Sustained Soluble Guanylate Cyclase Stimulation Offsets Nitric-Oxide Synthase Inhibition to Restore Acute Cardiac Modulation by Sildenafil

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ABSTRACT

Phosphodiesterase type 5 (PDE5) inhibitors are used to treat erectile dysfunction, and growing evidence supports potential cardiovascular utility. Their efficacy declines with reduced nitric-oxide synthase (NOS) activity common to various diseases. We tested whether direct soluble guanylate cyclase (sGC) stimulation restores in vivo cardiovascular modulation by PDE5 inhibition despite acute or chronically suppressed NOS activity. Mice (C57/B16; n = 62) were studied by in vivo pressure-volume analysis to assess acute modulation by the PDE5 inhibitor sildenafil (SIL; 100 μg/kg/min) of the cardiac response to isoproterenol (ISO) with or without NOS inhibition [N\textsuperscript{ω-}nitro-L-arginine methyl ester (L-NAME)] and cotreatment by the sGC stimulator BAY 41-8543. ISO-induced mild vasodilation but no basal cardiac effects and markedly blunted ISO-stimulated contractility. Acute BAY 41-8543 at a dose lacking cardiovascular effects did not alter ISO responses. However, after acute L-NAME, SIL ceased to influence cardiovascular function, but adding BAY 41-8543 fully restored SIL effects. After 1 week of L-NAME, neither SIL nor SIL + BAY 41-8543 acutely induced vasodilation or blunted ISO responses. However, sustained BAY 41-8543 despite concurrent NOS inhibition restored the cardiovascular efficacy of SIL. The disparity between acute and chronic NOS inhibition related to diffusion of PDE5 away from myocyte z-bands coupled with reduced protein kinase G activation. Both were restored by sustained sGC stimulation. Thus, PDE5 regulation of adrenergic reserve and systemic vasodilation depends upon NOS-induced cGMP/protein kinase G and can be enhanced by sustained low-level stimulation of sGC. This may prove beneficial for enhancing the efficacy of PDE5 inhibitors in conditions with chronically reduced NOS activity.

Phosphodiesterase type 5 (PDE5) is a cGMP-selective phosphodiesterase whose inhibition by pharmacological agents such as sildenafil is widely used to treat erectile dysfunction (Ghofrani et al., 2006). Although the cardiovascular effects of PDE5 inhibitors were first thought to be minor (Jackson et al., 2006), many studies have revealed their potential utility for a variety of conditions (for review, see Kass et al., 2007a), including pulmonary hypertension (Galiè et al., 2005), endothelial dysfunction (Gori et al., 2005; Guazzi et al., 2007b), suppression of ischemia/reperfusion injury (Kukreja et al., 2005), exertional intolerance in heart failure patients (Guazzi et al., 2007a; Lewis et al., 2007), and reduction of hypertrophy and cardiac dysfunction from sustained pressure overload (Takimoto et al., 2005b). Acute cardiac modulation has also been reported, with PDE5 inhibition (PDE5-I) blunting β-adrenergic-stimulated contractility in several species (Senzaki et al., 2001; Takimoto et al., 2005a), including humans (Borlaug et al., 2005).

The mechanism for these effects is generally thought to involve suppression of cGMP hydrolysis, thereby activating protein kinase G (PKG). This requires sufficient basal cyclase activity and may explain why PDE5-I is more influential under stress than rest conditions. Studies have further found that cGMP generated by nitric-oxide synthase (NOS) is par-
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particularly important for PDE5 cardiac (Takimoto et al., 2005a; Castro et al., 2006) and vascular (Piggott et al., 2006) regulation. Syndromes where NOS activity is compromised, such as diabetes and heart failure, blunt the efficacy of PDE5-I for inducing vasodilation (Bivalacqua et al., 2004) and altering cardiac function (Senzaki et al., 2001). In the heart, this interaction is further dictated by PDE5 myocyte localization, being normally concentrated at the z-band but becoming more diffuse if NOS is genetically absent or chronically inhibited (Takimoto et al., 2005a,b; Kass et al., 2007b). In such settings, PDE5-I has little impact on acute β-stimulation and acute exogenous NO cannot restore this (Takimoto et al., 2005a). Although chronic NO donors might be effective, these have been considered counterintended, being limited by both hypotension and tachyphylaxis.

Another approach is to directly stimulate soluble guanylate cyclase (sGC), a key downstream target of NO. A new class of drugs has been recently developed to achieve this (Evgenov et al., 2006; Stasch et al., 2006; Boerrigter et al., 2007). These agents induce dose-dependent vasodilation (Stasch et al., 2002a,b) by NO-independent sGC activation and an effect that is synergistic with NO-sGC stimulation (Priviero et al., 2005; Dumitrascu et al., 2006). This suggests one might be able to administer a nondilating dose of an sGC stimulator with minimal basal effects that could potentiate the efficacy of PDE5-I even when NOS activity was blunted. This might also reverse dislocated PDE5 and, thus, its cardiac regulation. Understanding this physiology has taken on increased relevance with the planned initiation of a clinical National Institutes of Health-sponsored trial of sildenafil for the treatment of heart failure with a preserved ejection fraction (RELAX trial). The present study tested these hypotheses, employing the sGC stimulator BAY 41-8543 (Stasch et al., 2002a,b). We examined interactions of BAY 41-8543 and sildenafil (sildenafil) on rest and -adrenergic-stimulated cardiac function in intact adult mice with and without acute or chronic NOS inhibition. The results confirm a synergy between nonhypotensive acute sGC stimulation and PDE5-I for restoring cardiovascular effects of PDE5-I after acute NOS inhibition and chronic (but not acute) efficacy of sGC stimulation when NOS suppression is more sustained.

Materials and Methods

Pharmaceuticals and Reagents. Sildenafil citrate (SIL; gift from Pfizer Central Research, Sandwich, Kent, UK), isoproterenol (Sigma-Aldrich, St. Louis, MO), BAY 41-8543 (Bayer AG, Wuppertal, Germany), and N-nitro-l-arginine methyl ester (l-NAME; Sigma-Aldrich) were used in this study.

In Vivo Studies. Male adult mice (n = 62; C57/B16, 8 weeks; The Jackson Laboratory, Bar Harbor, ME) were anesthetized, underwent thoracotomy, and instrumented for pressure-volume analysis (SPR-839 PV; Millar Instruments Inc., Houston, TX) as described previously (Georgakopoulos and Kass, 2001; Takimoto et al., 2005a). Isoproterenol (ISO; 20 ng/kg/min) with or without SIL (100 μg/kg/min) or BAY 41-8543 (5 μg/kg/min) was infused into a central jugular vein. The SIL dose yields a 37 ± 5.2 nM free plasma concentration (Takimoto et al., 2005a) similar to peak levels in humans after a therapeutic 100-mg dose (Borlaug et al., 2005). The BAY 41-8543 dose was selected as having negligible effects on resting hemodynamics based on an initial dose-response curve (Supplemental Table 1).

Heart function was assessed by pressure-volume (PV) loops at a fixed heart rate (550/min) or BAY 41-8543 (5 μg/kg/min) or ISO (100 μg/kg/min) was infused into a central jugular vein. The SIL dose yields a 37 ± 5.2 nM free plasma concentration (Takimoto et al., 2005a) similar to peak levels in humans after a therapeutic 100-mg dose (Borlaug et al., 2005). The BAY 41-8543 dose was selected as having negligible effects on resting hemodynamics based on an initial dose-response curve (Supplemental Table 1).

Heart function was assessed by pressure-volume (PV) loops at a fixed heart rate (550/min) or atrial pacing. The acute protocol consisted of assessing PV data at baseline, after ISO stimulation, re-baseline, drug intervention (either SIL, BAY 41-8543, or both), and then drug intervention with a second ISO exposure. Previous studies have confirmed high reproducibility of repeated ISO infusion studies alone (Takimoto et al., 2005a). In separate studies, animals were pretreated with the NOS inhibitor l-NAME (50 mg/kg i.p.) and 30 min later underwent the same pharmacological protocol. Other animals underwent chronic NOS inhibition (l-NAME, 1 mg/ml in drinking water × 1 week; 4–6 ml/day). This dose reverses the cardiovascular phenotype of mice overexpressing endothelial NOS (Ohashi et al., 1998) and inhibits NOS in WT mice (Ozaki et al., 2002). Mice were then studied acutely as above or studied after a 2nd week. In some cases, either l-NAME discontinued or continued but with BAY 41-8543 (30 mg/kg/day p.o.) added during the 2nd week. Four to eight mice were studied for each protocol.

Analysis of PDE5 Localization. To assay myocyte distribution of PDE5, immunohistochemistry was performed with either commercial antisera (1:250 dilution; Cell Signaling Technology Inc., Danvers, MA) or fluorescent histology employing a transfected fusion protein linking mouse PDE5 with De-Red. This probe was cloned from PDE5 coding sequence cDNA obtained from mouse lung (forward primer, 5'-ACGACCTGCTGATTGTGAGCAGGCCCCC- AACT-3'; reverse primer, 5'-ATATCCTGGCTGATCGTCCG- GTCGCCC-3'), and the polymerase chain reaction (PCR) product was run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe for 40 cycles run in duplicate, and GAPDH content was used to normalize PDE5 content of different samples. Reactions were performed with 900 nM of each specific primer pair and 250 nM of specific probe.
of amplification (denaturation at 95°C for 15 s and annealing/extension at 60°C for 60 s).

**Statistical Analysis.** Data are presented as mean ± S.E.M. Comparisons of ISO response with or without SIL and/or BAY 41-8543, and basal effect of SIL and/or BAY 41-8543 were performed by paired Student’s t test. Between-group comparisons were performed by one-way ANOVA with a Tukey multiple comparisons test. p Values < 0.05 were considered significant. The investigation conforms with guidelines for the care and use of laboratory animals as published by the National Research Council (1996).

**Results**

**Effects of PDE5 Inhibition, sGC Stimulation, and Both on Rest and ISO-Stimulated Function.** Summary hemodynamic data for control (and L-NAME treatment groups) are provided in Table 1, and effects of acute SIL, BAY 41-8543, or both on selected rest parameters are displayed in Fig. 1. In control mice, SIL slightly lowered systolic pressure and reduced arterial vascular resistance; however, cardiac function assessed using pressure-volume relations was not significantly altered (Fig. 1, left bars). End-diastolic volume also did not change significantly (data not shown). BAY 41-8543 alone had little impact on cardiac or vascular function, as expected given the dose chosen, and combining SIL + BAY 41-8543 (BAY) induced vasodilation similar to SIL alone but still did not significantly alter cardiac function (Fig. 1).

Left ventricular responses to ISO stimulation are shown in Fig. 2A. ISO enhanced systolic function reflected by the rise in ejection fraction and peak rate of pressure rise (dP/dtmax) and end-systolic elastance (Ees), and SIL inhibited this response as previously reported (Senzaki et al., 2001; Borlaug et al., 2005; Takimoto et al., 2005a). BAY 41-8543 itself did not alter the ISO response, and the combination again behaved like SIL alone. Summary data for contractility parameters are provided in the lower panels. ISO increased Ees by ~75% in controls and BAY 41-8543-treated hearts, but this was markedly reduced by SIL with or without concurrent sGC stimulation by BAY 41-8543. Similar findings were obtained with dP/dtmax.

**Synergy of cGC Stimulation and PDE5-I in Animals with Acute NOS Inhibition.** We next tested whether a decline in SIL modulation due to acute NOS inhibition was reversible by coadministration of an sGC stimulator. Acute L-NAME (Table 1) increased systemic vascular resistance (p < 0.05) with a borderline increase in peak systolic pressure (p = 0.06). dP/dtmax both declined (p < 0.001). Under this condition (Fig. 1, middle bars), SIL induced less arterial vasodilation, whereas BAY 41-8543 effects remained similar as in controls. Neither pharmaceutical alone significantly altered systolic function. However, their acute combination reversed these hemodynamic effects of acute L-NAME, lowering arterial resistance and systolic pressure and increasing dP/dtmax.

Acute-LNAME also prevented SIL-mediated suppression of ISO-stimulated contractility (Fig. 2B). BAY itself also did not alter this response, but an antiadrenergic effect was recovered when BAY was combined with SIL. Because the combination itself raised contractility (Fig. 1), one could question whether this obviated further changes with ISO. However, acute L-NAME itself increased the ISO contractile response 2-fold over control hearts (probably reflecting release of NO-cGMP negative feedback), and the basal rise in contractility with SIL + BAY was well below this ISO response, leaving substantial reserve that was still blocked by coadministration.

**Influence of Chronic NOS Inhibition.** We next tested whether modulation of cardiovascular function by PDE5 inhibition was similarly observed in mice subjected to chronic NOS inhibition. After 1 week of L-NAME, systolic pressure and resistance remained elevated and dP/dtmax less diminished than with acute L-NAME (p = 0.05) and baseline (p < 0.03; Table 1). LV mass was unchanged (heart weight/tibial length, 71 ± 1.4 in controls, 74 ± 1.5 mg/cm with chronic L-NAME, p = N.S.). In this condition, SIL and, importantly, the combination of SIL + BAY 41-8543 no longer significantly reduced systemic resistance, peak systolic pressure, or altered dP/dtmax (Fig. 1, right bars). ISO-stimulated cardiac function was not inhibited by SIL, BAY, or their combination (Fig. 2C). Both systemic resistance and cardiac ISO modulation responses with the combined treatment significantly differed between chronic L-NAME-treated animals and the other two groups.

To test whether the chronic L-NAME condition was reversible, mice were first administered L-NAME for 1 week, and then studied a week after its discontinuation (washout). In these animals, SIL effects on resting and ISO-stimulated contraction were restored to normal (Fig. 3A).

Although acute BAY 41-8543 administration did not recover PDE5-I regulation in hearts exposed to chronic L-NAME, we hypothesized that sustained sGC stimulation might be more effective. To test this, L-NAME was adminis-

**TABLE 1**

Baseline hemodynamics in control and with acute and chronic L-NAME treatment

p Values are for one-way ANOVA between conditions.

<table>
<thead>
<tr>
<th>Control (n = 17)</th>
<th>L-NAME (n = 17)</th>
<th>Chronic (n = 16)</th>
<th>ANOVA (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV peak-systolic pressure (mm Hg)</td>
<td>91.8 ± 2.0</td>
<td>99.2 ± 2.9*</td>
<td>103.4 ± 2.4*</td>
</tr>
<tr>
<td>SVR (mm Hg/ml/s)</td>
<td>572 ± 40</td>
<td>802 ± 77*</td>
<td>689 ± 47*</td>
</tr>
<tr>
<td>LVEDV (µl)</td>
<td>28.6 ± 2.2</td>
<td>30.3 ± 3.0</td>
<td>33.0 ± 2.4</td>
</tr>
<tr>
<td>SV (µl)</td>
<td>17.2 ± 1.6</td>
<td>13.8 ± 1.1</td>
<td>17.3 ± 0.8</td>
</tr>
<tr>
<td>Ees (mm Hg/µl)</td>
<td>9.1 ± 0.6</td>
<td>8.6 ± 0.7</td>
<td>7.7 ± 0.6</td>
</tr>
<tr>
<td>dP/dt_max (mm Hg/s)</td>
<td>12343 ± 621</td>
<td>8371 ± 515†</td>
<td>10574 ± 587§*</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>65 ± 3</td>
<td>48 ± 3*</td>
<td>54 ± 2*</td>
</tr>
</tbody>
</table>

* Post hoc comparison, p = 0.05 vs. control.
† Post hoc comparison, p = 0.06 vs. control.
‡ Post hoc comparison, p < 0.001 vs. control.
§ Post hoc comparison, p = 0.054 vs. acute L-NAME.
tered for 2 weeks, and BAY 41-8543 was provided concur-
rently (30 mg/kg/day p.o.) during only the 2nd week. Baseline
function was similar to controls (Table 2), but animals now
displayed normal acute SIL suppression of basal vascular
tone and ISO-stimulated cardiac contraction (Fig. 3B). Thus,
chronic but not acute sGC stimulation restored PDE5-I car-
diovascular modulation despite continued NOS inhibition.

Relation of Antiadrenergic Action of SIL to Myocyte
PDE5 Localization and PKG Activation. Prior studies
have shown that PDE5 adrenergic contractile regulation is
associated with changes in the myocyte localization of the protein, with normal positioning at the myocyte z-band being lost in myocytes where NOS is inactivated (Takimoto et al., 2005a). Figure 4A shows this was fully reversible if NOS function was restored after 1-week L-NAME wash-out. This was further tested in myocytes transfected in vivo with a fusion DsRed-PDE5 construct, avoiding the need for anti-
sera. As shown in Fig. 4B, PDE5 localized to z-bands (co-

Fig. 1. Influence of SIL, sGC stimulation (BAY 41-8543; BAY), or both on resting systemic vascular resistance (SVR), peak systolic blood pressure (SBP), and peak ven-

tricular dP/dt (dP/dt_max). Results are shown for three groups: control, acute L-NAME (A-LNM), and chronic L-NAME (C-LNM) and are displayed as percent change

from baseline. The absolute baseline values for these and other parameters are provided in Table 1. p Values shown beneath each set of bars are for one-way ANOVA (within

group) for drug effects (SIL, BAY, or their combination). Symbols below individual bars show post hoc (Tukey) test for between-treatment comparisons: ‡, p < 0.004 versus combined, p = 0.08 versus SIL; ¶, p < 0.005 versus BAY and SIL; and §, p < 0.05 versus combined. Symbols shown

above the bars identify comparisons between groups (e.g.,

control, acute, or chronic L-NAME) for a given treatment: *

p < 0.05 versus control; †, p < 0.002 versus other two groups.

Fig. 2. Left ventricular PV loops for baseline and ISO stimulation under control conditions and in mice coadministered SIL, BAY, or both. Top panels show pressure-volume loops and corresponding end-systolic pressure-volume relations (ESPVRs). Bottom graphs display summary data (n = 5–6 for

each) for absolute change in Ees (slope of ESPVR) and peak rate of pressure rise, dP/dt_max. Data are shown for control mice (A), mice pretreated acutely

with the NOS inhibitor, L-NAME (B), and mice pretreated with L-NAME for 1 week (C). See text for details. *, p < 0.05 versus the corresponding control

response (without drug).
whereas it was restored by either L-NAME washout or chronic L-NAME with concomitant BAY 41-8543 treatment. We further examined in vivo, became diffuse with NOS inhibition, and relocalized with activation of PKG. Gene and/or protein expression of PDE5 did not seem to contribute to the different responses (Fig. 5, C and D).

Discussion

The physiologic effectiveness of PDE5 inhibition depends upon sufficient generation of cGMP that can theoretically be derived from natriuretic peptide or NOS stimulation pathways. Here, we show that the regulation of arterial vasodilatory and cardiac β-adrenergic reserve by PDE5-I targets cGMP from soluble GC stimulation. The cGMP generated by this pathway was also shown to be important to a normal myocyte localization of PDE5 and to PKG activation induced by PDE5-I. Bypassing NOS and acutely stimulating sGC restored PDE5-I arterial and cardiac modulation when NOS was acutely but not chronically inhibited, the latter being associated with loss of normal myocyte PDE5 localization. However, sustained sGC stimulation was effective in offsetting the impact of chronic NOS inhibition. Given the expanding use of PDE5-I use for diseases in which NOS activity is chronically depressed, these findings suggest a therapeutic approach that may enhance their efficacy.

**PDE5 Regulation of Cardiovascular Function.** PDE5 is expressed in vascular smooth muscle and endothelium, with high levels in the corpus cavernosum and pulmonary...
vessels. Its cardiac expression is approximately 100-fold less than in lung (Takimoto et al., 2005a), and for many years, it was not thought to be an important role in the heart or cardiac disease. However, recent studies indicate involvement in a variety of myocardial stress responses (Salloum et al., 2003; Kukreja et al., 2005; Takimoto et al., 2005b) and acute β-adrenergic stimulation (Senzaki et al., 2001; Borlaug et al., 2005; Takimoto et al., 2005a). Basal cyclase activity and, thus, cGMP is low in normal resting heart, and PDE5-I has little impact under these conditions. Although two pathways can generate cGMP in vascular smooth muscle and cardiac myocytes, recent data suggest they are not interchangeable (Castro et al., 2006; Fischmeister et al., 2006; Takimoto et al., 2007b) and that NOS-NO stimulation is more selectively targeted by PDE5, at least in normal cells. The current findings showing that sGC stimulation rescues SIL-modulation despite acute NOS inhibition stand in marked contrast to a recent study where we showed ANP costimulation was totally ineffective in this regard (Takimoto et al., 2007b).

Reduction of NOS activity, by genetic knockout, pharmacological inhibition, or diseases such as diabetes or heart disease, subsequent lack of cGMP may lead to a loss of cyclic GMP (cGMP) production.
failure, suppresses the vasoactivity and cardiac modulation of PDE5 inhibitors (Zhao et al., 2001; Bivalacqua et al., 2004; Champion et al., 2005). In the vasculature, reduced NOS activity manifests as a diminished erectile (Bivalacqua et al., 2004) or pulmonary vasodilatator (Zhao et al., 2001) response, whereas in the heart, it blunts the negative modulation of β-adrenergic stimulation (Senzaki et al., 2001; Takimoto et al., 2005a) and, in preliminary studies, an ability to suppress pressure overload hypertrophy (Takimoto and Kass, 2005). Between 30 and 40% of patients with erectile dysfunction display little response to PDE5-I (Burnett, 2006), and although similar data for pulmonary hypertension or potential heart indications have yet to be reported, it seems probable that insufficient cGMP generation will apply to these syndromes as well. Thus, developing a method to circumvent this limitation is important.

The new drug class of sGC stimulators presents a potential solution. First developed in the mid-1990s (Evgenov et al., 2006), agents such as YC-1 (an indazole derivative) were found to be heme-dependent NO-independent sGC stimulators (Ko et al., 1994). BAY 41-8543, used in the current study, is based on the YC-1 structure, but is 2 orders of magnitude more potent (Stasch et al., 2002a,b). Its activity requires the sGC heme to be in its normally reduced form, and its effectiveness in the experiments reported here suggests this was indeed the form that was present. These drugs act to enhance NO-sGC stimulation, and because L-NAME does not fully inhibit NOS activity, their capacity to offset reduced but not absent NO probably played a role. However, this should apply similarly to acute and chronic NOS inhibition, yet for the latter, acute BAY 41-8543 + SIL was less effective because other mechanisms such as altered PDE5 localization/regulation applied. Another factor that may play a role in chronic hypertension or hypertrophy is sGC oxidation, which renders the enzyme less responsive to NO. In such cases, BAY 41-8543 would have less impact (Evgenov et al., 2006), whereas BAY 58-2667, which activates heme-oxidized or heme-free sGC, might be more effective (Evgenov et al., 2006; Stasch et al., 2006).

**Contrasting Effects of Acute and Chronic NOS Inhibition.** Chronic NOS inhibition led to a change in PDE5 distribution from z-bands, whereas this does not occur with acute inhibition (Takimoto et al., 2005a), and this localization seems to be important for PDE5-I to modulate adrenergic stimulation. The current study extends prior work by showing reversible localization changes, with concordance of results relying upon or independent of antisera binding specificity. Prior work identified NOS activity as central to this behavior (Takimoto et al., 2005a), and the current results further clarify cGMP synthesis rather than NO per se to be the primary signal. cGMP can bind to regulatory GAF domains in the N terminus of the PDE to stimulate hydrolytic activity, and PKG phosphorylation at S92 further activates the enzyme (Corbin et al., 2000; Smith et al., 2000; Zoraghi et al., 2005). It is intriguing to note that PKG can bind to troponin T via a leucine-zipper motif, and this has been proposed to be required for PKG phosphorylation of troponin I and subsequent desensitization of the myofilament to calcium (Yuasa et al., 1999). Loss of PDE5 localization at this site could then prevent its action of sarcomeric cGMP/PKG levels and, thus, contractile function, even if acutely fed the correct substrate.
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