Phosphorylation of the A-kinase-anchoring Protein Yotiao Contributes to Protein Kinase A Regulation of a Heart Potassium Channel

Lei Chen†, Junko Kurokawa§, and Robert S. Kass¶

From the †Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York 10032 and §Department of Bioinformational Pharmacology, Tokyo Medical and Dental University, Tokyo 101-0062, Japan

Regulation of the heart by the sympathetic nervous system, fundamental to the physiological response to stress and exercise, requires coordinated phosphorylation of multiple downstream molecular targets, including the I_{Ks} (slowly activating potassium current) channel. Sympathetic nervous system stimulation increases intracellular cAMP for which targeted regulation is directed in large part by distinct scaffold or anchoring proteins. Yotiao is an A-kinase-anchoring protein (AKAP) that recruits the cyclic AMP-dependent protein kinase (protein kinase A (PKA)) and protein phosphatase 1 to the carboxyl terminus of the I_{Ks} channel to form a molecular complex and control its phosphorylation state, crucial to the cardiac cellular response to sympathetic nervous system stimulation. Here we report that Yotiao itself is a substrate for PKA phosphorylation, and we identify a Yotiao amino-terminal (N-T) residue (Ser-27) that is PKA-phosphorylated in response to β-adrenergic receptor stimulation. The replacement of Ser-43 by Ala ablates the PKA phosphorylation of N-T Yotiao and markedly diminishes the functional response of the wild type and pseudo-phosphorylated I_{Ks} channel to cAMP but neither prevents the PKA phosphorylation of KCNQ1 nor its binding to Yotiao. These results suggest, for the first time, a critical role for the PKA phosphorylation of an AKAP in the functional regulation of an ion channel protein and postphosphorylation allosteric modulation of the I_{Ks} channel by Yotiao.

The second messenger cAMP transduces extracellular receptor-activated signals into a multitude of intracellular events, many of which are mediated by phosphorylation via cAMP-dependent protein kinase (PKA)1 (1). Intracellular specificity and the distribution of cAMP-dependent regulation are now recognized to be mediated largely by A-kinase-anchoring proteins (AKAPs), which together with specific substrates create macromolecular signaling complexes to control the PKA phosphorylation state of targeted proteins (2–4). AKAPs are a subset of functionally, rather than structurally, related scaffolding proteins. AKAPs form compartmentalized cellular environments by tethering distinct signaling molecules, such as the regulatory subunits PKA and protein phosphatases, and presenting them to select downstream targets (5–7). All AKAPs bind the type II PKA regulatory subunit with high affinity, but all AKAPs do not necessarily complex with protein phosphatases. Unique targeting domains on AKAPs mediate specific protein-protein interaction and ensure target specificity (8). Thus a key function of AKAPs is to fine-tune spatial and temporal regulation of the phosphorylation state of target proteins, particularly when rapid changes in response to physiologically mediated signals is needed (9).

The activity of the cardiac I_{Ks} (slowly activating potassium current) channel, critically important to the regulation of the cardiac action potential duration, particularly in the face of sympathetic nervous system stimulation (10, 11), is regulated by an AKAP-mediated alteration of its PKA phosphorylation state (12). The I_{Ks} channel forms a macromolecular complex consisting of an α subunit (KCNQ1), a regulatory subunit (KCNE1), and the AKAP Yotiao bound to the channel carboxyl-terminal domain (12). Disruption of the complex by mutation can render the channel functionally insensitive to cAMP-dependent regulation (12, 13) and elevates the risk of exercise-induced sudden death in mutation carriers (14–16). PKA phosphorylation of Ser-27 in the KCNQ1 amino terminus (N-T) accounts for most of the functional modulation of I_{Ks} by the sympathetic nervous system (13), and mutation of KCNQ1 residue Ser-27 to Asp (S27D) reconstitutes most, but not all, of the effects of PKA on expressed channel function (17).

Although it is clear that the assembly of this complex is necessary to reconstitute the functional regulation of the I_{Ks} channel by PKA, it is not clear whether phosphorylation of I_{Ks} alone, and not Yotiao as well, is sufficient to account for all of the important PKA-mediated functional changes in I_{Ks} channel activity. We recently reported that interactions between the phosphorylated KCNQ1 amino terminus and Yotiao bound to the KCNQ1 carboxyl terminus are required to alter I_{Ks} channel activity (17). Here we explore the possibility that Yotiao itself is a substrate for PKA phosphorylation and that phosphorylation of this adaptor protein participates in the overall functional response of the I_{Ks} channel complex to PKA. Precedence for this possibility has been established by recent studies that have shown that phosphorylation of the AKAP-Lbc precedes release of protein kinase D and that phosphorylation of the AKAP Gravin may contribute to β-adrenergic receptor resensitization (18, 19). Our results in fact provide evidence for a role of PKA.
phosphorylation of Yotiao in the functional response of the \( I_{Ks} \) channel complex to PKA-mediated phosphorylation and thus further evidence that Yotiao serves, at least in part, as an effector in regulating the activity of this important cardiac ion channel.

**MATERIALS AND METHODS**

**Cell Culture and Transfection**—Chinese hamster ovary (CHO) cells were maintained in Ham’s F12 culture media with 10% fetal bovine serum in a 37 °C incubator with 5% CO\(_2\). CHO cells were transfected with channel subunits, Yotiao and CD8, using Lipofectamine and Plus reagent (Invitrogen) when cells reached 20–30% confluence as described previously (12, 20). Transfected cells were identified by Dynabeads M-450 anti-CD8 beads (Dynal, Oslo, Norway).

**Molecular Biology**—Yotiao fusion protein fragments were generated by PCR and were subcloned into pGEX-4T1 vectors (Amersham Biosciences). Point mutations were introduced into the constructs using QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). All of the constructs were sequenced. GST fusion proteins of Yotiao fragments were then grown in DE3 cells as described previously (21).

**Western Blot, Immunoprecipitation, and Antibodies**—A phospho-specific Yotiao antibody (PYT2K) was raised against a phospho-peptide that encompasses the PKA consensus site at Yotiao N termini (Zymed laboratories). A phospho-specific Yotiao antibody was raised against a recombinant Yotiao fragment (residues 110–448) (Fusion Antibodies, Belfast, Northern Ireland). A phospho-S27 KCNQ1 antibody was raised as described previously (13). A commercial KCNQ1 antibody (C20) (Santa Cruz Biotechnology, Santa Cruz, CA) was used to detect total KCNQ1.

Western blot was done following a routine protocol. Immunoprecipitation experiments were carried out in modified radio-immune precipitation assay buffer (150 mM NaCl, 50 mM Tris, 1 mM EDTA, 0.25 mM Triton X-100, pH 7.5). Protein G beads were used to immobilize the immunocomplex. Immunoprecipitates were boiled in sample buffer and were then size-fractionated by SDS-PAGE.

**Phosphorylation Assays**—Yotiao fragment-GST fusion proteins were immobilized on GST beads. Full-length His-tagged Yotiao was immunoprecipitated with an anti-His antibody. Protein-bound GST beads or immunoprecipitates were washed three times with phosphorylation buffer (8 mM MgCl\(_2\), 10 mM EGTA, and 50 mM Tris/PiPES, pH 6.8) and then incubated with PKA catalytic subunits (5 units). Phosphorylation was initiated at room temperature with 33 \( \mu \)M Mg-ATP containing 10% \([\gamma-32P]\)ATP (PerkinElmer Life Sciences) and terminated after 5 min. Phosphorylation signals were assayed by autoradiography. Phosphorylation of Yotiao or KCNQ1 in intact CHO cells was induced by incubating the cells at 37 °C with 100 \( \mu \)M 8-bromo-cAMP for 15 min or 1 \( \mu \)M isoproterenol for 10 min (for cells co-transfected with \( \beta_1- \) or \( \beta_2\)-adrenergic receptors). 1 \( \mu \)M okadaic acid was always used to preserve phosphorylation. Cells were then lysed in lysis buffer (150 mM NaCl, 10 mM Tris, 1 mM EDTA, 1% Triton X-100, pH 7.4, and Complete protease inhibitor mixture (Roche Applied Science)) at 4 °C. Cell lysates were size-fractionated by SDS-PAGE. Phosphorylated proteins were detected by phospho-specific antibodies.

**Electrophysiology**—Whole-cell recordings using an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA) were performed 2 days after transfection, as described previously (13). Pipette resistance was 2–4 mehmohm. To study the maximal effects of PKA phosphorylation, 200 \( \mu \)M cAMP plus 0.2 \( \mu \)M okadaic acid (OA) (Calbiochem, San Diego, CA) was included in recording pipettes and dialyzed for 13 min before measurements were made. \( I_{Ks} \) (2-s isochronal) activation was determined by an analysis of deactivating tail currents recorded at –40 mV after a series of voltage steps (2-s, 20-mV increments). Tail currents were normalized to the tail current after the +80 mV testing pulse. Activation curves from individual experiments were then generated by plotting the normalized tail currents against test voltages. We fit the 2-s isochronal activation curves with Boltzmann functions to estimate the voltage at which half of the channels are available (\( V_{1/2} \)) providing a means for comparing the effects of Yotiao mutation on the voltage dependence of activation. The activation curves shown in Figs. 3A and 5 were generated with the mean Boltzmann parameters obtained from the individual experiments. Channel deactivation, recorded as current tails at –40 mV (2-s) following +60-mV activating voltage pulses (2-s), was fitted with a single exponential function to extract the deactivation time constant (\( \tau_d \)). Activation kinetics were characterized by the time (\( t_{1/2} \)) to reach the half-maximal current during 2-s activating pulses (+60 mV). Data were acquired and analyzed using pCLAMP 8.0 software (Axon Instruments). Recordings were made at room temperature. Data were shown as mean ± S.E. Statistical tests were carried out using EXCEL (Microsoft). Differences at \( p < 0.05 \) were considered to be significant (indicated by asterisks in Fig. 3, B and C).

**RESULTS**

The \( I_{Ks} \) channel macromolecular complex consists of the \( \alpha \) subunit KCNQ1, the regulatory subunit KCNE1, and the AKAP Yotiao (Fig. 1A) (12). The robust increase in \( I_{Ks} \) channel activity by sympathetic nervous system stimulation can be accounted for largely by PKA-mediated phosphorylation of KCNQ1 N-T Ser-27, but previous studies have not considered the possibility that Yotiao also may be a substrate for PKA phosphorylation. To determine whether Yotiao can be PKA-phosphorylated, His-tagged full-length Yotiao was expressed in...
CHO cells and immunoprecipitated using an anti-His antibody. The immunoprecipitated protein was then incubated with PKA and [32P]ATP. Fig. 1B shows that phosphorylated Yotiao can indeed be detected on autoradiography (lane 1). Negative control experiments (Fig. 1B) with mock immunoprecipitation (no Yotiao lysate (lane 2) or no immunoprecipitate antibody (lane 3)) confirmed the specificity of the autoradiograph and the conclusion that Yotiao can be PKA-phosphorylated. Sequence analysis of Yotiao revealed a PKA substrate consensus site in the Yotiao N-T domain between residues 37 and 48 (KKKRKTSSSKHDV) (Fig. 1C). This region contains two potential PKA phosphorylation substrates: Thr-42 and Ser-43 (Fig. 1C). We repeated the [32P] phosphorylation assay with GST fusion proteins of wild type and mutated Yotiao amino-terminal constructs in which both candidate residues were systematically replaced with alanine residues to identify candidate PKA substrates. Fig. 1C confirms that wild type Yotiao amino terminus, containing the intact PKA consensus site, can be PKA-phosphorylated (Fig. 1D, lane 1) but also shows that Ala substitution of both Thr-42 and Ser-43 ablates this response (Fig. 1D, lane 2). Similarly, the single substitution S43A but not T42A ablates PKA phosphorylation of the fusion protein (Fig. 1D, lane 3). These data suggest that Ser-43 is the key substrate for PKA phosphorylation of amino-terminal Yotiao.

We were next interested in determining whether or not Yotiao could be PKA-phosphorylated in intact CHO cells that could then be used to investigate possible functional consequences of Yotiao phosphorylation. To accomplish this, we raised an antibody (PYT2K) against a 9-mer peptide that includes the entire N-T PKA consensus sequence with a phosphorylated Ser-43 to probe motif-specific Yotiao phosphorylation. Fig. 2B illustrates the utility and the specificity of the phospho-Yotiao antibody (PYT2K) as well as evidence that full-length Yotiao was indeed phosphorylated in response to cAMP in CHO cells. In the experiments illustrated in Fig. 2A, CHO cells expressing Yotiao were incubated with membrane-permeable 8-bromo-cAMP (300 μM) and OA (1 μM) before obtaining cell lysates. Phosphorylated Yotiao can be detected by the phospho-Yotiao antibody. A Yotiao N-T peptide containing a phosphorylated Ser-43 successfully neutralized the phospho-Yotiao antibody, whereas a similar peptide containing a nonphosphorylated Ser-43 did not. This verified the phospho-specificity of the antibody. Fig. 2B shows that Yotiao is coupled to, and can be phosphorylated by, stimulation of β-adrenergic receptors. In these experiments, CHO cells were co-transfected with cDNA coding for Yotiao plus either β1 or β2 receptors, the predominant β-adrenergic receptor subtypes in heart and then treated with the nonselective β-adrenergic receptor agonist isoproterenol (1 μM) plus the phosphatase inhibitor OA (1 μM) to maximize possible β-adrenergic receptor-mediated Yotiao phosphorylation. As is the case for exposure to membrane-permeable cAMP, stimulation of β-adrenergic receptors results in Yotiao phosphorylation.

Does PKA-dependent phosphorylation of Yotiao have functional consequences on the regulation of the I\textsubscript{Ks} channel? To test for this possibility, CHO cells expressing KCNQ1/KCNE1 along with WT- or S43A-mutated Yotiao were dialyzed with pipette solutions containing 200 μM cAMP and 0.2 μM OA. The effects of cAMP/OA on I\textsubscript{Ks} channel activity were compared for cells expressing WT versus S43A Yotiao. Here we focused on the voltage dependence of I\textsubscript{Ks} activation and the time course of channel activation and deactivation because PKA regulation of I\textsubscript{Ks} has been demonstrated to affect these parameters (12, 16). Fig. 3 shows that the S43A Yotiao mutation, which removes the N-T Yotiao PKA substrate, markedly reduces the effects of cAMP/OA on these I\textsubscript{Ks} gating parameters. In this set of experiments, the half-maximal I\textsubscript{Ks} activation voltage (V\textsubscript{1/2}) for CHO cells expressing KCNQ1, KCNE1, and wild type Yotiao was 28.3 ± 2.1 mV (n = 11) (control, −cAMP/OA) and 15.8 ± 2.2 mV (n = 15) (+cAMP/OA) (p < 0.001) (Fig. 3A), consistent with previously reported effects of cAMP on I\textsubscript{Ks} activation (12). However, when we replaced WT by S43A Yotiao, the cAMP-dependent shift in I\textsubscript{Ks} activation was markedly reduced (V\textsubscript{1/2} = 22.9 ± 2.4 mV, n = 12, −cAMP/OA; V\textsubscript{1/2} = 19.5 ± 2.1 mV, n = 10, +cAMP/OA; NS). Substitution of S43A for WT Yotiao similarly greatly reduced the kinetic response of the I\textsubscript{Ks} channels to cAMP. As in previous studies (16), cAMP slowed I\textsubscript{Ks} deactivation by extracting the time constant (τ) of exponential current decay at −40 mV after a +60-mV conditioning pulse to activate channels (see “Materials and Methods”) (τ = 606 ± 25 ms, −cAMP/OA, n = 11; τ = 782 ± 62 ms, +cAMP/OA, n = 15, p < 0.05). When WT Yotiao was replaced by S43A Yotiao, the effect of cAMP on I\textsubscript{Ks} was no longer significant (τ = 732 ± 64 ms, −cAMP/OA, n = 12; τ = 760 ± 52 ms, +cAMP/OA, n = 10, NS). Similarly, replacement of WT by S43A Yotiao reduced the effect of cAMP/OA on I\textsubscript{Ks} activation kinetics assayed by measuring the time to half-maximal current (t\textsubscript{1/2}) during 2-s voltage steps to +60 mV (see “Materials and Methods”). In CHO cells expressing WT Yotiao, cAMP significantly sped I\textsubscript{Ks} activation as reported previously (16) (t\textsubscript{1/2} = 719 ± 38
of cAMP/OA. For cells expressing WT Yotiao, phosphorylation of KCNQ1 Ser-27 can be detected in CHO cells and Methods) in the absence (open bars) and presence (filled bars) of cAMP/OA. For cells expressing WT Yotiao, t½ was 696 ± 25 ms (−cAMP/OA, n = 11) and 782 ± 62 ms (+cAMP/OA, n = 15) (p < 0.05). For cells expressing S43A Yotiao, t½ was 732 ± 64 ms (−cAMP/OA, n = 12) and 760 ± 52 ms (+cAMP/OA, n = 10) (NS). C, the Yotiao Ser-43 mutation (S43A) reduces the cAMP-dependent acceleration of IKS channel activation. Activation t½ was measured at ±60 mV (see “Materials and Methods”) in the absence (open bars) and presence (filled bars) of cAMP/OA. For cells expressing WT Yotiao, t½ was 719 ± 38 ms (−cAMP/OA, n = 11) and 528 ± 35 ms (+cAMP/OA, n = 15) (p < 0.05). For cells expressing S43A Yotiao, t½ was 644 ± 31 ms (−cAMP/OA, n = 12) and 692 ± 30 ms (+cAMP/OA, n = 10) (NS).

ms, −cAMP/OA, n = 11; t½ = 528 ± 35 ms, +cAMP/OA, n = 15, p < 0.01) but not in CHO cells expressing S43A Yotiao (t½ = 644 ± 31 ms, −cAMP/OA, n = 12; t½ = 592 ± 30 ms, +cAMP/OA, n = 10, NS). Thus, the Yotiao mutation S43A, which ablates amino-terminal PKA phosphorylation of Yotiao, greatly reduces the functional kinetic response of the IKS channel to cAMP.

Ablation of Yotiao phosphorylation might alter the kinetic and voltage-dependent response of IKS channels to PKA by (i) disruption of amino-terminal KCNQ1 phosphorylation; (ii) disruption of the binding of Yotiao to the KCNQ1 carboxyl terminus; or (iii) changing the allosteric interactions with the phosphorylated KCNQ1 channel. Fig. 4 provides experimental evidence against possibilities (i) and (ii). Fig. 4A shows that phosphorylation of KCNQ1 Ser-27 can be detected in CHO cells expressing KCNQ1 plus WT Yotiao when exposed to membrane-permeable cAMP as reported previously by us (13). Replacement of WT Yotiao by Yotiao harboring the S43A mutation, which ablates N-T Yotiao phosphorylation, does not prevent KCNQ1 phosphorylation. Fig. 4B demonstrates that both WT Yotiao and S43A Yotiao can be co-immunoprecipitated with KCNQ1, suggesting that the KCNQ1 binding capacity of S43A Yotiao remains intact. These experiments rule out possibilities (i) and (ii), and taken together both experiments indicate that the role of Yotiao as an anchoring protein for the IKS channel is not compromised by S43A mutation.

To test for possibility (iii) we turned to a pseudo-phosphorylated KCNQ1 channel in which Ser-27 was replaced by a negatively charged residue (S27D). When co-expressed with WT Yotiao and KCNE1, pseudo-phosphorylated KCNQ1 reconsti-

FIG. 3. Phosphorylation of Yotiao Ser-43 contributes to cAMP-dependent regulation of IKS channels. IKS channel activity was measured in CHO cells expressing KCNQ1, KCNE1, and WT or S43A mutant Yotiao. Cells were dialyzed with or without cAMP (200 μM) and OA (0.2 μM), and recordings were compared after 13 min of dialysis. A, the Yotiao Ser-43 mutation (S43A) reduces the cAMP-dependent shift in IKS activation. Shown are IKS isochronal (2-s) activation curves (see “Materials and Methods”) in the presence (filled squares) or absence (open circles) of cAMP/OA. V½ for CHO cells expressing WT Yotiao (left panel) was 28.3 ± 3.1 mV (−cAMP/OA, n = 11) and 15.8 ± 2.2 mV (+cAMP/OA, n = 15) (p < 0.001). V½ for CHO cells expressing S43A Yotiao (right panel) was 22.9 ± 2.4 mV (−cAMP/OA, n = 12) and 19.5 ± 2.1 mV (+cAMP/OA, n = 10) (NS). The smooth curves are the Boltzmann relationships computed from these mean data (see “Materials and Methods”). Shown in insets are representative current traces recorded at 0 mV for both conditions. Scale bars: 40 pA/pF, 1 s (left panel); 20 pA/pF, 1 s (right panel). B, the Yotiao Ser-43 mutation (S43A) reduces the effect of cAMP on IKS deactivation. Deactivation time constants (τ) at −40 mV were measured (see “Materials and Methods”) in the absence (open bars) and presence (filled bars) of cAMP/OA. For cells expressing WT Yotiao, τ was 506 ± 25 ms (−cAMP/OA, n = 11) and 728 ± 62 ms (+cAMP/OA, n = 15) (p < 0.05). For cells expressing S43A Yotiao, τ was 732 ± 64 ms (−cAMP/OA, n = 12) and 760 ± 52 ms (+cAMP/OA, n = 10) (NS). C, the Yotiao Ser-43 mutation (S43A) reduces the cAMP-dependent acceleration of IKS channel activation. Activation t½ was measured at ±60 mV (see “Materials and Methods”) in the absence (open bars) and presence (filled bars) of cAMP/OA. For cells expressing WT Yotiao, t½ was 719 ± 38 ms (−cAMP/OA, n = 11) and 528 ± 35 ms (+cAMP/OA, n = 15) (p < 0.05). For cells expressing S43A Yotiao, t½ was 644 ± 31 ms (−cAMP/OA, n = 12) and 692 ± 30 ms (+cAMP/OA, n = 10) (NS).

FIG. 4. Replacement of Ser-43 by Ala does not disrupt Yotiao binding to or PKA phosphorylation of KCNQ1. A, KCNQ1 can be PKA-phosphorylated in the presence of either WT Yotiao or S43A mutant. CHO cells expressing KCNQ1 and WT Yotiao (left lanes) or S43A Yotiao (right lanes) were incubated with (+) or without (−) 300 μM 8-bromo-cAMP (cAMP) plus 1 μM OA. Lysates were prepared immediately after incubation as described under “Materials and Methods.” Phosphorylated KCNQ1 was detected by an antibody specific for phosphorylated KCNQ1 Ser-27 (Phospho-KCNQ1, upper panel). Total KCNQ1 was measured using a KCNQ1 antibody (C20) to control for loading (lower panel). B, KCNQ1 interacts with both WT Yotiao and S43A Yotiao. CHO cells were transfected with KCNQ1 and WT (left) or S43A Yotiao (right). The C20 KCNQ1 antibody was used to immunoprecipitate (IP) the KCNQ1-Yotiao complex, and co-precipitated Yotiao was immunoblotted using the antibody that recognizes basal Yotiao (see “Materials and Methods”). Control experiments (−) performed in the absence of immunoprecipitate antibody confirmed the specificity of the blots.
PKA phosphorylation of Yotiao modifies the activity of pseudo-
phosphorylated \( I_{\text{KC}} \) channels. Shown in Fig. 5 are currents and
activation curves measured in the absence and presence of intracellular cAMP/OA for cells expressing S27D KCNQ1, KCNE1 with WT (left), or S43A (right) Yotiao. Replacement of Ser-27 by Asp eliminates Ser-27 as a PKA substrate in these
right panel).

**DISCUSSION**

AKAPs play a central role in mediating cellular signal transduction by creating micro-signaling environments that present regulatory enzymatic molecules directly to the downstream targets (5, 6, 9, 22). The versatile nature of these proteins underlies the precise spatio-temporal control of cellular events. Often ignored, however, is the possibility that, when extracel-
ular or intracellular stimuli reach the subcellular compartment and activate signaling components, AKAPs themselves may be the targets of kinases or phosphatases. Here we provide evidence that the AKAP Yotiao itself is a PKA substrate. We show that Yotiao amino-terminal Ser-43 is no longer significant when WT Yotiao is replaced by S43A Yotiao in similar experiments (\( V_{1/2} = 24.5 \pm 1.8 \text{ mV}, n = 24, -c\text{AMP/OA}; V_{1/2} = 16.1 \pm 2.1 \text{ mV}, n = 13, +c\text{AMP/OA}; p < 0.01 \)). The effects of cAMP/OA on \( I_{\text{KC}} \) channels are mediated by phosphorylation of the Yotiao amino-terminal residue Ser-43.

**Yotiao phosphorylation alters the function of pseudo-phosphorylated \( I_{\text{KC}} \) channels.** Phosphorylation of KCNQ1 was simulated (pseudo-phosphorylated channels) by replacing KCNQ1 Ser-27 by Asp (S27D), and \( I_{\text{KC}} \) was measured in CHO cells expressing S27D KCNQ1, KCNE1, and WT (left) or S43A (right) Yotiao. PKA regulation of \( I_{\text{KC}} \) was tested by dialysis with cAMP (200 \( \mu \text{M} \)) and OA (0.2 \( \mu \text{M} \)) (see "Materials and Methods"). Isochronal \( I_{\text{KC}} \), activation curves (see "Materials and Methods") were measured in the absence (open circles) and presence (filled squares) of cAMP/OA and plotted as mean ± S.E. For cells expressing WT Yotiao, \( V_{1/2} \) was 24.5 ± 1.8 mV (−cAMP/OA, \( n = 24 \)) and 16.2 ± 2.1 mV (+cAMP/OA, \( n = 13 \)) (p < 0.01). For cells expressing S43A Yotiao, \( V_{1/2} \) was 28.6 ± 5.1 mV (−cAMP/OA, \( n = 4 \)) (NS). The smooth curves are the Boltzmann relationships computed from these mean data (see "Materials and Methods"). Shown in insets are representative current traces recorded at 0 mV under the indicated experimental conditions. Scale bars: 40 pA/pF, 1 s (left panel); 20 pA/pF, 1 s (right panel).
the $I_K$ channel complex. One possibility is that phosphorylation of Yotiao Ser-43 alters but does not ablate the interface between KCNQ1 and Yotiao and, in turn, affects conformation of the assembled channel complex. Other possibilities may involve allosteric intermolecular interactions between phosphorylated Yotiao and other intracellular segments of the KCNQ1/KCNE1 channel. These possibilities remain to be addressed in future studies.

In summary, our work shows that in the case of the $I_K$ channel complex, phosphorylation of an ion channel subunit (KCNQ1) together with the adaptor protein (Yotiao) that also coordinates the local signaling environment contributes to the postphosphorylation physiological functional regulation of the channel complex. The participation of the adaptor protein itself in the signaling response to external stimuli is not limited to the $I_K$ macromolecular complex because precedence exists for phosphorylation of other AKAPs such as AKAP-Lbc and AKAP 250 (Gravin) (18, 19). Hence the active participation of AKAPs in the functional regulation of targeted proteins may be a more universal role of these important signaling proteins than previously considered.

Acknowledgments—We thank Drs. Steven O. Marx and Guoxia Liu for help with the 32P phosphorylation assay. We thank Drs. Colleen E. Clancy and Cecile Terrenoire for reading and providing helpful suggestions for the manuscript.

REFERENCES

Downloaded from http://www.jbc.org/ by guest on November 15, 2017
Phosphorylation of the A-kinase-anchoring Protein Yotiao Contributes to Protein Kinase A Regulation of a Heart Potassium Channel
Lei Chen, Junko Kurokawa and Robert S. Kass

doi: 10.1074/jbc.M505191200 originally published online July 7, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M505191200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 27 references, 15 of which can be accessed free at http://www.jbc.org/content/280/36/31347.full.html#ref-list-1