cGMP Catabolism by Phosphodiesterase 5A Regulates Cardiac Adrenergic Stimulation by NOS3-Dependent Mechanism

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Abstract—β-Adrenergic agonists stimulate cardiac contractility and simultaneously blunt this response by coactivating NO synthase (NOS3) to enhance cGMP synthesis and activate protein kinase G (PKG-1). cGMP is also catabolically regulated by phosphodiesterase 5A (PDE5A). PDE5A inhibition by sildenafil (Viagra) increases cGMP and is used widely to treat erectile dysfunction; however, its role in the heart and its interaction with β-adrenergic and NOS3/cGMP stimulation is largely unknown. In nontransgenic (control) murine in vivo hearts and isolated myocytes, PDE5A inhibition (sildenafil) minimally altered rest function. However, when the hearts or isolated myocytes were stimulated with isoproterenol, PDE5A inhibition was associated with a suppression of contractility that was coupled to elevated cGMP and increased PKG-1 activity. In contrast, NOS3-null hearts or controls with NOS inhibited by N^G-nitro-L-arginine methyl ester, or soluble guanylate cyclase (sGC) inhibited by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one, showed no effect of PDE5A inhibition on β-stimulated contractility or PKG-1 activation. This lack of response was not attributable to altered PDE5A gene or protein expression or in vitro PDE5A activity, but rather to an absence of sGC-generated cGMP specifically targeted to PDE5A catabolism and to a loss of PDE5A localization to z-bands. Re-expression of active NOS3 in NOS3-null hearts by adenoviral gene transfer restored PDE5A z-band localization and the antiadrenergic efficacy of PDE5A inhibition. These data support a novel regulatory role of PDE5A in hearts under adrenergic stimulation and highlight specific coupling of PDE5A catabolic regulation with NOS3-derived cGMP attributable to protein subcellular localization and targeted synthetic/catabolic coupling. (Circ Res. 2005;96:100-109.)

Key Words: PDE5 ■ phosphodiesterase ■ sildenafil ■ nitric oxide synthase ■ contractility ■ z-band

Beta-adrenergic regulation of cardiac contraction is coupled to elevations in adenosine (cAMP) and guanosine (cGMP) cyclic nucleotides. Increased cAMP enhances contractility1,2 by activating protein kinase A (PKA), whereas concomitant stimulation of cGMP opposes this in part by activating protein kinase G (PKG-1).3,4 The latter response is thought to be attributable to stimulation of soluble guanylate cyclase (sGC) by NO.3-5 Cyclic GMP is also synthesized by receptor GC (rGC) coupled to natriuretic peptide stimulation, and both sources can modulate cardiac function and structure, particularly in hearts stimulated by neurohormones or mechanical stress.3-5,10-13 Cyclic GMP is also regulated by catabolic phosphodiesterases such as phosphodiesterase 5A (PDE5A), and PDE5A inhibition by sildenafil (Viagra; SIL) and similar compounds augments cGMP in vascular tissue and is the primary therapy for erectile dysfunction.14,15 However, the role for PDE5A in regulating cardiac function has remained unclear.16-18 Such clarification has become increasingly important because PDE5A inhibitors are poised to become chronic treatments for diseases such as pulmonary hypertension.19,20

One intriguing feature of cGMP catabolic regulation by PDE5A is that in vascular tissues, it appears coupled to NO synthase (NOS3)–dependent synthetic pathways. For example, SIL counters hypoxia-induced pulmonary hypertension more effectively in nontransgenic (NTG) mice than in animals lacking the NOS3 isoform (NOS3−/−).21 Similarly, SIL enhances erectile tone less effectively in diabetic rats than...
Male wild-type (WT) and NOS3−/− mice (C57BL6; 6 to 8 weeks old; The Jackson Laboratory) were studied. PDE5A was inhibited in vivo with or lacking the NOS3 gene or with NOS pharmacologically inhibited, we found that β-adrenergic contractile stimulation is suppressed by PDE5A inhibition, and this regulation requires NOS3 activity. This dependence on NOS3 is attributable to specific targeting of sGC/cGMP to PDE5A and to a role of NOS/NO in properly localizing PDE5A to z-band regions within cardiomyocytes.

Materials and Methods

Male wild-type (WT) and NOS3−/− mice (C57BL6; 6 to 8 weeks old; The Jackson Laboratory) were studied. PDE5A was inhibited in vivo with SIL (100 μg/kg per minute; 37 ± 5.2 nmol/L free plasma concentration); or EMD-360527/5 (160 to 300 μmol/L for purified PDE5A (versus 1 to 20 μmol/L for PDE1 or PDE3). In vitro studies used 0.1 to 1 μmol/L SIL, 0.05 μmol/L tadalafil (prepared in 0.1% dimethyl sulfoxide and EMD in 0.1% dimethyl sulfoxide and EMD in 10 nmol/L for purified PDE5A) and this regulation requires NOS3 activity. This dependence on NOS3 is attributable to specific targeting of sGC/cGMP to PDE5A and to a role of NOS/NO in properly localizing PDE5A to z-band regions within cardiomyocytes.

In Vivo Studies

Isoproterenol (ISO; 20 ng/kg per minute intravenous infusion for 5 minutes) with or without PDE5A inhibitor was given to anesthetized intact mice and in vivo heart function assessed by pressure–volume (PV) relations23 at a fixed atrial pacing rate of 600 to 650 minutes⁻¹. Data were measured at baseline, with ISO, reseline, PDE5A inhibition, and PDE5A inhibition ISO. The ISO-only response was highly reproducible.

Isolated Myocyte Studies

Excised hearts were retroperfused by buffer containing 2,3-butanediol monoxime (1 mg/mL) and taurine (0.628 mg/mL) for 3 minutes, 0.9 mg/mL collagenase (type 2; 299 U/mg; Worthington), and 0.05 mg/mL protease (Sigma) for 6 to 7 minutes. Ventricles were gently chopped, filtered (150-micron mesh), centrifuged (500 rpm×1 minute), and rinsed in Tyrode’s solution with increasing calcium (final 1.8 mM Ca²⁺). Cells were incubated with 5 μmol/L Indo-1 AM (Molecular Probes), rinsed, and studied at 27°C by field stimulation in an inverted fluorescence microscope (Diaphot 200; Nikon). Sarcomere length (IonOptix) and whole-cell calcium transient were measured. After baseline, cells were exposed to 10 mMOL ISO, then ISO+SIL, or ISO+EMD-360527/5 at pH 7.45. SIL was diluted in 0.1% dimethyl sulfoxide and EMD in 0.001% propanediol; control solutions contained similar vehicle concentrations.

Gene and Protein Expression

PDE5A gene expression was assessed by quantitative real-time polymerase chain reaction (PCR). Residual genomic DNA was removed from mRNA by treatment with DNase I and cDNA synthesized with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Relative abundance of PDE5A mRNA was determined by SYBR Green I assay (Quantitect SYBR Green PCR; Qiagen) using the following primers: PDE5A (GenBank No. NM_153422.1) upper-primer-1493 5′-TGAGCAGTTCTCTGGAGAAGCCT-3′, lower-primer-1596 5′-ATGCACCACATCTGTGGC-3′, product 104 bp; GAPDH (NM_008084.1) upper-primer-263 5′-ACCATCTTCAGGAGCGAGAC-3′, lower-primer-363 5′-GCCTTCTCATGGTGTTGAA-3′, product 101 bp; with a GeneAmp 5700 Sequence Detection System (Applied Biosystems). PCR samples were run in triplicate and GAPDH content used to normalize PDE5A content of different samples. Reactions (20 μL) were performed with 300 nmol/L of the specific primer pairs for 40 cycles of amplification (denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s). Amplification specificity of PCR products was confirmed by melting curve analysis.24 Subsequent to the final PCR cycle, reactions were heat denatured over a 35°C temperature gradient at 0.03°C/s from 60°C to 95°C. Protein lysates from whole myocardium and isolated cardiac myocytes were extracted in lysis buffer (No. 9803; Cell Signaling) with miniprotease inhibitor (No. 1 to 836-153; Roche) and 5% Triton (Sigma). After12 000g centrifugation 30 minutes, protein was quantified (No. 23235; Pierce), NuPAGE lithium dodecyl sulfate sample buffer added (No. 161 to 0737; Bio-Rad), and lysates electrophoresed on NuPAGE 4% to 12% Bis-Tris polyacrylamide gels (Invitrogen). Membranes were incubated with rabbit polyclonal antibodies raised against purified bovine lung PDE5A (1:5000; Cell Signaling), the amino terminal PDE5A domain (1:5000; gift from Chelski, Cell Signaling) and imaged 18 to 24 hours after transfection as described.27 Images (50- to 80-μm exposure) were acquired every 10 seconds using custom software and processed by ImageJ (NIH). Fluorescence resonance energy transfer (FRET) was the change in 480 nm/545 nm emission intensities (ΔR) on 430 nm excitation28 expressed as percentage change more than the basal

Baseline Hemodynamic Effects of PDE5A Inhibition by SIL in NTG Mice and NOS3−/−

<table>
<thead>
<tr>
<th></th>
<th>NTG (Baseline)</th>
<th>SIL (Baseline)</th>
<th>P</th>
<th>NTG (Baseline)</th>
<th>SIL (Baseline)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>94 ± 5</td>
<td>86 ± 6</td>
<td>&lt;0.005</td>
<td>120 ± 2</td>
<td>111.8 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>CO (mL/min)</td>
<td>10.6 ± 3</td>
<td>11.1 ± 2.3</td>
<td>NS</td>
<td>9.8 ± 1.2</td>
<td>10.4 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>EF (%)</td>
<td>62 ± 12</td>
<td>64 ± 7</td>
<td>NS</td>
<td>54 ± 10</td>
<td>59 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>dp/dt max (mg Hg/s)</td>
<td>11 885 ± 2758</td>
<td>11 604 ± 2387</td>
<td>NS</td>
<td>10 107 ± 1338</td>
<td>11 211 ± 1498</td>
<td>NS</td>
</tr>
<tr>
<td>dp/dt (sec⁻¹)</td>
<td>201 ± 53</td>
<td>227 ± 56</td>
<td>NS</td>
<td>125 ± 21</td>
<td>143 ± 23</td>
<td>NS</td>
</tr>
<tr>
<td>dp/dt (mg Hg/s)</td>
<td>-9623 ± 946</td>
<td>-8405 ± 209</td>
<td>NS</td>
<td>-11 134 ± 1298</td>
<td>-11 618 ± 1418</td>
<td>NS</td>
</tr>
<tr>
<td>PMX/EDV (mg Hg/s)</td>
<td>35.3 ± 6.1</td>
<td>33.6 ± 8.3</td>
<td>NS</td>
<td>29.2 ± 4.1</td>
<td>31.8 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>Tau (ms)</td>
<td>3.9 ± 0.5</td>
<td>3.4 ± 0.8</td>
<td>NS</td>
<td>5.0 ± 0.8</td>
<td>4.6 ± 0.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; CO, cardiac output; EF, ejection fraction; IP, instantaneous pressure at time of dp/dtmax; PMX/EDV, maximal ventricular power normalized to end-diastolic volume; Tau, time constant of pressure relaxation.
intensity (R). Cells were bathed in HEPES-buffered Ringer’s modified saline (1 mmol/L CaCl2) at room temperature (20°C to 22°C). For NOS inhibition studies, cells were preincubated with 1 mmol/L N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) for 30 minutes at 37°C.

### PDE5A and PKG-1 Activity Analysis

Total low Km cGMP phosphodiesterase activity was assayed at 1 μmol/L substrate by fluorescence polarization (Molecular Devices) under linear conditions or a 2-step radiolabeled method,\textsuperscript{18} with or without added SIL (0.1 to 1 μmol/L), tadalafil (50 nmol/L), or isobutyl-methylxanthine (IBMX; 50 μmol/L). PDE assays at 1 μmol/L cGMP detected several high-affinity cGMP-PDEs (PDE5A and PDE9A) and dual-specificity PDEs (eg, PDE1C, PDE3A, PDE10A, and PDE11A).

PKG-1 activity was assayed by colorimetric analysis (CycLex) performed in whole myocytes incubated with or without added ISO (10 nmol/L), SIL (1 μmol/L), tadalafil (50 nmol/L), or sGC inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoline-1-one (3 μmol/L; Sigma). After 10 minutes, cells were lysed and PKG-1 activity determined.

### Immunofluorescent Histology

WT and NOS3\textsuperscript{−/−} cardiomyocytes were fixed in 50% methanol/50% acetone and incubated overnight with sequence-specific PDE5A antibody (gift from K. Omori, Tanabe Seiyaku Co, Saitama, Japan) at 1:5000 dilution and either mouse monoclonal \alpha-actinin (1:500 dilution; Chemicon) or NOS3 (1:3000; Transduction Laboratories). Secondary incubation used anti-rabbit Alexa 488 and anti-mouse Alexa 546 (1 hour; 27°C; Molecular Probes). Cells were imaged on a Zeiss inverted epifluorescence microscope with argon-krypton laser confocal scanning system (Ultra VIEW; PerkinElmer Life Sciences).

### In Vivo Adenoviral Gene Transfer

In vivo intact heart adenovirus gene transfer of NOS3 to NOS3\textsuperscript{−/−} mice was performed as described.\textsuperscript{29} Hearts were exposed by limited thora-cootomy, animals cooled to 18°C to 20°C, the distal thoracic aorta clamped, and recombinant adenovirus vector (30 mL) containing a cytomegalovirus promoter coupled to either a marker gene (β-galactosidase) or NOS3 (10\textsuperscript{9} particles) injected into the left ventricular (LV) cavity. The aorta was clamped for 9 to 10 minutes, then released, the chest closed, and animals rewarmed. Hemodynamic studies were performed 3 to 5 days later (peak adenoviral gene expression).

### Results

**Minimal Effect of SIL (PDE5A inhibition) on Basal Function**

SIL had minimal impact on basal cardiovascular function in both and NOS3\textsuperscript{−/−} animals (Table). A <10% decline in

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Figure 1. A, PDE5A inhibition (SIL) blunts ISO-enhanced sarcomere shortening without changing the calcium transient in NTG myocytes but has no effect in NOS3\textsuperscript{−/−} cells (example tracings on left, summary data on right; n=12 from 4 hearts; *P<0.01). B, Pretreatment of myocytes with ODQ prevents antiadrenergic effect of PDE5A inhibition (EMD-368527/5) on ISO response (n=10; example shown). C, LV PV loops for baseline and ISO stimulation with or without concurrent PDE5A inhibition (SIL). ISO-enhanced contractility was blocked by SIL in NTG but not NOS3\textsuperscript{−/−} hearts. D, Summary data for maximal rate of pressure rise (dP/dt\textsubscript{max}) in NTG but not NOS3\textsuperscript{−/−}. The bottom panel shows percentage change in ISO response by SIL. SV indicates stroke volume; SW, stroke work; PMXI, maximal power index. *P<0.001 vs control groups.
systolic pressure was observed in NTG animals (similar to humans\(^3\)), but this did not occur in NOS3\(^{-/-}\) mice. Treatment of myocytes with SIL alone slightly enhanced basal shortening (12\(\%\); \(P<0.04\)) but did not alter calcium transients.

**Blunting of \(\beta\)-Adrenergic Cardiac Stimulation by PDE5A Inhibition Requires NOS3**

In contrast to rest function, ISO-stimulated contractility was blunted by PDE5A inhibition in isolated myocytes and intact hearts, but this required the presence of active NOS3. In NTG myocytes, ISO increased sarcomere shortening (>200\%), and this declined by more than half with coexposure to SIL (Figure 1A). There was no accompanying change in the calcium transient supporting a myofilament Ca\(^{2+}\) densitization mechanism. In contrast, ISO-stimulated shortening in NOS3\(^{-/-}\) myocytes was unaltered by SIL. Because NOS3-derived NO modifies cell function via sGC/cGMP signaling,\(^9\) we next tested the effect of inhibiting sGC activity by ODQ\(^1\) (20 mmol/L) in NTG myocytes (Figure 1B). ODQ prevented the decline in ISO response from PDE5A inhibition.

Figure 1C and 1D shows results from in vivo heart studies. In NTG animals, ISO stimulation shifted the LV PV loop leftward, increasing its width and area consistent with greater contractility (Figure 1C, top left). Cotreatment with SIL suppressed this response (Figure 1C, top right). In contrast, NOS3\(^{-/-}\) hearts showed a similar rise in contractility from ISO before and after SIL infusion (Figure 1C, bottom panels). Figure 1D provides summary data for LV maximal rate of pressure increase (dP/dt\(_{max}\)) and other ejection-phase measures of systolic function.

**NOS3 Is Required for PDE5A Inhibition to Stimulate PKG-1 and cGMP**

We next tested whether PDE5A inhibition stimulated PKG-1 in adult myocytes and whether this was lacking in NOS3\(^{-/-}\) cells (Figure 2A). PDE5A inhibition slightly enhanced PKG-1 activity under basal conditions (\(\approx 10\%\); \(P<0.05\)). ISO also increased PKG-1 activity, but both combined raised this activity \(\approx 70\%\). However, these interventions did not change PKG-1 activity in cells lacking NOS3. Coincubation of NTG cells with ODQ also prevented PKG-1 activation by ISO or ISO+SIL, further supporting a central requirement of cGMP derived by NOS-stimulated sGC for SIL modulation.

To directly monitor intracellular cGMP production, FRET analysis was performed in normal neonatal rat myocytes (Figure 2B and 2C). ISO, SIL, and the NO donor diethylamine NONOate/NO all enhanced the FRET signal, providing the first direct demonstration that PDE5A inhibition enhances cGMP in myocytes. However, pretreatment of myocytes with the NO inhibitor L-NAME blocked the cGMP rise by SIL, but not with the other stimuli.
Lack of NOS3 Does Not Alter PDE5A Expression or In Vitro Activity

PDE5A mRNA expression was 100-fold lower in whole heart than in lung and even lower in isolated myocytes. However, expression levels were similar in NTG and NOS3⁻/⁻ tissues (Figure 3A). Protein expression was also similar between genotypes in whole heart (Figure 3B) and myocytes (Figure 3C), with a prominent band observed at ≈95 kDa that matched that in lung. A second ≈70-kDa band was consistently observed in heart tissue that either reflected a splice variant or proteolytic fragment. Similar findings were obtained with alternative antibodies (data not shown). In vitro PDE5A activity (~30% of...
total cGMP–esterase activity) was also similar between genotypes (Figure 3D). Coincubation with IBMX (positive control) lowered activity by \( \approx 90\% \). Similar results for PDE5A-dependent cGMP–esterase activity were obtained by radio enzyme assay18 (32±7.3% NTG \( [n=9] \); 37.9±8.2% NOS3\(^{-/-} \) \([n=8]\); \( P=NS \)).

Lack of NOS3 or Chronic NOS Activity Alters Myocyte Localization of PDE5A

Given similar gene/protein expression and in vitro activity, we next tested whether PDE5A cellular localization was altered by the lack of NOS3. In NTG cells, PDE5A was present throughout the cell but also localized to z-band striations (Figure 4A, green PDE5A; red α-actinin). PDE5A Immunostaining was inhibited by specific blocking peptide (4B, left), whereas this same peptide did not block PDE1C staining (4C, left), supporting assay specificity. PDE5A displayed colocalization with NOS3 (4D through 4F) at z-band striations. However, in NOS3\(^{-/-} \) cells, PDE5A distribution was diffuse (Figure 4G) with less localization to z-bands (Figure 4H, α-actinin; Figure 4I, colocalization).

To test whether altered PDE5A localization resulted from the absence of NOS3 protein or NOS activity, L-NAME (1 mg/mL in drinking water or 50 mg/kg IP for acute) was administered to NTG mice for varying periods and myocytes then isolated and stained for PDE5A and α-actinin. With subacute NOS inhibition, PDE5A remained localized to z-bands, whereas it shifted away from these structures (diffuse) with sustained L-NAME exposure (Figure 5A). Z-bands remained present (Figure 5B). Thus, chronic NOS activity was the key requirement for maintaining normal myocyte PDE5A localization.

Because 1 to 2 hours of L-NAME exposure did not alter PDE5A z-band localization but inhibited NOS activity, this protocol was used to define the role each played in modulating the efficacy of PDE5A inhibition (Figure 5C). As in NOS3\(^{-/-} \) hearts, acute NOS inhibition eliminated the antiadrenergic effect of SIL. This was partially rescued by coadministration of an exogenous NO donor (DEA/NO; 4 μg/kg per minute) supporting NO-generated cGMP as central to PDE5A-catabolic regulation. However, DEA/NO did not restore SIL efficacy in NOS3\(^{-/-} \) mice. Thus, both NOS activity and PDE5A z-band localization were required for adrenergic modulation by PDE5A inhibitors.

Re-Expression of NOS3 in NOS3-Null Heart Restores PDE5A Localization and Function

To further test the importance of chronic NOS3 expression and activity on PDE5A cardiac regulation, we re-expressed...
NOS3 in NOS3-null hearts by means of in vivo whole heart adenoviral gene transfer. NOS3 was primarily expressed in myocytes by gene transfer. D, Western blot for NOS3 in control (WT), NOS3−/− transfected with β-galactosidase (Adβgal), and NOS3−/− transfected with NOS3 (AdNOS3). Bottom panel shows immunoprecipitate to NOS3 probed for caveolin-3 (cav3). E, NOS activity assessed by arginine–citrulline conversion was restored to NTG levels in NOS3−/− + AdNOS3. F through H, Confocal images of PDE5A (F, green), NOS3 (G, red), and colocalization of both proteins (H, yellow) in myocyte from NOS3−/− heart transfected with AdNOS3. NOS3 restoration led to PDE5A being relocalized to z-bands and colocalizing with NOS3. I, Restored effectiveness of PDE5A-I (SIL) for blunting the contractile (dp/dtmax) response to ISO.

Discussion

This study reveals a strong interdependence between PDE5A-mediated cGMP catabolism and cGMP synthesis by NOS3 in regulating adrenergic-stimulated cardiac contractility. First, NOS3 and consequent NOcG activity would appear to provide the critical substrate targeted to catabolism by PDE5A. Second, chronic NOS activity is required for PDE5A to be properly localized to z-band regions near adrenergic receptor-complex signaling proteins. Loss of either component was sufficient to inhibit antiadrenergic effects of PDE5A inhibitors, and chronic lack of NOS activity combined both changes. These data provide the most comprehensive analysis of PDE5A physiology in cardiac myocytes and the intact heart to date and reveal a novel mechanism to explain variable responses to PDE5A inhibitors in patients with impaired NO synthesis.

NO3,4,9 and β-adrenergic stimulation6,7 enhance cGMP, and thereby PKG-I activity, and this was confirmed in the
current study (Figure 2). However, these are not the sole stimuli because natriuretic peptides acting through rGC also increase cGMP to activate PKG-1. In the NOS3 heart, this pathway may even be somewhat upregulated to help maintain basal myocardial cGMP and PKG-1 activity (Figure 2A). Yet we found that lack of NOS3 was sufficient to prevent PKG-1 activation and antiadrenergic effects of PDE5A inhibitors. Similar findings with acute NOS or sGC inhibition further support a specific interaction between NOS-derived cGMP and PDE5A catabolism.

The antiadrenergic effect of PDE5A inhibition likely resulted from PKG-1 activation, which depresses myofilament calcium sensitivity by enhancing troponin I phosphorylation.\(^3,4\) This was indicated by the lack of Ca\(^{2+}\)-transient change despite blunted sarcomere shortening when SIL was added to ISO. cGMP also activates dual-sensitivity PDEs that lower adrenergic stimulated cAMP,\(^34\) but one would expect a change in calcium transient by this mechanism. As has been shown for NOS-induced cGMP synthesis,\(^8,9,33,35\) effects of PDE5A inhibition on contraction were negligible at rest but increased during \(\beta\)-adrenergic costimulation. This likely stems from increased cGMP synthesis and PKG-1 activation because of calcium-activated NOS,\(^6\) \(\beta_3\)-G\(_{\text{c}}\)–coupled signaling,\(^7\) and cAMP inhibition of cGMP-PDEs.\(^36\) The FRET analysis found cGMP increased similarly with ISO with or without L-NAME, although PKG-1 activation with ISO (±SIL) was absent in myocytes lacking NOS3 and those with sGC inhibited by ODQ. The latter suggests coupling of NOS activity to adrenergic stimulation was important.

NOS3 activity and cardiomyocyte PDE5A localization were altered in NOS3 hearts and NTG hearts exposed to chronic NOS inhibition, and either mechanism was sufficient to explain loss of a SIL response. When NOS was acutely inhibited, PDE5A remained localized to z-bands, yet the antiadrenergic effect of SIL was lost. NO infusion partially restored a SIL response in NTG hearts yet had no impact in NOS3 hearts because the latter still had PDE5A shifted away from the z-bands. Figure 7 summarizes our proposed model. After activation of the \(\beta\)-adrenergic receptor, cAMP and cGMP are stimulated, the latter principally because of activation of NOS3 and its target sGC. The effect of this cGMP on PKG-1 activation is contained within a strategic compartment by the localization of PDE5A, which targets the cGMP–PKG activity to a region strategically linked to adrenergic regulation. Acute PDE5A inhibition enhances localized cGMP and PKG activity to blunt adrenergic stimulation. Acute NOS3 blockade by L-NAME removes the required cGMP substrate and thus efficacy of PDE5A inhibition, but exogenous NO can still restore efficacy. With genetic loss of NOS3 or with chronic NOS inhibition (right), PDE5A moves away from z-bands (left). This removes its physiologic regulation of acute adrenergic signaling, and exogenous NO can no longer restore the efficacy of PDE5A inhibition.

Figure 7. Model of NOS3-PDE5A coregulation of \(\beta\)-adrenergic stimulated contractility in the heart. In a wild-type (WT) normal myocyte (left), \(\beta\)-adrenergic stimulation triggers adenylate–cyclase (AC)-coupled activation of cAMP and NOS3/sGC generation of cGMP. cAMP enhances intracellular calcium and activates PKA to enhance contractility. cGMP signaling is compartmentalized by regional placement of PDE5A, which targets the cGMP–PKG activity to a region strategically linked to adrenergic regulation. Acute PDE5A inhibition enhances localized cGMP and PKG activity to blunt adrenergic stimulation. Acute NOS3 blockade by L-NAME removes the required cGMP substrate and thus efficacy of PDE5A inhibition, but exogenous NO can still restore efficacy. With genetic loss of NOS3 or with chronic NOS inhibition (right), PDE5A moves away from z-bands (left). This removes its physiologic regulation of acute adrenergic signaling, and exogenous NO can no longer restore the efficacy of PDE5A inhibition.
inhibition were enhanced. The data also suggest that NOS3 or its product could play a direct role in PDE5A localization, helping PDE5A interact with a scaffold protein that anchors it to z-bands. A-Kinase anchoring proteins perform such a role by targeting PKA to specific kinases, PDEs, and other signaling proteins to localize signaling.10–22 Corresponding cGMP-dependent protein kinase anchoring proteins remain poorly defined, but such proteins might play an analogous role in localizing PDE5A activity. Intriguingly, a similar loss of PDE5A from z-band regions has been observed in canine hearts with dilated cardiomyopathy, suggesting that altered localization and accompanying physiologic regulation may contribute to cardiac disease.

Previous studies have reported low levels of PDE5A expression in the myocardium43,44 and minimal effects of PDE5A inhibition on resting heart function.16–18,30 This has led to the conclusion that PDE5A plays little role in the heart. The current results support this under basal conditions, but they reveal a very different situation during β-stimulation. Importantly, having a low expression level does not imply a lack of physiologic impact, particularly if strategic intracellular placement can confer targeted signaling effects. The current results are supported by previous in vivo canine heart data,18 and testing in humans would seem warranted.

Increasing cGMP in the heart by natriuretic peptide–coupled synthesis can counter stress-response remodeling/cardiac hypertrophy.11,12 The current data raise the possibility that a similar benefit may be obtained by manipulating cGMP catabolic pathways. They further imply that therapeutic efficacy of a PDE5A inhibitor depends on NOS3 expression and activity, and this could underlie hyporesponsiveness in many patients receiving this treatment. As the use of PDE5A inhibitors expands to diseases such as pulmonary hypertension, these considerations will take on more clinical significance. Strategies to upregulate NOS3 activity may prove a valuable approach to augment the influence of PDE5A and its inhibitors.

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