Protein Kinase A Phosphorylation Alters Kvβ1.3 Subunit-mediated Inactivation of the Kv1.5 Potassium Channel

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The human Kv1.5 potassium channel forms the IKur current in atrial myocytes and is functionally altered by coexpression with Kvβ subunits. To explore the role of protein kinase A (PKA) phosphorylation in β-subunit function, we examined the effect of PKA stimulation on Kv1.5 current following coexpression with either Kvβ1.2 or Kvβ1.3, both of which coassemble with Kv1.5 and induce fast inactivation. In Xenopus oocytes expressing Kv1.5 and Kvβ1.3, activation of PKA reduced macroscopic inactivation with an increase in K+ current. Similar results were obtained using HEK 293 cells which lack endogenous Kv+ channel subunits. These effects did not occur when Kv1.5 was coexpressed with either Kvβ1.2 or Kvβ1.3 lacking the amino terminus, suggesting involvement of this region of Kvβ1.3. Effects of phosphorylation appeared to be electrostatic, as replacement of serine 24 with a negatively charged amino acid reduced β-mediated inactivation, while substitution with a positively charged residue enhanced it. These results indicate that Kvβ1.3-induced inactivation is reduced by PKA activation, and that phosphorylation of serine 24 in the subunit NH2 terminus is responsible.

Voltage-gated K+ channels play a critical role in the normal physiology of excitable cells. K+ currents contribute to action potential repolarization in cardiac cells (1, 2) while in vascular smooth muscle, regulation of membrane potential by K+ currents is a major determinant of vascular tone (3, 4). Kv1.5 is one of the more cardiovascular-specific K+ channel isoforms identified to date, although it has been found in other tissues (1, 5–7). Upon heterologous expression, Kv1.5 generates a rapidly activating delayed rectifier K+ current which is sensitive to block by 4-aminopyridine (1). Substantial data indicate that Kv1.5 forms at least in part the molecular basis for an ultrafast block by 4-aminopyridine (1). Substantial data indicate that Kv1.5 current following coexpression with either Kvβ1.2 or Kvβ1.3 is modulated by PKA stimulation which activates cAMP-dependent protein kinase (PKA),1 with an increase in K+ current amplitude (10). In addition, Kv1.5 likely encodes a channel responsible for a 4-aminopyridine-sensitive, delayed rectifier K+ current (IKdr) in vascular smooth muscle, which is also enhanced by stimulation of PKA (3, 7, 11).

Kvβ subunits such as Kv1.5 coassemble as homo- or heterotetramers with α subunits to form functional K+ channels (1). In addition, smaller β subunits modify channel function (1, 12). Two β subunits cloned from ferret and human heart (13–17), Kvβ1.2 and Kvβ1.3, represent splice variants from the same gene. These two proteins are identical in the carboxyl 292 amino acids, and this portion appears to be responsible for physical interaction or binding with the α subunit (12, 18). In contrast, the NH2 termini show little identity (~25%), consistent with the concept that this region accounts for differences in β subunit function (1, 12). Coexpression of either Kvβ1.2 or Kvβ1.3 with Kv1.5 alters channel function, with the development of rapid although partial inactivation, slowed deactivation, and a hyperpolarizing shift in the voltage sensitivity of activation (13, 16). Additional studies have demonstrated that as with other Shaker-type K+ channel α-β interactions, both of these β subunits bind to a specific region on the Kv1.5 NH2 terminus (17–19).

The amino acid sequences of both Kvβ1.2 and Kvβ1.3 contain consensus sites for phosphorylation by PKA. The purpose of this study was to determine whether this α-β interaction is modulated by PKA stimulation. We found that coexpression of Kvβ1.3, but not Kvβ1.2, enabled a response to kinase activation, with marked slowing of fast inactivation and an increase in K+ current. These effects can be attributed to phosphorylation of a specific consensus site by PKA in the NH2 terminus of the Kvβ1.3 subunit.

EXPERIMENTAL PROCEDURES

Materials—Reagent grade chemicals, as well as 8-chlorophenylthio cAMP, 3-isobutyl-1-methylxanthine, and forskolin were obtained from Sigma, while 8-bromo-cAMP was purchased from Calbiochem. Tissue culture media and reagents, including LipofectAMINE, were obtained from Life Technologies, Inc. (Grand Island, NY). Enzymes and buffers were obtained from Roche Molecular Biochemicals (Indianapolis, IN), Promega (Madison, WI), and New England Biolabs (Beverly, MA). The source of other materials is specified below.

1 The abbreviations used are: PKA, protein kinase A; IRES, internal ribosome entry sequence.
**K⁺ Channel Expression**

*Xenopus laevis* Oocytes—DNA constructs of Kv1.5, Kvβ1.2, and Kvβ1.3 (each in a modified pSP64T vector (13, 16)) were linearized with EcoRI and cRNA transcribed using the SP6 RNA polymerase (SP6 Cap-Scribe, Roche Molecular Biochemicals, Indianapolis, IN). Defolliculated *Xenopus* oocytes were prepared as described previously and injected with approximately 40 nl of RNA (20). Kv1.5 cRNA was diluted with RNAse-treated water so that currents for experimentation did not exceed 5 nA. This was combined with an excess of uncleaved RNA in ratios which achieved maximal effect as assessed by K⁺ current characteristics during electrophysiological recordings (13, 16, 21).

**HEK 293 Cells**—For coexpression of Kv1.5 and Kvβ1.3 in HEK 293 cells, human Kv1.5 (–22–1894 nucleotides) and human Kvβ1.3 (–53–1500 nucleotides) were inserted in tandem into a modified pBKCMV (m-pBK) vector that had the β-galactosidase ATG at position 151 removed to increase expression efficiency. The Kv1.5 subunit was placed 3' to Kvβ1.3 and behind an internal ribosome entry sequence (IRES), thus generating a dual cistronic mRNA. A 590-base pair IRES was subcloned into EcoRI/EcoRV prepared pBSKS⁺. The above mentioned subunit fragments were blunt and subcloned into blunt XbaI sites of the pBS polylinker that flanked the subcloned IRES. This construct was then digested with SauI, blunt, and then digested with NcoI to release the Kvβ1.3/IRES/Kv1.5 sequence. This fragment was then inserted into NotI/Smal digested m-pBK. This construct consistently generated currents showing complete Kvβ1.3 effects. Kvβ1.3 mutants were also expressed using this m-pBK IRES construct.

Recently thawed HEK 293 cells (ATCC number 1573-CRL) were maintained in culture and transiently transfected using LipofectAMINE as described previously (22), with coexpression of green fluorescent protein to identify cells for voltage clamp analysis. For transfections, 2.5 μg of hKv1.5-pBK, 4 μg of Kvβ1.3/IRES/Kv1.5-pBK, and 0.5 μg of GFP-pPCICMV were mixed with 25 μl of LipofectAMINE reagent and applied overnight, after which the standard culture medium was maintained. Cells were removed from the dish using brief trypsinization, washed twice with maintenance medium, and stored at room temperature for recording within the next 12 h.

**Electrophysiological Recording and Data Analysis**

Oocyte recordings were performed using the two-microelectrode voltage-clamp technique as described previously (20, 21). Membrane potentials were controlled by a high-compliance voltage-clamp amplifier (Clampator, Dagan Instruments, Minneapolis, MN) with voltage command potentials generated by a 12-bit digital-to-analog converter controlled by a PC using pClamp software (Axon Instruments, Foster City, CA). Pipettes were filled with 3M KCl, and a standard extracellular bath solution was utilized (in mM: NaCl 96, KCl 2, CaCl₂ 1.8, MgCl₂ 1, HEPES 5, pH 7.5). Oocytes that demonstrated endogenous currents greater than 1% of expressed currents were not utilized. Data were sampled at 10–20 kHz and filtered at 2–5 kHz. The holding potential was −80 mV and the cycle time for all pulse protocols was 10 s or slower to allow full recovery from inactivation between pulses unless otherwise specified. To calculate cell membrane electrical capacitance, the capacitive transient was recorded during a small voltage step (−80 to −70 mV) during which K⁺ currents were not activated. Integration of the leak-corrected transient yielded the charge (Q) transferred during the voltage step of V from which capacitance (C) was calculated: \( C = Q/V \). All experiments were conducted at room temperature (22 ± 2 °C).

K⁺ current recordings in HEK 293 cells were obtained with an Axopatch 200B amplifier (Axon Instruments) using the whole cell configuration of the patch clamp technique (22). The intracellular pipette solution contained (in mM): KCl 110, HEPES 10, K₂BAPTA 5, K₂ATP 5, and MgCl₂ 1 (pH 7.2); the extracellular bath solution contained (in mM): NaCl 130, KCl 4, CaCl₂ 1.8, MgCl₂ 1, HEPES 10, glucose 10 (pH 7.35). Currents were sampled at 1–10 kHz and filtered at 0.5–5 kHz. Data acquisition and command potentials were controlled by pClamp software.

Analysis of data was performed using either custom programs that were designed to read and analyze pClamp data files or Clampfit 6.04. Activation curves were constructed from deactivating tail currents and were fitted with a Boltzmann equation. The time course of macroscopic K⁺ current inactivation was fitted with an exponential function using a nonlinear least squares algorithm. Comparison of the voltage-dependent and kinetic properties of K⁺ currents after PKA stimulation to control values was performed using a paired t test. Results are presented as mean ± S.E.

**Fig. 1. Modulation of K⁺ current derived from Kv1.5 + Kvβ1.3 by PKA stimulation.** A, K⁺ currents are shown following co-injection of cRNA for Kv1.5 and Kvβ1.3 in Xenopus oocytes under control conditions (left panel) and after bath superfusion of PKA activators (right panel); voltage is stepped from −80 mV to a maximal potential of +50 mV, with repolarization to −30 mV. Stimulation of PKA caused marked slowing of macroscopic inactivation associated with an increase in K⁺ current. B, similar data are presented following coexpression of Kv1.5 and Kvβ1.2. Under these conditions, there was no effect of kinase stimulation on K⁺ currents. C, the time course of normalized peak current (at +50 mV) is shown after the start of bath superfusion of PKA activators (arrow, time 0). The increase in K⁺ current amplitude seen with Kvβ1.3 coexpression (▲) is not present with Kvβ1.2 (●).

**Mutagenesis**

For some experiments, Kv1.5 was coexpressed with an NH₂-terminal deletion mutant of Kvβ1.3 (ΔN50Kvβ1.3). This mutant was constructed as described previously (21) by changing the serine at position 50 to a methionine. A COOH-terminal deletion mutant of Kv1.5 (ΔC57Kv1.5) (23) was generated by transfer from pGEM into wild-type pBK using EcoRI and HindIII, followed by transfer into the IRES construct using StuI. Point mutations were constructed in the Kvβ1.3 sequence in which serines were converted to alanines at position 24 (S24A), 39 (S39A), or 164 (S164A) using either overlap extension polymerase chain reaction mutagenesis (24) or the Quick-Change Site-directed mutation kit from Stratagene. Multiple independent recombinant clones were analyzed by restriction digestion to verify correct assembly and to screen for the presence of the mutation (creation of a new restriction site). Mutant inserts were fully sequenced and clones lacking polymerase errors were chosen for electrophysiologic studies.

**RESULTS**

Co-expression of Kv1.5 and Kvβ1.3: Effects of PKA Activation—In Xenopus oocytes, coexpression of Kvβ1.3 with Kv1.5 produced K⁺ currents that displayed partial inactivation and slowed deactivation as previously reported (16, 21) (Fig. 1A). With sustained depolarization to +50 mV, peak K⁺ current declined by 44 ± 2% over a 50-ms period, with a rapid time...
channel activation was shifted to hyperpolarized potentials (midpoint or $V_{50} = -22.0 \pm 1.3 \text{ mV}$) compared with Kv1.5 alone ($V_{50} = -5.2 \pm 1.0 \text{ mV}; n = 19$).

In order to test PKA, cells were perfused with a combination of kinase activators (8-chlorophenylthio-cAMP, 200 μM; 3-isobutyl-1-methylxanthine, 1 μM; and forskolin, 10 μM) which activates the cyclic fibrosis conductance regulator in a rapid, potnet manner (25). As illustrated in Fig. 1A, activation of PKA caused marked slowing of macroscopic inactivation for $K^+$ currents derived from Kv1.5 + Kvβ1.3 ($\tau = 4.2 \pm 0.1 \text{ ms}$ before and $6.2 \pm 0.3 \text{ ms}$ after PKA activation; $p < 0.01$), with a reduction in the overall extent of $K^+$ current inactivation induced by the $\beta$ subunit (21 ± 2% at +50 mV, compared with 44 ± 2% before kinase activation). In addition, $K^+$ current deactivation became more rapid, consistent with reduced $\beta$-mediated effects (deactivation $\tau$ at $-40 \text{ mV}$ was 63 ± 6 ms before and 47 ± 4 ms after PKA). The loss of $K^+$ current inactivation was associated with a significant, sustained increase in current amplitude ($+40 \pm 3\%$ at $+50 \text{ mV}$ in 15 min; $n = 17$). The time course of these effects was rapid, as shown by normalized $K^+$ current values during an individual experiment in Fig. 1C.

To further investigate the mechanisms of these effects by PKA, activation curves were constructed from deactivating tail currents. Stimulation of PKA did not change the midpoint of this curve ($V_{50} = -22.0 \pm 1.3 \text{ mV}$ before and $-22.8 \pm 1.1 \text{ mV}$ after PKA), indicating that the voltage dependence of channel opening was not affected. In addition, there was no associated change in cell membrane electrical capacitance with kinase activation (+1 ± 2% at 15 min), suggesting that significant changes in cell surface area were not involved in the effect observed.

$K^+$ currents derived from coexpression of Kvβ1.3 with Kv1.5 demonstrate not only development of fast voltage-dependent inactivation, but also enhancement of the slow or C-type inactivation that is present with Kv1.5 alone (21). The extent of slow inactivation was studied with a twin pulse protocol during which the duration of the first pulse (+70 mV) was progressively lengthened, followed by a 50 ms hyperpolarizing step to $-80 \text{ mV}$ prior to the second pulse (+70 mV) in order to permit full recovery of fast inactivation while minimizing recovery of slow inactivated channels (21). The fraction of slow inactivation was calculated as $1 - (P_c/P_t)$, where $P_c$ is the peak current of the second pulse and $P_t$ is the peak current of the first pulse. With a prepulse of 2 s, this value was 0.32 ± 0.2 under control conditions, and 0.30 ± 0.02 after PKA stimulation (data not shown). These data indicate that despite the effect of PKA to reduce fast inactivation, the extent of slow inactivation was not altered.

**Lack of PKA Response following Coexpression of Kvβ1.2 with Kv1.5**—Because Kvβ1.2 can also coassemble with Kv1.5, we investigated whether coexpression of this subunit led to a similar effect with PKA activation. As demonstrated in Fig. 1B, $K^+$ currents following coexpression of Kvβ1.2 with Kv1.5 in Xenopus oocytes demonstrated partial inactivation (50 ± 2% at $+50 \text{ mV}; n = 11$), slowed deactivation, and an altered voltage dependence of channel opening ($V_{50} = -20.4 \pm 2.0 \text{ mV}$) compared with Kv1.5 alone. With stimulation of PKA, there was no effect on either $K^+$ current amplitude ($+3 \pm 2\%$ at $+50 \text{ mV}$ in 15 min; $n = 11$), kinetics of inactivation ($\tau = 4.0 \pm 0.1 \text{ ms}$ before and $4.0 \pm 0.1 \text{ ms}$ after PKA at $+50 \text{ mV}$), or extent of inactivation (50 ± 3% after PKA). A comparison of the typical response to kinase activation when Kv1.5 was coexpressed with the two different $\beta$ subunits is demonstrated in Fig. 1C.

**Effects of PKA in Mammalian Cells**—In order to confirm the relevance of our findings for $K^+$ channel regulation in mammalian cells, we also examined the effects of PKA activation in HEK 293 cells. Similar results were obtained when the $K^+$ channel subunits under study were expressed in this mammalian cell line. Following exposure to the cAMP analog 8-bromo-cAMP, there was marked slowing in the time course of macroscopic fast inactivation for cells coexpressing Kv1.5 and Kvβ1.3 ($\tau = 3.7 \pm 0.2 \text{ ms}$ before and $5.9 \pm 0.3 \text{ ms}$ after PKA stimulation; $n = 7$), so that current at the end of a 250-ms voltage step was increased by 39 ± 8% (Fig. 2A). Analogous to results in oocytes, there was no significant effect on slow inactivation ($\tau_{slow} = 402 \pm 100 \text{ ms}$ before and $468 \pm 78 \text{ ms}$ after PKA). On the other hand, there was greater variability in the effect of PKA to increase $K^+$ current amplitude in HEK 293 cells than was seen in oocytes. Peak $K^+$ current increased in most but not all cells (+29 ± 4% in 5 of 7 cells) expressing Kv1.5 + Kvβ1.3 (Fig. 2A), with no significant change for the group as a whole (+1 ± 4%). As in oocytes, there was no change in the voltage dependence of channel opening as assessed by deactivating tail currents (Fig. 2B; $V_{50} = -22 \pm 1 \text{ mV}$ before and $-23 \pm 1 \text{ mV}$ after kinase stimulation).

**Role of Serine 24 in Kvβ1.3 in the PKA Response—Kvβ1.2 and Kvβ1.3 share a common consensus PKA phosphorylation site in the conserved COOH-terminal region of the proteins (serine 164 in Kvβ1.3). However, two additional sites are present in the unique NH$_2$ terminus of Kvβ1.3 (serine 24 and serine 39) which are not present in Kvβ1.2. Additional experiments were performed to test the hypothesis that one of these potential sites in the variable NH$_2$ terminus of the protein was responsible for the effects of PKA activation which occurred with Kvβ1.3 but not Kvβ1.2. The NH$_2$-terminal 50 amino acids of Kvβ1.3 were removed and the mutant construct (ΔNS50Kvβ1.3) was coexpressed with Kv1.5 in Xenopus oocytes. Deletion of this portion of the Kvβ1.3 NH$_2$ terminus eliminated $\beta$-mediated inactivation as described previously (21). Moreover, the PKA response seen with wild-type Kvβ1.3 was abolished, with no effect on $K^+$ current amplitude ($-4 \pm 2\%$ at $+50 \text{ mV}$ in 15 min; $n = 6$) or voltage dependence of channel opening ($V_{50} = -18 \pm 2 \text{ mV}$ before and $-19 \pm 2 \text{ mV}$ after PKA) following stimulation of PKA (data not shown). These results implicated the Kvβ1.3 NH$_2$ terminus in the effects of kinase activation and suggested that the observed increase in peak current with PKA activation was probably due to decreased inactivation.

To determine if one or both unique NH$_2$-terminal consensus PKA sites in Kvβ1.3 were responsible for the effects of PKA, each site was removed individually by changing serine to an alanine (Kvβ1.3S24A and Kvβ1.3S39A). $K^+$ currents following coexpression of Kvβ1.3S24A with Kv1.5 in oocytes resembled wild-type currents, except that both the time course and extent
FIG. 3. Involvement of serine 24 in Kvβ1.3 and effect of charge mutations at this position. A, using site-directed mutagenesis, each of the two unique NH2-terminal putative PKA sites in Kvβ1.3 was removed individually by changing the serine in the consensus site to alanine (Kvβ1.3S24A and Kvβ1.3S39A). Coexpression of Kvβ1.3S24A with Kv1.5 produced inactivating K+ currents (left panel) which did not respond to activation of PKA (right panel). B, the time course of normalized peak current (+50 mV) for representative experiments with each mutant is shown in the lower panel. PKA activation had no effect on K+ current resulting from Kv1.5 + Kvβ1.3S24A (+0 ± 1% at 15 min; n = 7), while coexpression of the Kvβ1.3S39A mutant with Kv1.5 produced currents which retained the wild-type response to PKA (+36 ± 3% at 15 min; n = 8). C, upon substitution of a negatively charged amino acid for serine 24 (Kvβ1.3S24D), fast inactivation was dramatically reduced (left panel), while replacement with a positively charged amino acid (Kvβ1.3S24K) caused enhancement of the time course and extent of macroscopic inactivation (right panel) compared with wild-type Kvβ1.3.

of fast inactivation were enhanced (Fig. 3A). At +50 mV, inactivation τ was 3.4 ± 0.2 ms (versus 4.2 ± 0.1 ms for wild-type) with a fall in peak K+ current of 58 ± 2% over 50 ms (versus 44 ± 2% for wild-type; n = 7). In addition, activation of PKA had no effect on the extent of inactivation (54 ± 2% after PKA), the speed of macroscopic current decay (τ = 3.5 ± 0.1 ms after PKA), or K+ current amplitude (+0 ± 1% at +50 mV in 15 min) in the presence of this mutation (Fig. 3A and B). K+ currents generated by the Kvβ1.3S39A mutation demonstrated characteristic response to PKA stimulation (Fig. 3B) which was similar to that seen with wild-type Kvβ1.3. Upon exposure to PKA activators, K+ currents demonstrated slowing of fast inactivation (τ = 4.5 ± 0.2 ms before and 5.9 ± 0.3 ms after PKA) and a reduction in the extent of this process (41 ± 2% before and 23 ± 3% after PKA), associated with a rapid increase in peak K+ current amplitude (+36 ± 3% at +50 mV in 15 min; n = 8). Taken together, these data indicate that coexpression of Kvβ1.3 modulates the response of Kv1.5 to PKA activation by phosphorylation at a specific site, serine 24, on the NH2 terminus of the β subunit protein. Because inactivation is enhanced with the Kvβ1.3S24A mutant, it is likely that wild-type Kvβ1.3 is phosphorylated to some extent under basal or unstimulated conditions.

As demonstrated in Fig. 4, data obtained in HEK 293 cells further confirmed the mutagenesis experiments conducted in Xenopus oocytes. There was no effect of PKA activation on K+ currents derived from Kv1.5 + Kvβ1.3S24A (Fig. 4A) with respect to fast inactivation (τfast = 4.8 ± 0.3 ms before and 5.1 ± 0.3 ms after PKA; n = 7). On the other hand, stimulation of PKA caused substantial relief of inactivation when Kv1.5 was coexpressed with the Kvβ1.3S39A mutant (Fig. 4B; τfast = 4.7 ± 0.2 ms before and 7.6 ± 0.4 ms after PKA; n = 6). A similar effect was observed with a mutation which removed the conserved COOH-terminal Kvβ1.3 PKA consensus site, serine 164, as shown in Fig. 4C (inactivation τfast = 5.9 ± 0.4 ms before and 7.3 ± 0.4 ms after PKA; n = 6). Finally, because previous reports showed that phosphorylation of sites in the COOH terminus of the Kv1.1 α subunit modulate Kvβ1.1-mediated inactivation (26, 27), a Kv1.5 mutant lacking the COOH-terminal 57 amino acids and the two PKA consensus sites (serine 555 and serine 578) in the channel was studied. When this mutant was coexpressed with Kvβ1.3, a wild-type response to PKA activation was observed (Fig. 4D; inactivation τfast = 5.0 ± 0.5 ms before and 10.0 ± 0.4 ms after PKA; n = 5). These results further substantiate the data in oocytes which implicate phosphorylation of serine 24 in Kvβ1.3 as the sole molecular mechanism responsible for the PKA reduction in fast inactivation.

Role of Charge at Position 24 in Kvβ1.3-mediated Inactivation—To determine whether the effects of PKA resulted directly from the negative charge imparted by phosphorylation, the serine at position 24 in Kvβ1.3 was mutated to the negatively charged amino acid aspartate (Kvβ1.3S24D). Co-expression of Kv1.5 with Kvβ1.3S24D in Xenopus oocytes produced

FIG. 4. Effects of mutations in Kvβ1.3 and Kv1.5 on the PKA response following coexpression in HEK 293 cells. A, K+ currents elicited by 250 ms voltage steps to +50 mV are shown before and after exposure to 8-bromo-cAMP after transfection of Kv1.5 and Kvβ1.3S24A into HEK 293 cells. In the presence of the β subunit mutation, the cAMP analog had no effect on fast inactivation. B and C, experiments similar to that shown in panel A for the Kvβ1.3S39A and Kvβ1.3S164A mutations, respectively. When coexpressed with Kv1.5, K+ currents retained the wild-type response to stimulation of PKA, with marked slowing of fast inactivation. D, expression of the COOH-terminal 57-amino acid deletion mutant of Kv1.5 with wild-type Kvβ1.3 produced K+ currents which also responded to activation of PKA with relief of fast inactivation.
K+ currents in which inactivation was substantially reduced (19 ± 3%; n = 5) compared with wild-type Kv1.3 as shown in Fig. 3C (left panel). Nonetheless, the β-mediated hyperpolarizing shift in the voltage dependence of channel activation was largely preserved (V1/2 = −15 ± 1 mV), indicating that the loss of inactivation associated with this mutation was not due to loss of β subunit expression. To further assess the role of charge at this location, serine 24 was then mutated to the positively charged residue lysine (Kv1.3S24K). This mutation had the opposite effect of Kv1.3S24D, with enhancement of the extent (65 ± 1%; n = 5) and time course (τ = 2.6 ± 0.1 ms) of inactivation with respect to wild-type Kv1.3 (Fig. 3C, right panel). These data indicate that the mechanism whereby phosphorylation relieves β-mediated inactivation is electrostatic in nature, and that the local charge at this position in Kv1.3 is critical to the α-β interaction which confers inactivation to Kv1.5 current.

**DISCUSSION**

Our findings demonstrate that activation of PKA modulates K+ current derived from coexpression of Kv1.5 and Kv1.3, with relief of inactivation that is associated with an increase in current size. A reduction in fast inactivation would be predicted to cause some increase in K+ current amplitude. While PKA activation always enhanced K+ current in Xenopus oocytes, this response was somewhat more variable in HEK 293 cells, with an increase in current in most (5 of 7) but not all cells. A possible explanation for this discrepancy is the fact that voltage clamp of the K+ currents under study is faster in HEK cells than in oocytes. Activation is rapid for Kv1.5 currents and in the presence of β subunit coexpression, the inactivation which is induced is also fast. If initial clamp of activating currents is slower in oocytes, there could be more overlap in time between the processes of activation and inactivation. Under these conditions, any change in the degree of inactivation is more likely to alter current amplitude because of concomitant activation. This discrepancy was the only experimental difference between results in the two expression systems, with other findings in oocytes essentially duplicated in HEK 293 cells.

Removal of the PKA phosphorylation site at serine 24 in the Kv1.3 subunit abolished the PKA modification of fast inactivation. Moreover, this effect was mimicked by substitution of an acidic amino acid which caused reduced inactivation, while mutation to a basic amino acid enhanced it. Thus, we conclude that the molecular basis of the PKA effect is direct phosphorylation of the Kv1.3 subunit NH2 terminus at this position. The location of the phosphorylation site responsible for the PKA effect is not surprising, since our previous work has shown that it is the NH2 terminus of Kv1.3 which confers inactivation upon Kv1.5 current through a possible open channel blocking mechanism (21). Removal of the first 10 amino acids of Kv1.3 abolishes fast inactivation, while attaching the first 87 amino acids of Kv1.3 to the NH2 terminus of Kv1.5 restores it (21). Our data suggest that when the NH2 terminus of Kv1.3 assumes a three-dimensional structure, serine 24 must be positioned in a region critical for β-mediated inactivation, given the effect of charge mutations at this location. It is possible that serine 24 is positioned close to one or more basic residues which are important for the pore blocking effect of the NH2 terminus, and that phosphorylation or negative charge can shield these positively charged residues. This mechanism has been demonstrated previously for kinase modulation of Kv3.4, which encodes a rapidly inactivating A-type channel (28-30). Stimulation of protein kinase C removes fast inactivation by phosphorylation of NH2-terminal residues which are in close proximity to a group of basic amino acids in the inactivation particle.

Our results here indicate that despite the effect of PKA to reduce Kv1.3 fast inactivation, the extent of slow inactivation was not altered. We have previously proposed that the enhanced slow inactivation observed with Kv1.3 was directly linked to the β-induced fast inactivation, since NH2-terminal deletions in Kv1.3 that removed fast inactivation also removed the enhanced slow inactivation (21). However, the data presented here suggest the effect of Kv1.3 to promote slow inactivation is more complex and not solely coupled to β-induced fast inactivation. If it was, one would predict that PKA relaxation of fast inactivation would also reduce slow inactivation.

As noted above, prior studies have shown that the inactivation conferred onto Kv1.1 by Kvβ1.1 is modulated by a subunit phosphorylation (26, 27). Based upon our data, it is now apparent that phosphorylation of the β subunit itself also regulates β-mediated effects. The functional response of different K+ channel subunits to activation of protein kinases represents not only a means to modulate subunit interactions, but also another mechanism for K+ current diversity in vivo. Modulation of α-β function by kinase systems also further complicates the correlation of native currents with those obtained from cloned proteins in heterologous systems.