well-working range of PGI2. These results suggest that basally produced PGI2 could exhibit an antihypertrophic effect on the heart on a pressure overload and that increased production of PGI2 via COX-2 induction in failing hearts would further contribute to the suppression of cardiac hypertrophy.
Because pressure overload–induced cardiac hypertrophy was accompanied by both cardiomyocyte hypertrophy and cardiac fibrosis, we next investigated whether the antihypertrophic role of IP depends on its effect on cardiomyocytes or noncardiomyocytes. In wild-type noncardiomyocytes, cicaprost significantly suppressed PDGF-induced proliferation; this growth factor was reportedly implicated in the development of cardiac hypertrophy and fibrosis.31 This result is in good agreement with a report that PGI₂ was a major prosta-
noid produced by cultured cardiac fibroblasts and that an IP agonist inhibited fibroblast proliferation and expression of collagen type I and III mRNAs.13 In addition, antifibrotic effects of PGI₂ have also been reported in extracardiac tissues, such as blood vessels and the kidney.22,33 These results indicate that stimulation of IP reduces the proliferation of noncardiomyocytes and suggest that stimulation of IP on fibroblasts accounts for its suppressive effect on pressure overload–induced cardiac fibrosis.

In wild-type cardiomyocytes, cicaprost failed to reduce the cardiotrophin-1–induced hypertrophy, agreeing with a previous report that PGI₂ did not show a direct antihypertrophic action on cultured rat cardiomocytes.12,17 It is noteworthy that angiotensin II–induced cardiomyocyte hypertrophy was totally dependent on several factors secreted from noncardiomyocytes, such as endothelin and cardiotrophin-1.34,35 Accordingly, the action of IP on noncardiomyocytes may be involved in the underlying mechanism of its suppressive effects on cardiomyocyte hypertrophy in vivo. In support of this idea, PGI₂ was reported to be capable of attenuating hypertrophy of cardiomyocytes when they were cocultured with noncardiomyocytes, although it did not have a direct antihypertrophic effect on cardiomocytes.12 These results suggest that PGI₂ acts on noncardiomyocytes and inhibits their release of hypertrophy-inducible factors, leading to the suppression of cardiomyocyte hypertrophy.

In the present study the expression of IP mRNA and the IP-mediated increase in [cAMP], were apparently much greater in noncardiomyocytes than in cardiomyocytes, suggesting further that activation of IP on noncardiomyocytes would be more important in the suppression of cardiac hypertrophy. However, we could not exclude a role of IP on cardiomyocytes in the suppression of cardiac hypertrophy because IP mRNA was expressed in cardiomyocytes and because its stimulation induced a small but significant increase in [cAMP]. Further studies would be required to clarify the detailed mechanisms of antihypertrophic role of IP in pressure overload–induced cardiac hypertrophy.

We found the expression of mRNAs for EP₂, EP₃, EP₄, FP, and TP and increased in that for IP in the heart. In a model of pressure overload–induced cardiac hypertrophy, however, the degree of cardiac hypertrophy in EP₂⁻/⁻, EP₃⁻/⁻, EP₄⁻/⁻, EP₂⁻/⁻, FP⁻/⁻, or TP⁻/⁻ mice was not significantly different compared with that in their respective wild-type mice. This result suggests that neither EP subtype, FP, or TP plays a major role in the present model of pressure overload–induced cardiac hypertrophy. In contrast, there are several reports suggesting hypertrophic effects of exogenously administered PGF₂α on rat cardiomyocyte both in vivo and in vitro,17,18 although there has been no report showing the hypertrophic effect of endogenous PGF₂α. The apparent discrepancy in the effect of PGF₂α may be derived from a species difference, as reported; the hypertrophic effect of PGF₂α on cultured cardiomyocytes found in rats was not observed in mice.36 Alternatively, amounts of endogenously produced PGF₂α may be insufficient to affect pressure overload–induced cardiac hypertrophy in vivo.

PGI₂ is a major prostanoid in the cardiovascular system and exerts a variety of actions in the system. It exhibits a
cardioprotective effect in ischemia/reperfusion injury and plays a part in the late phase of ischemic preconditioning. A recent study has shown that neonatal infarction in the carotid artery after endothelial injury is markedly enhanced in IP−/− mice, suggesting an antiproliferative effect of PGI2 on vascular smooth muscle cells. In agreement with this effect, PGI2 analogues have long been used for the treatment of primary pulmonary hypertension. Recently, a gene therapy using PGI2 synthase gene transfection has been tested in rat models of cardiovascular diseases, such as pulmonary hypertension, vascular remodeling, and peripheral vascular occlusion, and has been shown to be promising. Furthermore, the present study showed a novel role of IP in pressure overload–induced cardiac hypertrophy, emphasizing, along with previous reports, a potent therapeutic potential of PGI2 for cardiovascular diseases. It should be noted, however, that there may be a species difference in the effect of IP, as shown in the present study for the effect of FP on cardiomyocyte hypertrophy, indicating that the usefulness of PGI2 for the treatment of cardiac hypertrophy and heart failure remains to be determined in future studies.

In conclusion, the present study clearly showed that IP-mediated signaling suppresses the development of pressure overload–induced cardiac hypertrophy via its inhibition of both cardiomyocyte hypertrophy and cardiac fibrosis. The finding should contribute to better understanding of the mechanism underlying cardiac hypertrophy.

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References


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