Phosphorylation and Putative ER Retention Signals Are Required for Protein Kinase A–Mediated Potentiation of Cardiac Sodium Current

Jingsong Zhou, Hyeon-Gyu Shin, Jianxun Yi, Wangzhen Shen, Christine P. Williams, Katherine T. Murray

Abstract—Activation of protein kinase A (PKA) increases Na\(^+\) current derived from the human cardiac Na\(^+\) channel, hH1, in a slow, nonsaturable manner. This effect is prevented by compounds that disrupt plasma membrane recycling, implying enhanced trafficking of channels to the cell membrane as the mechanism responsible for Na\(^+\) current potentiation. To investigate the molecular basis of this effect, preferred consensus sites (serines 483, 571, and 593) and alternative sites phosphorylated by PKA in the rat heart isoform (serines 525 and 528) were removed in the I-II interdomain linker, a region in the channel previously implicated in the PKA response. Our results demonstrate that the presence of either serine 525 or 528 is required for Na\(^+\) current potentiation. The role of amino acid sequences that can mediate channel-protein interactions was also examined. Removal of a PDZ domain-binding motif at the carboxy terminus of H1 did not alter the PKA response. The I-II interdomain linker of the channel contains 3 sites (479RRKR481, 533RRR535, and 659RQR661) with the sequence RXR, a motif known to mediate retention of proteins in the endoplasmic reticulum (ER). The PKA-mediated increase in Na\(^+\) current was abolished when all 3 sites were eliminated, with RRR at position 533 to 535 primarily responsible for this effect. These results demonstrate that both α-subunit phosphorylation and the presence of putative ER retention signals are required for the PKA-mediated increase in cardiac Na\(^+\) current, an effect that likely involves interaction of the I-II interdomain linker with other proteins or regions of the channel. (Circ Res. 2002;91:540–546.)

Key Words: Na\(^+\) channel • protein kinase • heart • protein trafficking

Agonists such as hormones and neurotransmitters regulate the activity of plasma membrane proteins by activating protein kinases, which phosphorylate target proteins to modify their function. In addition, there is increasing evidence that receptor activation of cellular signaling systems can modulate membrane proteins by regulating their trafficking into and out of the plasma membrane.\(^1\) One of the best-studied examples of this phenomenon is the response of the glucose transporter GLUT4 to insulin signaling.\(^2\) With agonist stimulation, tyrosine phosphorylation of the insulin receptor triggers a chain of intracellular events leading to recruitment of GLUT4 from intracellular stores to the plasma membrane to effect glucose transport. Trafficking of both presynaptic serotonin transporters and the γ-aminobutyric acid (GABA) transporter GAT1 is altered by protein kinase C (PKC) activation, with relocation of transporters from the plasma membrane into intracellular compartments.\(^3\) The cystic fibrosis transmembrane conductance regulator CFTR has been shown to rapidly redistribute from the cytoplasm to the cell surface after cAMP stimulation.\(^6\) It is likely that other ion channels are subjected to similar regulation, although little information is available regarding this, or the process of channel trafficking in general.

In the heart, voltage-gated Na\(^+\) channels are critical for the normal conduction of electrical impulses,\(^7\) whereas dysfunction of these channels can lead to the generation of life-threatening cardiac arrhythmias in humans.\(^8\) For these reasons, modulation of Na\(^+\) channel activity at the plasma membrane can have important consequences from both a physiological and pathophysiological standpoint. Results of previous studies in mammalian cardiac myocytes examining the effects of β-adrenergic stimulation, which activates cAMP-dependent protein kinase (PKA), on Na\(^+\) current (\(I_{\text{Na}}\)) have been controversial, with conflicting results.\(^10\)–\(^12\) Both an increase and decrease in Na\(^+\) current have been observed, with variable shifts in the voltage dependence of channel gating. However, more recent data obtained in myocytes\(^12\)–\(^13\) and with recombinant channels\(^14\)–\(^16\) have demonstrated con-
sistent enhancement in \( I_{\text{Na}} \). Using the recombinant human cardiac Na\(^+\) channel, hH1, we recently reported that activation of PKA caused a slow increase in cardiac Na\(^+\) current that was nonsaturable over nearly 1 hour, with a concomitant shift in the voltage dependence of channel activation and inactivation to more negative voltages.\(^{17}\) Preincubation of cells with compounds that disrupt plasma membrane recycling (chloroquine or monensin) prevented the PKA-mediated rise in \( I_{\text{Na}} \), indicating that this effect likely resulted from an increase in the number of Na\(^+\) channels at the plasma membrane. Chimeric constructs of hH1 and the skeletal muscle Na\(^+\) channel, hSKM1 (a channel that lacks the PKA response), were used to identify the I-II interdomain linker as a region in hH1 required for this effect.

In this study, we have investigated further the molecular basis of PKA-mediated potentiation of cardiac Na\(^+\) current. Given that the I-II interdomain linker is required, we hypothesized that phosphorylation of one or more sites in this cytoplasmic region is essential for the effect. Therefore, we examined the role of favored consensus sequences for PKA, as well as sites (serine 525 and 528) known to be phosphorylated in the conserved rat heart isoform. In addition, considerable evidence indicates that the targeting or trafficking of ion channels within a cell is frequently mediated by interaction with other proteins through specific amino acid motifs present in the channel sequence. Based on recent evidence for other channels,\(^{18–20}\) we also sought to determine whether the carboxy (C) terminus, which contains a PDZ domain-binding motif, and putative ER retention signals in the I-II interdomain linker are required for the PKA response.

### Materials and Methods

#### Materials

Reagent grade chemicals, as well as 8-chlorophenylthio cAMP (8-cpt-cAMP), 3-isobutyl-1-methylxanthine (IBMX), and forskolin were obtained from Sigma. Enzymes and buffers were purchased from Roche and Promega. Both IBMX and forskolin were dissolved in DMSO to generate 400 and 20 mmol/L stock solutions, respectively. Reagent grade chemicals, as well as 8-chlorophenylthio cAMP (8-cpt-cAMP) was generated by inserting a premature stop codon in the protein syntrophin.\(^{24}\) Therefore, to eliminate this site in hH1, a C-terminal deletion mutant lacking the final 10 amino acids (SPDRDRESIV) was generated by inserting a premature stop codon using PCR.

#### Electrophysiological Recording and Data Analysis

Na\(^+\) current recordings were performed using the 2-microelectrode voltage clamp technique as previously described.\(^{17}\) Pipettes were filled with 3 mol/L KCl, and a standard extracellular bath solution was used (in mmol/L: NaCl 96, KCl 2, CaCl\(_2\) 1.8, MgCl\(_2\) 1, HEPES 5; pH 7.5). Oocytes that demonstrated endogenous, voltage-activated currents (eg, Ca\(^{2+}\)-dependent Cl\(^{-}\) current)\(^{2}\) greater than 1% of expressed currents were not utilized. The holding potential was \(-120\) mV and the cycle time for all pulse protocols was 1 second or slower to allow full recovery from inactivation between pulses, unless otherwise specified. Cell membrane electrical capacitance was calculated as described.\(^{17}\) All experiments were conducted at room temperature (22±2°C).

Analysis of data was performed using custom programs that were designed to read and analyze pClamp data files. The reversal potential was estimated using a linear fit to the ascending limb of the current-voltage relationship to construct activation curves. Activation and inactivation curves were fitted with a Boltzmann equation. Comparison of Na\(^+\) current properties between mutant and wild-type channels was performed using an independent sample \( t \) test, whereas values before and after PKA stimulation were analyzed using a paired \( t \) test. The effect of PKA on normalized \( I_{\text{Na}} \) derived from different constructs was compared using 1-way analysis of variance (ANOVA), with the Tukey and Bonferroni multiple comparison procedures. Results are presented as mean±SEM.

#### Mutagenesis

Amino acids in the sequence of the I-II interdomain linker of hH1 were replaced as described in the Results using overlap extension polymerase chain reaction (PCR) mutagenesis.\(^{23}\) The mutant PCR products were ligated into the TOPO TA cloning vector (Invitrogen) for sequencing. Polymerase error-free mutagenic segments were reinserted into the original hH1 construct using restriction enzyme sites. For a given mutation, multiple clones were sequenced and used for experimentation. The extreme C-terminus of hH1 contains a sequence motif predicted to bind PDZ domains in other proteins.\(^{18}\) For both cardiac and skeletal muscle channels, a C-terminal peptide 10 amino acids in length is sufficient for binding to a PDZ domain in the protein syntrophin.\(^{24}\) Therefore, to eliminate this site in hH1, a C-terminal deletion mutant lacking the final 10 amino acids (SPDRDRESIV) was generated by inserting a premature stop codon using PCR.

#### Results

**Preferred Consensus PKA Sites in the I-II Interdomain Linker**

Inspection of the amino acid sequence in the I-II interdomain linker reveals the presence of 13 consensus sites for phosphorylation of a serine or threonine by PKA (indicated by the bold, underlined residues in Figure 1), as defined by the sequence (R/K)\(_2\)(S/T).\(^{25}\) Previous studies have shown that the sequence (R/K)\(_2\)X(S/T) is a preferred phosphoacceptor site for PKA compared with other sequence possibilities.\(^{25}\) Three sites are present in the I-II interdomain linker of hH1 having this preferred or strong consensus sequence, represented by serines 483, 571, and 593 (designated by the crosses in Figure 1). Therefore, we initially eliminated these putative PKA sites in hH1 by mutating all 3 serines to alanines (S483/571/593A). Heterologous expression of the mutant construct resulted in Na\(^+\) currents that were essentially identical to wild-type hH1 currents with respect to kinetics and voltage dependence of channel gating (Figure 2A, and online Table found in the online data supplement available at http://www.circresaha.org). On bath superfusion with 8-cpt-cAMP, IBMX, and forskolin (a combination of

---

**Zhou et al Potentiation of Cardiac Sodium Current 541**

---

Downloaded from http://circ.ahajournals.org/ by guest on July 10, 2017
Figure 1. Sequence of the I-II interdomain linker of the rat (rH1) and human (hH1) cardiac Na\(^+\) channels. Consensus sites for phosphorylation by PKA having the motif (R/K)\(_2\)(S/T) are indicated by the bold, underlined amino acids for S/T. Preferred consensus PKA sites of the (R/K)\(_2\)(X)\(_2\)(S/T) sequence are indicated by the solid box above the motif. RXR sites are designated by the asterisks. RXR sites are previously observed for hH1. These results indicate that phosphorylation sites, including 2 sites in the I-II interdomain linker, are not required for the effect of PKA to modulate either current amplitude or channel gating.

**Figure 2.** Effect of PKA activation on Na\(^+\) currents derived from the S483/571/593A hH1 mutant channel. A, Na\(^+\) currents are shown under control conditions (left) and after bath superfusion of PKA activators (right; voltage: 0 to 100 mV). B, Using the voltage clamp protocol described in A, peak Na\(^+\) currents are plotted as a function of the test potential under control conditions (●) and after PKA stimulation (○). C, Time course of effect of PKA activators on peak Na\(^+\) current (●; at −20 mV) and cell membrane capacitance (○) is illustrated. Average data are expressed normalized to predrug values. Time 0 (arrow) indicates the start of drug superfusion. D, Activation curves on the right (generated from the data in B), and inactivation curves on the left (generated by 100 ms prepulses from 10 to 20 mV) are shown for each condition (● and ○). The data are expressed as a percentage of control values. 

**Role of Serines 525 and 528**

It has been previously demonstrated that PKA stimulation enhanced Na\(^+\) current derived from the rat heart channel (hH1), with a slow time course similar to that seen for hH1. This effect persisted despite removal of multiple potential phosphorylation sites, including 2 sites in the I-II interdomain linker. Subsequent work by Murphy and colleagues demo-
strated that rH1 is selectively phosphorylated in vitro and in vivo at two sites, serine 526 and 529 in the I-II interdomain linker27 (neither of which was removed in the previous study). Having the sequence motif RX(S/T), these 2 PKA consensus sites are conserved in hH1 as serines 525 and 528 (indicated by the asterisks in Figure 1), although the functional significance of phosphorylation at these locations has not been previously determined. To investigate the role of these sites in potentiation of hH1 current by PKA, another mutant construct was generated in which both sites were eliminated by alanine replacement (S525/528A). Na\textsubscript{\textit{v}} currents derived from expression of this channel were similar to wild-type currents (Figure 3A and online Table), except for slight slowing of recovery from inactivation (τ = 5.1±0.1 ms versus 4.7±0.1 ms for hH1; P<0.05). However, PKA stimulation did not increase I\textsubscript{\textit{Na}} for this mutant (−9±5% in 55 minutes; Figures 3A, 3B, and 4A), indicating that 1 or both of these 2 residues is required for the PKA response, presumably due to phosphorylation. In addition, the shift in the voltage dependence of channel activation and inactivation to more negative potentials that typically occurs with PKA stimulation was also lost (Figure 3C and Table), suggesting that phosphorylation in this region can modify channel gating.

To determine which of these residues is required for the PKA response, additional mutant channels were generated in which serine 525 or serine 528 was individually eliminated (S525A, S528A; online Table). For both constructs, Na\textsuperscript{+} currents increased in response to PKA stimulation (+27±5% and +20±5% for S525A and S528A, respectively, in 55 minutes; Figure 4A and Table), with an effect similar in magnitude to that for wild-type hH1. Thus, the presence of either serine 525 or 528 alone can enable the PKA response. With respect to activation and inactivation curves for the single mutation channels, in general PKA stimulation had little effect, although this was not always the case (Table). These results demonstrate that phosphorylation at serines 525 and 528 can modulate channel gating, although these subtle effects are complex and not dependent on a single residue. Often, the effects of phosphorylation can be mimicked by the substitution of a negatively charged amino acid for the involved serine/threonine. If serines 525 and 528 are the only substrates modified by PKA to increase hH1 current, negative charge substitution at these positions should mimic constitutively-active stimulation with maximal current potentiation, and therefore loss of the invoked PKA response. However, when both serines were replaced with a glutamate (S525/528E; online Table), Na\textsuperscript{+} currents increased in a wild-type manner following PKA activation (+24±8% in 55 minutes, Figure 4A and Table). These results demonstrate that the effect of PKA stimulation to increase cardiac I\textsubscript{Na}
requires at least 2 processes: phosphorylation of consensus sites in the I-II interdomain linker, and one or more additional molecular events mediated by the kinase, that could include phosphorylation of other substrates/proteins.

C-Terminus of hH1

Like brain and skeletal muscle channels, cardiac Na\(^+\) channels including hH1 have a highly conserved motif (R[K/Q][E|R][S/T][A/V]) at the C-terminus predicted to bind PDZ domains in other proteins.\(^{18}\) Indeed, Na\(^+\) channels with these sequences have been shown to bind the PDZ domain of syntrophin, a cytoplasmic protein that is part of the dystrophin-associated protein complex.\(^{24,28}\) Given that a number of ion channels are targeted to certain membrane sites by proteins. However, as recently described for assembly of the sequence KKXX, are found only at the extreme C-terminus of hH1.\(^{17}\) It is now recognized that ER retention signals can also play an important role in the trafficking of many plasma membrane proteins.\(^{17}\) Although the time course of Na\(^+\) channels, we hypothesized that this region of the C-terminus might also participate in PKA-regulated enhancement of hH1 current. To test this hypothesis, a C-terminal deletion mutant lacking the terminal PDZ domain binding region was constructed (hH1ΔC). Expression of this mutant generated Na\(^+\) currents that resembled wild type, although the time course of Na\(^+\) current inactivation was slowed (online Table; \(\tau\) at \(-20\ mV\)=1.7±0.1 ms versus 1.2±0.1 ms; \(P<0.05\)) and the inactivation curve was shifted by \(-5\ mV\) (\(V_{1/2}\)=-69±1 mV versus \(-64±1\ mV\); \(P<0.05\) compared with hH1). The PKA response of Na\(^+\) current derived from hH1ΔC (+36±8%; Figure 4B) was not statistically different from that seen with hH1 (+26±5%). Thus, whereas the C-terminus of hH1 may participate in targeting of the channel within the cell, its presence is not required to increase hH1 current amplitude on PKA stimulation.

ER Retention Signals

It is now recognized that ER retention signals can also play an important role in the trafficking of many plasma membrane proteins to the cell surface.\(^{19,20,29,30}\) These amino acid sequences function to retain newly formed proteins in the ER, probably by binding to resident chaperone proteins. This “brake” in expression can be relieved by binding of another protein to mask the signal, permitting exit from the ER and trafficking of the protein complex to the plasma membrane. For some ion channels, binding of channel subunits to these sites is a mechanism to facilitate channel expression.\(^{19,20}\) A number of ER retention signals, including the classical sequence KKKX, are found only at the extreme C-terminus of proteins. However, as recently described for assembly of the ATP-sensitive K\(^+\) channel, the RXR motif can be located in other cytoplasmic regions of a protein. Inspection of the hH1 amino acid sequence reveals the presence of 6 RXR sites, 3 of which are located in the I-II interdomain linker (indicated by the solid boxes in Figure 1): RKR at positions 479 to 481, RRR at 533 to 535, and RQR at 659 to 661. To investigate the role of these sites in the PKA-mediated enhancement in \(I_{\text{Na}}\), they were removed by mutating the 3 amino acids to alanines (AAA; online Table).\(^{19}\) On elimination of all 3 sites (RXR-AAA), \(I_{\text{Na}}\) was unchanged after PKA activation (−4±10%). For the 533RRR-AAA535 individual mutation, the effect of PKA to increase Na\(^+\) current was markedly reduced (+4±2% in \(I_{\text{Na}}\) at 55 minutes; Figure 4B and Table). For the 479RKR-AAA481 mutant, a wild-type increase in Na\(^+\) current was seen with PKA stimulation (+31±8%, whereas for the 659RQR-AAA661 channel, an intermediate response was observed (+15±5%). Thus, in addition to α-subunit phosphorylation, putative ER retention signals in the I-II interdomain linker are required for PKA to increase cardiac Na\(^+\) current amplitude, with the site at positions 533 to 535 playing a major role. Of note, \(V_{1/2}\) of the inactivation curve for the 533RRR-AAA535 mutant demonstrated a −5-mV shift compared with wild-type hH1 (\(P<0.05\); online Table). In addition, removal of any of the RXR sites eliminated the negative shift in the voltage dependence of channel activation. These findings also suggest a role for residues in the I-II interdomain linker to modulate hH1 gating.

Discussion

For both rat and human recombinant cardiac Na\(^+\) channels, activation of PKA causes a slow increase in Na\(^+\) current amplitude.\(^{14,15,17}\) There is substantial evidence that this effect results from enhanced trafficking of Na\(^+\) channels to the plasma membrane: (1) the time course is considerably slower than an effect to increase ionic current due to altered channel gating;\(^{31}\); (2) for the human channel, the effect of PKA is eliminated by exposure to compounds that disrupt recycling of plasma membrane;\(^{32}\) and (3) in rat cardiac myocytes, the PKA-mediated increase in \(I_{\text{Na}}\) is accompanied by an increase in channel number by single-channel analysis.\(^{32}\) Our results demonstrate that for hH1, PKA-mediated potentiation of \(I_{\text{Na}}\) is a complex process that requires at least 2 steps: (1) phosphorylation of serine 525 and/or 528 in the I-II interdomain linker; and (2) one or more additional molecular events mediated by the kinase.

Although trafficking of many membrane proteins can be regulated by activation of protein kinases, the structural requirements for this process are largely unknown and only now being elucidated. Ligand occupancy can influence internalization of serotonin transporters in response to PKC activation, an effect associated with phosphorylation of the transporter.\(^{3}\) For the GABA transporter GAT1, a leucine heptad repeat sequence (leucine zipper) is involved in the regulated trafficking of this protein in response to PKC activation.\(^{32}\) PKA-mediated phosphorylation of aquaporin 2 is essential for regulated movement of water channels to the plasma membrane on elevation of intracellular cAMP.\(^{33}\) With respect to other voltage-gated ion channels, activation of PKA has been shown to modulate K\(^+\) current derived from the Kv1.1 channel by both enhanced channel synthesis and redistribution of previously synthesized channels to the plasma membrane.\(^{34}\) The latter effect was dependent on phosphorylation of a specific PKA site in the C-terminus of the channel. Similar to Kv1.1, our results indicate that α-subunit phosphorylation of hH1 is a required feature of the PKA response.

For a growing number of proteins, functional activity at the plasma membrane is influenced by the presence of ER retention signals, and our results demonstrate that this is also the case for hH1. For the ATP-sensitive K\(^+\) channel, RXR...
sites are present in Kir6.1, Kir6.2, and SUR1 subunits and play an important role in channel assembly and expression. A similar ER retention signal is present in the GABA<sub>B1</sub> subunit, which is masked on assembly with GABA<sub>B2</sub> subunits to permit expression of functional GABA<sub>B</sub> receptors. Thus, given the evidence to date with other proteins, involvement of these sites in the PKA response for hH1 provides further support that kinase activation enhances trafficking of cardiac Na<sup>+</sup> channels to the plasma membrane. Based on our findings, we hypothesize that one or more RXR sites in the I-II interdomain linker mediates interaction of the α-subunit with some peptide, such as another region of the Na<sup>+</sup> channel or an adaptor protein. This interaction can be modified by phosphorylation, possibly to promote assembly of the protein complex, with masking of RXR sites to modulate channel expression. Analogous to our results for hH1, trafficking of NMDA receptors to the cell surface in neurons is controlled by both an RXR motif in the NR1 subunit, as well as phosphorylation of the channel in close proximity to this site. In addition, phosphorylation of the AMPA receptor GluR2 subunit by PKC differentially regulates interaction of the C-terminus with PDZ domains in other proteins (eg, GRIP1 and PICK1), an effect that likely modulates receptor expression during synaptic plasticity.

It is noteworthy that the RXR sequences implicated in hH1 do not function as typical ER retention sites. Removal of such sites should enhance basal protein trafficking to the plasma membrane, with a marked increase in baseline expression. Although our studies were not designed to compare Na<sup>+</sup> channel expression for different constructs at baseline, for at least one mutant (533RRR-AAA535), elimination of an RXR site appeared to reduce Na<sup>+</sup> channel expression, as described in the Materials and Methods. These data suggest that PKA activation may recruit a pool of Na<sup>+</sup> channels to the plasma membrane that is distinct from the ER. Further support for this concept comes from the recent report that in rat ventricular myocytes, caveolae can mediate presentation of Na<sup>+</sup> channels to the plasma membrane in response to β-adrenergic receptor stimulation. Caveolae are small invaginations in the plasma membrane enriched with cholesterol, sphingolipids, and the scaffolding protein caveolin. Caveolins interact with multiple signaling molecules to concentrate a variety of cellular signaling proteins in caveolae, including G-protein α-subunits such as G<sub>α</sub>, β-adrenergic receptors, adenylyl cyclases, and protein kinases including PKA and PKC isoforms. Caveolae also possess the molecular transport machinery required for vesicle formation and can pinch off to form intracellular vesicles. In addition to Na<sup>+</sup> channels, caveolae can medulate activity of water channel proteins and volume-regulated Cl<sup>-</sup> channels at the plasma membrane. Whether activation of β-adrenergic receptors and PKA recruits cardiac Na<sup>+</sup> channels from other intracellular compartments besides caveolae is currently not known.

Based on available data, we hypothesize that the I-II interdomain linker may be involved in regulated trafficking of other Na<sup>+</sup> channel isoforms. Certainly for the rat heart isoform as shown in Figure 1, there is complete conservation of the phosphorylation and RXR sites required in hH1. Unlike Na<sup>+</sup> current in native cardiac myocytes, <i>I<sub>Na</sub></i> in neurons is consistently reduced by PKA activation. With respect to recombinant brain channels, Na<sup>+</sup> current derived from the rat brain (rBIIA) channel is modulated by activation of PKA in a complex manner, resulting in either a decrease or increase in Na<sup>+</sup> current amplitude. The PKA-mediated reduction in Na<sup>+</sup> current involves phosphorylation of specific consensus PKA sites in the I-II interdomain linker. Removal of these sites leads to Na<sup>+</sup> current potentiation with PKA activation. This increase in Na<sup>+</sup> current is slow and reminiscent of our data with hH1, with a continual rise in <i>I<sub>Na</sub></i> over a 20-minute interval. Interestingly, as for hH1, the I-II interdomain linker of the rBIIA channel is required for Na<sup>+</sup> current potentiation. However, the specific sites required for the PKA response have not been identified. Rather, potentiation was found to be dependent on regions of sequence found throughout the length of the I-II interdomain linker. These findings suggest at least some degree of conservation in the molecular features required for the PKA response among Na<sup>+</sup> channel isoforms.

In conclusion, we have identified structural features in the human cardiac Na<sup>+</sup> channel protein that are required for PKA-mediated potentiation of Na<sup>+</sup> current, including α-subunit phosphorylation, additional unidentified molecular event(s) resulting from kinase activation, and putative ER retention signals. These studies provide an initial step toward unraveling the molecular mechanisms whereby cells can acutely regulate the number of Na<sup>+</sup> channels in the plasma membrane in response to PKA stimulation.

Acknowledgments

This work was supported by grants from the US Public Health Service (HL55665 and HL46681), the American Heart Association, Tennessee Affiliate, and a postdoctoral fellowship grant from the American Heart Association, Southeast Affiliate (J.Z.). We thank Dr Al George for helpful discussions and critical manuscript review.

References


Phosphorylation and Putative ER Retention Signals Are Required for Protein Kinase A-Mediated Potentiation of Cardiac Sodium Current

Jingsong Zhou, Hyeon-Gyu Shin, Jianxun Yi, Wangzhen Shen, Christine P. Williams and Katherine T. Murray

Circ Res. 2002;91:540-546; originally published online August 15, 2002;
doi: 10.1161/01.RES.0000033598.00903.27

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/91/6/540

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2002/09/14/91.6.540.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/