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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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