Phosphodiesterase Type 5 Is Highly Expressed in the Hypertrophied Human Right Ventricle, and Acute Inhibition of Phosphodiesterase Type 5 Improves Contractility

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Background—Sildenafil was recently approved for the treatment of pulmonary arterial hypertension. The beneficial effects of phosphodiesterase type 5 (PDE5) inhibitors in pulmonary arterial hypertension are thought to result from relatively selective vasodilatory and antiproliferative effects on the pulmonary vasculature and, on the basis of early data showing lack of significant PDE5 expression in the normal heart, are thought to spare the myocardium.

Methods and Results—We studied surgical specimens from 9 patients and show here for the first time that although PDE5 is not expressed in the myocardium of the normal human right ventricle (RV), mRNA and protein are markedly upregulated in hypertrophied RV (RVH) myocardium. PDE5 also is upregulated in rat RVH. PDE5 inhibition (with either MY-5445 or sildenafil) significantly increases contractility, measured in the perfused heart (modified Langendorff preparation) and isolated cardiomyocytes, in RVH but not normal RV. PDE5 inhibition leads to increases in both cGMP and cAMP in RVH but not normal RV. Protein kinase G activity is suppressed in RVH, explaining why the PDE5 inhibitor–induced increase in cGMP does not lead to inhibition of contractility. Rather, it leads to inhibition of the cGMP-sensitive PDE3, explaining the increase in cAMP and contractility. This is further supported by our findings that, in RVH protein kinase A, inhibition completely inhibits PDE5-induced inotropy, whereas protein kinase G inhibition does not.

Conclusions—The ability of PDE5 inhibitors to increase RV inotropy and to decrease RV afterload without significantly affecting systemic hemodynamics makes them ideal for the treatment of diseases affecting the RV, including pulmonary arterial hypertension. (Circulation. 2007;116:238-248.)

Key Words: contractility • hypertension, pulmonary • hypertrophy • inhibitors • inotropic agents

The failing right ventricle (RV) is a common clinical problem, complicating pulmonary arterial hypertension (PAH), pulmonary thromboembolism, heart/lung transplantation surgery, or surgery for congenital heart disease. To be clinically effective, an ideal candidate therapy should increase RV inotropy, dilate the pulmonary circulation (its afterload), and not affect the systemic vasculature or the left ventricle (LV). RV remodeling and contractility are surprisingly understudied; currently, there are no RV-specific standard or experimental therapies.1,2 Phosphodiesterase type 5 (PDE5) inhibitors like sildenafil are relatively selective pulmonary vasodilators and were just approved for the treatment of PAH.3 PDE5 is thought to be expressed in the coronary vessels but not in the human myocardium.4 Within the first year of the use of sildenafil for erectile dysfunction, a number of cardiac deaths were reported; it was soon realized that most were related to the interaction of sildenafil with nitrates, often required for intercourse-induced angina, resulting in profound hypotension. However, these early deaths led to extensive efforts to study potentially direct effects of sildenafil on the heart. PDE5 inhibitors were clearly shown to lack any significant direct effects on the myocardium of normal human and animal hearts in vitro,5,6 in healthy volunteers,7 or even in patients with coronary artery disease in vivo.8 This was supported by the finding that PDE5 is not expressed in the...
normal myocardium.6 Expert panels and professional bodies like the American College of Cardiology/American Heart Association writing group published position statements on the effects of sildenafil, clearly stating that it lacks primary effects on the myocardium: “Furthermore, PDE5 is not present in cardiac myocytes, and sildenafil has been shown to have no direct inotropic effects.”4

Therefore, when PDE5 inhibitors started being studied for the treatment of PAH, they were thought to affect only the pulmonary vasculature. Their acute and chronic effects were thought to result solely from their ability to increase cGMP levels preferentially in the pulmonary artery smooth muscle cells, thereby inducing relatively selective pulmonary vasodilation, in addition to antiinflammatory and proapoptotic effects on the vessel wall.10–14 In one of the first reports of the hemodynamic effects of sildenafil in patients with PAH, we showed that a single oral dose of sildenafil (75 mg) and maximal dose of inhaled nitric oxide (80 ppm) had similar effects on systemic and pulmonary hemodynamics, but only sildenafil improved cardiac output.10 This suggested a primary inotropic effect of sildenafil on the RV (which is hypertrophied and/or failing in most patients with PAH) because the effects on the systemic vasculature were not significant. However, although we could not rule out that some of the increase in cardiac output could be due to the decrease in the LV afterload, this finding was obscured by the then-prevailing dogma that PDE5 was absent in the human heart. In addition, Lepore et al13 followed a similar protocol but suggested that sildenafil did not affect myocardial function. We now hypothesize that PDE5 is upregulated in the hypertrophied RV (RVH) and that this upregulation is physiologically significant.

Methods
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. All experiments on human tissues and rats were done with permission from the University of Alberta committees on human ethics and animal policy and welfare, respectively.

Animal Model of RVH
We studied RVH using a well-validated PAH model created by intraperitoneal injection of monocrotaline, an alkaloid from crotalaria spectabilis, in adult Sprague Dawley rats (Charles River Laboratories, St. Constant, Quebec, Canada).15,16 Monocrotaline is selectively toxic to the pulmonary arterial endothelium and causes significant PAH within 3 weeks of injection. This is associated with significant RVH, a finding that we have validated with extensive hemodynamic and echocardiographic studies.16,17 We euthanized the rats between 3 and 4 weeks after injection, at a time when the rats, despite the RVH, do not have severe right heart failure based on the absence of signs like significant edema or ascites.

Immunohistochemistry, confocal microscopy, immunoblot laser-capture microdissection, and quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) were performed as recently described16,17; for details, see the online Data Supplement.

Isolated RV Langendorff Perfusion
Rats were anesthetized with intraperitoneal injection of 60 mg/kg pentobarbital. A midline sternotomy was performed, and within 1 minute, the heart was isolated and the aorta was cannulated and perfused with Krebs’ buffer at 12 to 13 cm/min. The hearts had a mean intrinsic rate of 180 to 190 bpm (hearts with a native rate <160 bpm were not used). A 0.03-cm³ latex balloon (Harvard Apparatus, Saint-Laurent, Quebec, Canada) filled with water and attached to a pressure transducer (Cobe, Richmond Hill, Ontario, Canada) was placed in the RV via the right atrium, and pressure waves were sampled at a rate of 1000 Hz by PowerLab. Pressure readings were converted into first-derivative traces to give dp/dt and were analyzed with Chart 5.4 software (ADInstruments Inc, Colorado Springs, Colo).

cGMP and cAMP Levels
RV free walls were isolated from rat hearts (with or without pretreatment with oral sildenafil). cAMP and cGMP levels were determined with cAMP and cGMP EIA kits (Biomedical Technologies Inc, Stoughton, Mass) and expressed as picomoles per weight of myocardium.

PDE Activity Assays
Total cGMP-PDE activity and cAMP-PDE activity were assayed at 1 μmol/L substrate (fluorescein-labeled derivatives of cGMP and cAMP, respectively). We used a fluorescence polarization assay (Molecular Devices, Sunnyvale, Calif)18 under linear conditions with and without a PDE5 inhibitor (sildenafil 1 μmol/L) or a PDE3 inhibitor (milrinone 10 μmol/L).

Protein Kinase G Activity
Protein kinase G (PKG-1) activity was assayed in RV free wall tissues with colorimetric analysis (CycLex, Ina, Nagano, Japan) in which a peroxidase-coupled anti–phospho-G-kinase substrate monoclonal antibody is used as a reporter molecule in a 96-well ELISA format.19,20 Activity also was studied by measuring the phosphorylation of VASP, a myocardial PKG-1 target, using immunoblot densitometry (over GAPDH expression) was performed and presented as previously described.16,17

### Patient Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Tissue</th>
<th>RV Thickness, cm</th>
<th>Medications</th>
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<tbody>
<tr>
<td>1</td>
<td>19 y</td>
<td>F</td>
<td>Left atrial sarcoma, heart transplantation</td>
<td>Normal LV, normal RV</td>
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<td>Asa</td>
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<td>54 y</td>
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<td>Intractable angina, moderate LVH, heart transplantation</td>
<td>LVH, normal RV</td>
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<td>Metoprolol, statin, ramipril, nitrates, ASA, ticlopidine</td>
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<td>3</td>
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<td>RVH</td>
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<td>PAH, RV biopsy and transplantation</td>
<td>RVH</td>
<td>1.8</td>
<td>Sildenafil, flosan</td>
</tr>
</tbody>
</table>

ASA indicates aspirin.
Cell-shortening studies in RV myocytes were obtained as described previously. For details, see the online Data Supplement.

### Statistical Analysis

Data are expressed as mean±SEM, and significant differences were evaluated with Student t test for unpaired data or 1-way ANOVA, followed by post-hoc Fisher PLSD as appropriate (SPSS 11, SPSS Inc, Chicago, Ill). Values of P<0.05 were considered significant.

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.

### Results

We studied hearts (surgical resection or biopsy specimens) from 9 patients with either normal RVs or RVH and from a patient with LV hypertrophy (but normal RV) (the Table). RV free wall samples were studied. The diagnosis of RVH was based on standard echocardiographic criteria. Confocal microscopy and multiple-staining technique was used to detect the expression of PDE5 and to colocalize this enzyme with myosin heavy chain (MHC) in the myocardium or smooth muscle actin (SMA) in coronary vessels. In both the normal LV and RV, PDE5 was expressed only in the coronary artery media, not in the myocardium. In contrast, PDE5 was markedly upregulated in the myocardium of all the hypertrophied ventricles studied (Figure 1 and Data Supplement Figure IA).

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**Figure 1.** PDE5 expression is increased in the hypertrophied but not the normal human RV. Multiple-staining immunohistochemistry technique and multiphoton confocal microscopy show that PDE5 protein is expressed only in the media of the coronary vessels (colocalization with SMA) in the normal RV and LV and is markedly upregulated in the myocardium (colocalization with MHC) of the hypertrophied ventricles. The left column (red) shows expression of either SMA or MHC. The middle column (green) shows PDE5 expression on the same slide. The third column is a merged picture of the red and green channels plus a nuclear stain with DAPI (blue). Patient numbers correspond to the Table. All magnifications are ×40 except for patient 9 (right lower corner), for whom a ×150 image shows a single cardiomyocyte.
Patient 2 had hypertrophied LV but normal RV and showed PDE5 expression only in the LV myocardium. Although we did not systematically study PDE5 expression in the LV, we show both the LV and RV tissue from this patient because it suggests that the PDE5 upregulation is restricted only to the pressure-overloaded chamber and is likely not induced by circulating factors. Further confirmation that our hearts had significant hypertrophy came from the fact that all of these hearts, but not the normal hearts, showed a marked upregulation of brain natriuretic peptide in the myocardium (Figure 1).

To determine whether PDE5 mRNA also was upregulated, we studied whole-RV tissue using qRT-PCR. PDE5 mRNA levels were significantly higher in RVH compared with normal RV (Figure 2A). In contrast, PDE3 mRNA (a phosphodiesterase known to be highly expressed in the myocardium) did not differ between normal RVs and RVH. To compartmentalize PDE5 mRNA in the RV (vascular versus myocardial), we used laser-captured microdissection to selectively isolate myocardium (characterized by high MHC and low SMA expression) and coronary vessels (characterized by low MHC and high SMA expression) in human RVs. PDE5 mRNA is significantly upregulated in the RVH myocardium, whereas it is found in minimal amounts in the normal RV myocardium. Conversely, PDE5 mRNA is found in the coronary arteries from both the normal and RVH hearts, in agreement with our immunohistochemistry (Figure 2B and 2C). These human data were reproduced in the rat RVs (Data Supplement Figure III).

Is the PDE5 upregulation physiologically significant? This is difficult to assess in vivo because PDE5 inhibition also will decrease pulmonary vascular resistance, and the decreased RV afterload itself will improve RV function. PDE5 inhibitors also may decrease venous tone, decreasing preload. In addition, the small decrease in systemic pressure that might occur with PDE5 inhibitors may increase sympathetic input to the RV, also indirectly improving function. To exclude these confounding factors, we used a modification of a Langendorff-isolated, perfused rat heart model; the RV contractile function was studied while the pressure was recorded in the RV by a balloon, the pulmonary artery (afterload) was occluded, and the preload (balloon volume) was kept constant (Figure 3A).

Within 3 weeks after monocrotaline injection, the rats developed severe PAH and RVH, which we and others have previously characterized using invasive hemodynamic and echocardiographic measurements (Figure 3B). Immunohistochemistry confirmed that, as in the human RV, PDE5 was significantly expressed only in RVH and not in normal RV or LV (Figure 3C). We also used laser-captured microdissection and qRT-PCR (as we did in human RVs; Figure 2) on rat RVs and demonstrated that the expression of PDE5 mRNA was upregulated in the rat RVH myocardium in a...
manner similar to that in human RVH (Data Supplement Figure IV).

The β-agonist isoproterenol caused similar increases in RV developed pressure in normal RV and RVH. However, although MY-5445, a relatively specific PDE5 inhibitor, did not affect the normal RV, it caused a significant dose-dependent increase in the developed pressure and both maximum and minimum contractility (dP/dt) in the RVH (Figure 4). The native heart rate in the perfused hearts was not different between the normal RVs (192±18 bpm) and RVH (185±14 bpm) and was not altered by the PDE5 inhibitor.

To mimic clinical conditions, we used sildenafil (50 mg PO) and fed normal versus RVH rats 60 minutes before running the isolated hearts. Because of differences in the metabolism of sildenafil in rats, this dose gives serum levels similar to those seen in humans. RVs from normal sildenafil-treated rats showed no difference in baseline contractile pressure compared with the nontreated controls. In contrast, hypertrophied RVs from sildenafil-treated rats showed a significant increase in the baseline developed pressure (Data Supplement Figure IV) and dP/dt (not shown) compared with the RVH from untreated controls. MY-5445 had no additional effects on the RVs from the sildenafil-treated rats, whether normal or hypertrophied, suggesting that the sildenafil-treated rats had maximal PDE5 inhibition (Data Supplement Figure IV).

To confirm that PDE5 inhibitors have primary inotropic effects in individual RV cardiomyocytes, we performed cell-shortening experiments. As in the whole-heart experiments, sildenafil (10−6 mol/L) increased contractility in the cardiomyocytes from the RVH but not the normal RVs (Figure 5A).

Normally, PDE5 inhibition causes an increase in cGMP and activation of PKG. This is associated with a decrease in intracellular Ca2+ and would predict a decrease in contractility. However, PKG activity has been reported to be decreased in the hypertrophied LV myocardium. This suggests that the PDE5 inhibition–induced increase in cGMP might not be able to activate the PKG pathway downstream in the RVH; instead, it might preferentially inhibit the cGMP-sensitive PDE3, increasing cAMP and activating PKA, which leads to increases in intracellular Ca2+ and enhanced inotropy, a mechanism exploited clinically by the PDE3 inhibitor milrinone. We proceeded to examine this hypothesis using several pharmacological tools as presented schematically in Figure 5B.

Treatment with sildenafil causes both an increase in cGMP levels and interestingly an increase in cAMP only in RVH,
although there is no effect on cGMP or cAMP levels in normal RVs because the target enzyme, ie, PDE5, is not expressed in the normal myocardium. Isoproterenol, as expected, caused an increase in cAMP levels in both control RV and RVH without any significant effect on cGMP levels. Interestingly, isoproterenol and sildenafil increased cAMP to a similar degree (Figure 6A).

We then measured total cGMP and cAMP phosphodiesterase activities, and by using the relatively specific inhibitors sildenafil and milrinone, we were able to measure the components of the total activity that were due to PDE5 and PDE3, respectively. At baseline, cGMP-PDE total activity was significantly increased in RVH compared with normal RV. This increase in overall cGMP-PDE activity in RVH was contributed almost entirely by PDE5 because sildenafil caused a decrease in activity to a level similar to the normal RV (Figure 6B). As expected, milrinone had no effect on cGMP-PDE activity. In contrast, cAMP-PDE total activity was similar at baseline in RVH versus normal RV. Interestingly and as hypothesized, sildenafil significantly inhibited cAMP-PDE5 activity in RVH in a manner similar to milrinone (Figure 6B). As expected, milrinone decreased cAMP-PDE activity in the normal RV (where PDE3 is expressed), but sildenafil had no effects (because PDE5 is not significantly expressed).

We then directly measured PKG activity and showed a significant decrease in RVH compared with normal RV (Figure 6C). In addition, we showed that the levels of phosphorylated vasodilator–stimulated phosphoprotein (VASP, a PKG-1 substrate in the myocardium) were decreased in RVH compared with normal RVs, further confirming a suppression of PKG activity in RVH (Data Supplement Figure V). Despite causing a significant increase in cGMP in the RVH myocardium (Figure 6A), sildenafil only slightly and nonsignificantly increased PKG1 activity (*P<0.01) (Data Supplement Figure V).

In rat whole-RV tissue, the small amount of PDE5 protein expression seen in the normal RV is likely due mostly to its expression in the coronary vessels (Figures 1 and 2). In contrast, PDE3 expression is similar in both RVH and normal RV. The expression patterns of the PDE5 and PDE3 are in agreement with their activities (Figure 6B). On the other hand, the expression of PKG1 does not appear to be significantly decreased, suggesting a functional inhibition of its activity in RVH (Figure 6C). In agreement with our immunohistochemistry data in the rat RV (Figure 3), PDE5 expression measured by immunoblots is significantly increased in RVH (Figure 6D).

We then studied the functional significance of the model proposed in Figure 5B in RV contractility by using pharma-
cological dissection of the pathway distal to PDEs (Figure 7 and Data Supplement Figure VB). PKG inhibitors (Rp-8-CPT-cGMPS and KT 5823) slightly increased contractility in the normal RV (suggesting that PKG might have some tonic negative inotropic effect) but had no significant effects on RVH. They did not inhibit the inotropic effects of isoproterenol (which are mediated by cAMP/PKA) but also did not inhibit the inotropic effects of sildenafil and MY-5445. This was in agreement with our hypothesis that the inotropic effects of PDE5 inhibitors are not mediated by cGMP-PKG but rather by cAMP-PKA. Indeed, PKA blockade (H89) completely inhibited the effects of PDE5 inhibitors and isoproterenol in RVH. Interestingly, there was a decrease in contractility beyond baseline in RVH treated with both a PDE5 inhibitor and H89 (Figure 7). This suggests that perhaps some negative inotropic effect of PKG might be exposed if PKA is inhibited.

**Discussion**

We report for the first time that PDE5 is markedly upregulated in human RVH. We also show that in the rat PDE5 inhibition with sildenafil or MY-5445 increases contractility (developed pressure, dP/dtmax, and myocardial cell shortening) in RVH but not in normal RV, which lacks PDE5 expression. PDE5 inhibition in the RVH is associated with an increase in cGMP, which would normally activate PKG (leading to a decrease in intracellular Ca^{2+}) but also inhibit the cGMP-sensitive PDE3. Because overall PKG activity is inhibited in the RVH, the pathway is preferentially shifted toward inhibition of PDE3, leading to an increase in cAMP, activation of PKA, increased intracellular calcium, and increased contractility (Figure 5B). The PDE5 inhibitor–induced increase in both contractility and cAMP levels in RVH is significant and similar in magnitude to isoproterenol (Figures 4, 6A, and 7C and Data Supplement Figure V). Our findings have immediate clinical applications; PDE5 inhibition might be a new means of enhancing RV function, which has repeatedly been shown to be a critical predictor of functional status in many cardiovascular diseases.1,2

Despite its important role, the RV has been understudied compared with the LV. Extrapolating findings from the LV to the RV is not appropriate because their physiology1,2 and embryology32 are quite different. Similarly, extrapolating findings from the normal to the hypertrophied ventricle also is inappropriate given the dramatic molecular and metabolic changes that take place in the hypertrophied or failing myocardium.2 Very recently, Borlaug et al33 hypothesized that despite its very low expression in the normal LV, PDE5 is strategically compartmentalized within the myocytes34 and that its inhibition might alter heart function. Using load-independent echocardiographic parameters, they showed that a single dose of sildenafil in healthy volunteers blunted the

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**Figure 5.** Isolated single RV cardiomyocyte cell-shortening experiments and proposed mechanism for the inotropic effects of PDE5 inhibitors in RVH. A, Representative and mean data of single RV cardiomyocyte cell shortening for normal (nRV) and RVH cardiomyocytes before and after sildenafil treatment (n=5 rats per group, 16 to 18 cells per group; P<0.01). B, Our proposed mechanism for acute PDE5 inhibition causing increased inotropy in RVH. The site of action of drugs used (see below) is shown in red.
systolic response of the LV to β-adrenergic stimulation, ie, dobutamine infusion. Interestingly, they also showed a small but significant increase in the baseline, unstimulated contractility of the LV; they speculated that this might be the result of either reflex sympathetic activation, consequent to a slight systemic vasodilatation, or a direct effect on the LV, but they did not study the RV. Because the RV Langendorff model uses a constant afterload and preload and eliminates circulating factors and autonomic input, our data suggest that the increased contractility of the rat RVH is due to direct effects of the PDE5 inhibitors on the myocardium (Figures 4, 6, and 7). This is further supported by the direct effects of sildenafil on isolated cardiomyocytes (Figure 5A).

Our proposed mechanism of the inotropic effects of PDE5 inhibitors on the RVH (Figure 5B) is supported by the pharmacological dissection of the pathway distal to PDEs. For example, as our model predicted, the effects of sildenafil and MY5445 in RVH were inhibited by a PKA inhibitor and not by a PKG inhibitor (Figure 7 and Data Supplement Figure V). Clearly, the role of PKG-1 activity is crucial for this proposed mechanism; although protein expression of PKG1 is not altered in RVH (Figure 6D), its activity is clearly suppressed in RVH compared with normal RV, as shown by 2 different techniques (Figure 6C and Data Supplement Figure V). However, there is a trend (P=0.08) toward an increase in PKG-1 activity in the RVH myocardium when treated with sildenafil (Data Supplement Figure V). This is not surprising given the presence of PKG-1 protein in the RVH myocardium (Figure 6D) and the large sildenafil-induced increase in cGMP levels in the RV (Figure 6A). We propose that the relative balance of the cGMP-PKG versus cAMP-PKA axis will determine the acute response of the myocardium to sildenafil. This might vary among different heart chambers, perhaps species, and even stages of myocardial disease. In RVH (at least during relatively compensated RV dysfunction), the predominant axis in this response is cAMP-PKA.

Other mechanisms also might be involved in the beneficial acute effects of PDE5 inhibitors in the hypertrophied RV. For example, PDE5 inhibition might improve coronary perfusion (PDE5 inhibitors were first developed as antianginal agents), which might be important in the relatively ischemic hypertrophied RV, further improving function, particularly diastolic relaxation (dP/dtmin; Figure 4B).

Takimoto et al showed recently that chronic PDE5 inhibition improved LV function in a mouse model with LV

Figure 6. Enzymatic activity and expression of PDE3, PDE5, and PKG in normal RV and RVH. A, cGMP and cAMP levels in the RV (normal vs RVH) from untreated rats, sildenafil-treated hearts (50 mg gavage 60 minutes before death), and isoproterenol-perfused hearts (n=5 per group; *P<0.01 vs nRV, †P<0.01 vs untreated). B, cGMP- and cAMP-PDE activities in normal and hypertrophied RVs treated acutely with vehicle (perfusate), sildenafil, or milrinone (n=6 per group; *P<0.01 vs nRV, †P<0.01 vs vehicle). C, PKG-1 activity in RV homogenates from normal controls and rats with RVH (n=6 per group; *P<0.01). D, Immunoblots of rat normal and hypertrophied RVs. There is a marked upregulation of PDE5 expression in the hypertrophied RV, whereas PDE3 and PKG-1 expression is not significantly different.
hypertrophy resulting from transverse aortic constriction. LV hypertrophy regressed and LV contractility improved in mice treated with PDE5 inhibitors despite the persistence of aortic constriction. The authors showed that PDE5 inhibition inactivated a number of genes of the fetal/hypertrophy heart gene program, which is pathologically activated in LV hypertrophy. If similar mechanisms take place in the RV, then PDE5 inhibitors might cause regression of RVH and improvement of RV function, in addition to their effects on decreasing the RV afterload. In a double-blind randomized trial, Wilkins et al. showed that sildenafil (but not bosentan, an endothelin receptor antagonist also approved for the treatment of PAH) decreased RVH (studied by magnetic resonance imaging), although the relative importance of decreasing afterload versus direct antihypertrophic effects on the RV is difficult to determine in vivo. Because of its effectiveness, excellent toxicity profile, and relatively low price compared with the other available therapies, sildenafil use is rapidly increasing in PAH patients, and its potential benefit in a number of cardiovascular disorders is being investigated.

The increased mortality, mainly a result of ventricular arrhythmias, in patients with LV failure treated with PDE3 inhibitors (compared with placebo) might at first appear concerning because, according to our model, PDE5 inhibition translates into PDE3 inhibition in RVH. PDE3 inhibitor studies were performed mostly on patients with diseased LVs from ischemic heart disease. However, the LVs of patients with PAH do not typically have significant coronary disease. Furthermore, because the LV in these patients also is not hypertrophied, PDE5 is not significantly expressed in the LV

Figure 7. Effects of PKG and PKA inhibition on the PDE5 inhibitor- and isoproterenol-induced inotropy in normal and hypertrophied RVs. A, Representative trace of contractile pressure in a normal RV (top) and RVH (bottom) at baseline and with sildenafil and concomitant PKG inhibitor (KT-5823). B, Representative trace of contractile pressure in a normal RV (top) and RVH (bottom) at baseline and with sildenafil and concomitant PKA inhibitor (H-89). C, Mean data of developed RV pressures from modified Langendorff-perfused hearts (n=6 per group; *P<0.01 vs baseline, †P<0.01 vs sildenafil, ‡P<0.01 vs isoproterenol) in the presence of different drug combinations (see Results).
myocardium (Figure 3C), so PDE5 inhibition should not affect the LV myocardium (because the target of the drug is absent). In that sense, PDE5 inhibitors are truly RVH chamber specific. PDE5 inhibitors have been used for a number of years in PAH patients; in the Sildenafil Use in Pulmonary Arterial Hypertension (SUPER) trial, in which long-term follow-up of at least 1 year was documented, there were no reports of increased mortality, ventricular arrhythmias, or cardiac-related deaths.3

Study Limitations

More work is needed to study the long-term effect of PDE5 inhibitors in both compensated and decompensated RV disease. We studied human RVs mostly from patients with RVH and relatively compensated RV dysfunction who did not have signs of overt RV failure (except patient 9) and underwent elective surgery. Similarly, our rat RVs showed hypertrophy (not dilated or thin walled), and the rats did not have signs of severe right heart failure. It is possible that the regulation of PDE5 expression and function and the effects of sildenafil might be different in the RVs of patients with advanced disease or are perhaps dilated and thin walled; our results cannot necessarily be extrapolated to patients with late stages of RV failure.

Conclusions

Our findings on human hearts and a well-established animal model raise a number of intriguing possibilities that need to be considered by clinicians treating PAH patients with PDE5 inhibitors or designing trials with this class of drugs. PDE5 inhibitors might have the very desirable combination of primary inotropic, anti hypertrophic,30 and afterload-reducing effects on the RV without significantly affecting systemic hemodynamics,10,38 making them very attractive for the treatment of many diseases involving the RV.

Sources of Funding

Dr Michelakis was funded by grants from the Canadian Institutes of Health Research, Alberta Heart and Stroke Foundation, Alberta Heritage Foundation for Medical Research, Canadian Foundation for Innovation, and Canada Research Chairs program. Dr Nagendran was funded by the Canadian Institutes of Health Research, Alberta Heritage Foundation for Medical Research, and University of Alberta Clinical Investigator Program.

Disclosures

Dr Michelakis has received consulting fees from Pfizer. The other authors report no conflicts.

References


**CLINICAL PERSPECTIVE**

We describe for the first time a significant upregulation of phosphodiesterase-5 (PDE5) in the hypertrophied but not the normal human and rat right ventricle (RV). We also show that although PDE5 inhibitors do not alter the contractility of the normal RV, they significantly increase contractility of the hypertrophied RV. Our findings might have direct implications for the growing patient population with RV dysfunction, including patients with pulmonary arterial hypertension, chronic thromboembolic disease, or congenital heart disease and those after lung transplantation. PDE5 inhibitors may improve RV contractility without affecting systemic hemodynamics in patients with normal left ventricles because PDE5 is minimally expressed in the normal left ventricular myocardium. Thus, PDE5 inhibitors may be one of the first examples of an RV-specific therapy. The dual beneficial action of PDE5 inhibitors on the right-sided circulation (ie, pulmonary vasodilatation plus direct RV inotropy) makes these drugs superior to other therapies for pulmonary hypertension that work only on the pulmonary vasculature. The documented beneficial effects of sildenafil in patients with pulmonary arterial hypertension (which led to its approval by the Food and Drug Administration) might also be a result of its so-far unrecognized effects on the RV. Some of the end points in pulmonary arterial hypertension clinical trials with PDE5 inhibitors, eg, the 6-minute walk or the functional class, might also be positively affected by the increased RV contractility. However, whether such beneficial effects of PDE5 inhibitors are sustained after long-term therapy or are even present in failing RVs remains to be determined.
Phosphodiesterase Type 5 Is Highly Expressed in the Hypertrophied Human Right Ventricle, and Acute Inhibition of Phosphodiesterase Type 5 Improves Contractility