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Phosphodiesterase-2 Is Up-Regulated in Human Failing Hearts and Blunts β-Adrenergic Responses in Cardiomyocytes

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Objectives This study investigated whether myocardial phosphodiesterase-2 (PDE2) is altered in heart failure (HF) and determined PDE2-mediated effects on beta-adrenergic receptor (β-AR) signaling in healthy and diseased cardiomyocytes.

Background Diminished cyclic adenosine monophosphate (cAMP) and augmented cyclic guanosine monophosphate (cGMP) signaling is characteristic for failing hearts. Among the PDE superfamily, PDE2 has the unique property of being able to be stimulated by cGMP, thus leading to a remarkable increase in cAMP hydrolysis mediating a negative cross talk between cGMP and cAMP signaling. However, the role of PDE2 in HF is poorly understood.

Methods Immunoblotting, radioenzymatic- and fluorescence resonance energy transfer–based assays, video edge detection, epifluorescence microscopy, and L-type $Ca2^{+}$ current measurements were performed in myocardial tissues and/or isolated cardiomyocytes from human and/or experimental HF, respectively.

Results Myocardial PDE2 expression and activity were ~2-fold higher in advanced human HF. Chronic β-AR stimulation via catecholamine infusions in rats enhanced PDE2 expression ~2-fold and cAMP hydrolytic activity ~4-fold, which correlated with blunted cardiac β-AR responsiveness. In diseased cardiomyocytes, higher PDE2 activity could be further enhanced by stimulation of cGMP synthesis via nitric oxide donors, whereas specific PDE2 inhibition partially restored β-AR responsiveness. Accordingly, PDE2 overexpression in healthy cardiomyocytes reduced the rise in cAMP levels and L-type $Ca2^{+}$ current amplitude, and abolished the inotropic effect following acute β-AR stimulation, without affecting basal contractility. Importantly, PDE2-overexpressing cardiomyocytes showed marked protection from norepinephrine-induced hypertrophic responses.

Conclusions PDE2 is markedly up-regulated in failing hearts and desensitizes against acute β-AR stimulation. This may constitute an important defense mechanism during cardiac stress, for example, by antagonizing excessive β-AR drive. Thus, activating myocardial PDE2 may represent a novel intracellular antiadrenergic therapeutic strategy in HF. (J Am Coll Cardiol 2013;62:1596–606) © 2013 by the American College of Cardiology Foundation

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Heart failure (HF) is among the most common causes of morbidity and mortality in the Western world. Regardless of the underlying cause, HF patients show a hyperactive sympathetic nervous system with elevated plasma catecholamine levels and long-term activation of the beta-adrenergic receptor (β-AR) signaling pathway. This leads to desensitization and attenuated β-AR responsiveness due to decreased β_1-AR number and function (1,2). These changes may participate in the progression of HF by further compromising contractile performance. On the other hand, some of the functional abnormalities of failing cardiomyocytes, particularly β-AR desensitization, may also be adaptations that protect from detrimental effects of long-term β-AR stimulation, for example, arrhythmias and myocardial hypertrophy (2). Accordingly, drugs intended to reverse or bypass β-AR desensitization (catecholamines or phosphodiesterase [PDE] inhibitors), cause symptomatic improvement in the short term, but demonstrate long-term increased mortality due to sudden cardiac death (3). Conversely, large-scale clinical trials have shown that β-AR blockers reduce mortality in HF (4–6).

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There is recent evidence that higher myocardial levels of the second messenger cyclic guanosine monophosphate (cGMP) may be beneficial, partly by antagonizing the cyclic adenosine monophosphate (cAMP)-mediated effects of β-AR overstimulation (7–9). Cyclic GMP is synthesized by either soluble guanylyl cyclases activated by nitric oxide (NO) or particulate guanylyl cyclases activated by the natriuretic peptides atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) (10). In the heart, cAMP and cGMP levels are regulated in a highly specific and stimulus-dependent manner by cyclic nucleotide degrading PDEs (11,12). Among the PDE superfamily, the PDE2 isoform is unique in being activated by cGMP via binding to the regulatory N-terminal GAF-B domain (13,14). cGMP functions as an allosteric regulator of PDE2, and occupancy of this binding site changes the kinetics for cyclic nucleotide hydrolysis, stimulating the hydrolysis of cAMP 5- to 30-fold. This may provide a negative cross-talk mechanism between cAMP and cGMP signaling pathways. Indeed, we showed previously that cGMP activation of PDE2 locally depletes cAMP in the vicinity of L-type Ca^{2+} channels, thus strongly antagonizing β-AR or cAMP stimulation of the L-type Ca^{2+} current ($I_{Ca,L}$) (15–17). In the human atrium, where cAMP turnover is more important, constitutive PDE2 activity controls basal $I_{Ca,L}$ (18), and activation of this PDE2 by cGMP also decreases stimulated $I_{Ca,L}$ (19). In neonatal cardiomyocytes, local PDE2 is coupled to a β-AR–stimulated pool of adenylyl cyclases and cAMP-dependent protein kinase type-II isoforms, antagonizing β-AR inotropic responses partly via the β_3-AR/NO/cGMP axis (9,20).

However, despite the unique function of PDE2 in cGMP and cAMP signaling, and the well-known perturbations in β-AR and NO/natriuretic peptide signaling pathways, it is unknown whether PDE2 has a significant pathophysiological role in failing hearts. Here, we show that myocardial PDE2 is markedly upregulated in HF and that specific PDE2 inhibition restores β-AR responsiveness in diseased cardiomyocytes, whereas PDE2 overexpression blunts catecholamine responsiveness and protects against pathological hypertrophy. These data establish PDE2 as a new member of the group of proteins contributing to the well-known phenomenon of β-AR desensitization in chronic HF and implicates PDE2 as a key downstream element that might be a target for the development of novel antiadrenergic therapeutic approaches.

Abbreviations and Acronyms

- **Ad-βGal** = adenovirus encoding β-galactosidase
- **ANP** = atrial natriuretic peptide
- **BAY** = BAY 60-7550
- **BNP** = B-type natriuretic peptide
- **β-AR** = beta-adrenergic receptor
- **cAMP** = cyclic adenosine monophosphate
- **cGMP** = cyclic guanosine monophosphate
- **EGF** = green fluorescent protein
- **FRET** = fluorescence resonance energy transfer
- **HF** = heart failure
- **$I_{Ca,L}$** = L-type Ca^{2+} current
- **ISO** = isoprenaline
- **LV** = left ventricular
- **NO** = nitric oxide
- **PDE2** = phosphodiesterase-2
- **SNP** = sodium nitroprusside

Methods

The study conformed to the principles outlined in the Declaration of Helsinki, and all procedures were approved by the ethics committees of our institutions. Patient characteristics and drugs, as well as all methods used, including preparation of protein extracts, PDE activity assays, myocyte isolation, and adenoviral transductions, cAMP measurements by fluorescence resonance energy transfer (FRET), Ca^{2+} transients and cell shortening (IonOptix, Milton, Massachusetts), $I_{Ca,L}$ measurements, and data analysis and statistics are detailed in the supplemental methods section in the Online Appendix.

Results

Myocardial PDE2 is increased in end-stage HF. We measured PDE2 protein levels in left ventricular (LV) myocardium from patients with terminal HF (ejection fraction ≤37%) and compared them with nonfailing donor hearts (n = 6). Each sample was normalized to calsequestrin

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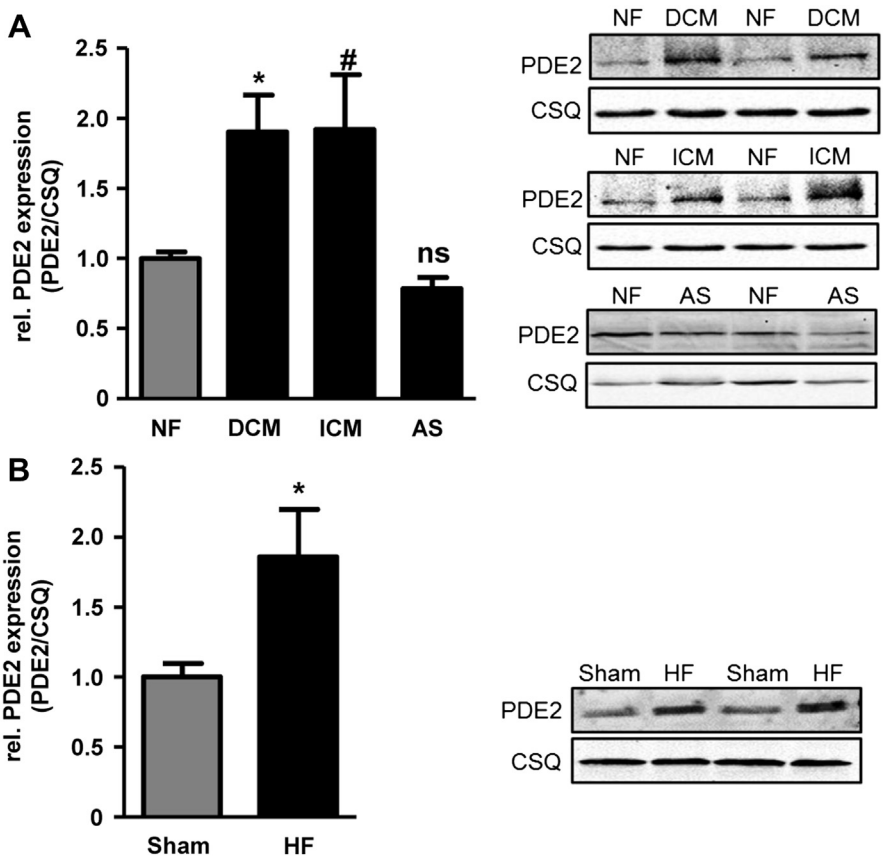

Figure 1 PDE2 Is Up-Regulated in Failing Hearts

(A) Phosphodiesterase-2 (PDE2) protein levels in left ventricular (LV) myocardium from patients with end-stage dilated cardiomyopathy (DCM) or ischemic cardiomyopathy (ICM), or aortic stenosis (AS) with myocardial hypertrophy but preserved cardiac function compared to nonfailing donor hearts (NF); n = 5 to 6. **(B)** PDE2 protein levels in LV myocardium from dogs subjected to rapid pacing–induced heart failure (HF) compared with Sham-operated controls (Sham); n = 7 to 9. PDE2 expression was normalized to calsequestrin (CSQ). *p < 0.05 versus NF or Sham, and #p = 0.05 versus NF. rel. = relative.

as an internal control. Immunoblots demonstrated greater signal intensity in the failing hearts (~2-fold) with dilated (n = 5) and ischemic (n = 5) cardiomyopathy (Fig. 1A; see Online Table 1 for patient characteristics). This was independent of β-blocker treatment before heart transplantation (Online Fig. 1). The protein data were confirmed at a functional level by a radioenzymatic assay in the presence and absence of the specific PDE2 inhibitor BAY 60-7550 (BAY Cayman Chemical Company, Ann Arbor, Michigan) (100 nmol/l) showing about a 2-fold greater PDE2 activity toward cAMP in failing hearts compared with nonfailing donor heart controls (Online Fig. 2). Interestingly, PDE2 expression did not differ between nonfailing controls and hypertrophied LV myocardium from patients with preserved cardiac function (ejection fraction ≥50%) who underwent aortic valve replacement due to aortic stenosis (n = 6) (Fig. 1A, Online Table 1). Given the well-known limitations of human heart tissue samples, including partly uncontrolled clinical parameters, we re-evaluated PDE2 expression in a well-established large-animal HF model of dogs subjected to ventricular tachycardia pacing (n = 9). Immunoblotting confirmed our findings in human HF showing ~2-fold greater PDE2 protein expression in failing dog hearts compared with sham controls (n = 7) (Fig. 1B).

Increased myocardial PDE2 expression/activity results from excessive adrenergic drive in vivo. We tested whether PDE2 up-regulation is like β_1-AR down-regulation a consequence of pathological β-AR overstimulation. As expected, long-term isoprenaline (ISO, Sigma-Aldrich (Saint Quentin, France)) infusion (2.4 μg/g/day for 4 days) in rats induced cardiac hypertrophy (as indicated by an increased heart-to-body weight ratio) (Fig. 2A) and induction of the fetal gene expression program (as indicated by a >8-fold increase in transcript levels of the cardiac fetal gene *ANP*) (Fig. 2B). In line with our hypothesis, this was accompanied by an ~2-fold increase in PDE2 protein levels (Fig. 2C). Next, we determined PDE2 mRNA levels by quantitative reverse-transcription polymerase chain reaction and found 4-fold higher levels in the ISO-infused hearts compared with control hearts, indicating that PDE2 is regulated at the

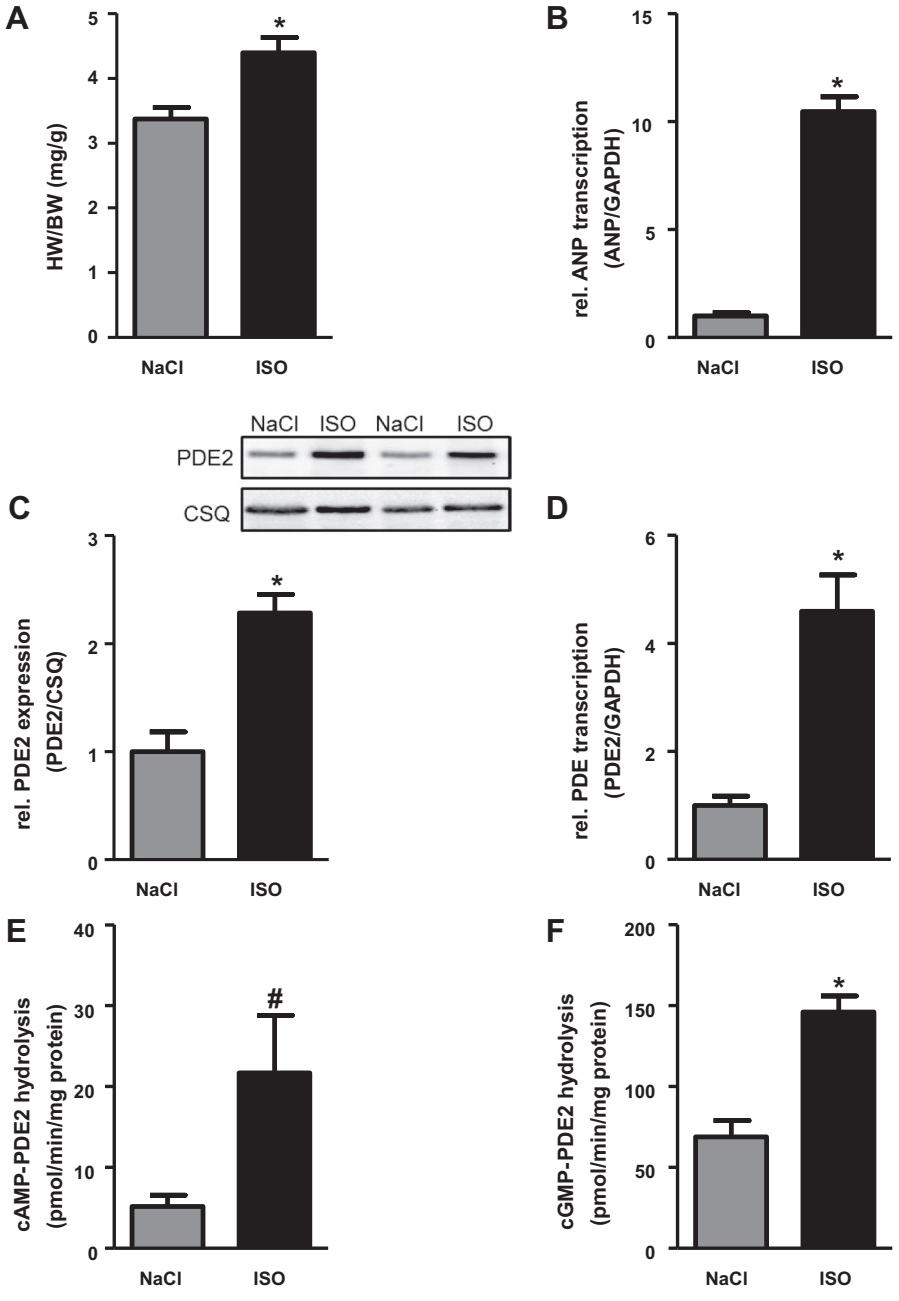

Figure 2 PDE2 in a Rat Model of Long-Term ISO Exposure: Elevated Expression and Activity

Male Wistar rats were either treated with isoprenaline (ISO) (2.4 μg/g/day) or vehicle (0.9% NaCl) for 4 days. ISO-mediated induction of cardiac hypertrophy was analyzed by determining **(A)** heart-weight to body-weight ratio (HW/BW); n = 14, and **(B)** LV atrial natriuretic peptide (ANP) mRNA levels normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH); n = 4 to 6. **(C)** Representative immunoblot and quantification of PDE2 protein expression in LV homogenate. Values are normalized to CSQ; n = 8. **(D)** LV PDE2 mRNA levels normalized to CSQ; n = 5 to 6. **(E and F)** PDE2 activity in LV tissue toward cyclic adenosine monophosphate (cAMP)/cyclic guanosine monophosphate (cGMP) was measured by radioenzymatic assay and defined as the fraction of total cAMP/cGMP-PDE activity inhibited by 100 nmol/l BAY 60-7550 (BAY); n = 6. *$p < 0.05$ and #$p = 0.06$ versus Vehicle. Abbreviations as in Figure 1.

transcriptional level (Fig. 2D).To determine whether the increased LV PDE2 protein expression is translated into greater activity, we analyzed PDE2-dependent cAMP and cGMP hydrolytic activities. As shown in Figures 2E and 2F, relative cAMP-PDE2 activity was ~4-fold higher and cGMP-PDE2 activity was ~2-fold greater in LV homogenates from the ISO- versus vehicle-treated hearts.

Higher cAMP-PDE2 hydrolytic activity in diseased cardiomyocytes. To further evaluate changes in PDE2 at the cardiomyocyte level, ventricular cardiomyocytes were

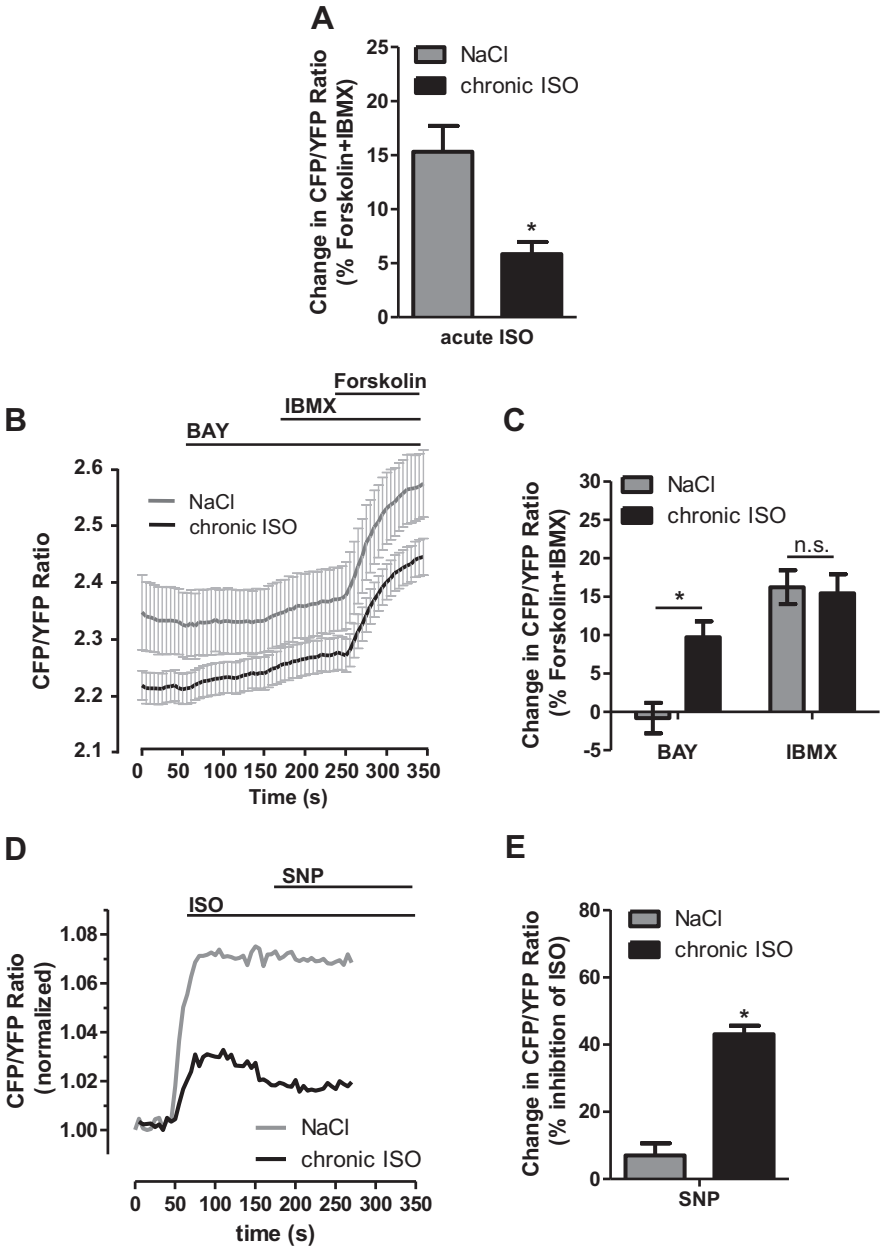

Figure 3 **Higher PDE2 cAMP Hydrolytic Activity in Hypertrophied and β-Adrenergically Desensitized Cardiomyocytes**

After 4 days ISO or NaCl treatment, cardiomyocytes were isolated from the sacrificed animals and transduced for 48 h with recombinant adenovirus encoding the cAMP sensor Epac2-camps. Adult rat ventricular myocytes were stimulated with the indicated substances and cyan fluorescent protein (CFP)/yellow fluorescent protein (YFP) ratio was determined upon 436-nm excitation (emission filters: YFP 535 ± 15 nm, CFP 480 ± 20 nm). **(A)** Average of the responsiveness to 1 nmol/l ISO as percentage of maximal response (forskolin + IBMX). **(B)** Fluorescence resonance energy transfer (FRET) traces presented as CFP/YFP ratio of cardiomyocytes treated with 100 nmol/l BAY, 100 μmol/l IBMX, and 10 μmol/l forskolin. **(C)** Percentage of maximal (forskolin + IBMX) response; NaCl n = 7, ISO n = 11. **(D)** Representative traces normalized to basal CFP/YFP ratio of cardiomyocytes treated with 100 nmol/l ISO and 50 μmol/l sodium nitroprusside (SNP). **(E)** Percentage of maximal (ISO) response; NaCl n = 7, ISO n = 7. *$p < 0.05$ versus NaCl. Abbreviations as in Figures 1 and 2.

isolated from adult rats either subjected to long-term ISO or vehicle infusion, and cAMP-PDE2 hydrolytic activity was monitored using the adenovirally expressed exchange protein directly activated by cAMP– cAMP sensor (Epac2-camps) FRET biosensor for cAMP. Acute application of ISO (1 nmol/l) produced a clear increase in cAMP in control cardiomyocytes, which was markedly attenuated in cardiomyocytes from ISO-treated hearts (−70%) (Fig. 3A), as expected from the known β-AR desensitization in this model. The basal FRET ratios were lower in cells isolated from ISO-treated rats (Fig. 3B). Application of BAY had no significant effect on cAMP accumulation in control cardiomyocytes, in

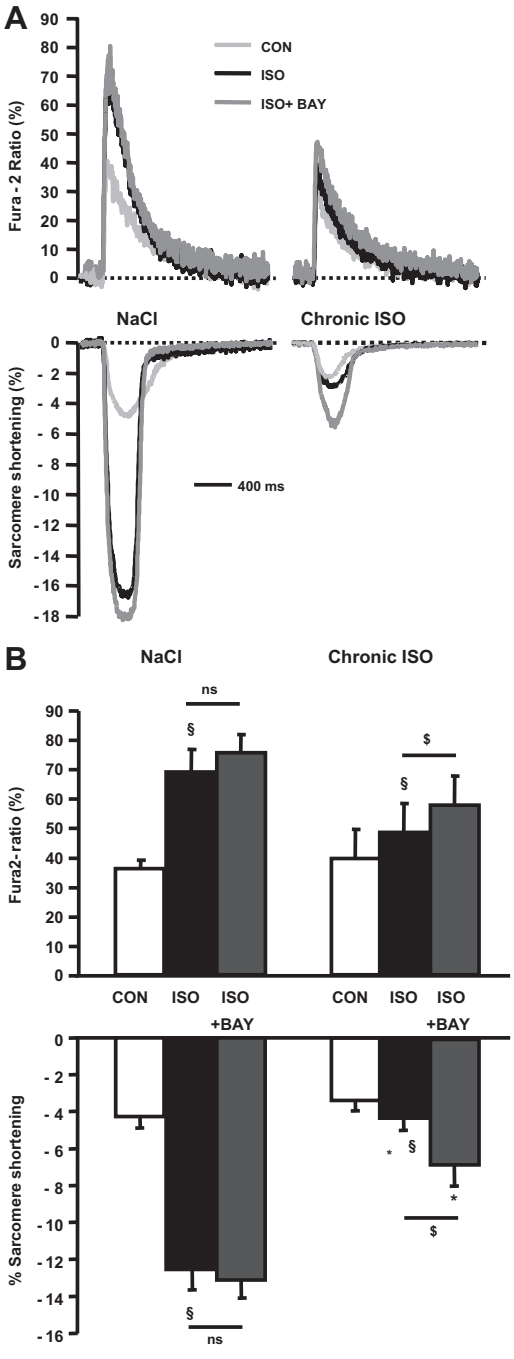

Figure 4 **PDE2 Inhibition Increases β-AR Stimulation of Ca^{2+} Transients and Sarcomere Shortening in Hypertrophied and β-Adrenergically Desensitized Cardiomyocytes**

Ventricular cardiomyocytes were isolated from adult rat hearts subjected to either long-term ISO or NaCl. **(A)** Representative traces of Ca^{2+} transients **(top)** and sarcomere shortening **(bottom)** recorded in cardiomyocytes paced at 0.5 Hz and loaded with Fura-2 AM (5 μmol/l) showing the effect of a β-adrenergic receptor (β-AR) stimulation with 1 nmol/l ISO, and of PDE2 inhibition by BAY (100 nmol/l) on top of ISO. **(B)** Mean absolute values of Fura-2 ratio **(upper graphs)** and sarcomere shortening **(lower graphs)** in control conditions, or in the presence of ISO or ISO + BAY (n = 15 to 18). §p < 0.05 versus control (Con); $p < 0.05 versus ISO; *p < 0.05 versus NaCl. Abbreviations as in Figures 2 and 3.

line with the fact that PDE2 accounts for only 3% of the total cAMP-hydrolyzing activity under normal conditions (20). By comparison, application of the nonselective PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX, Sigma-Aldrich [Saint Quentin, France]) (100 μmol/l) or the adenylyl cyclase-activator forskolin (10 μmol/l) in the continued presence of BAY produced a similar net increase in cAMP (Fig. 3B). The absolute cAMP levels (cyan fluorescent protein/yellow fluorescent protein ratio in Fig. 3B) were lower in ISO cardiomyocytes versus control ($p = 0.05$). However, in long-term ISO–treated cardiomyocytes, application of BAY alone produced a significant increase in cAMP levels (Figs. 3B and 3C). The overall maximal changes in cAMP levels from basal to forskolin/IBMX maximum were similar in both groups, suggesting comparable rates of cAMP production. These data indicate a stronger contribution of PDE2 to cAMP hydrolysis in diseased cardiomyocytes.

Because PDE2 is activated by cGMP, we tested whether higher PDE2 activity in diseased cardiomyocytes could be further enhanced by cGMP synthesis. To this end, we challenged cardiomyocytes with the NO donor sodium nitroprusside (SNP Sigma-Aldrich, Munich, Germany) (50 μmol/l) after acute β-AR stimulation with ISO. SNP reduced the ISO response in long-term ISO cardiomyocytes, but had no significant effect in vehicle controls (Figs. 3D and 3E). Therefore, further activation of up-regulated PDE2 is still possible in diseased cardiomyocytes. Interestingly, these data point to a predominant effect of the cGMP-induced PDE2 activation over the cGMP-mediated PDE3 inhibition in disease.

To investigate how increased PDE2 expression translates into function, Ca^{2+} transients (Fura-2 ratio) and sarcomere shortening were measured in isolated cardiomyocytes with the IonOptix system. ISO (1 nmol/l) produced a large increase in the Fura-2 ratio and sarcomere shortening in NaCl myocytes, but only a modest increase in cardiomyocytes from ISO-treated hearts (Fig. 4), again confirming the β-AR desensitization. BAY alone produced a small but significant effect on both parameters in vehicle-treated cardiomyocytes (Online Table 2). However, in long-term ISO–treated cardiomyocytes, BAY produced a 2-fold larger effect (Online Table 2). Importantly, the application of BAY in addition to ISO had no significant effect on vehicle-treated cardiomyocytes, but increased 2- to 3-fold the ISO effect on Ca^{2+} transients and sarcomere shortening in the long-term ISO group (Fig. 4B). Thus, PDE2 inhibition partially restores β-AR responsiveness, which indicates that PDE2 is in fact playing a functional role in diseased myocytes.

Overexpression of PDE2 blunts responses to acute β-AR stimulation and protects from norepinephrine-induced hypertrophy. To dissect further the specific role of up-regulated PDE2, we infected adult rat ventricular myocytes with either an adenovirus encoding hemagglutinin-tagged mouse PDE2A2 (Ad-PDE2) or a control adenovirus encoding β-galactosidase (Ad-βGal) or green fluorescent

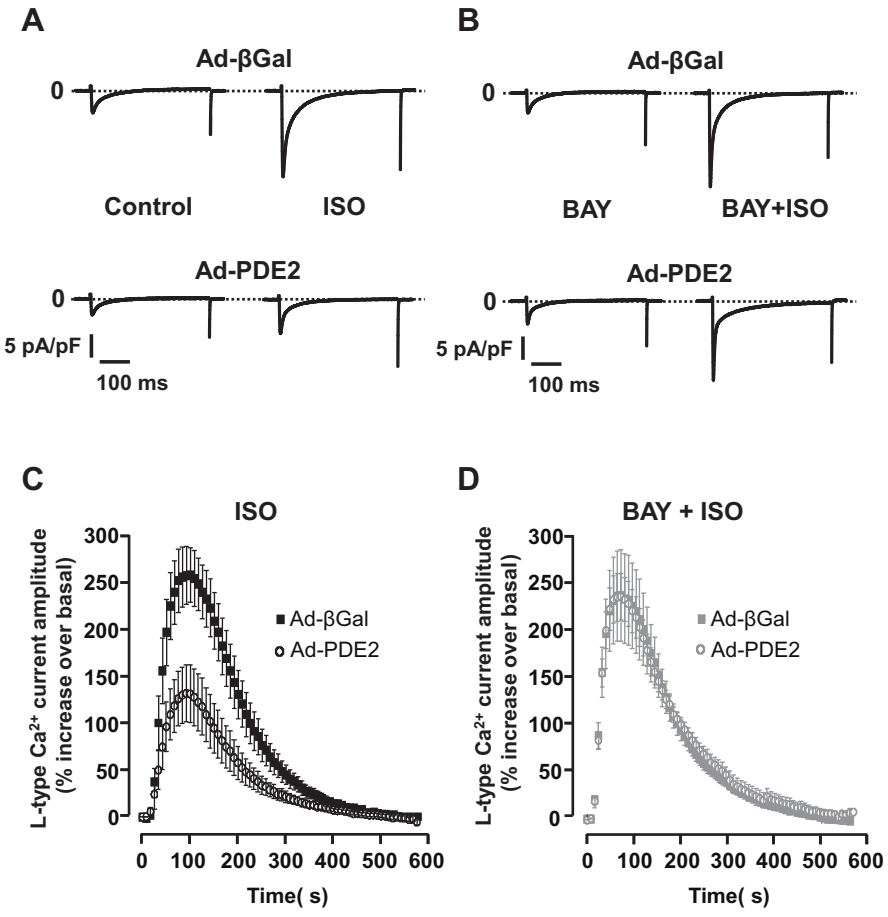

Figure 5 **PDE2 Overexpression Decreases β-AR Stimulation of $I_{Ca,L}$ in Cardiomyocytes**

The whole-cell configuration of the patch-clamp technique was used to record the L-type Ca^{2+} current ($I_{Ca,L}$). The cells were depolarized every 8 s from −50 to 0 mV during 400 ms. The use of −50 mV as the holding potential allowed the inactivation of voltage-dependent Na^+ currents. K^+ currents were blocked by replacing all K^+ ions with external and internal Cs^+. **(A and B)** Representative examples of individual $I_{Ca,L}$ current traces showing the effect of ISO (100 nmol/l, 15 s) alone **(A)** or in the presence of the PDE2 inhibitor BAY (100 nmol/l) **(B)** on $I_{Ca,L}$ in adult rat ventricular myocytes infected with either adenovirus encoding β-galactosidase (Ad-βGal) **(top traces)** or Ad-PDE2 **(bottom traces)**. To correct for differences in cell size, the current traces were normalized to cell membrane capacitance and are presented as current densities in pA/pF. **(C)** Normalized average time course of $I_{Ca,L}$ amplitude following ISO pulse stimulation (100 nmol/l, 15s) in each group; n = 7 to 8. **(D)** Effect of ISO (100 nmol/l, 15 s) in the presence of BAY (n = 5). Abbreviations as in Figures 1 to 4.

protein (EGFP). Immunoblot detection of overexpressed PDE2 and, at the functional level, higher PDE2 hydrolysis activity toward cAMP and cGMP in Ad-PDE2–infected cardiomyocytes are shown in Online Fig. 3. We then determined the consequences for cAMP changes in response to β-AR stimulation. For this, cardiomyocytes coexpressing Epac2-camps and either β-Gal or PDE2 were challenged with a short application of ISO (100 nmol/l, 15 s) either alone or in the presence of BAY (100 nmol/l). As monitored by FRET, PDE2 overexpression markedly decreased intracellular cAMP levels generated by β-AR stimulation. This β-AR response was almost completely restored when the cardiomyocytes were additionally challenged with BAY (Online Fig. 4).

Next, we examined whether PDE2 overexpression modifies the β-AR regulation of excitation–contraction coupling. Although overexpression of PDE2 had no significant effect on basal $I_{Ca,L}$ density (4.8 ± 0.7 pA/pF in Ad-PDE2, n = 7 vs. 3.2 ± 0.7 pA/pF in Ad-βGal, n = 8), the response to a pulse application of 100 nmol/l ISO was markedly blunted in myocytes infected with Ad-PDE2 (Figs. 5A and 5C). However, inhibition of PDE2 with BAY restored a normal response to ISO (Figs. 5B and 5D). In another series of experiments, sarcomere shortening and the Fura-2 ratio were simultaneously recorded (Fig. 6A). Under basal conditions, the amplitude and relaxation kinetics of sarcomere shortening were not significantly different between Ad-βGal and Ad-PDE2 cardiomyocytes (Figs. 6B and 6C). Ad-PDE2 cardiomyocytes developed significantly smaller Ca^{2+} transients than Ad-βGal, yet with no difference in the relaxation kinetics. When the cells were challenged with ISO (1 nmol/l), Ad-βGal cardiomyocytes responded with a strong increase in both

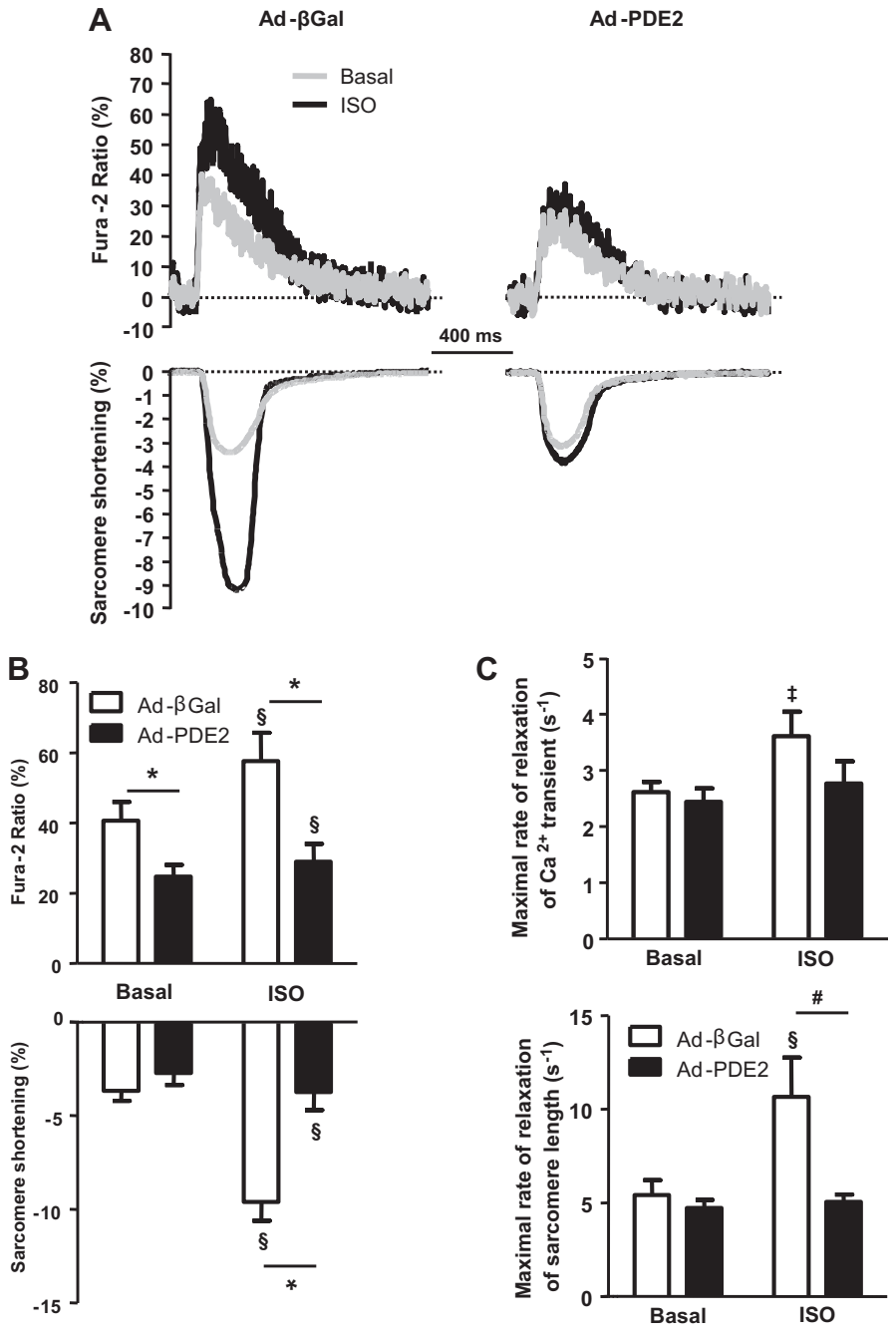

Figure 6 PDE2 Overexpression Blunts β-AR Stimulation of Ca^{2+} Transients and Sarcomere Shortening in Cardiomyocytes

Adult rat ventricular myocytes were loaded with Fura-2 and stimulated at a frequency of 0.5 Hz. Sarcomere length and Fura-2 ratio were recorded using an IonOptix System. **(A)** Representative traces of basal and ISO (1 nmol/l) stimulated Ca^{2+} transient and sarcomere shortening in Ad-βGal and Ad-PDE2–infected cells. **(B)** Average amplitudes of Ca^{2+} transients and sarcomere shortening. **(C)** Maximal rates of relaxation and Ca^{2+} reuptake; n = 8 to 11; §$p < 0.05$ versus basal; ‡$p = 0.05$ versus basal; *$p < 0.05$ versus Ad-βGal; #$p = 0.05$ versus Ad-βGal. Abbreviations as in Figures 1, 2, 4, and 5.

contraction and Ca^{2+} transient amplitudes (Fig. 6B), accompanied by an acceleration in their relaxation kinetics (Fig. 6C). In PDE2-overexpressing cardiomyocytes, however, these responses were markedly attenuated. To test sarcoplasmic reticulum function at the molecular level, we assayed phosphorylated phospholamban (P-PLB). Immunoblots showed that overexpression of PDE2 does not change the phosphorylation level of PLB under basal conditions, but significantly decreases the P-PLB/total PLB ratio after stimulation with 1 nmol/l ISO (Online Fig. 5). These results

suggest that PDE2 overexpression does not affect sarcoplasmic reticulum function under basal conditions and is consistent with our findings that the β-AR response of Ca^{2+} transients and sarcomere shortening was inhibited by PDE2 overexpression without any negative effect on basal contractility. Collectively, our findings highlight a direct and potent involvement of PDE2 in β-AR responsiveness in HF.

Cardiac hypertrophy is 1 of the first stages of stress-induced HF. To examine the effect of up-regulated PDE2 on cardiomyocyte hypertrophy, we determined the increase in cell size in cultured adult cardiomyocytes in response to 24-h stimulation with norepinephrine or phenylephrine. As shown in Figure 7, PDE2 overexpression attenuated significantly the norepinephrine- and phenylephrine-induced increase in cell surface area. These data indicate that PDE2 has protective effects under conditions of α- and/or β-AR–induced pathological hypertrophy.

Discussion

The major findings of the present study are: 1) myocardial PDE2 is up-regulated in human and experimental HF; 2) specific PDE2 inhibition restores β-AR responsiveness in diseased cardiomyocytes; 3) increased PDE2 activity is further enhanced by cGMP stimulation via NO donors; and 4) PDE2 overexpression abolishes β-AR responsiveness without affecting basal contractility and protects against pathological hypertrophy.

PDE2 is up-regulated in failing hearts. Consistent with the literature, our study shows rather low protein expression of PDE2 in healthy hearts (Fig. 1), and accordingly, inhibition of PDE2 has little effect on cAMP hydrolytic activity in healthy cardiomyocytes (Figs. 3B and 3C). However, our data indicate that in HF, with enhanced adrenergic drive and marked perturbations in the β-AR and NO/natriuretic peptide signaling, PDE2 becomes up-regulated and, by mediating cGMP-dependent cAMP degradation, contributes to the imbalance between these 2 signaling pathways. This is consistent with recent findings showing disorganization of cAMP-dependent protein kinase (PKA) regulatory subunits and A-kinase anchoring proteins, which showed increased capturing of cGMP-dependent protein kinase (PKG) and PDE2 (21). By contrast, the expression and activity of other cAMP-hydrolyzing PDEs (e.g., PDE3) is reduced in human and experimental HF (22,23). PDE regulation in vivo is complex, and 1 transduction signal may affect several PDEs differently. For instance, cGMP activates PDE2, but competitively inhibits PDE3 (24). Through the latter mechanism, an increase in cGMP levels inhibits cAMP degradation. Thus, it is likely that under HF conditions, both the lower PDE3 and higher PDE2 activity could be a consequence of increased cGMP signaling that is further amplified by increased PDE2 and/or reduced PDE3 expression. In this context, it is interesting to note that the cGMP-hydrolyzing PDE5 is also increased in failing human hearts (8). This may partly compensate for the PDE2/PDE3 alterations.

β-AR signaling is a biologically dynamic system that adapts quickly and reversibly by desensitization in response to overstimulation. Accordingly, β-blockers "resensitize" the failing heart and are associated with normalization of β-ARs (1,2). However, β-blocker therapy did not appear to normalize up-regulated myocardial PDE2 expression in our samples, pointing to different regulatory mechanisms of altered β-ARs and PDE2 in HF. In addition, it was intriguing to observe that PDE2 expression was still normal in LV tissue samples from aortic stenosis patients with myocardial hypertrophy and preserved cardiac function (Fig. 1A). This indicates an important role for PDE2 in decompensated rather than earlier stages of myocardial remodeling.

PDE2 up-regulation and its consequences after long-term excessive β-AR drive. The long-term ISO infusion model recapitulates key features of human HF, particularly pathological hypertrophy and the well-known phenomenon of β-AR desensitization (25,26). In this model, we observed an increase in PDE2 protein expression, mRNA transcription, and PDE2 cGMP/cAMP hydrolytic activity. Specific PDE2 inhibition partially restored β-AR responsiveness in diseased cardiomyocytes, indicating that PDE2 up-regulation contributes significantly to the well-known phenomenon of β-AR desensitization, considered to be a major protective mechanism in HF. A central translational question is whether potentially beneficial PDE2 up-regulation in HF can be further amplified by coactivation of cGMP synthesis. Indeed, diseased cardiomyocytes showed a significant decrease in cAMP levels when challenged with the NO donor SNP, further validating the feasibility of PDE2 activation in the context of HF (see the following text).

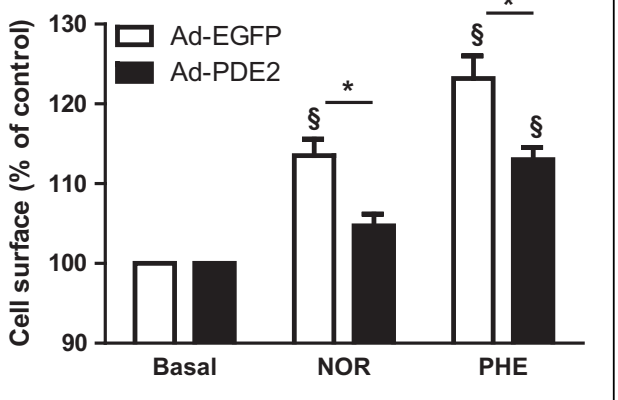

Figure 7 PDE2 Overexpression Protects Cardiomyocytes Against Hypertrophy Induced by α/β-AR Stimulation

Cardiomyocytes were stimulated with 10 μmol/l norepinephrine (NOR) or 10 μmol/l phenylephrine (PHE) for 24 h, and the cellular surface area was determined by microscopy; n = 5 to 6 preparations. §$p < 0.05$ versus basal; *$p < 0.05$ adenovirus encoding enhanced green fluorescent protein (Ad-EGFP) versus Ad-PDE2.

PDE2 abolishes β-AR–mediated inotropic effects via cAMP degradation and reduction of $I_{Ca,L}$ amplitude. In cardiomyocytes isolated from long-term ISO rats, β-AR stimulation of cell shortening and Ca^{2+} transients was severely blunted due to PDE2 overexpression. Moreover, adenoviral PDE2 overexpression markedly blunts ISO-induced inotropy, cAMP generation, and the increase in $I_{Ca,L}$ and Ca^{2+} transients in adult cardiomyocytes. Importantly, all effects were reversible upon specific PDE2 inhibition with BAY. This is the first direct evidence that PDE2 overexpression inhibits the increase in $I_{Ca,L}$ by reducing cAMP concentrations and indicates that PDE2 regulates a specific cAMP pool that modulates β-AR signaling. Our findings are consistent with previous reports showing indirectly that activation of PDE2 is responsible for the inhibitory effect of intracellular cGMP on $I_{Ca,L}$ in frog ventricular (16,27) and human atrial myocytes (19) and that, via stimulation of soluble guanylyl cyclase with NO donors, attenuates β-AR stimulation in frog (27) and neonatal rat cardiomyocytes (20).

PDE2 does not affect basal contractility but protects against pathological hypertrophy. NO/natriuretic peptides have been shown to exert both beneficial cGMP-dependent antihypertrophic and potentially detrimental negative inotropic effects on the failing heart. Both effects have been largely attributed to activation of cGMP-dependent PKG (28,29), with uncertain contribution of cGMP-dependent PDE2 activation. Our findings in PDE2-overexpressing cardiomyocytes suggest no negative effect on basal contractility or $I_{Ca,L}$ (Figs. 5B and 6B), but beneficial effects of PDE2 up-regulation in the context of pathological hypertrophy induced by norepinephrine and phenylephrine (Fig. 7). The antihypertrophic properties together with the maintained basal contractility suggest that the up-regulated PDE2 in HF is beneficial despite limiting the contractile reserve upon β-AR stimulation, a profile that is quite similar to β_1-AR down-regulation in HF.

Potential limitations. To study PDE function, we evaluated different species, including humans. However, the role played by each PDE isoform varies among mammalian species (11,12), and accordingly, our results may not recapitulate the situation in humans in all details. In addition, selective PDE2 inhibitors or activators for in vivo use have not been developed, and PDE2 knockout mice have not yet been generated to study the precise role of PDE2 in the healthy and diseased heart. Therefore, we studied the function of cardiac PDEs using specific inhibitors or adenoviral overexpression in vitro, but this might not reflect the complex regulation of the PDEs in vivo, particularly of PDE2, which is a dual esterase that is positively regulated by cGMP.

Novelty and clinical significance. The current therapeutic strategies for HF are complex and only moderately efficient. Clearly, improved HF therapies are necessary, but a lack of understanding of the pathophysiological mechanisms underlying HF has hindered the development of more effective, rational therapeutic approaches. Several studies demonstrated beneficial effects of cGMP stimulation in HF, and multicenter trials are currently testing the therapeutic value of inhibition of cGMP hydrolyzing PDEs (PDE5) or activation of soluble guanylyl cyclase in patient populations (30). The rationale of these studies is consistent with our hypothesis that augmentation of cGMP signaling may serve as a myocardial brake, blunting the detrimental effects of excessive catecholamines in HF. Despite substantial advances in the field, there are still controversies regarding the therapeutic value of activated cGMP signaling via PDE5 inhibition, and the clinical translation of increasing cGMP synthesis has been limited by hypotension and tolerance development with nitrates and rather neutral responses to natriuretic peptides (30).

Conclusions

To the best of our knowledge, this study is first to show that PDE2 is up-regulated in HF and constrains the effects of long-term β-AR stimulation on cardiomyocyte Ca^{2+} handling and contractility in HF. Moreover, the up-regulated PDE2 expression appears unaffected by β-blocker therapy, but could be further enhanced in diseased cardiomyocytes and protects against hypertrophic stimuli without negatively affecting basal contractility, clearly suggesting that activation of PDE2 could be a novel and feasible approach for the treatment of HF. In addition, cAMP hydrolysis through PDE2 has been shown to mediate the ANP/cGMP-induced decreases of aldosterone production in adrenal glomerulosa cells, suggesting synergistic beneficial effects on the cardiorenal axis (31). Overall, our data suggest that increased myocardial PDE2 levels may constitute an important defense mechanism in end-stage HF by antagonizing β-AR/cAMP–mediated cardiac toxicity. Thus, augmenting myocardial PDE2 activity in early stages of HF may represent a new subcellular antiadrenergic therapeutic strategy in HF. Further extensive studies in large-animal HF, as well as the development of specific PDE2 activators for in vivo use, is required to define precisely the specific contributions of PDE2 to HF pathophysiology and to validate the therapeutic value of enhanced PDE2 activity in HF patients.

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REFERENCES

1. Bristow MR, Ginsburg R, Minobe W, et al. Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. N Engl J Med 1982;307:205–11.

2. El-Armouche A, Eschenhagen T. beta-Adrenergic stimulation and myocardial function in the failing heart. Heart Fail Rev 2008;14:225–41.
3. Packer M, Carver JR, Rodeheffer RJ, et al., The PROMISE Study Research Group. Effect of oral milrinone on mortality in severe chronic heart failure. N Engl J Med 1991;325:1468–75.
4. Bristow MR. beta-adrenergic receptor blockade in chronic heart failure. Circulation 2000;101:558–69.
5. Packer M, Bristow MR, Cohn JN, et al. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. N Engl J Med 1996;334: 1349–55.
6. MERIT-HF Study Group. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF). Lancet 1999;353(9169): 2001–7.
7. Mika D, Leroy J, Vandecasteele G, Fischmeister R. PDEs create local domains of cAMP signaling. J Mol Cell Cardiol 2012;52:323–9.
8. Pokreisz P, Vandenwijngaert S, Bito V, et al. Ventricular phosphodiesterase-5 expression is increased in patients with advanced heart failure and contributes to adverse ventricular remodeling after myocardial infarction in mice. Circulation 2009;119:408–16.
9. Stangherlin A, Gesellchen F, Zoccarato A, et al. cGMP signals modulate cAMP levels in a compartment-specific manner to regulate catecholamine-dependent signaling in cardiac myocytes. Circ Res 2011; 108:929–39.
10. Su J, Scholz PM, Weiss HR. Differential effects of cGMP produced by soluble and particulate guanylyl cyclase on mouse ventricular myocytes. Exp Biol Med (Maywood) 2005;230:242–50.
11. Zaccolo M, Movsesian MA. cAMP and cGMP signaling cross-talk: role of phosphodiesterases and implications for cardiac pathophysiology. Circ Res 2007;100:1569–78.
12. Fischmeister R, Castro LR, Abi-Gerges A, et al. Compartmentation of cyclic nucleotide signaling in the heart: the role of cyclic nucleotide phosphodiesterases. Circ Res 2006;99:816–28.
13. Francis SH, Blount MA, Corbin JD. Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions. Physiol Rev 2011;91:651–90.
14. Martinez SE, Beavo JA, Hol WG. GAF domains: two-billion-year-old molecular switches that bind cyclic nucleotides. Mol Interv 2002;2: 317–23.
15. Hartzell HC, Fischmeister R. Opposite effects of cyclic GMP and cyclic AMP on Ca2+ current in single heart cells. Nature 1986;323: 273–5.
16. Mery PF, Pavoine C, Belhassen L, Pecker F, Fischmeister R. Nitric oxide regulates cardiac Ca2+ current. Involvement of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. J Biol Chem 1993;268:26286–95.
17. Dittrich M, Jurevicius J, Georget M, et al. Local response of L-type Ca(2+) current to nitric oxide in frog ventricular myocytes. J Physiol 2001;534:109–21.
18. Rivet-Bastide M, Vandecasteele G, Hatem S, et al. cGMP-stimulated cyclic nucleotide phosphodiesterase regulates the basal calcium current in human atrial myocytes. J Clin Invest 1997;99:2710–8.
19. Vandecasteele G, Verde I, Rucker-Martin C, Donzeau-Gouge P, Fischmeister R. Cyclic GMP regulation of the L-type Ca(2+) channel current in human atrial myocytes. J Physiol 2001;533:329–40.
20. Mongillo M, Tocchetti CG, Terrin A, et al. Compartmentalized phosphodiesterase-2 activity blunts beta-adrenergic cardiac inotropy via an NO/cGMP-dependent pathway. Circ Res 2006;98: 226–34.
21. Aye TT, Soni S, van Veen TA, et al. Reorganized PKA-AKAP associations in the failing human heart. J Mol Cell Cardiol 2012;52: 511–8.
22. Ding B, Abe J, Wei H, et al. Functional role of phosphodiesterase 3 in cardiomyocyte apoptosis: implication in heart failure. Circulation 2005; 111:2469–76.
23. Abi-Gerges A, Richter W, Lefebvre F, et al. Decreased expression and activity of cAMP phosphodiesterases in cardiac hypertrophy and its impact on beta-adrenergic cAMP signals. Circ Res 2009;105:784–92.
24. Degerman E, Belfrage P, Manganiello VC. Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). J Biol Chem 1997;272:6823–6.
25. Wittkopper K, Fabritz L, Neef S, et al. Constitutively active phosphatase inhibitor-1 improves cardiac contractility in young mice but is deleterious after catecholaminergic stress and with aging. J Clin Invest 2010;120:617–26.
26. El-Armouche A, Wittkopper K, Degenhardt F, et al. Phosphatase inhibitor-1-deficient mice are protected from catecholamine-induced arrhythmias and myocardial hypertrophy. Cardiovasc Res 2008;80: 396–406.
27. Mery PF, Pavoine C, Pecker F, Fischmeister R. Erythro-9-(2-hydroxy-3-nonyl)adenine inhibits cyclic GMP-stimulated phosphodiesterase in isolated cardiac myocytes. Mol Pharmacol 1995;48:121–30.
28. Rosenkranz AC, Woods RL, Dusting GJ, Ritchie RH. Antihypertrophic actions of the natriuretic peptides in adult rat cardiomyocytes: importance of cyclic GMP. Cardiovasc Res 2003;57:515–22.
29. Fiedler B, Lohmann SM, Smolenski A, et al. Inhibition of calcineurin-NFAT hypertrophy signaling by cGMP-dependent protein kinase type I in cardiac myocytes. Proc Natl Acad Sci U S A 2002;99:11363–8.
30. Lee DI, Kass DA. Phosphodiesterases and cyclic GMP regulation in heart muscle. Physiology (Bethesda) 2012;27:248–58.
31. MacFarland RT, Zelus BD, Beavo JA. High concentrations of a cGMP-stimulated phosphodiesterase mediate ANP-induced decreases in cAMP and steroidogenesis in adrenal glomerulosa cells. J Biol Chem 1991;266:136–42.

Key Words: β-adrenoceptor signaling ■ cAMP ■ cGMP ■ heart failure ■ phosphodiesterase 2.

APPENDIX

For supplemental figures and tables, and an expanded Methods section, please see the online version of this paper.