Independent and Exclusive Modulation of Cardiac Delayed Rectifying K⁺ Current by Protein Kinase C and Protein Kinase A

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Abstract—Expression of minK in Xenopus oocytes results in a current similar to the cardiac slow delayed rectifying K⁺ (Iₖₛ) current. Modulation of the Iₖₛ current in cardiac myocytes has been studied extensively because of its role in shaping the cardiac action potential. The human and cat minK cDNA have been cloned, but their regulation by protein kinases has not been characterized. We report here on the complex modulation of human and cat Iₖₛ currents by protein kinase C (PKC) and protein kinase A (PKA). Activation of PKC by phorbol ester (100 nmol/L phorbol 12,13-didecanoate [PDD]) produces an increase in Iₖₛ current that peaks after 20 minutes and then subsequently decreases to ≈50% of the control level after 1 hour. PKA activation only produces a sustained increase in Iₖₛ current. Interestingly, premodulation by PKC prevents Iₖₛ current modulation by PKA, and PKC has no effect on Iₖₛ current after potentiation by PKA. This shows that the Iₖₛ current is modulated by PKC and PKA in a mutually exclusive manner and suggests that multiple interacting phosphorylation sites are involved. Activation of PKC by diacylglycerol analogues only produces a slow decrease in Iₖₛ current. The biphasic effects of PKC on Iₖₛ current activated by PDD can also be separated by dose and duration. Low doses of PDD (5 nmol/L) or brief applications (5 minutes) of 100 nmol/L PDD only produces Iₖₛ current activation. These data suggest that there are at least 2 independent PKC phosphorylation sites in the minK-KvLQT1 channel. Additionally, long-term activation of PKC strongly attenuates the Iₖₛ current expression even when the corresponding changes in capacitance are taken into account. (Circ Res. 1998;83:995-1002.)

Key Words: minK current • Iₖₛ • phosphorylation • protein kinase C • protein kinase A • Xenopus oocyte

Delayed rectifying K⁺ channels are important in determining the shape and repolarization of cardiac action potentials. One component of the cardiac delay rectifier is termed the K⁺ (Iₖₛ) current. Recent data strongly suggest that Iₖₛ is composed of 2 molecular entities: KvLQT1 and minK.1,2 Mutations in both components are responsible for the most common forms of inherited long-QT syndrome in humans.1-3 Iₖₛ plays an important role in cardiac electrophysiology and its modulation should be equally important, particularly in determining the cardiac action potential duration. Iₖₛ is modulated in the heart by β-adrenergic receptors through a cAMP pathway and by α-adrenergic receptors by the PKC pathway.4 There is also evidence that Ca²⁺, released intracellularly by IP₃ after activation of either α-adrenergic or M3 muscarinic receptors, may modulate Iₖₛ.6 Iₖₛ is also found in the inner ear, and mutations in minK are found in certain forms of congenital deafness.7

When RNA for minK is injected into Xenopus oocytes, the resulting outward current has most of the characteristics of the Iₖₛ current. This includes a positive midpoint of activation, slow activation and deactivation kinetics, and sensitivity to various blockers such as tetraethylammonium chloride (TEA), Ba²⁺, and azimilide.8-10 It was speculated initially that minK may form homomeric ion channels; however, it is now known that the Iₖₛ current in oocytes is the functional expression of the exogenous minK together with the endogenous KvLQT1 in frogs.2 The complementary DNA of minK has been cloned from many species, including mouse,13 rat,8 guinea pig,14 cat,15 rabbit,16 and human.17 Iₖₛ current, expressed in oocytes from all of these species, was upregulated by PKA activation.18 Mouse and rat Iₖₛ currents were decreased by PKC activation,19 whereas guinea pig Iₖₛ current was activated by PKC.14 In mouse and rat minK there is a putative PKC phosphorylation site at serine 102. Amino acid sequence comparison reveals that guinea pig minK protein has an asparagine residue at the same position. Mutating the guinea pig asparagine into serine creates a mutant guinea pig Iₖₛ current that is upregulated by PKC. On the other hand, mutating the mouse serine 102 and neighboring residues produces a mutant mouse Iₖₛ current that is upregulated by PKC.19 These results suggest that position 102 in the minK protein is critical in determining the effect of PKC. Both human and cat minK have a serine residue at position 102. It could be predicted, therefore, that PKC would downregulate Iₖₛ currents from human and cat minK. However, we report here that Iₖₛ...
currents from human and cat minK are both up- and down-regulated by PKC activation. This biphasic effect depends on the dose, duration, and method of PKC activation. We also observe that long-term activation of PKC leads to irreversible downregulation of $I_{Ks}$ current. In addition, we report that $I_{Ks}$ current is resistant to potentiation by PKA after prior modulation by PKC, and conversely that PKC modulation will not occur after PKA activation. Our results suggest that PKC and PKA play an important role in modulating $I_{Ks}$ current and that their modulatory effects are mutually exclusive.

**Materials and Methods**

**Oocyte Expression**

Adult female *Xenopus laevis* (Xenopus 1; Ann Arbor, MI) were anesthetized with 0.3% tricaine (Sigma Chemical), and ovarian lobes were removed. The follicular layers were digested by incubation with 2 mg/mL collagenase (type 1A; Sigma Chemical) in O2R solution (in mmol/L: NaCl 82.5, KCl 2, MgCl$_2$ 1, HEPES 5; pH 7.4) for 2 hours at room temperature. After digestion, healthy stage V–VI oocytes were manually selected based on size and uniformity of color. Oocytes were cultured in a modified Barth’s medium (in mmol/L: NaCl 88, KCl 1, NaHCO$_3$ 2.4, CaCl$_2$ 0.4, Ca(NO$_3$)$_2$ 0.3, MgSO$_4$ 0.8, HEPES 20; 5% fetal bovine serum and 150 μg/mL gentamycin) at 15°C to 18°C. cRNA was dissolved in diethylpyrocarbonate H$_2$O in a concentration of 1 μg/μL. One day after oocytes isolation, human minK, cat minK, or HERG cRNA (20 to 50 ng) were injected by an automatic injector (Drummond Scientific). Oocytes were studied 2 to 12 days after RNA injection.

**Electrophysiology**

Currents were measured with the conventional 2-microelectrode voltage-clamp technique with a Turbo Tec-10c amplifier (NPI Electronic). Electrodes were fabricated from borosilicate glass (World Precision Instrument) by a flaming/Brown micropipette puller (Sutter Instrument). The recording electrodes were filled with 3 mol/L KCl, and the typical resistance was 1 MΩ. Oocytes were continuously perfused with ND96 (in mmol/L: NaCl 96, KCl 2, MgCl$_2$ 1, CaCl$_2$ 1.8, HEPES 5; pH 7.4) at room temperature (23°C to 25°C). Holding potential was −80 mV in all experiments. Currents were acquired at 1 kHz and filtered at 400 Hz for presentation. Pulse/Pulsefit software (version 8.01, HEKA Electronic) was used for data acquisition and analysis and a P/2 protocol was used for leakage subtraction. The voltage-clamp protocol for measuring $I_{Ks}$ currents was as follows: a 5-second depolarizing pulse to 30 mV was used to activate the current, followed by a 5-second repolarizing pulse to −70 mV for tail current measurement. $I_{Ks}$ current amplitudes were measured at the end of the 5-second pulse at 30 seconds. This standard voltage protocol was repeated every 30 to 60 seconds. Current-voltage (I-V) relationships were constructed by measuring the tail current amplitudes (at −70 mV) after a test pulse from −50 to −60 mV and normalized to the maximal control current ($I_{Ks}$). I-V curves from the same oocytes were measured −20 minutes ($\Delta$) and −60 minutes ($\Delta$) after 100 nmol/L PDD application and scaled to the normalized control current. Lines indicate Boltzmann fits with voltage of half-activation (V_{1/2}) of 7.6±1.7 mV for control (○), 10.95±1 mV 20 minutes after PDD (△), and 12.6±1 mV 60 minutes after PDD (△); slope factors were 13.8±0.7 mV (○), 15.6±1 mV (△), and 15.2±1 mV (△), respectively.

The final DMSO concentration in ND96 did not exceed 0.5%, and DMSO alone had no effect on minK or HERG currents. Pseudosubstrate peptide inhibitors of PKA (PKAI5–24) and PKC (PKCI19–36) were purchased from Peninsula Laboratories. The kinase inhibitory peptides were dissolved in sterile water and injected into oocytes at a concentration of 250 μmol/L for PKCl and 120 to 250 μmol/L for PKAI, assuming the volume of oocyte to be 1 μL. 20 Oocytes were allowed to recover for 8 to 12 hours before experiments. $I_{Ks}$ current from oocytes injected with cat minK is referred to as $I_{Ks}$ (hmink) and $I_{Ks}$ current from oocytes injected with human minK.

**Results**

Expression of human $I_{Ks}$ (hmink) or cat $I_{Ks}$ (cminK) cRNA in *Xenopus* oocytes elicited an outwardly rectifying $K^+$ current.
Short-Term Modulation of $I_{Ks}$ by PKC

In oocytes expressing $I_{Ks}$, current, activation of PKC by 100 nmol/L PDD induced a biphasic effect on the currents. Figure 1A shows a typical experiment in which $I_{Ks}$ (hminK) current response to PDD was assessed by pulsing repetitively to 30 mV every 30 seconds during PDD application. The $I_{Ks}$ (hminK) current amplitude was transiently increased by 100 nmol/L PDD, reached a peak at about 20 minutes, and then declined to below control levels in the continued presence of 100 nmol/L PDD. This biphasic effect induced by 100 nmol/L PDD was seen in all hminK- or cminK-injected oocytes examined (n = 16). The inset shows current traces from a single oocyte expressing $I_{Ks}$ (hminK) current before, at the peak of the increase, and 1 hour after PDD application. During the potentiation phase the $I_{Ks}$ (hminK) and $I_{Ks}$ (cminK) current amplitudes reached a peak of 148.5 ± 12.2% of control level (n = 16) 20 minutes after the start of PDD perfusion. The $I_{Ks}$ currents then declined to an amplitude of 52 ± 7% (n = 16) of control level after 1 hour of PDD application. The amplitude of $I_{Ks}$ currents continued to decline in the presence of 100 nmol/L PDD (≥100 min). The capacitance of the oocytes was not significantly altered after 1 hour of PDD application (141 ± 20 nF in control and 114 ± 12 nF in PDD; n = 10 each).

The enhancement of guinea pig $I_{Ks}$ current by activators of PKC has been attributed to a negative shift in the voltage dependence of activation.

We therefore examined the effect of PDD on $I_{Ks}$ current activation. $I_{Ks}$ tail currents with 5 nmol/L PDD had a much smaller effect than the first PDD application (5 nmol/L PDD (≥100 min)). The capacitance of the oocytes was not significantly altered after 1 hour of PDD application (141 ± 20 nF in control and 114 ± 12 nF in PDD; n = 10 each).

5-minute PDD application increased $I_{Ks}$ (hminK) current further: a 148 ± 6% increase compared with the first PDD-induced peak and a 263 ± 55% increase compared with the control current (n = 3). Then 100 nmol/L PDD was applied continuously and $I_{Ks}$ (hminK) current decreased to 61 ± 6.6% of control current (n = 3). It was also possible to separate the potentiation of $I_{Ks}$ current from the suppression by use of low doses of PDD. Figure 2B shows a representative experiment in which $I_{Ks}$ (cminK) current was upregulated by application of 5 nmol/L PDD for more than 100 minutes with no evidence for suppression. Similar effects were seen in 4 other oocytes, expressing either human or cat minK and exposed to 5 nmol/L PDD for a duration of 75 to 100 minutes. The average potentiation by 5 nmol/L PDD was 135 ± 11% above control level when measured 75 minutes after PDD perfusion (n = 5). The increase in $I_{Ks}$ currents with 5 nmol/L PDD had a much slower time course than the transient increase seen with 100 nmol/L PDD (compare Figure 1A and 2A with Figure 2B).

Continuous application of 20 nmol/L PDD modulated $I_{Ks}$ current in a biphasic manner similar to that seen with 100 nmol/L PDD.
Figure 3. Effects of various PKC activators on \(I_{k_s}\) currents. A, Representative experiment showing that 100 \(\mu\)mol/L DOG decreased \(I_{k_s}\) (hminK) current without an initial potentiating phase. Inset shows current records at times indicated. Scale bars=500 nA and 2 seconds. B, Summary plot showing the effects of different PKC activators on \(I_{k_s}\) currents. Currents were measured at the end of a test pulse to 30 mV for 5 seconds at the times shown after application of PKC activators. These values were normalized to control currents before application of PKC activators. The inactive enantiomer of PDD (4\(\alpha\)-PDD) was included as a negative control. Number of experiments is shown above each bar.

Figure 4. Effects of PKC and PKA activation on \(I_{k_s}\) currents. A, PKA stimulates \(I_{k_s}\) (hminK) current. \(I_{k_s}\) (hminK) current was increased in the presence of 20 \(\mu\)mol/L forskolin plus 500 \(\mu\)mol/L IBMX. B, \(I_{k_s}\) (hminK) current modulation by PKC. Representative experiment shows that 1-hour application of PDD decreased \(I_{k_s}\) (hminK) current. C, Recordings from another oocyte shows that 1-hour application of PDD decreased \(I_{k_s}\) (cminK) current, and that subsequent application of PKA activators had little additional effect. D, Application of PKA activators increased \(I_{k_s}\) (hminK) current 2-fold, but subsequent application of PDD for 60 minutes has no additional effect. PDD indicates 100 nmol/L PDD; PKA, 20 \(\mu\)mol/L forskolin plus 500 \(\mu\)mol/L IBMX. Scale bars=500 nA and 2 seconds.

PKC and PKA Interact in Their Modulation of \(I_{k_s}\)

\(I_{k_s}\) current is modulated by both PKC and PKA, we investigated the effects of PKC and PKA comodulation. After downregulation of \(I_{k_s}\) (hminK) current by 1-hour exposure to 100 nmol/L PDD, subsequent activation of PKA did not significantly increase \(I_{k_s}\) (hminK) current amplitude (Figure 4C). \(I_{k_s}\) current amplitudes were 49\(\pm\)5.5% of control after 1-hour of PDD application, and 54\(\pm\)6% of control in the presence of PDD and PKA activators (50 minutes after addition of forskolin plus IBMX; \(n=9\)). Likewise, in the continuous presence of forskolin and IBMX, 100 nmol/L PDD did not have any additional effect on \(I_{k_s}\) (hminK).
I activators increased 
repetitive brief applications (5 minutes) of 100 nmol/L PDD bars 
PKA, 20 
mol/L IBMX. Scale bars~500 nA and 2 seconds.

Figure 5. PDD modulation of I_{Ks} current is strictly PKC dependent. A, An oocyte injected with 250 
mol/L PKA inhibitory peptide (PKAI), PDD elicited an increase in I_{Ks} (hminK) current within 12 minutes. I_{Ks} (hminK) current then decreased to below control level after 46 minutes of PDD perfusion. B, Another oocyte injected with 250 
mol/L of a PKCI and perfused with 100 nmol/L PDD for 46 minutes produced no effect on I_{Ks} (cminK) current. C, In an oocyte injected with 250 
mol/L PKCl, PKA activators increased I_{Ks} (hminK) current 2-fold. PDD, 100 nmol/L PDD; PKA, 20 
mol/L forskolin plus 500 
mol/L IBMX. Scale bars~500 nA and 2 seconds.

currents even after 1-hour exposure (Figure 4D). PKA activation increased I_{Ks} current amplitudes to 273±57% of control (n=8), and subsequent PKC activation did not significantly alter the current magnitude (202±31% of control; n=4). The activation curves were not significantly altered by either treatment: with V_{1/2}=-6.9±4.9 mV for control, -6.0±2.3 mV for PKA activation, and -9.5±5.6 mV for both PKA and PKC modulation of I_{Ks} (cminK) current. When repetitive brief applications (5 minutes) of 100 nmol/L PDD (which induce I_{Ks} current potentiation) were followed by addition of PKA activators, no additional potentiation could be seen (162±9% of control after brief PDD application and 187±26% after subsequent PKA activation; n=4). The results show that the modulatory effects of PKC and PKA on I_{Ks} current are nonadditive and mutually exclusive.

To study the comodulatory roles of PKA and PKC in more detail, specific protein kinase inhibitors were injected into oocytes expressing I_{Ks} (hminK) or I_{Ks} (cminK) currents. Injecting 120 to 250 
mol/L of the pseudosubstrate peptide inhibitors of PKA or PKC 8 to 12 hours before recording did not alter the expression level. I-V relationships, or the activation or deactivation kinetics of I_{Ks} currents (data not shown). Figure 5A shows that injection of a PKA-specific inhibitory peptide (PKAI) had no effect on PDD modulation of I_{Ks}, (hminK) current, because 100 nmol/L PDD still elicited the typical biphasic response. In 4 PKAI-injected oocytes, I_{Ks} currents increased to 140±20% of control and then decreased to 73±27.5% of control after 1 hour of 100 nmol/L PDD. This is similar to results obtained in oocytes not injected with the inhibitory peptides. However, injection of a PKC-specific inhibitory peptide (PKCI) completely abolished both the up- and downregulatory effects of 100 nmol/L PDD (Figure 5B). In 6 PKCI-injected oocytes, I_{Ks} currents did not respond to 45- to 90-minute perfusion of 100 nmol/L PDD (105.5±3.2% of control 1 hour after PDD). The results confirm that PDD modulates I_{Ks} current selectively by activation of PKC and that the stimulatory effect of PDD is not caused by activation of PKA. Injection of PKCI did not affect the action of PKA on I_{Ks} currents, because forskolin plus IBMX still elicited a 161.5±13% increase of I_{Ks} current in the presence of PKCI (Figure 5C; n=5). Injection of PKAI did eliminate the stimulatory effect of forskolin and IBMX on I_{Ks} currents (91.5±28%, n=2), confirming the specificity of PKAI. These results suggest that PDD increases I_{Ks} current in a PKC-specific, but PKA-independent manner.

### Long-Term Downregulation of I_{Ks} by PKC

Continuous application of 100 nmol/L PDD decreased I_{Ks} (hminK) and I_{Ks} (cminK) currents with a very slow time course. A steady-state level of I_{Ks} current was not reached even after 2.5 hours application of PDD. We therefore examined the long-term effect of PDD on I_{Ks} current expression. Figure 6A and 6B show that overnight incubation (12 to 20 hours) of I_{Ks} (hminK)-expressing oocytes with 100 nmol/L PDD chronically reduced the I_{Ks} (hminK) current density. In 2 independent experiments, I_{Ks} (hminK) current densities were 45.8±11% and 37.7±4% of control currents after overnight treatment with 100 nmol/L PDD (n=25). The I-V relationships of I_{Ks} (hminK)-expressing oocytes treated overnight with 100 nmol/L PDD were not different from control hminK-injected oocytes, although oocyte capacitance was significantly decreased from 153±15 nF to 78±11 nF (n=15). Similar results were seen for oocytes expressing I_{Ks} (cminK). Overnight incubation with 100 
mol/L OAG also decreased I_{Ks} (hminK) current densities, although to a lesser extent (63±15% of control current; n=7). There was no effect on I_{Ks} (hminK) current density or oocyte capacitance after overnight incubation with either 5 nmol/L PDD (n=4) or 100 nmol/L 4α-PDD (Figure 6A; n=3). After overnight incubation of 100 nmol/L PDD and extensive wash with control solution (>30 min), 20 
mol/L forskolin plus 500 
mol/L IBMX still increased the remaining I_{Ks} current amplitude by 153±9% (n=2), indicative of normally functioning I_{Ks} channels.

We further addressed the specificity of the PDD long-term effect by using several membrane-permeable PKC inhibitors. Oocytes were incubated with different PKC inhibitors 2 to 3 hours before overnight PDD incubation. Figure 6B shows that nonspecific kinase inhibitors such as staurosporine, as well as more selective PKC inhibitors such as calphostin C and chelerythrine, antagonized the downregulation of I_{Ks} current by overnight treatment of 100 nmol/L PDD. The oocyte capacitance was not altered significantly by overnight exposure to 100 nmol/L PDD in the presence of PKC inhibitors (n=18). Incubation of hminK-injected oocytes with chelerythrine alone had no effect on the current amplitude nor the voltage dependence of activation (n=11). To explore the PKC downregulation on a different K⁺ channel, we treated oocytes expressing HERG with 100 nmol/L PDD for 18 to 22 hours. The HERG current density, kinetics, and the oocyte capacitance, were similar for the PDD-treated and control HERG-expressing oocytes (Figure 6C). Our results suggest
that long-term PKC activation specifically and irreversibly attenuates the $I_{Ks}$ current.

**Discussion**

This is the first report of an increase in $I_{Ks}$ current by PKC modulation in a species with a serine at position 102; however, the modulation of $I_{Ks}$ (hminK) and $I_{Ks}$ (cminK) currents has not been examined to date. Other species may have a similar pattern of modulation by PKC. We observe no change in the voltage-dependence of activation of $I_{Ks}$ (hminK) and $I_{Ks}$ (cminK) after modulation by PKC, in contrast with the negative 6 to 10 mV shift seen in guinea pig minK, $I_{Ks}$ current. 14 Our results are similar to the results in guinea pig myocytes in which an increase in $I_{Ks}$ current by phorbol ester has been reported previously. 1

PKC Modulation of $I_{Ks}$ Appears to Involve Multiple Phosphorylation Sites

A surprising finding was that OAG and DOG, which also activate PKC, only produce a downregulation of the $I_{Ks}$ current. It has been reported that in rat cardiac myocytes, OAG and phorbol esters have different potency and mechanisms of action on the L-type Ca$^{2+}$ channels. This was postulated to reflect different forms of PKC being activated by the different PKC activators. 32 Multiple forms of Ca$^{2+}$-dependent and independent PKC enzymes are found in oocytes 27 and in cardiac myocytes. 28,29 It is possible that specific PKCs may have different sensitivities to activation by various PKC activators, 30,31 and that once activated, different PKCs may phosphorylate different sites. According to this hypothesis, PDD could activate both high-and low-sensitivity PKC isoforms (sensitivity relative to PDD), and OAG/DOG would only be able to activate the low-sensitivity PKC isoform. The high-sensitivity PKC isoform may phosphorylate a unique site on minK-KvLQT1 that would produce potentiation of current. On the other hand, the low-sensitivity PKC isoform may phosphorylate a site common to PDD and OAG/DOG activation that would downregulate the current. This model would explain why low concentrations of PDD upregulate the current, by activating the high-sensitivity PKC isoform and phosphorylating the unique potentiating site. The low-sensitivity isoform of PKC would require higher concentrations of PDD and longer exposure for its activation. This is supported by the data showing that brief repeated applications of 100 nmol/L PDD only elicit potentiation of $I_{Ks}$ current, but brief applications of 100 μmol/L OAG and DOG have no effect. Biphase regulation of a different K$^+$ channel by PKC has been reported previously. PKC activation leads to an upregulation followed by a downregulation of the *Aplysia* Kv1.1a channel, similar to our data on $I_{Ks}$ current. It was found that 2 different phosphorylation sites are involved in the modulation of Kv1.1a. 31

**Mechanisms for Long-Term Downregulation of $I_{Ks}$ by PKC**

There was a specific decrease in oocyte capacitance in oocytes whose $I_{Ks}$ current has been downregulated by prolonged treatment with 100 nmol/L PDD, suggesting that the

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**Figure 6.** Long-term effects of PDD on $I_{Ks}$ and HERG currents. PDD (100 nmol/L) was added to the oocyte incubation medium for 16 to 22 hours. A, Overnight, PDD incubation decreased $I_{Ks}$ (hminK) current density by 45.8±11% of control oocytes (without PDD). Overnight incubation with 100 nmol/L 4α-PDD, an inactive form of phorbol ester, had no significant effects on $I_{Ks}$ (hminK) currents. Representative current traces from a control and a PDD-treated oocyte are shown on the right. Scale bars=1 μA and 2 seconds. B, Oocytes injected with hminK cRNA were treated with various PKC inhibitors 2 hours before overnight 100 nmol/L PDD incubation. Chele indicates 20 μmol/L chelerythrine; calp, 4 μmol/L calphestin C; and stau, 4 μmol/L staurosporine. C, Overnight PDD incubation (100 nmol/L) had no effect on the expression of HERG channels. Current traces showing control and PDD-treated oocytes are shown on the right. Tail current amplitudes were measured at −70 mV after a test pulse to 30 mV for 5 seconds. Scale bars=1 μA and 2 seconds.
decrease in \( I_{Ks} \) current expression may be related to the internalization of surface membrane. In support of this, overnight incubation with 5 nmol/L PDD, or 100 nmol/L 4α-PDD, or inclusion of PKC inhibitors with 100 nmol/L PDD, all of which do not reduce \( I_{Ks} \) current, also produce no decrease in oocyte capacitance. If PKC phosphorylation of minK-KvLQT1 channels leads to internalization of the channels, perhaps the mechanism is similar to the phosphorylation-induced desensitization of the \( \beta \)-adrenergic receptors. \( \beta \)-Adrenergic receptors can be phosphorylated at multiple sites by several kinases such as \( \beta \)ARK and PKA.\(^{33} \) The phosphorylated receptor first exhibits a reduction in the agonist affinity, and then the receptors are internalized in the continued presence of agonist. However, it should be noted that in the present study, although there is a 49% (\( n=15 \)) decrease in oocyte capacitance; most of the reduction in \( I_{Ks} \) current is not occurring through this mechanism, because current density levels (normalizing for this reduction in membrane area), as shown on Figure 6, still decrease dramatically with long-term treatment with PDD. Other possible mechanisms to explain this reduction in current density include a turnover of \( I_{Ks} \) channels through synthesis and internalization modulated by PKC stimulation so that there is a large reduction in channel synthesis in addition to enhanced internalization or degradation. Whatever the precise mechanism, it appears to be specific for \( I_{Ks} \), because oocytes expressing HERG and treated overnight with PDD show no effects on the \( I_{Ks} \) current or the oocyte capacitance.

**Mutually Exclusive Modulation of \( I_{Ks} \) by PKC and PKA**

An interesting finding was the lack of comodulation by PKC and PKA on \( I_{Ks} \) current. We observed potentiation of both \( I_{Ks} \) (hminK) and \( I_{Ks} \) (cminK) currents by activators of PKA.\(^{15} \) Because there is no consensus PKA phosphorylation site on minK, it is likely that the PKA phosphorylation site(s) is on KvLQT1. It has been shown recently that KvLQT1, expressed alone or with minK, is stimulated by PKA.\(^{25} \) Our results show that after PKC modulation of \( I_{Ks} \), current, the current is no longer sensitive to PKA. The opposite is also true, because after \( I_{Ks} \) current potentiation by PKA, PKC is unable to modulate the current. Convergent modulation of ion channels by different protein kinases have been reported. For example, the type II \( Na^+ \) channel can be modulated by PKA after it is first phosphorylated at a PKC site.\(^{34} \) Nevertheless, the mutually exclusive actions of PKA and PKC on \( I_{Ks} \) current appears to be a novel mechanism of ion channel regulation. This exclusivity ensures that \( I_{Ks} \) current is modulated by either PKA or PKC, but not by both at the same time. Moreover, the order of PKA or PKC modulation is pivotal in determining the effect seen. In the partial sequence of *Xenopus* KvLQT1, a putative PKA site (serine 390) is flanked by 2 putative PKC sites (threonine 381 and serine 399).\(^{2} \) Because the PKA and PKC sites are located close to one another, steric hindrance of the negatively charged phosphate groups may prevent all sites from being phosphorylated. This would be one way to explain the mutually exclusive effects of PKC and PKA activation on \( I_{Ks} \) current. Alternatively, a conformational change caused by phosphorylation by a kinase may hide or block other sites from additional phosphorylation.

PKA activation by the \( \beta \)-adrenergic receptors stimulates \( I_{Ks} \) in cardiac myocytes of various species.\(^{6,16} \) It has also been shown that PDD activates \( I_{Ks} \) in guinea pig myocytes,\(^{22,25} \) whereas PDD decreases \( I_{Ks} \) in mouse myocytes.\(^{15} \) It is less clear how \( I_{Ks} \) is regulated in higher mammals, such as the cat and the human. Our results imply that regulation of human and cat \( I_{Ks} \) by PKC may be different from other species. It appears that although the serine/asparagine 102 site is probably involved, the exact molecular mechanism of PKC modulation of \( I_{Ks} \) currents is likely to be more complicated.

In summary, we have investigated the modulation of human and cat minK \( I_{Ks} \) currents by phosphorylation. PKC modulation of \( I_{Ks} \) currents is complex and depends on the time and concentration of PDD. Our results suggest that there are at least 2 functionally distinct PKC sites on KvLQT1-minK. One that potentiates \( I_{Ks} \) current, and another that downregulates it. Long-term exposure to PKC activators leads to irreversible downregulation of \( I_{Ks} \) current. PKA increases \( I_{Ks} \) current amplitude, and the effects of PKA and PKC are mutually exclusive. This suggests that PKA and PKC may phosphorylate common sites, or that phosphorylation of one site occludes phosphorylation of other sites. Given the importance of \( I_{Ks} \) in cardiac action potential repolarization, and its role in long-QT syndrome, understanding the regulation of human \( I_{Ks} \) may shed light on its physiological and pathophysiological roles. The ability to up- or downregulate \( I_{Ks} \) could be a means of controlling the duration of action potential in human heart. Further studies on the regulation of minK-KvLQT1 from different species coexpressed in mammalian cells should provide a better understanding of the modulation of this important cardiac current.

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1002  Modulation of $I_{Ks}$ by Protein Kinases C and A


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