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Regulation of Sarcoplasmic Reticulum Ca\(^{2+}\) ATPase 2 (SERCA2) Activity by Phosphodiesterase 3A (PDE3A) in Human Myocardium

PHOSPHORYLATION-DEPENDENT INTERACTION OF PDE3A1 WITH SERCA2*

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Background: PDE3A is part of a SERCA2 signaling complex in cardiac myocytes.

Results: PDE3, not PDE4, regulates the activation of SERCA2 by PKA in human myocardium; phosphorylation of PDE3A1 at Ser-292/Ser-293 promotes its integration into the SERCA2 signaling complex.

Conclusion: PDE3A1 regulates cAMP-mediated control of SERCA2 through its phosphorylation-dependent interaction with SERCA2.

Significance: Targeted disruption of the PDE3A1-SERCA2 interaction may provide a new therapeutic approach for heart failure.

Cyclic nucleotide phosphodiesterase 3A (PDE3) regulates cAMP-mediated signaling in the heart, and PDE3 inhibitors augment contractility in patients with heart failure. Studies in mice showed that PDE3A, not PDE3B, is the subfamily responsible for these inotropic effects and that murine PDE3A1 associated with sarcoplasmic reticulum Ca\(^{2+}\) ATPase 2 (SERCA2), phospholamban (PLB), and AKAP18 in a multiprotein signalosome in human sarcoplasmic reticulum (SR). Immunohistochemical staining demonstrated that PDE3A co-localizes in Z-bands of human cardiac myocytes with desmin, SERCA2, PLB, and AKAP18. In human SR fractions, cAMP increased PLB phosphorylation and SERCA2 activity; this was potentiated by PDE3 inhibition but not by PDE4 inhibition. During gel filtration chromatography of solubilized SR membranes, PDE3 activity was recovered in distinct high molecular weight (HMW) and low molecular weight (LMW) peaks. HMW peaks contained PDE3A1 and PDE3A2, whereas LMW peaks contained PDE3A1, PDE3A2, and PDE3A3. Western blotting showed that endogenous HMW PDE3A1 was the principal PKA-phosphorylated isoform. Phosphorylation of endogenous PDE3A by rPKAe increased cAMP-hydrolytic activity, correlated with shift of PDE3A from LMW to HMW peaks, and increased co-immunoprecipitation of SERCA2, cav3, PKA regulatory subunit (PKARII), PP2A, and AKAP18 with PDE3A. In experiments with recombinant proteins, phosphorylation of recombinant human PDE3A isoforms by recombinant PKA catalytic subunit increased co-immunoprecipitation with rSERCA2 and rat rAKAP18 (recombinant AKAP18). Deletion of the recombinant human PDE3A1/PDE3A2 N terminus blocked interactions with recombinant SERCA2. Serine-to-alanine substitutions identified Ser-292/Ser-293, a site unique to human PDE3A1, as the principal site regulating its interaction with SERCA2. These results indicate that phosphorylation of human PDE3A1 at a PKA site in its unique N-terminal extension promotes its incorporation into SERCA2/AKAP18 signalosomes, where it regulates a discrete cAMP pool that controls contractility by modulating phosphorylation-dependent protein-protein interactions, PLB phosphorylation, and SERCA2 activity.

Enzymes in the cyclic nucleotide phosphodiesterase 3 (PDE3)\(^2\) family of cyclic nucleotide phosphodiesterases have an important role in regulating cAMP-mediated signaling in cardiac myocytes. Inhibitors of these enzymes are used to increase the force of contraction in patients with heart failure. The observation that inotropic responses to PDE3 inhibition are preserved in PDE3B-KO mice but not in PDE3A-KO mice indicates that PDE3A isoforms are specifically involved in the

\(^*\) The abbreviations used are: PDE3, cyclic nucleotide phosphodiesterase 3; AKAP, A-kinase anchoring protein; rAKAP, recombinant AKAP; HMW, high molecular weight; LMW, low molecular weight; SR, sarcoplasmic reticulum; signalosome, macromolecular regulatory complex; PKAc, PKA catalytic subunit; rPKAc, recombinant PKAc; PLB, phospholamban; PP2A, protein phosphatase 2A; PP1, protein phosphatase 1; rhPDE3A, recombinant human PDE3A; SERCA2, sarcoplasmic reticulum Ca\(^{2+}\) ATPase 2; rSERCA2, recombinant SERCA2; rAKAP18, recombinant AKAP18; CT domain, C-terminal domain; S6, Superose 6; aa, amino acid(s).
regulation of contractility (1, 2). Furthermore, in mouse myocardium, the PDE3A1 isoform was found to be a component of a SERCA2-PLB-AKAP18 multiprotein complex or “signalosome” that regulates the transport of cytoplasmic Ca\(^{2+}\) into the sarcoplasmic reticulum (2–3). By potentiating cAMP-dependent phosphorylation of phospholamban in this signalosome, PDE3 inhibition stimulates Ca\(^{2+}\) uptake and increases the amplitude of intracellular Ca\(^{2+}\) cycling. In renal collecting duct principal cells, incorporation of PDE4D into a signalosome containing AKAP18 and aquaporin-2 (AQP2) plays an important role in cAMP/PKA-mediated insertion of the AQP2 water channel into the cell membrane and regulation of vasopressin-induced water reabsorption (4).

Many of the interactions of PDE3 isoforms with other proteins have been shown to be dependent upon their phosphorylation. This has been well established in the case of PDE3B (5–12). Some of the protein–protein interactions of PDE3A isoforms have also been shown to be phosphorylation-dependent, including those with 14–3–3 (13–15). More recently, it has become clear that different isoforms of PDE3A, whose amino acid sequences are identical except for varying lengths of N-terminal sequence, differ significantly with respect to these interactions (15). PDE3A1 is found exclusively in intracellular membranes of cardiac myocytes and is preferentially phosphorylated at the 14–3–3-binding site Ser-312 in response to PKA activation, whereas PDE3A2, which lacks the N-terminal 154 amino acid sequence of PDE3A1, is preferentially phosphorylated at the alternative 14–3–3-binding site Ser-428 in response to PKC activation (15). PKA activation and PKC activation were found to promote the association of PDE3A1 and PDE3A2 with distinct protein interactomes (15).

In the experiments described below, we identified components of the SERCA2-PLB-AKAP18 signalosome in human myocardium. We showed that PDE3, and not PDE4 (that has also been shown to interact with PLB in human cardiac myocytes (16)), regulates the phosphorylation of PLB and the stimulation of SERCA2 activity and Ca\(^{2+}\) transport by cAMP in human SR fractions. The integration of PDE3A1 into the SERCA2-regulatory signalosome involves direct interactions with both AKAP18 and SERCA2 and is phosphorylation-dependent. Interaction of PDE3A1 with SERCA2 is dependent upon the phosphorylation of Ser-292/Ser-293, a multikinase-dependent role in cAMP/PKA-mediated insertion of the AQP2 water channel into the cell membrane and regulation of vasopressin-induced water reabsorption (4).

**Materials**

Use of human heart samples in these studies was approved by review committees at NHLBI, NIH, and the University of Utah.

**Materials**

\[\text{[\gamma}^{32}\text{P}]\text{ATP (3000 Ci/mmol) was obtained from MP Biomedicals (Solon, OH); rPKAc (catalog #14-440) and rSERCA2 (catalog #H00000488-P01) were obtained from Calbiochem and Abnova, respectively. Anti-PLB (ab2865), -p-PLB (ab15000), -AKAP-LBC (ab56917), and -AKAP18 (ab30987) antibodies were from Abcam (Cambridge, MA). Antibodies to PP2A (610556), PKA regulatory subunit (PKARII; 610626), and PKAc (610981) were from BD Biosciences. Anti-PP1 antibody (sc7482) and anti-caveolin-3 antibody (sc5310) were from Santa Cruz Biotechnology; anti-SERCA2 antibody (MA3-910) was from Affinity Bioreagents. Anti-phospho-PKA substrate antibody (9621) was from Cell Signaling; anti-desmin antibody (M0760) was from Dako. Protein G magnetic beads (88488) were from Thermo Fisher Scientific (Rockford, IL); mouse anti-FLAG® M2 magnetic beads (M8823) and mouse anti-FLAG M2-peroxidase HRP antibody (A8592) were from Sigma; mouse anti-His mAb magnetic beads (L00275) were from GenScript. Anti-myomesin antibody (mMac myomesin B4) was obtained from Developmental Studies Hybridoma Bank (University of Iowa). Rabbit polyclonal antibody to human PDE3A (accession number AAA35912) was generated against peptide corresponding to amino acids 1127–1141 (GKPRGEEIPTQRKPDQ) CT domain (C-terminal domain) and was affinity-purified as described below. For immunohistochemical studies, the secondary antibodies, Alexa Fluor 488 or Alexa Fluor 594, were from Molecular Probes. For SDS-PAGE and Western blots, HRP-labeled secondary antibody and SuperSignal® Westpico and Westfento chemiluminescent reagents were from Thermo Fisher Scientific. Signals were detected with Image reader LAS3000 (GE Healthcare). Other materials were obtained as indicated.**

**Methods**

**Afinity Purification of Anti-human PDE3A-CT Antibody—Approximately 30 mg of PDE3A-CT peptide (GPKRGEIEPTQKRDPDQ) was coupled to 3 ml of Affi-Gel 10 (Bio-Rad) beads that were used to purify anti-PDE3A-CT rabbit polyclonal antibody. Immunized serum was diluted (10 times) with 10 mM Tris buffer, pH 7.5. Anti-GF-Gel 10-PDE3A-CT-peptide beads and the diluted serum were rotated end-over-end for 16 h at 4 °C. After centrifugation, the immunobeads were washed (3 times, 20 bed volumes) with 10 mM Tris, pH 7.5. Anti-PDE3A antibody was eluted from the beads by adding 5 ml of elution buffer (100 mM glycine buffer, pH 2.5) and by rotating the mixture for 3 min. The eluted antibody was neutralized immediately with 1 M tris, pH 7.5, and dialyzed at 4 °C against 1 liter of PBS overnight. Diaclzed antibody was reconstituted with 50% glycerol and kept at −80 °C. Affinity-purified anti-PDE3A-CT antibodies were used for all studies in this report.**

**Immunohistochemistry—**Frozen heart blocks were prepared from snap-frozen normal human heart samples obtained from Capital Biosciences, Inc., and sections were made at a thickness of 10 μm using a microtome at 25 °C. Paraformaldehyde-fixed cryostat heart sections were washed in PBS (3 × 5 min) and blocked and permeabilized (6 h, 4 °C) in blocking buffer (PBS containing 10% donkey serum and 0.05% Triton X-100). Slides were incubated in blocking buffer with primary antibody overnight and washed with PBS (3 × 5 min) before incubating in blocking buffer for 2 h with the secondary antibodies, Alexa Fluor 488 or Alexa Fluor 594. Anti-PDE3A-CT antibody, generated against human PDE3A aa 1127–1141 (GKPRGEEIPTQKRDPDQ), was used in these studies. As controls, slides were incubated with nonimmune IgG or with primary anti-PDE3A-CT antibodies incubated with blocking (immunizing) peptide before staining with secondary antibody. For peptide blocking/competition experiments, ~20 μg of anti-PDE3A-CT antibody was combined with 100 μg of blocking peptide in a
small volume (500 μl) of PBS and incubated (2 h at room temperature or overnight at 4 °C). After blocking with the immunizing peptides, antibody/peptide mixtures were diluted into blocking buffer and used for staining of samples. Slides were viewed with a Zeiss LSM510 laser scanning confocal microscope.

Preparation of Subcellular Fractions of Human Myocardium—Human myocardium was obtained from the left ventricular free wall of explanted hearts from patients with idiopathic dilated cardiomyopathy undergoing cardiac transplantation (University of Utah). Normal human heart samples were also obtained from Capital Biosciences, Inc. (Rockville, MD). Heart tissues were quickly washed in ice-cold PBS, chopped with scissors, and homogenized (4 ml/g of tissue) at 4 °C in buffer A (0.29 M sucrose, 10 mM MOPS, 2 mM EGTA, Roche Applied Science protease inhibitor mixture, pH 7.0) with a rotor-stator homogenizer (Omni International, Marietta, GA) at 30,000 rpm (60–80 s on ice) followed by homogenization (on ice, 20 strokes in a glass Dounce homogenizer). Homogenates were centrifuged (11,000 × g, 15 min) in a Beckman JA-20 rotor. Supernatants were further centrifuged (150,000 × g, 1 h) in a Beckman 55.2 Ti rotor, yielding cytosolic fractions and pellets. Pellets were resuspended by hand homogenization (glass-glass) in two volumes (relative to starting material) of buffer A without EGTA. After recentrifugation (150,000 × g for 1 h), pellets, i.e. “myocardial membrane fractions,” were suspended in buffer A (without EGTA) using a Dounce homogenizer and stored at −80 °C. Each preparation was made from combined tissues from at least three different explanted hearts. For some experiments myocardial membrane fractions were suspended in buffer B (50 mM HEPES, 50 mM sucrose, 1 mM EDTA, 10 mM pyrophosphate, 5 mM NaF, 100 mM NaCl, 5 mM MgCl₂, 0.1 mM okadac acid, Roche Applied Science protease inhibitor mixture, pH 7.5). Myocardial membranes were then solubilized by homogenization (using a Dounce homogenizer, 20 strokes) and incubation/rotation of homogenates with Nonidet P40 (v/v, 1% final) (Thermo Fisher Scientific) for 1 h at 4 °C. The final washed pellets contain-
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pH 7.0, for Ca\(^{2+}\) uptake and SERCA activity assays as described below.

**Measurement of \(^{45}\text{Ca}^{2+}\) Uptake into SR Vesicles**—For experiments in Fig. 2, D–F, oxalate-dependent Ca\(^{2+}\) uptake was quantified as described previously (19). As described above, after incubation (30 min, 30 °C) without or with various additions, SR vesicles were collected and resuspended in a sucrose/K-PIPES buffer. Then portions (50 μl, 10 μg of protein) were incubated at 37 °C for 1 min in 0.4 ml of Ca\(^{2+}\) uptake buffer consisting of 50 mM imidazole-HCl, pH 7.0, 100 mM KCl, 6 mM MgCl\(_2\), 10 mM NaCl (to inhibit mitochondrial Ca\(^{2+}\) uptake), 10 mM potassium oxalate, 20 μM ruthenium red (to inhibit SERCA2 activity), 0.1 mM EGTA, \(45\text{CaCl}_2\) (10,000 dpm/nmol) (PerkinElmer Life Sciences, catalog #NE013), and unlabeled CaCl\(_2\) (0.5 μM free Ca\(^{2+}\)). Uptake was initiated by adding 50 mM ATP (50 μl), and reactions were terminated by filtration through 0.45-μm Millipore filters. After washing four times with 4 ml of buffer containing 140 mM KCl, 10 mM NaCl, 2 mM MgCl\(_2\), 1 mM CaCl\(_2\), and 50 mM imidazole-HCl, pH 7.0, radioactivity retained on the filters was quantified by liquid scintillation counting. Free Ca\(^{2+}\) was calculated using a MAXCHELATOR program obtained at Stanford University.

**SERCA Activity Assay**—In Fig. 2F, Ca\(^{2+}\)-ATPase activity in SR fractions was determined by measuring the amount of \(\text{Pi}\) released after the addition of ATP using the malachite green ATPase method (20). Briefly, as described above, after incubation (30 min, 30 °C) without or with 250 units of rPKAc, SR vesicles were isolated and suspended in sucrose/K-PIPES buffer. Then samples (50 μl, 10 μg of protein) were assayed for SERCA2 enzymatic activity in the presence or absence of thapsigargin (10 μM), as SERCA2 activity was determined as that portion of total activity inhibited by thapsigargin. The assay mixture (total volume, 125 μl) contained 0.125 M KCl, 20 mM imidazole, pH 7.0, 0.1 mM EGTA, 0.103 mM CaCl\(_2\), 5 mM MgCl\(_2\), 1 μM ionomycin, and 10 μg of SR. To initiate the reaction, 25 μl of substrate (1.25 mM ATP) was added to a final concentration of 0.25 mM. The mixture was incubated at room temperature for 3 min. The reaction was terminated by adding 25 μl of 250 g/liter TCA, vortexed quickly, and centrifuged (8000 × g, 3 min). Supernatants (20 μl) were added to 96-well plates followed by color reagent (100 μl/sample), which consisted of 54 mM ammonium molybdate and 0.73 mM malachite green. After 1 min sodium citrate (340 g/liter, 10 μl) was added with gentle shaking (room temperature for >20 min), after which plates were scanned at 650 nm. \(\text{Pi}\) was calculated by converting 0.650 to nanomoles by means of a standard curve.

**Gel Filtration Chromatography on Superose 6 Columns**—Solubilized myocardial membranes (1.0 ml, 3 mg of total protein), prepared as described above, were applied to a Superose 6 HR 10/30 column (AKTA FPLC System, GE Healthcare) that was equilibrated and eluted with buffer C (buffer B without sucrose containing 150 mM NaCl and 1% Nonidet P-40) (Fig. 3A). Cytosolic fractions (1.0 ml, 3 mg of total protein), prepared as described above, were also applied to Superose 6 that was equilibrated and eluted with buffer C (without Nonidet P-40) (Fig. 3B). Portions of the fractions (0.5 ml) were used for assay of PDE3A activity or SDS-PAGE/Western blotting (using anti-PDE3A-CT antibodies) (Fig. 3, A and B) as described above. In some experiments (Fig. 3C), portions of eluted HMW (H) and LMW (L) peaks from solubilized membrane fractions (M) and cytosolic fractions (C) were pooled and concentrated. As described above (see “Immunoprecipitation and Immunoblotting”), fractions were cleared by incubation with 5 μg of rabbit non-immune IgG and then with 50 μl of Protein G magnetic beads for 30 min before placing the tubes into a magnetic stand to collect the beads against the side of the tube. As presented in Fig. 3C, cleared fractions were transferred to new tubes and incubated overnight at 4 °C with 10 μg of non-immune IgG (IgG lane) or with 10 μg of anti-PDE3A-CT (PDE3A lanes) followed by incubation (1 h, 4 °C) and immunoprecipitation with 50 μl of fresh Protein G magnetic beads before placing the tubes into a magnetic stand to collect the beads. Immunoprecipitated proteins bound to the magnetic beads were washed (3×, buffer B) and then eluted by boiling (5 min) in 200 μl of Laemml SDS sample buffer. After boiling, magnetic beads were separated from the eluted proteins by placing the tubes in a magnetic stand. Samples of eluted proteins were subjected to SDS-PAGE and immunoblotting with anti-pPKA substrate or anti-PDE3A-CT (PDE3A) antibodies (Fig. 3C) as described above.

**In Vitro Phosphorylation of Endogenous PDE3A**—For experiments in Fig. 3D, solubilized membrane fractions (500 μg) were cleared with 5 μg of rabbit non-immune IgG and 50 μl of Protein G magnetic beads, and PDE3A was immunoprecipitated using 10 μg of anti-PDE3A-CT and 50 μl of Protein G magnetic beads as described above. Immunoprecipitated PDE3A bound to the magnetic beads was incubated for 1 h at 30 °C in phosphorylation buffer (50 mM HEPES, pH 7.5, 5 mM MgCl\(_2\), 100 mM NaCl, 1 mM EDTA, 10 mM PP\(_i\), 5 mM NaF, 0.1 μM okadaic acid) with 200 μM ATP or 200 μM ATP supplemented with [γ-\(^{32}\text{P}\)]ATP (10 μCi per reaction; 3000 Ci/mmol stock) in the absence or presence of 250 units of rPKAc or rPKAc plus 10 μM PKAc inhibitor (PKI). After the incubation, tubes were placed in a magnetic stand to separate the beads from the reaction mixtures, and beads (with bound immunoprecipitated PDE3A) were washed (3×, buffer B).

As indicated in Fig. 3D (lower panel) immunoprecipitated PDE3A (not incubated with [γ-\(^{32}\text{P}\)]ATP) was assayed for PDE3 activity using \(^{3}\text{H}\)cAMP as substrate. The assays were terminated, and tubes were placed in a magnetic stand to separate the beads from the reaction mixtures. In the separated reaction mixtures, the product 5′-\(^{3}\text{H}\)AMP was converted to \(^{3}\text{H}\)adenosine, which was quantified as described in our published method (18).

As shown in Fig. 3D (upper panel), after incubation of immunoprecipitated PDE3A with rPKAc and [γ-\(^{32}\text{P}\)]ATP, the beads were separated from the reaction mixtures and washed as described above. \(^{32}\text{P}\)-Labeled PDE3A bound to the magnetic beads was eluted by boiling the beads (5 min) in Laemmli SDS sample buffer and then by placing the tubes in a magnetic stand to separate the eluted \(^{32}\text{P}\)-labeled PDE3A from the magnetic beads. Samples of eluted \(^{32}\text{P}\)-labeled PDE3A were subjected to SDS-PAGE. γ-\(^{32}\text{P}\) phosphorylation of PDE3A was detected by scanning the wet gels by phosphorimaging (GE Healthcare). Proteins on wet gels were then electrophotographically transferred to nitrocellulose membranes, and PDE3A was identified by
Western blotting using anti-PDE3A antibody (Fig. 3D, middle panel). PK1 blocked rPKAc-induced phosphorylation and activation of PDE3A.

In other experiments (Fig. 4) chromatography of solubilized myocardial membranes (3 mg) on Superose 6 was repeated 2 times and LMW fractions (#24–34, Fig. 3A) were pooled and concentrated via Centriprep YM-3 (centrifugal filter units with Ultracel-3 membranes (nominal molecular weight limit, >3 kDa)). The concentrated fractions were split, and after incubation for 1 h at 30 °C without (LMW control) or with (LMW + PKA) rPKAc in phosphorylation buffer containing 200 μM ATP and 5 mM MgCl₂ were re-chromatographed on Superose 6 (Fig. 4). Portions of the fractions (0.5 ml) were used for assay of PDE3 activity or SDS-PAGE/Western blotting (using anti-PDE3A-CT and other indicated antibodies) as described above.

Co-immunoprecipitation of PDE3A with Components of SERCA2 Regulatory Signalosomes from Pooled LMW Fractions after Incubation with or without rPKAc—For these studies (Fig. 5), pooled LMW Superose 6 fractions from solubilized myocardial membranes (two experiments) were prepared as described above (cf. Figs. 3A and 4). These LMW fractions were concentrated and split into three fractions that were then incubated (1 h, 30 °C) in phosphorylation buffer containing 200 μM ATP and 5 mM MgCl₂ without (IgG, Control) or with (PKA-C) rPKAc.

To study co-immunoprecipitation of PDE3A with components of the SERCA2/AKAP18 signalosome (Fig. 5), at the completion of these reactions the three LMW fractions were cleared with rabbit non-immune IgG (5 μg) and Protein G magnetic beads (50 μl) as described above and then incubated (overnight, 4 °C) with anti-PDE3A-CT antibody (10 μg) (control, PKA-C) before incubation (1 h, 4 °C) with Protein G magnetic beads. Tubes were placed in a magnetic stand to separate the beads from the reaction mixtures. Immunoprecipitated proteins bound to Protein G magnetic beads were washed and eluted as described above, and portions of eluted samples were subjected to SDS-PAGE transferred to nitrocellulose membranes, and immunoblotted with indicated antibodies (Fig. 5). Total membrane proteins (10 μg, input) were loaded on gels as controls.

Co-immunoprecipitation of FLAG-tagged Recombinant Human PDE3A (rhPDE3A) Variants and rSERCA2 after Incubation with or without rPKAc—FLAG-tagged rhPDE3A1 (open reading frame, accession number NP_0009912) and its phosphorylation site mutants (rhPDE3A1-S292A/S293A (P1), rhPDE3A1-S312A (P2), rhPDE3A1-S428A (P3), rhPDE3A1-S438A (P4), and rhPDE3A1-S292A/S293A/S312A/S438A (P5)) (Fig. 6B) were synthesized by Genscript, Inc (Piscataway, NJ), subcloned into pAcSG2 baculoviral expression vector, and expressed in SF21 cells (21). A comparison of the translated sequences of human AKAP18γ (accession number NP_057461 and AK300587; 348 aa residues) and rat AKAP18δ (accession number Q6JP77; 353 aa residues) indicate that 8 of the first 12 aa residues of human AKAP18γ and rat AKAP18δ are identical and that the proteins are homologous (~74% aa identity). SF21 cell supernatants, containing His-tagged rAKAP18δ, were prepared as described above. FLAG-tagged rhPDE3A1 (usually 50 arbitrary units) was incubated (30 min, 30 °C) with rAKAP18δ (50 ng) in phosphorylation buffer containing 2 mM DTT, 5 mM MgCl₂, and 200 μM ATP (final volume, 300 μl) with or without different concentrations of rPKAc (Fig. 7B) and with or without 50 units of rPKAc (Fig. 7C). As a control, in Fig. 7C, rhPDE3A1 (50 units) was incubated with or without rPKAc (50 units) and with SF21 cell supernatants that contained or did not contain rAKAP18δ. Reactions were stopped by dilution of reaction mixtures to 1.0 ml with buffer B containing 1 mg/ml SF21 cell supernatants and cleared by incubation with 5 μg of mouse

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non-immune IgG and then with 50 μl of Protein G magnetic beads as described above. Anti-His mAb magnetic beads incubated with SF21 cell supernatants as described above for anti-FLAG M2 magnetic beads were used for immunoprecipitation of recombinant proteins (25 μl, 2 h, 4 °C). Tubes were placed in a magnetic stand to separate the beads from the reaction mixtures, which were removed. Immunoprecipitated proteins bound to anti-His mAb magnetic beads were washed and eluted by boiling in Laemmli SDS sample buffer (200 μl). As described above, samples of eluted immunoprecipitated proteins as well as of reaction mixtures (“input proteins”) were subjected to SDS-PAGE and immunoblotted with indicated antibodies.

RESULTS

PDE3A Co-localizes with PLB, SERCA2, and AKAP18 in the Z-bands of Human Cardiac Myocytes—The intracellular distribution of PDE3A in cryostat sections of normal human left ventricle was examined by immunostaining with peptide affinity-purified anti-PDE3A-CT antibodies (Fig. 1). Consistent with our previous studies (2, 23), co-staining with anti-PDE3A-CT and anti-desmin antibodies demonstrated that PDE3A is distributed in a striated pattern in the Z-lines of human cardiac myocytes (Fig. 1, A and B), with little co-localization with myomesin (M-line marker protein) (Fig. 1B). PDE3A co-localized with PLB, SERCA2, and an AKAP18 variant (detected by antibodies to human AKAP18 (Abcam: Ab 30987)) (Fig. 1, A and B).

PDE3 Inhibition Potentiates cAMP-dependent Phosphorylation of PLB and Its Stimulation of Ca2+ Uptake in Human Myocardial Membranes—The effects of PDE3 inhibition on PLB phosphorylation and Ca2+ uptake were examined in SR fractions prepared from human left ventricular myocardium. At 0.1 μM cAMP, phosphorylation of PLB at Ser-16 by endogenous PKA was stimulated by the PDE3-selective inhibitor cilostamide (Fig. 2, A and B). Because PDE4 has also been found to co-immunoprecipitate with PLB in subcellular preparations from human myocardium (16), we tested the effect of the PDE4-specific inhibitor rolipram on PLB phosphorylation and found no effect (Fig. 2, A and B). This result corresponds to the lower amount of rolipram-sensitive cAMP hydrolytic activity relative to cilostamide-sensitive cAMP hydrolytic activity in these SR preparations (Fig. 2C). cAMP increased ATP-dependent, oxalate-supported Ca2+ uptake (Fig. 2D), and this effect was potentiated by cilostamide (Fig. 2E). The addition of rPKAc stimulated both Ca2+ uptake and SERCA2 activity (Fig. 2F). These results indicate that PDE3 has a specific role in regulating phosphorylation of PLB by cAMP/PKA and the consequent stimulation of Ca2+ uptake in human myocardium.

Gel Filtration Chromatography of PDE3A Isoforms from Human Myocardium—In previous studies we showed that the phosphorylation of rPDE3A isoforms in transfected HEK293 cells promotes their interactions with other proteins (15). To address whether phosphorylation might promote the integra-
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FIGURE 2. cAMP, PKA, and PDE3 inhibition increase SERCA2 activity and Ca$^{2+}$ uptake in human SR fractions. A, as described under “Methods,” after incubation of SR fractions (20 μg) in the absence or presence of the indicated concentrations of ATP and cAMP without or with cilostamide (Cil) or rolipram (Rol), endogenous PLB, phosphorylated PLB, and β-actin were detected after SDS-PAGE and immunoblotting. Results are presented as the mean ± S.E. (n = 3 independent experiments). **P < 0.01 compared with control (Ctrl) or rolipram (Rol) or cilostamide (Cil). B, bar graph summarizing pSer16PLB/PLB total ratios. Cilostamide significantly enhanced the effect of 0.1 μM cAMP on phosphorylation of PLB (*, p < 0.01 versus control (n = 3 independent experiments). C, PDE activity in human cardiac SR fractions was assayed as described under “Methods” and expressed as specific activity (pmol of cAMP hydrolyzed/min/mg). Results are presented as the mean ± S.E. (n = 3 preparations). PDE3 activity was determined as the calsequestrin-sensitive fraction, and PDE4 activity was determined as the rolipram-sensitive fraction. PDE3 activity was significantly higher than PDE4 activity (*, p < 0.001). D, after incubation of SR fractions without or with the indicated concentrations of cAMP (0–10 μM), 45Ca$^{2+}$ uptake was measured in the presence of 0.5 μM free Ca$^{2+}$ as described under “Methods.” Results are presented as % increase due to cAMP, with basal Ca$^{2+}$ uptake (0.4 ± 0.8 nmol/mg/min; n = 3) taken as 100%. cAMP significantly enhanced 45Ca$^{2+}$ uptake (*, p < 0.01; **, p < 0.04). E, after incubation of SR fractions with or without cAMP (3 μM) in the presence or absence of cilostamide (1 μM), 45Ca$^{2+}$ uptake (0.5 μM free Ca$^{2+}$) was assayed as described under “Methods.” Results are presented as the mean ± S.E. (n = 3). Cilostamide significantly enhanced the effect of cAMP on 45Ca$^{2+}$ uptake (*, p < 0.02). F, Ca$^{2+}$ uptake (upper panel) and SERCA activity (lower panel) were assayed in reaction mixtures containing Mg$^{2+}$ and ATP in the presence or absence of rPKAc as described under “Methods.” Results are presented as pmol (Ca$^{2+}$ or P)/min/mg (mean ± S.E.) (n = 3); rPKAc significantly enhanced 45Ca$^{2+}$ uptake and SERCA2 activity (*, p < 0.001).

tion of PDE3A into multiprotein SERCA2 signalosomes in cardiac myocytes, we analyzed cytosolic and Nonidet P-40-solubilized membrane proteins from human left ventricular myocardium by Superose 6 gel filtration chromatography (Fig. 3). As seen in Fig. 3A, PDE3 activity in solubilized myocardial membrane proteins was recovered in distinct HMW (∼3000 kDa) and LMW (∼700 kDa) peaks. Western blotting with anti-PDE3A antibodies indicated that the HMW and LMW peaks contained all three PDE3A isoforms. In contrast, upon Superose 6 chromatography of cytosolic fractions, from which PDE3A1 is absent, PDE3A2 and PDE3A3 eluted in a single LMW peak (Fig. 3B). As seen in Fig. 3C, immunoprecipitation of PDE3A from pooled HMW or LMW peaks of solubilized membranes followed by Western blotting with anti-phospho PKA substrate and anti-PDE3A-CT antibodies demonstrated that endogenous PDE3A1 in HMW peaks was the most highly PKA-phosphorylated PDE3A isoform. These results suggested that phosphorylation of endogenous PDE3A1 by PKA may be involved in its incorporation into HMW multiprotein complexes.

To confirm these findings, studies of the phosphorylation of endogenous PDE3A by rPKAc in the absence or presence of [γ-32P]ATP or unlabeled ATP were carried out in vitro using immunoprecipitated PDE3A from solubilized myocardial membranes. As seen in Fig. 3D (upper panels), after immunoprecipitated PDE3A was incubated with [γ-32P]ATP in the absence (Ctrl) or presence of rPKAc or rPKAc plus PKI peptide, phosphorimaging of wet gels after SDS-PAGE combined with Western blotting demonstrated that both PDE3A1 and PDE3A2 could be markedly phosphorylated by rPKAc, with little if any increase in the phosphorylation of PDE3A3. PKI blocked phosphorylation of PDE3A1 and PDE3A2. As seen in Fig. 3D (lower panel), phosphorylation of immunoprecipitated PDE3A by rPKAc was accompanied by stimulation of PDE3A hydrolytic activity (35% compared with controls, p < 0.01). PKI blocked rPKAc-induced activation of PDE3A.
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PDE3A Associates Phosphorylation-dependently with PLB, SERCA2, and AKAP18 — We examined the effects of phosphorylation by rPKAc on the interaction of endogenous PDE3A with PLB, SERCA2, and AKAP18 (Figs. 4 and 5). Pooled and concentrated membrane LMW Superose 6 fractions (analogous to fractions 24–34 in Fig. 3A) were divided and incubated in phosphorylation buffer containing ATP and MgCl2 in the absence or presence of rPKAc and then re-chromatographed on Superose 6 (S6) columns (Fig. 4) or immunoprecipitated with anti-PDE3A–CT antibody (Fig. 5). As seen in Fig. 4, PDE3 activity in control-pooled membrane LMW fractions exhibited an apparent molecular mass of 700 kDa during re-chromatography on S6 columns (Fig. 4, LMW Control). After incubation with rPKAc, however, the apparent molecular mass of most of the eluted PDE3A1 and PDE3A2 was shifted to from LMW fractions to ≈3000 kDa (Fig. 4, LMW + PKA). Most of the PDE3A3, which was not phosphorylated or only weakly phosphorylated by rPKAc (Fig. 3), remained in the LMW peak. This indicates that phosphorylation by rPKAc (cf. Fig. 3D) correlates with the shift in elution of PDE3A1 and PDE3A2. As also seen in Fig. 4, shifts from LMW to the HMW fractions were observed as well for AKAP18, PLB, PKA-RII, and PKA catalytic subunits as well as PP1 and caveolin 3.

As seen in Fig. 5, incubation of pooled and concentrated membrane LMW fractions with rPKAc increased the co-immunoprecipitation of PDE3A with AKAP18 (not AKAP-LBC), SERCA2, PP2A, PP1, and cav3 but reduced the co-immunoprecipitation of PLB. These results suggest that phosphorylation of...
membrane-associated PDE3A promotes its integration into signalosomes containing AKAP18, SERCA2, and other proteins involved in the regulation of Ca\(^{2+}\) transients and myocardial contractility by cAMP. The decreased association of PLB with these proteins after phosphorylation by PKA is consistent with the idea that the integration of PLB into this complex is dependent upon its interactions with SERCA2 and AKPA18 and that these interactions are reduced by phosphorylation of PLB by PKA at Ser-16 (2, 3, 24).

In Fig. 4, the shift of the PDE3A3 isoform from LMW to HMW fractions after incubation with PKA cannot be explained by phosphorylation of PDE3A3, as it is not a substrate for PKA (cf. Fig. 3). Although PDE3A2 can be readily phosphorylated by rPKAc in vitro (Figs. 3D and 5) and this phosphorylation is correlated with its shift from LMW to HMW fractions (Fig. 4), endogenous HMW PDE3A2 is less highly phosphorylated than PDE3A1. It is possible that PDE3A3 forms heteromeric complexes with PDE3A1 and/or PDE3A2 and that phosphorylation of multiple proteins contributes to the incorporation of PDE3A isoforms into the endogenous SERCA2 regulatory signalosome. In addition, phosphorylation at more than one site in PDE3A isoforms may contribute to their shift in migration, as our previous work indicates that PDE3A isoforms interact directly or indirectly with a large number of other proteins (15).

Because murine PDE3A1 is a component of a SERCA2 regulatory signalosome in murine myocardium (2, 3) and because endogenous PDE3A1 is the most highly PKA-phosphorylated isoform in human myocardium (Fig. 3C), we studied its phosphorylation-dependent interactions with SERCA2 and AKAP18 in more detail using recombinant forms of these proteins. The co-immunoprecipitation of both proteins with rhPDE3A1 was increased by phosphorylation of rhPDE3A1 by rPKAc (Figs. 6C and 7).

To gain insight into molecular mechanisms contributing to the phosphorylation-dependent interaction of rhPDE3A1 and rSERCA2, we generated PDE3A1 constructs with C- and N-terminal deletions and serine-to-alanine mutations at PKA sites in the PDE3A1 N terminus. As seen in Fig. 6C, rSERCA2 also co-immunoprecipitated with phosphorylated rhPDE3A-RD. rPDE3A-RD contains only the N-terminal portion of PDE3A1 (aa 146 – 484), including both its unique N-terminal extension and some of its shared sequence with PDE3A2). SERCA2 did not co-immunoprecipitate with rhPDE3A-Δ510, which is a recombinant form from which the first 510 aa of the PDE3A open reading frame were deleted and which is not phosphorylated by rPKAc. This indicated that interactions with rSERCA2 involved the N terminus of rhPDE3A1.
### DISCUSSION

PDEs have critical roles in the compartmentation of cAMP signaling (25–29). A number of studies involving many different PDEs have demonstrated that these enzymes are recruited to specific intracellular multiprotein complexes (signalosomes) through protein-protein interactions and that as a result of this localization individual PDEs are able to regulate specific cAMP-mediated signaling pathways with great precision (25–29). Understanding the mechanisms by which PDEs are localized to signalosomes and the consequences of this localization is especially important in cardiac muscle, where inhibitors of the PDE3 family of enzymes are used to increase myocardial contractility in patients with heart failure. In earlier work we showed that inotropic responses to PDE3 inhibition in mice, which correlated with increases in intracellular Ca\(^{2+}\) transients, are attributable specifically to isoforms in the PDE3A subfamily and that PDE3A1 is a component of a murine SERCA2-regulatory signalosome (1–3). In the experiments described here, we have confirmed that human PDE3A1 is part of a similar SERCA2-, PLB- and AKAP18-containing signalosome localized to sarcomeric Z-bands in human cardiac myocytes. We also showed that the integration of PDE3A isoforms into this signalosome is phosphorylation-dependent and that the interaction of PDE3A1 with SERCA2 is dependent upon the phosphorylation of PDE3A1 at Ser-292/Ser-293, a sequence in its unique N-terminal extension.

Although both PDE3 and PDE4 have been found to co-immunoprecipitate with PLB in complexes from mouse and human myocardium (16), our studies show that PDE3-selective inhibition (but not PDE4 inhibition) potentiates the phosphorylation of PLB by endogenous PKA and stimulation of SERCA2 activity and Ca\(^{2+}\) uptake in SR-enriched vesicles prepared from human myocardium. This most likely reflects the higher amount of PDE3 activity relative to PDE4 activity in SR preparations from human myocardium. Taken together, our observations provide evidence for the physiologic and therapeutic importance of the association of PDE3A with the SERCA2-regulatory signalosome.

As noted above, previous studies of PDE3B showed a role for N-terminal phosphorylation in integrating adipocyte PDE3B into signalosomes in response to insulin or \(\beta_3\)-adrenergic receptor agonists (5, 6, 10). N-terminal phosphorylation in response to PKA and PKC activation has also been shown to regulate the interactions of PDE3A isoforms with 14-3-3 and with other (as-yet-unidentified) proteins (15). The interactions with 14-3-3 were consequences of the phosphorylation of two sites in the N-terminal sequence common to both PDE3A1 and PDE3A2, Ser-312 and Ser-428; there was no evidence for the interaction of PDE3A1 with SERCA2 and AKAP18 in a phosphorylation-dependent manner. The interaction with SERCA2 is dependent upon the phosphorylation of Ser-292/Ser-293, a site unique to PDE3A1 that was not known to have a role in regulating protein-protein interactions. The fact that PDE3A2, which lacks this site, also interacts phosphorylation-dependently with SERCA2 indicates that phosphorylation of other sites in its sequence is responsible for this interaction and/or that the lack of the unique N terminus in PDE3A2 alters its phosphorylation and/or interactions with other signaling proteins. These findings add significantly to our understanding of the mechanisms by which PDEs are localized to signalosomes and the consequences of this localization.

### FIGURE 5

**PKAc promotes interactions of PDE3A with components of the SERCA2 regulatory signalosome.** Solubilized myocardial membranes were prepared (3 mg of protein, 1 ml) and subjected to chromatography on Superose 6 columns as in Fig. 3A. Membrane LMW fractions were pooled from two different experiments (Fig. 3A) and concentrated via Centriprep YM-3. Pooled, concentrated fractions were split into three fractions and incubated in phosphorylation buffer with 200 \(\mu\)M ATP and 5 mM MgCl\(_2\), for 1 h at 30 °C in the absence (IgG, Control) or presence (PKA-C) of PKAc. At the completion of these reactions, fractions were cleared with non-immune IgG and Protein G magnetic beads as described above and then incubated (overnight, 4 °C) with non-immune IgG (IgG, Control) or anti-PDE3A-CT antibody (PKA-C) before incubation and immunoprecipitation (IP) with Protein G magnetic beads (1 h, 4 °C). Proteins associated with the Protein G magnetic beads were eluted by boiling in 200 μl of Laemmli SDS sample buffer. Samples (15 μl) were subjected to SDS/PAGE and immunoblotted (IB) with specific antibodies as shown. Input membrane proteins (10 μg) were also loaded on the gels as positive controls. Representative results from three independent experiments are shown. Similar amounts of PDE3A were immunoprecipitated in the control fractions and in fractions incubated with PKAc. Band intensities of immunoprecipitated PDE3A and its interacting signaling molecules were analyzed using an LAS3000 analyzer and presented as binding percentage ratios from human myocardium. This most likely reflects the higher amount of PDE3 activity relative to PDE4 activity in SR preparations from human myocardium. Taken together, our observations provide evidence for the physiologic and therapeutic importance of the association of PDE3A with the SERCA2-regulatory signalosome.

### TABLE

<table>
<thead>
<tr>
<th>IP: PDE3A</th>
<th>Control</th>
<th>PKA-C</th>
<th>% change (PKAc/control) ((p&lt;0.01, n=3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE3A A1</td>
<td>217</td>
<td>123</td>
<td>101 +/- 0.28</td>
</tr>
<tr>
<td>PDE3A A2</td>
<td>198</td>
<td>123</td>
<td>198 +/- 0.9 *</td>
</tr>
<tr>
<td>PDE3A A3</td>
<td>61</td>
<td>123</td>
<td>61 +/- 0.4 *</td>
</tr>
<tr>
<td>SERCA2</td>
<td>165</td>
<td>123</td>
<td>165 +/- 0.4 *</td>
</tr>
<tr>
<td>PLB</td>
<td>165</td>
<td>123</td>
<td>165 +/- 0.9 *</td>
</tr>
<tr>
<td>AKAP18</td>
<td>161</td>
<td>123</td>
<td>161 +/- 0.6 *</td>
</tr>
<tr>
<td>AKAP</td>
<td>186</td>
<td>123</td>
<td>186 +/- 0.5 *</td>
</tr>
<tr>
<td>LBC</td>
<td>202</td>
<td>123</td>
<td>202 +/- 1.3 *</td>
</tr>
<tr>
<td>PKAc</td>
<td>178</td>
<td>123</td>
<td>178 +/- 0.7 *</td>
</tr>
<tr>
<td>PP2A</td>
<td>167</td>
<td>123</td>
<td>167 +/- 1.0 *</td>
</tr>
<tr>
<td>PP1</td>
<td>259</td>
<td>123</td>
<td>259 +/- 3.0 *</td>
</tr>
</tbody>
</table>

With at least four PKA consensus sites (RRX(S/T) in the rhPDE3A1 N terminus, we inserted serine-to-alanine mutations in these sites: S292A/S293A (P1), S312A (P2), S428A (P3), S438A (P4), and S292A/S293A/S312A/S438A (P5). As seen in Fig. 6D, P1 and P5 mutations markedly diminished PKA-stimulated interactions with rSERCA2. PDE3A1 mutants P2 (S312A) and P3 (S428A) showed slightly decreased phosphorylation-dependent interactions with rSERCA2, whereas the PDE3A1 P4 mutation (S438A) had no effect (Fig. 6). These data indicate that Ser-292/Ser-293 is the major PKA site regulating the interaction of rhPDE3A1 with rSERCA2.

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*Note: The table and figure are placeholders and should be replaced with actual data and visual elements.*
of the molecular mechanisms through which PDE3A1 is localized to intracellular signalosomes that control intracellular Ca\(^{2+}\) handling and suggest further that the interactions of each PDE3A isoform with SERCA2 can be selectively targeted.

As noted earlier, the sequences of PDE3A1 and PDE3A2 are identical save for the presence in the former of a unique 154-amino acid N-terminal extension. Understanding how this extension affects the function of PDE3A1 is, therefore, central to understanding the unique roles of the two isoforms. A growing body of evidence indicates that several molecular mechanisms are involved. The hydrophobic loops within this sequence restrict PDE3A1 distribution to cellular and intracellular membranes. Within these membranes, the protein-protein interactions of each isoform are clearly different; the 5-hydroxytryptamine receptor, for example, binds to PDE3A1 but not to PDE3A2 (30). The N-terminal extension also affects the tertiary structure of the downstream sequence; its presence converts Ser-428 from being a strong PKC site in PDE3A2 to a weaker one in PDE3A1. This contributes to the selective regulation of these isoforms by preferential phosphorylation of PDE3A1 at the protein binding site Ser-312 by PKA and preferential phosphorylation of PDE3A2 by PKC at the protein binding site Ser-428 (15). Because phosphorylation promotes the association of each isoform with different proteins, this is likely to be a factor in the two isoforms having distinct interactomes. In addition, phosphorylation by PKB of the Ser-292/Ser-293 site, which is found within the N-terminal extension unique to PDE3A1, stimulates cAMP hydrolytic activity, providing another mechanism by which PDE3A1 can be selectively regulated (31).
Our results showing that the interaction of PDE3A1 with SERCA2 is dependent upon the phosphorylation of Ser-292/Ser-293 reveal a new and important mechanism whereby the unique N-terminal extension of PDE3A1 regulates its function. The amino acid sequence surrounding this site, aa 288–294, is RRRRSSSS, and all three serines can be phosphorylated in vitro under different conditions (31); hence, it is likely this site can be phosphorylated by different kinases activated in response to different upstream signals. Phosphorylation at Ser-292/Ser-293 phosphorylated by different kinases activated in response to agonists as inotropic agents. Because PP2A and PP1 are thought to be the principal phosphatases responsible for dephosphorylation of PKA substrates and PLB (36), the presence of PPI and PP2A in the complex would be expected to catalyze the dephosphorylation of PDE3A, PLB, and other PKA substrates.

Our findings add to the understanding of the mechanisms whereby PDE3A, a component of a SERCA2-containing signalosome, regulates cAMP-mediated changes in contractility in cardiac myocytes. Phosphorylation of PLB on Ser-16 causes its dissociation from SERCA2, relieving its inhibition of SERCA2 activity and increasing Ca\(^{2+}\) transport into the SR (24, 32, 33). These actions increase the amplitude of intracellular Ca\(^{2+}\) transients, which are attenuated in dilated cardiomyopathy (34). PKA-catalyzed phosphorylation of PLB at Ser-16, induced by isoproterenol, forskolin, or isobutylmethylxanthine, correlated with increased cardiac relaxation (24, 35). The interaction of PLB with AKAP18 is also reduced by phosphorylation of Ser-16 (3). Phosphorylation of PDE3A1 at Ser-292/Ser-293, which correlates with its recruitment into the SERCA2 regulatory signalosome, would tend to counteract this effect by reducing the concentration of cAMP in the proximity of this complex (Fig. 8). This combination of stimulation and inhibition of SERCA2 activity in response to PKA activation through separate mechanisms may permit a greater degree of “fine-tuning” of SR Ca\(^{2+}\) handling in response to \(\beta\)-adrenergic receptor agonists. It may also explain the synergism observed when PDE3 inhibitors are used in combination with \(\beta\)-adrenergic receptor agonists as inotropic agents. Because PP2A and PP1 are thought to be the principal phosphatases responsible for dephosphorylation of PKA substrates and PLB (36), the presence of PPI and PP2A in the complex would be expected to catalyze the dephosphorylation of PDE3A, PLB, and other PKA substrates.

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**FIGURE 7.** rPKAc-induced phosphorylation of rhPDE3A increases its interaction with rat rAKAP18. A, schemes representing hPDE3A1 protein domains (NHR1, trans-membrane domain; NHR2, membrane association domain; CCR, conserved C-terminal catalytic region) and rAKAP18 protein domains, with RII binding site (aa 301–314) and a unique N terminus (aa 1–26). B and C, His-tagged rAKAP18 (100 ng) and 50 units of FLAG-tagged rhPDE3A1 were incubated for 30 min at 30 °C in phosphorylation buffer containing 200 μM ATP and 5 mM MgCl\(_2\) in the absence or presence of the indicated concentrations (units) of rPKAc (B) or in the absence or presence of 50 units of rPKAc (C). In either case, our results suggest that an agent capable of binding to PDE3A1 in its Ser-292/Ser-293-phosphorylated conformation may be able to inhibit its protein-protein interactions without affecting those of PDE3A2, thereby selectively inhibiting the integration of PDE3A1 into specific signalosomes.

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FIGURE 8. Model of the regulation of SERCA2 activity by cAMP and the AKAP18 and PLB-containing signalosome. A, components of the AKAP18/SERCA2/PLB signalosome are shown. B, in the absence of cAMP, SERCA2 was inhibited by its interaction with PLB. Activation of PKA by cAMP resulted in the phosphorylation of PLB and PDE3A (and, most likely, other molecules in the signalosome). The former dissociates from SERCA2, increasing SERCA2 activity, but the integration of phosphorylated PDE3A into the signalosome limits this effect by increasing hydrolysis of cAMP. PKA and P2A in the signalosome would be expected to catalyze the dephosphorylation of PDE3A, PLB, and other PKA substrates and return the SERCA2 complex to its basal state. C, PDE3 inhibition potentiates the effect of cAMP on SERCA2.

substrates and return the SERCA2 complex to its basal state. Of note, AKAP18 associates with inhibitor I and thereby can control PP1 activity (37).

Our results may have therapeutic implications. Selective inhibition of the PDE3A isoforms associated with SERCA2 might allow the inotropic benefits of stimulating Ca\(^{2+}\) transport into the sarcoplasmic reticulum without the harmful effects of global inhibition of PDE3 activity (2, 38). Currently available PDE3 inhibitors, however, have little selectivity for PDE3A versus PDE3B isoforms, whose catalytic domains are similar, and no selectivity for individual PDE3A isoforms, whose catalytic domains are identical (39). Blocking the integration of PDE3A1 into SERCA2 regulatory complexes either by blocking its phosphorylation or by blocking the interactions of phosphorylated PDE3A1 with SERCA2 may be a way of targeting PDE3 activity in a specific microdomain in cardiac myocytes to stimulate SR Ca\(^{2+}\) uptake and produce inotropic actions without the adverse consequences that accompany diffuse increases in intracellular cAMP content (29, 38).

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PDE3A Regulates SERCA2 Signaling in Human Myocardium


Signal Transduction: Regulation of Sarcoplasmic Reticulum Ca\(^{2+}\) ATPase 2 (SERCA2) Activity by Phosphodiesterase 3A (PDE3A) in Human Myocardium: PHOSPHORYLATION-DEPENDENT INTERACTION OF PDE3A1 WITH SERCA2

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