Phosphorylation of the Consensus Sites of Protein Kinase A on $\alpha_{1D}$ L-type Calcium Channel*

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The novel $\alpha_{1D}$ L-type Ca$^{2+}$ channel is expressed in supraventricular tissue and has been implicated in the pacemaker activity of the heart and in atrial fibrillation. We recently demonstrated that PKA activation led to increased $\alpha_{1D}$ Ca$^{2+}$ channel activity in tsA201 cells by phosphorylation of the channel protein. Here we sought to identify the phosphorylated PKA consensus sites on the $\alpha_1$ subunit of the $\alpha_{1D}$ Ca$^{2+}$ channel by generating GST fusion proteins of the intracellular loops, N terminus, proximal and distal C termini of the $\alpha_1$ subunit of $\alpha_{1D}$ Ca$^{2+}$ channel. An in vitro PKA kinase assay was performed for the GST fusion proteins, and their phosphorylation was assessed by Western blotting using either anti-PKA substrate or anti-phosphoserine antibodies. Western blotting showed that the N terminus and C terminus were phosphorylated. Serines 1743 and 1816, two PKA consensus sites, were phosphorylated by PKA and identified by mass spectrometry. Site directed mutagenesis and patch clamp studies revealed that serine 1743 solely to indicate this fact.

L-type Ca$^{2+}$ channels are essential for the generation of normal cardiac rhythm, for induction of rhythm propagation through the atrioventricular node and for the contraction of the atrial and ventricular muscles (1–5). L-type Ca$^{2+}$ through the atrioventricular node and for the contraction of the mal cardiac rhythm, for induction of rhythm propagation and distal C termini of the $\alpha_1$ subunit of $\alpha_{1D}$ Ca$^{2+}$ channel. An in vitro PKA kinase assay was performed for the GST fusion proteins, and their phosphorylation was assessed by Western blotting using either anti-PKA substrate or anti-phosphoserine antibodies. Western blotting showed that the N terminus and C terminus were phosphorylated. Serines 1743 and 1816, two PKA consensus sites, were phosphorylated by PKA and identified by mass spectrometry. Site directed mutagenesis and patch clamp studies revealed that serine 1743 solely to indicate this fact.

The modulation of $\alpha_{1C}$ Ca$^{2+}$ channel by cAMP-dependent PKA phosphorylation has been extensively studied, and the C terminus of $\alpha_1$ was identified as the site of the modulation (20–22). Our group was the first to report that 8-bromo-cAMP (8-Br-cAMP), a membrane-permeable cAMP analog, increased $\alpha_{1D}$ Ca$^{2+}$ channel activity using patch clamp studies (2). However, very little is known about potential PKA phosphorylation consensus motifs on the $\alpha_{1D}$ Ca$^{2+}$ channel. We therefore hypothesized that the C terminus of the $\alpha_1$ subunit of the $\alpha_{1D}$ Ca$^{2+}$ channel mediates its modulation by cAMP-dependent PKA pathway.

EXPERIMENTAL PROCEDURES

Subcloning of Intracellular Loops, N Terminus, Proximal or Distal C Terminus of the $\alpha_1$ Subunit of the Rat $\alpha_{1D}$ Ca$^{2+}$ Channel into pGEX-6P-1 Vector—pCMV6b/rat $\alpha_{1D}$ plasmid was kindly provided by Dr. Susumu Seino from Kobe University, Japan. Two sets of primers were used to amplify each of the intracellular loops, proximal, or distal C terminus of $\alpha_1$ subunit of rat $\alpha_{1D}$ Ca$^{2+}$ channel. Forward primer had a BamHI site and a reverse primer had a Sall site. Intracellular loop 1: forward: 5’-cgcggatcgggtccttagtggagaattc-3’; reverse: 5’-acgcgtcgacgacaacggacagc-3’. Intracellular loop 2: forward: 5’-cggcggatccttctctgacctg-3’; reverse: 5’-agcggacagcgacgtagcttcgtctgcaacctgtg-3’. Intracellular loop 3: forward: 5’-cgcggatcgggtccttagtggagaattc-3’; reverse: 5’-acgcgtcgacgacaacggacagc-3’. Proximal C terminus: forward: 5’-cgcggatcgggtccttagtggagaattc-3’; reverse: 5’-acgcgtcgacgacaacggacagc-3’. Distal C terminus: forward: 5’-cgcggatcgggtccttagtggagaattc-3’; reverse: 5’-acgcgtcgacgacaacggacagc-3’. The abbreviations used are: SA, sinoatrial; AV, atrioventricular; PKA, cAMP-dependent protein kinase; GST, glutathione S-transferase; WT, wild type; SNS, sympathetic nervous system.

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positive control. Untransformed DH5α E. coli and uninduced cultures of the transformed DH5α E. coli served as a negative control.

**Western Blot with Anti-GST Antibody**—Equal amounts of GST fusion proteins were loaded on 10% SDS-polyacrylamide gel. The samples were transferred to a nitrocellulose membrane, and the membrane was blocked with 5% nonfat dry milk and 3% bovine serum albumin in 0.1% Tween-20 TBS (TBST). The membrane was washed with 0.1% TBST, and was then probed with mouse monoclonal anti-GST at a dilution of 1:1000. The membrane was washed again with 0.1% TBST before being probed with rabbit anti-mouse horseradish peroxidase conjugate antibody at 1:6000. The membrane was washed with 0.1% TBST before development with ECL Plus Western blotting detection system (Amersham Biosciences) and Kodak developer.

**In Vitro Kinase Assay for PKA**—The kinase assay was performed on immobilized GST fusion proteins as described by the manufacturer (PKA assay kit, 17-134, Millipore). Briefly, immobilized GST fusion proteins were washed five times with 1× Tris-buffered saline (TBS). Then 25 μl of kinase assay mixture was added to each tube. The mixture consisted of 5 μl of 5× reaction buffer, 2 ng of recombinant PKA catalytic subunit, 7.5 μl of double distilled water, and diluted 1:1 magnesium/ATP (PKA assay kit, 17–134, Millipore). The reaction was allowed to occur for 30 min at 30 °C in a water bath with shaking. The reaction was stopped by adding 2× SDS loading buffer, and boiling for 5 min. Negative controls consisted of GST fusion proteins subjected to an in vitro kinase assay without the catalytic subunits of PKA.

**Western Blot with Anti-PKA Substrate and Anti-phosphoserine Antibody**—Equal amounts of phosphorylated GST fusion proteins or negative controls of GST fusion proteins, as outlined in the in vitro kinase assay, were loaded on 10% SDS-polyacrylamide gel. The samples were transferred to a nitrocellulose membrane, and the membrane was blocked with 5% nonfat dry milk and 3% bovine serum albumin in 0.1% TBST. The membrane was washed with PBS, and with mouse monoclonal anti-GST antibody at 1:200 for negative controls and at 1:500 for the remaining blot (PhosphoDetect™, 700 μg/ml, Calbiochem) or with mouse monoclonal anti-phosphoserine antibody at 1:500 for negative controls and at 1:1000 for the remaining blot (mouse ascites fluid, Sigma). The membrane was washed with 0.1% TBST before being probed with rabbit anti-mouse

3′, reverse: 5′-acgcgtgcctacaaggtggtgatgcaaa-3′. For the subcloning of the N terminus of α1 subunit of rat α1D Ca2+ channel into pGEX 6P-1 vector, the forward primer had a BamHI site, and the reverse primer had an EcoRI site. Primers: forward: 5′-ccggaaacgcgtcgacctacaaggtggtgatgcaaa-3′; reverse: 5′-ccggaaacgcgtcgacctacaaggtggtgatgcaaa-3′.

All amplified fragments were then subcloned into a glutathione S-transferase (GST) bacterial expression vector pGEX-6P-1. The DH5α bacterial strain of Escherichia coli was transformed with the recombinant pGEX-6P-1 vectors, and sequencing of clones with recombinant vectors were confirmed by Genemed (South San Francisco, CA).

**Expression of GST Fusion Proteins of Intracellular Loops, N Terminus, Proximal and Distal C Terminus of α1 Subunit of Rat α1D Ca2+ Channel**—GST fusion proteins were expressed under the induction of the lactose analog isopropyl β-D-thiogalactoside as described before (Handbook of GST Gene Fusion System, 18-1157-58, Amersham Biosciences). Purification of the fusion proteins was performed as previously described (23). All samples were run on 12% Bis-acrylamide gel under reducing conditions and the gel was stained with Coomassie Blue. To check the success of the isopropyl-1-thio-β-D-galactopyranoside induction, DH5α transformed with pGEX-6P-1 served as a

**FIGURE 1. Generation of GST fusion proteins of intracellular loops, N terminus and C terminus of the α1 subunit of α1D Ca2+ channel.** A, a sketch of the α1 subunit of α1D Ca2+ channel with the four domains, N terminus, proximal and distal C terminus. B, PKA consensus sites as determined by scansite on intracellular loops, proximal and distal C terminus of the α1 subunit of α1D Ca2+ channel. IC Loop, intracellular loop.
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Site-directed Mutagenesis—Site-directed mutagenesis was performed on pCMV6b/rat α1D using QuikChange™ site-directed mutagenesis kit from Stratagene (La Jolla, CA) according to the manufacturer’s instructions. Serine residues were replaced by alanine in all substitution mutants. The following mutations were introduced by mutagenic primers: S1743A, S1816A, and S1964A. The presence of mutations was confirmed by sequencing at the Laval University sequencing facility (Quebec, Canada).

Transfection of the tsA201 Cell Line—Mammalian tsA201 cells were grown in high glucose Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1-glutamine (2 mM), penicillin G (100 units/ml), and streptomycin (10 mg/ml, Invitrogen) from our laboratory. Cells were incubated in 5% CO2, humidified atmosphere. The tsA201 cells were transfected using the Ca2+ phosphate method with the following modification: to identify transfected cells, 7 μg of EBO/CD8 plasmid was cotransfected with 7 μg of each of the subunits of α1D, Ca2+ channel, α1, β, and α1/δ cDNAs. For patch clamp experiments, 2–3 days post-transfection cells were incubated for 2 min in a medium containing anti-CD8a-coated beads (Dynabeads, M-450 CD8a). The unattached beads were removed by washing with extracellular solution (in mM): 135 choline chloride, 1 MgCl2, 2 CaCl2, and 10 HEPES; adjusted to pH 7.4 with tetraethylammonium hydroxide (TEA-OH). Cells expressing CD8a and therefore binding beads were distinguished from non-transfected cells by light microscopy.

Electrophysiology—Ca2+ currents were recorded in whole cell configuration of the patch clamp technique (pClamp 9, Axon Instrument) (2, 24). The internal solution contained (in mM): 135 CsCl, 4 MgCl2, 4 ATP, 10 HEPES, 10 EGTA, and 1 EDTA; adjusted to pH 7.2 with TEA-OH. Data were digitized at 5 kHz with an analog-to-digital converter. The recordings were filtered with a low-pass corner frequency of 2 kHz. For the time course, α1D, Ca2+ current was continuously recorded at a test potential of 10 mV from a holding potential of 100 mV.

Statistics—Data were expressed as means ± S.E. Percent of inhibition was calculated as difference of the current amplitude by the intervention over the control value. When indicated,
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such a way that the GST tag was on the N terminus of different fragments of the α₁ subunit of the α₁D Ca²⁺ channel protein (Fig. 1B). GST fusion proteins spanning the intracellular loops, N terminal and C terminus of the α₁ subunit of α₁D Ca²⁺ channel were then generated (Fig. 2A). The expected size of GST fusion proteins of N terminus, proximal and distal C terminus is 43, 69, and 68 kDa, respectively. The expected size of GST fusion proteins of intracellular loops 1, 2, and 3, is 49, 44, and 36 kDa, respectively. Coomassie Blue staining showed that GST fusion proteins of N terminus, proximal and distal C terminus were migrating at higher than 40 kDa on polyacrylamide gel (Fig. 2A). Coomassie Blue stained gels also showed that GST fusion proteins of intracellular loops 1, 2, and 3 were migrating at 49, close to 49 and 36 kDa, respectively (Fig. 2A). Western blots showed the expression of GST fusion proteins of N terminus, proximal and distal C terminus, and intracellular loops (Fig. 2, B–D, respectively).

**GST Fusion Proteins Were Phosphorylated**—After successfully generating the GST fusion proteins of intracellular loops and the N terminus and C terminus of the α₁ subunit of α₁D Ca²⁺ channel, these fusion proteins were subjected to an in vitro PKA kinase assay followed by Western blot to determine which of the PKA consensus sites is phosphorylated by PKA. The phosphorylation status of the GST fusion proteins was tested by two antibodies. The first antibody was an anti-PKA substrate antibody that is capable of detecting phosphorylated serines only in a PKA consensus site (arginine-arginine-x-serine, where x can be any amino acid). The second antibody was an anti-phosphoserine antibody that can detect phosphorylated serines regardless of the consensus site.

**RESULTS**

**Generation of GST Fusion Proteins**—Scansite showed that the α₁ subunit of the α₁D Ca²⁺ channel has a number of PKA consensus sites, mainly on the C terminus (Fig. 1A). To identify which of the potential PKA consensus sites is phosphorylated by PKA, constructs of GST fusion protein were designed in such a way that the GST tag was on the N terminus of different fragments of the α₁ subunit of the α₁D Ca²⁺ channel protein (Fig. 1B). GST fusion proteins spanning the intracellular loops, N terminal and C terminus of the α₁ subunit of α₁D Ca²⁺ channel were then generated (Fig. 2A). The expected size of GST fusion proteins of N terminus, proximal and distal C terminus is 43, 69, and 68 kDa, respectively. The expected size of GST fusion proteins of intracellular loops 1, 2, and 3, is 49, 44, and 36 kDa, respectively. Coomassie Blue staining showed that GST fusion proteins of N terminus, proximal and distal C terminus were migrating at higher than 40 kDa on polyacrylamide gel (Fig. 2A). Coomassie Blue stained gels also showed that GST fusion proteins of intracellular loops 1, 2, and 3 were migrating at 49, close to 49 and 36 kDa, respectively (Fig. 2A). Western blots showed the expression of GST fusion proteins of N terminus, proximal and distal C terminus, and intracellular loops (Fig. 2, B–D, respectively).

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Phosphorylated Sites on Proximal C-terminus of α1D Ca Channel by PKA

A

1509 DN FYLTRGLDD YGPHILHDET KRISWSEYDPKA KGRKLEKELVV TLLEIKQPL 1560
1561 QGKLCIRHRV ACKDVDANYM FAPDQGTVMP KAIIFLAVYY ALIKXKGELDLLQKEKSLAV 1520
1623 TPKINNKKQEM KKDQVYPPA GGDSDTVGKF YATLPQIDYPY FFQKPRKREGG UYCKYPAMNT 1680
1681 TIALQAGLRT LHSIGIEKKR AICSIQNQGD PFDKQPEKKVF PKRNGAGLGF YSVKVRN ND 1740
1741 HFDCLQKTTT HRLP VQDQPS IPAPMNDEKP LFPPAGNSVC EHHNNHNYG KGVPSTMBAN 1800
1801 INHANKSFAK XGRFDIGIDL HIWSFVHWS YKSIDRELQKR SSTKRAFLYY TISRSEQSEF 1860
1861 QL.

B

Mass Spectrum of Peptide containing Phosphorylated Serine 1743

C

Mass Spectrum of Peptide containing Phosphorylated Serine 1743

D

No Phosphorylated Sites on GST by PKA

FIGURE 4. Phosphorylation of serine 1743 and serine 1816 identified by mass spectrometry on the proximal C terminus of the α1D subunit of α1D Ca\textsuperscript{2+} channel. Trypsinized proximal C terminus of the α1D subunit of α1D Ca\textsuperscript{2+} channel was subjected to mass spectrometry. A, amino acid sequence of proximal C terminus of α1D subunit of α1D Ca\textsuperscript{2+} channel with phosphoroloyed amino acid residues. Five sites were found to be phosphorylated by PKA (amino acids in red). Mass spectrometry covered 82% of the proximal C terminus of the α1D subunit of α1D Ca\textsuperscript{2+} channel. B and C, mass spectra for peptides containing serines 1716 and 1743, respectively. D, amino acid sequence of GST. No phosphorylated sites were detected on GST. Mass spectrometry covered 92% of the C-terminal results. In contrast, the intracellular loops of the α1D subunit of α1D Ca\textsuperscript{2+} channel were found to be minimally phosphorylated, both with the anti-PKA substrate and with the anti-phosphoserine antibodies (Fig. 3, C and D), while GST protein (negative control) was not found to be phosphorylated (Fig. 3, E and F).

Mass Spectrometry Identified Phosphorylated Sites on GST Fusion Proteins—To identify the phosphorylated PKA consensus sites on the α1D subunit of α1D Ca\textsuperscript{2+} channel, mass spectrometry was performed on GST fusion proteins phosphorylated by PKA. The phosphorylated GST fusion proteins were run on a polyacrylamide gel, stained with Coomassie Blue, and the corresponding bands were excised. We performed an in-gel digestion with trypsin and chymotrypsin. Mass spectrometry identified two PKA consensus sites phosphorylated on the proximal C terminus of the α1D subunit of the α1D Ca\textsuperscript{2+} channel: serines 1743 and 1816 (Fig. 4, A–C). Mass spectrometry identified three more non-PKA consensus sites not identified by scansite and these were: serine 1703, serine 1788, and threonine 1795 (Fig. 4A). Mass spectrometric analysis did not detect any phosphorylated residues on GST (Fig. 4D) in agreement with our prior findings (Fig. 3, E and F).

The same approach applied for the proximal C terminus (mass spectrometry), we found that serine 1964 was phosphorylated. This residue is a PKA consensus site, phosphorylated in trypsinized digests of the distal C terminus of the α1D subunit of α1D Ca\textsuperscript{2+} channel. In addition, from our mass spectrometry studies, we identified a number of non-PKA consensus sites that were phosphorylated on the N terminus and distal C terminus of the α1D subunit of the α1D Ca\textsuperscript{2+} channel. These included serines 1944, 2152, 2165, and threonine 2147 phosphorylated on the distal C terminus (Fig. 5A). Likewise, we found that serines 45, 46, 52, 121, and threonine 49 were all phosphorylated on the N terminus (Fig. 5B). These findings suggest that there are non-PKA consensus sites that can be phosphorylated by PKA on the N terminus and distal C terminus of the α1D subunit of the α1D Ca\textsuperscript{2+} channel.

We also found non-PKA consensus sites on intracellular loop 1 of the α1D subunit of the α1D Ca\textsuperscript{2+} channel (Fig. 5C). On the other hand, we did not detect any phosphorylated residues on double digests of the other two loops. However, we identified phosphorylated non-PKA consensus sites on trypsinized digests of intracellular loop 2 of the α1D subunit of α1D Ca\textsuperscript{2+} channel. The phosphorylated residues on intracellular loop 1 were: serines 517, 519 and threonines 443, 504. For trypsinized digests of intracellular loop 2 the phosphorylated amino acid residues were: serines 857, 923, 929, and threonines 863.

Negative Controls of Intracellular Loop 1, N Terminus, Proximal and Distal C Terminus Were Not Phosphorylated—To test the antibodies and the mass spectrometric approaches, we performed an in vitro kinase assay without the catalytic subunit of PKA. No phosphorylated peptides were detected by Western blot (Fig. 6) nor by mass spectrometry.

Site-directed Mutagenesis and Patch Clamp Studies Showed That Serine 1816 Is Functionally Important—Of the nine PKA sites identified by scansite, 3 were found to be phosphorylated...
Phosphorylation Sites on N-terminal and distal C terminus of α1D Ca2+ channel by PKA

We then measured the basal current of these cells with or without the presence of 50 μM 8-Br-cAMP, which activates PKA in tsA201 cells (2). To minimize the variation of the current measurements, all current comparisons were made under similar conditions, which included using the same set of tsA201 cells, same amount of plasmids and same post transfection recording time.

The first deletion mutant with a stop codon at amino acid residue 1517 of the C terminus of the α1D Ca2+ channel resulted in the abolition of α1D ICa-L (data not shown). The second deletion mutant had a stop codon at amino acid residue 1917 of the C terminus of α1D Ca2+ channel. Cells expressing this deletion mutant showed an increase of ICa-L in response to 50 μM 8-Br-cAMP similar to that observed in cells expressing the wild-type (WT) α1D channel. These findings further support the unique importance of the proximal region of the C terminus of α1D Ca2+ channel.

We finally examined the modulation of single site mutants in α1D Ca2+ channel with 8-Br-cAMP (3). While treatment of tsA201 cells expressing WT α1D Ca2+ channel with 8-Br-cAMP caused a significant increase in ICa-L (Fig. 7A), substitution of serine 1743 by alanine did not show any alteration in 8-Br-cAMP-induced current increase (10.70 ± 3.3%) (Fig. 7C). Substitution of serine 1816 by alanine did not show any alteration in 8-Br-cAMP-induced current increase (10.70 ± 3.3%) (Fig. 7D).

**DISCUSSION**

Here we provide a systematic biochemical and proteomic approach for identifying phosphorylated PKA consensus sites on the α1D subunit of the α1D Ca2+ channel. We have identified serines 1743 and 1816 as two major functional PKA consensus sites. We also showed that serine 1816 has a major functional role in the α1D Ca2+ channel response to PKA as compared with serine 1743.

We also showed that the distal C terminus is phosphorylated by PKA, but no functional site was identified. We found that serine 1964 by alanine did not show any alteration in 8-Br-cAMP-induced enhancement of ICa-L (data not shown). Averaged data for WT α1DCa2−1D/Ca2−1D/S1743A and α1D/S1816A are shown in Fig. 7D.
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FIGURE 7. α1D/S1743A and α1D/S1816A mutants had a decreased response to 8-Br-cAMP in tsA201 cells. Time course for the effect of 8-Br-cAMP (50 μM) on I_{Ca-L} of wild-type α1D Ca^{2+} channel, α1D/S1743A and α1D/S1816A expressed in tsA201 cell, respectively. A, 8-Br-cAMP increased I_{Ca-L} of wild-type α1D Ca^{2+} channel. B, response to 8-Br-cAMP was decreased in mutant α1D/S1743A Ca^{2+} channel. C, response to 8-Br-cAMP was almost completely abolished in mutant α1D/S1816A Ca^{2+} channel. D, averaged data of the increase of α1D I_{Ca-L} by 8-Br-cAMP (50 μM) in tsA201 cells. Differences were deemed significant at a p value of < 0.05.

alanine the response of the α1D Ca^{2+} channel to 8-Br-cAMP was almost abolished.

The weak phosphorylation seen for intracellular loop 3 on Western blots can be accounted for by the presence of serines 1263 and 1264, two non-PKA consensus sites that were not detected by mass spectrometry. This occurred because treatment of the intracellular loop 3 peptide with trypsin or chymotrypsin generates small peptides that are of low mass to be detected. For example, trypsin digestion of the intracellular loop 3 peptide results in a small peptide, KFWYVVNSS, which has a molecular mass close to the detection threshold for mass spectrometry. This occurred because treatment of the intracellular loop 3 peptide with trypsin or chymotrypsin generates small peptides that are of low mass to be detected. For example, trypsin digestion of the intracellular loop 3 peptide results in a small peptide, KFWYVVNSS, which has a molecular mass close to the detection threshold for mass spectrometry. Chymotrypsin generates peptides that are even smaller than the tryptic peptides.

Physiological Significance—The α1D Ca^{2+} channel has a more negative activation voltage (between −60 to −40 mV) than the α1C Ca^{2+} channel. This enables the channel to play an important role in phase 4 diastolic depolarization of the pacemaker (12, 15, 19, 25, 26). The restricted expression of the α1D Ca^{2+} channel to atria, SA, and AV nodes supports such a notion (2, 3, 10). Sinus bradycardia and second degree AV block seen in the α1D Ca^{2+} channel knock-out mice, shows that deletion of the α1D Ca^{2+} channel seems to affect heart rate and conduction (12, 16, 17, 19). Furthermore, the α1D Ca^{2+} channel knock-out mice were prone to atrial fibrillation (12, 16, 17, 19).

The phosphorylation process constitutes one of the major regulatory pathways for cardiac L-type Ca^{2+} channels. Because the SA node is heavily innervated by the sympathetic nervous system (SNS) (29), stimulation of the SNS in response to exercise or stress insults results in a rapid and dramatic increase in heart rate. CAMP-dependent PKA is the point of convergence for the regulation of Ca^{2+} channels by many neurotransmitters and hormone receptors including the β1- and β2-adrenergic receptors and the serotonergic 5-HT{sub 4} receptor, all of which are present in atrial cells (27, 28). PKA is a serine/threonine kinase that can be stimulated by extracellular signals that elevate intracellular cAMP concentrations which, in turn, can induce phosphorylation of the α1D L-type Ca^{2+} channel, leading to increased channel activity (30).

Identifying consensus PKA phosphorylation sites α1D L-type Ca^{2+} channel is important to the understanding of cardiac rhythm regulation by the SNS.

A—In summary, the present work establishes that serines 1743 and 1816, two PKA consensus sites, are phosphorylated by PKA and are functionally relevant. The present data are the first to identify serine 1816 as a PKA consensus site that can be phosphorylated by PKA and is functionally relevant. Not all PKA consensus sites are functionally relevant, i.e. phosphorylation of these sites does not alter the activity of α1D Ca^{2+} channel in response to PKA activation and phosphorylation.

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