Original Article

Functional study of the effect of phosphatase inhibitors on KCNQ4 channels expressed in Xenopus oocytes

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Aim: KCNQ4 channels play an important part in adjusting the function of cochlear outer hair cells. The aim of this study was to investigate the effects of ser/thr phosphatase inhibitors on human KCNQ4 channels expressed in Xenopus laevis oocytes.

Methods: Synthetic cRNA encoding human KCNQ4 channels was injected into Xenopus oocytes. We used a two-electrode voltage clamp to measure the ion currents in the oocytes.

Results: Wild-type KCNQ4 expressed in Xenopus oocytes showed the typical properties of slow activation kinetics and low threshold activation. The outward K+ current was almost completely blocked by a KCNQ4 blocker, linopirdine (0.25 mmol/L). BIMI (a PKC inhibitor) prevented the effects of PMA (a PKC activator) on the KCNQ4 current, indicating that PKC may be involved in the regulation of KCNQ4 expressed in the Xenopus oocyte system. Treatment with the ser/thr phosphatase inhibitors, cyclosporine (2 µmol/L), calyculin A (2 µmol/L) or okadaic acid (1 µmol/L), caused a significant positive shift in V1/2 and a decrease in the conductance of KCNQ4 channels. The V1/2 was shifted from -14.6±0.5 to -6.4±0.4 mV by cyclosporine, -18.8±0.5 to -9.2±0.4 mV by calyculin A, and -14.1±0.5 to -0.7±0.6 mV by okadaic acid. Moreover, the effects of these phosphatase inhibitors (okadaic acid or calyculin A) on the induction of a positive shift of V1/2 were augmented by further addition of PMA.

Conclusion: These results indicate that ser/thr phosphatase inhibitors can induce a shift to more positive potentials of the activation curve of the KCNQ4 current. It is highly likely that the phosphatase functions to balance the phosphorylated state of substrate protein and thus has an important role in the regulation of human KCNQ4 channels expressed in Xenopus oocytes.

Keywords: KCNQ4 channel; phosphatase inhibitor; calyculin A; okadaic acid; protein kinase C; phosphorylation; electrophysiology; Xenopus oocyte

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Introduction

KCNQ4, a novel potassium channel, plays a part in regulating the membrane potential and function of various cell types in the body[1]. The KCNQ4 current is a low-threshold, slow activating and noninactivating current that is expressed in the outer hair cells of the cochlea, brain, and heart. Mutations in KCNQ4 give rise to an inherited syndrome of deafness[2]. Therefore, the regulatory pathways of KCNQ4 channels play an important role in adjusting the function of outer hair cells. The KCNQ gene subfamily is composed of five K+ channels, KCNQ1 to KCNQ5. The KCNQ4 channel was included in the Kv nomenclature as Kv 7.4 (voltage-gated potassium channel subunit Kv7.4)[3]. The heteromers of KCNQ2/KCNQ3 underlie the neuronal M-current, which modulates neuronal excitability. Many intracellular messengers, eg, PIP2, IP3, diacylglycerol (DAG), calmodulin, calcineurin, activators/inhibitors of PKC, tyrosine kinases, and myosin light chain kinase, have been reported to modulate M currents[4–6]. Moreover, the A-kinase-anchoring protein AKAP150, which binds PKC, facilitates the inhibition of KCNQ2 current[7]. Analysis of recombinant KCNQ2 channels suggests that targeting of PKC through association with AKAP150 is important for inhibition. However, the effect of PKC activation on KCNQ channels is still controversial[8]. The inhibition of the metabolism of DAG by PKC kinase blockers cannot mimic the effect of muscarinic modulation by muscarinic agonist, and it has been suggested...
that activated PKC and its targets are not essential players in the acute muscarinic modulation of KCNQ channels in mammalian expression systems[8]. By contrast, the activation of PKC induced a large positive shift in the conductance-voltage curve for KCNQ channels expressed in *Xenopus* oocytes[9]. The activation of PKC had a different effect on KCNQ channels expressed in mammalian cells and *Xenopus* oocytes, which could be due to the different intracellular environment and basal levels of channel phosphorylation.

The electrophysiological properties of KCNQ4 channels are similar to those of heteromers of KCNQ2/KCNQ3, such as the shifting effect of the conductance-voltage response curve induced by M1 receptor stimulation[10], but the information about interactions between PKC and KCNQ4 is not clear. PKC, by phosphorylating its target protein and modulating its function, could interact with phosphatases. Protein phosphatases mediate the physiological dephosphorylation of target proteins, an activity that might be expected to reverse the effect induced by PKC. However, the inhibition of phosphatases can sometimes enhance the effect induced by a PKC activator. Therefore, the ser/thr phosphate inhibitors, cyclosporine, okadaic acid, and calyculin A, were used in this study to investigate the role of phosphatase on the activity of KCNQ4 channels expressed in *Xenopus* oocytes. Our results demonstrated that phosphatase inhibitors induced a shift in the voltage dependence of KCNQ4 channels to more positive potentials. Furthermore, the PKC activator PMA potentiates the effects of okadaic acid and calyculin A in the modulation of KCNQ4 channels. Thus, we propose that endogenous phosphatases play a role in the regulation of KCNQ4 channels and balance the excess activity of PKC in the *Xenopus* oocytes expression system.

**Materials and methods**

**Preparation of RNA**

The molecular biological procedures were performed much as previously described[11]. The plasmid cDNA encoding the human KCNQ4 channel was a generous gift from Prof Thomas J JENTSCH. The cDNA had been previously subcloned into the expression vector pTLN, which contains the 5' and 3' regions of the *Xenopus* ß-globin gene to boost expression in oocytes. Plasmid DNA was linearized with *HpaI* (Gibco BRL, USA). For synthesis of cRNA, the mMessage mMachine SP6-polymerase kit (Ambion, USA) was used. The nucleotide sequence of the construct was verified by automated DNA sequencing.

**Preparation of Xenopus oocytes**

*Xenopus* oocytes were collected from frogs anesthetized in 0.1% 3-aminobenzoate, methanesulfonic acid salt, Sigma-Aldrich). In brief, a lobe of an ovary was surgically removed from the frog’s abdominal cavity through a small incision and placed in modified Barth’s solution (MBS; in mmol/L: 88 NaCl, 1 KCl, 2.4 NaHCO3, 0.82 MgSO4, 0.41 CaCl2, 0.33 Ca(NO3)2, and 15 HEPES-Tris; adjusted to pH 7.6 with NaOH). Isolated oocytes were defolliculated enzymatically by incubation in collagenase (2 mg/mL, type I, Gibco, USA) in sterile MBS for 1–2 h followed by several washes in MBS containing 0.1% BSA (Sigma). Stage V–VI oocytes were then incubated and kept overnight at 18 °C. Healthy oocytes were selected, and cRNA (10–15 ng/50 nL) was microinjected into each oocyte using a Nanolject microinjector (Drummond, USA). Injected oocytes were maintained at 18 °C for 2–4 d in MBS supplemented with gentamicin (50 mg/L). The MBS was replaced with fresh medium once a day before electrophysiological recordings. All animal care and experimental procedures were performed according to the guidelines of the Animal Care Committee of the Chung Shan Medical University.

**Electrophysiology**

The two-electrode voltage clamp (TEVC) technique was used to record whole cell KCNQ4 currents in *Xenopus* oocytes at room temperature (22–26 °C) using an AxoClamp-2B amplifier (Molecular Devices, San Francisco, USA). One of the electrodes was connected to the HS-2x1L headstage to record voltage, while the other was connected to the HS-2x10 MG headstage to record current. Glass electrodes were made from capillary tubing on a vertical electrode puller (Model PP-830, Narishige Scientific Instrument Lab, Japan) and had a tip resistance of 0.5 to 2.0 MΩ when filled with 3 mol/L KCl. Whole-cell K+ currents were studied on the oocytes 2–4 d after the cRNA injection. During the experiments, oocytes were placed in an RC-3Z recording chamber (Warner Instruments, USA) and immersed in ND 96 solution consisting of (mmol/L) 96 NaCl, 1 KCl, 1 MgCl2, 1 CaCl2, and 5 Heps; the pH was 7.4. The chamber solution was connected through two 3 mol/L KCl 1% agar-bridged, Ag/AgCl reference electrodes to a virtual ground belonging to a current monitor headstage (VG-2A-x100, Molecular Devices, San Francisco, CA, USA). The condition of each single oocyte was checked before measurements by recording membrane potentials. Only oocytes with membrane potentials below -30 mV were used for current recordings. The data were digitized at 5 kHz and stored using Digidata 1322A (Molecular Devices, San Francisco, USA), and analysis was accomplished with pClamp 9.0 software (Molecular Devices, San Francisco, USA). To determine the conductance-voltage (G–V) relations, a step protocol was employed, whereby the oocytes were clamped at -80 mV for 3 s and depolarized at +60 mV with 20 mV increments to -100 mV, followed by a tail pulse at -30 mV for 2 s.

**Data analysis**

The tail current protocol was used to generate steady-state activation curves (conductance-voltage curves), which were fitted to a two-state Boltzmann function as follows

\[
I_{\text{tail} (V_m)} = I_{\text{tail} (\text{max})}/[1-\exp\left((V_{1/2} - V_m)/k\right)]
\]

where \(V_{1/2}\) is the voltage that produces half-maximal activation of conductance, \(V_m\) is the membrane potential, and \(k\) is the slope factor. \(I_{\text{tail} (\text{max})}\) is the maximal tail current amplitude. Data are presented as means±SEM. Statistical significance was determined using Student’s t test with one-way analysis of...
variance (ANOVA), and P values < 0.05 were taken to indicate statistically significant difference.

**Chemicals**

PMA (phorbol 12-myristate 13-acetate), cyclosporine, okadaic acid and calyculin A were obtained from LC Laboratories (USA). BIMI (bisindolylmaleimide I) was obtained from Calbiochem (San Diego, CA, USA). Linopirdine was obtained from Sigma (St Louis, MO, USA). PMA, okadaic acid and calyculin A were dissolved as stock solutions in dimethylsulfoxide (DMSO). Linopirdine was dissolved in 0.1 mol/L HCl as a 25 mmol/L stock solution.

**Results**

**Human KCNQ4 currents in Xenopus oocytes**

The KCNQ4-expressed oocytes were voltage clamped at -60 mV and stepped to potentials ranging from -80 to +60 mV, which produced slow-activated and low-threshold currents as shown in Figure 1A (upper traces). Application of linopirdine (0.25 mmol/L), a blocker of KCNQ4, to the external bath solution completely abolished the K⁺ outward current (Figure 1B). The inhibitory effect of linopirdine was reversible by washout (Figure 1C). Figure 1D shows the current-voltage curves of no injection, RNase-free water and KCNQ4 cRNA-injected oocytes. Only the KCNQ4 cRNA group currents show sensitivity to linopirdine. The time course of the peak tail current amplitudes is shown in Figure 1E. The application of linopirdine and washout are indicated by arrows. There was very little run-down of KCNQ4, as indicated by the control curve (without treatment).

**Effect of PKC activator on the KCNQ4 current**

PKC is an important regulator of KCNQ channels, as shown in previous work[9]. To test whether activation of PKC leads to a functional change of the KCNQ4 current in a Xenopus oocyte model system, PMA (a PKC activator) and BIMI (a PKC inhibitor) were used in this study. Bath application of PMA (2 µmol/L) caused a significant inhibitory effect on the amplitude of the KCNQ4 currents (Figure 2A) and a shift in $V_{1/2}$ (half-maximum of the conductance-voltage curve) to more positive potentials (Figure 2C). The $V_{1/2}$ before and after PMA treatment are from -17.7±0.9 mV to -6.5±0.7 mV ($P<0.05, n=15$). The time course of the tail current amplitudes showed that the effect of PMA is reversible by washout (Figure 2D). An inactive form of PMA, α-PMA, was found to exert an insignificant effect on the $V_{1/2}$ (-18.3±0.9 mV to -16.4±1.2 mV, $n=3$). Moreover, pretreatment with the PKC inhibitor BIMI (2 µmol/L) significantly attenuated the positive shift of $V_{1/2}$ of KCNQ4 induced by PMA. There was no significant difference between the steady-state conductance-voltage curves of BIMI and BIMI plus PMA (Figure 2B and 2E). These results suggested that the effect of PMA on the KCNQ4 channel was through the activation of PKC.

**Effect of phosphatase inhibitors on the KCNQ4 current**

Since the inhibition of phosphatase would maintain a higher
level of the phosphorylation of substrate proteins, the effect of ser/thr phosphatase inhibitors might increase the phosphorylation level of KCNQ4 channels. To test whether phosphatase inhibitors (cyclosporine, calyculin A, and okadaic acid) could cause changes in the electrophysiological properties of channels, we applied these phosphatase inhibitors to KCNQ4-expressed oocytes. Bath administration of cyclosporine (2 μmol/L) for 10 min produced a significant inhibitory effect on current amplitude (Figure 3A) and a positive shift of $V_{1/2}$ (from -14.6±0.5 to -6.4±0.4 mV; $n=5$) (Figure 3B). Similar results were also obtained from the application of calyculin A (2 μmol/L) and okadaic acid (1 μmol/L). Typical current traces are shown.
in Figures 4A to C for calyculin A (A, before treatment; B, after calyculin A; C, Calyculin A+PMA) and in Figures 4D to F for okadaic acid (D, before treatment; E, after okadaic acid; F, okadaic acid+PMA). The peak tail current amplitudes of KCNQ4 (at -20 mV) were inhibited by calyculin A (from 2.05±0.08 µA decrease to 1.45±0.13 µA; \( P<0.05, n=5 \)) and okadaic acid (from 1.64±0.05 µA decrease to 1.2±0.04 µA; \( P<0.05, n=4 \)). The inhibition effects of calyculin A and okadaic acid were significantly enhanced by the subsequent addition of 2 µmol/L PMA (from 1.45±0.13 µA to 0.76±0.07 µA for calyculin A; from 1.2±0.04 µA to 0.80±0.04 µA for okadaic acid). Detailed information on the half-activation voltage \( (V_{1/2}) \) can be obtained through the analysis of conductance-voltage (G–V) curves. The effects of calyculin A and okadaic acid on the conductance-voltage (G–V) curves of KCNQ4 are shown in Figure 4G and 4H, respectively. Both calyculin A and okadaic acid produced a shift in the voltage of half-maximal activation \( (V_{1/2}) \) to a more positive potential (calyculin A: from -18.8±0.5 to -9.2±0.4 mV; \( P<0.05, n=5 \); okadaic acid: from -14.1±0.5 to -0.7±0.6 mV; \( P<0.05, n=4 \)). Subsequently, the application of PMA after phosphatase inhibitor (calyculin A or okadaic acid) treatment induced a further positive shift in the \( V_{1/2} \) of KCNQ4 channels.

In other words, the change in electrophysiological parameters induced by a combined treatment with PKC activator (PMA) and phosphatase inhibitor on KCNQ4 was greater than that of either alone.

**Discussion**

In a previous study, Gamper et al.[12] found that the inhibition of tyrosine phosphatase reduces the conductance of KCNQ channels expressed in transfected Chinese hamster ovary cells. In the present study, we demonstrate that ser/thr phosphatase inhibitors can inhibit the conductance of KCNQ4 channels and shift the \( V_{1/2} \) (midpoint of conductance-voltage curve) to a more positive potential in a *Xenopus* oocyte expression system. This implies that the endogenous phosphatase is required for maintaining KCNQ4 channel activities in the *Xenopus* expression system. Moreover, the combination treatment of PMA (a PKC activator) and a phosphatase inhibitor showed a greater inhibitory effect than that of each alone, indicating that the most important inhibition in channel activity was due to the enhanced phosphorylation of KCNQ4. Thus, we propose that KCNQ4 channel activity is regulated by a balance between phosphorylation and dephosphorylation by the protein kinase.
and phosphatase.

PMA, a PKC activator, has been shown to produce a positive shift in the voltage dependence of KCNQ2 channels, while chelerythrine, a PKC inhibitor, attenuated the shift induced by muscarinic stimulation in the *Xenopus* oocytes expression system. In results consistent with the previous studies, we also found that the effect of PMA on the KCNQ4 channels was antagonized by pretreatment with a PKC inhibitor, BIMI. Interestingly, PKA can reduce the effects of elevated Ca2+ on run-down of expressed KCNQ4 channels. The different modulating effect by PKA and PKC may be due to the PKC phosphorylation sites that do not overlap with those phosphorylated by PKA.

A detailed characterization of the sites of phosphorylation of KCNQ2/KCNQ3 channels has been identified by mass spectrometry. The phosphorylation sites are the S4-S5 intracellular loop and the domain for tetramerization in the C terminus. Taken together, these studies suggest that the cytoplasmic phosphorylation sites of KCNQ channels may play an important role in channel inhibition through the activation by PKC. However, it has been reported that the stimulation of the diacylglycerol-PKC pathway does not play an essential role in the acute modulation of the KCNQ channel in a mammalian expression system. One possible explanation for the different results between *Xenopus* oocytes and mammalian expression systems is that a DAG-insensitive PKC (atypical PKC) exists in mammalian cells; another possibility is that the amount of active PKC or/and auxiliary protein differs depending on the intracellular environment. A recent study concerning the adaptor/auxiliary protein AKAP150 has found that it can interact simultaneously with both KCNQ2 and PKC, promoting the PKC-induced serine phosphorylation of KCNQ2 in a mammalian expression system. A mutant form of AKAP150 that is unable to bind PKC attenuates the current inhibition by M1-receptor activation. These observations support the hypothesis that the auxiliary protein AKAP150 is required for PKC-induced inhibition of KCNQ channels by M1-receptor activation.

Another possible way to modulate the activity of KCNQ channels is with phosphatases. The dephosphorylation of phosphorylated KCNQ proteins may be due to an initial stimulation of the membrane phosphatases, which would be expected to antagonize the inhibitory effect induced by PKC stimulation on KCNQ channels. In this case, the inhibition of phosphatase activity accounts for the altered balance between kinases and phosphatases, which in turn probably contributes to increasing the phosphorylated state of KCNQ4. Phosphatase inhibitors would be expected to cause a positive shift in *V*1/2 potentials if the endogenous phosphatase activity is present in this model system. Among the phosphatases, protein phosphatases 1 and 2A are major ser/thr protein phosphatases involved in many cellular events, including regulation of the cell cycle of *Xenopus* laevis oocytes. Cyclosporine, okadaic acid, and calyculin A are potent inhibitors of protein phosphatase. In this study, we show that treatment of *Xenopus* oocytes expressing human KCNQ4 with cyclosporine, okadaic acid, or calyculin A produced a positive shift of *V*1/2. These results indicate that endogenous phosphatase may modulate the human KCNQ4 channels expressed in *Xenopus* oocytes. A combination treatment of PMA plus phosphatase inhibitor produced a more positive shift of *V*1/2 than that of phosphatase inhibitor alone, indicating that the maintenance of a higher level of the phosphorylated proteins would result in a more dramatic positive shift in the *V*1/2 of KCNQ4 channels. Although our studies did not reveal the phosphorylation or dephosphorylation sites of KCNQ4, the inhibition of the dephosphorylation of phosphorylated proteins by phosphatase inhibitors did cause a significant change in the channel properties of KCNQ4. In summary, we demonstrated that phosphatase inhibitors induce a positive shift of the activation curve and an inhibition of channel conductance in human KCNQ4 expressed in *Xenopus* oocytes. Phosphatase may interact with substrates such as KCNQ4 or other auxiliary proteins to balance the phosphorylation level induced by protein kinase and thus play an important role in the regulation of KCNQ4 channel activity in a *Xenopus* oocyte expression system.

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**Author contribution**

Tzu-rong SU performed research. Cay-huyen CHEN analyzed data and preparations. Shih-jen HUANG analyzed data and reagents. Chun-yi LEE did part of preparations. Mao-chang SU performed TEVC recording. Gwan-hong CHEN performed oocytes isolation and TEVC recording. Shuan-yow LI, Jian-njou YANG wrote part of introduction. Min-jon LIN wrote the paper and performed part of research.

**References**

1. Kharkovets T, Hardelin JP, Safieddine S, Schweizer M, El-Amraoui A, Petit C, et al. KCNQ4, a K+ channel mutated in a form of dominant deafness, is expressed in the inner ear and the central auditory pathway. Proc Natl Acad Sci USA 2000; 97: 4333–8.
2. Kubisch C, Schroeder BC, Friedrich T, Ludjohann B, El-Amraoui A, Martin S, et al. KCNQ4, a novel potassium channel expressed in sensory outer hair cells is, mutated in dominant deafness. Cell 1999; 96: 437–46.
3. Gutman GA, Chandy KG, Adelman JP, Aiyar J, Bayliss DA, Clapham DE, et al. International Union of Pharmacology. XLI. Compendium of voltage-gated ion channels: potassium channels. Pharmacol Rev 2003; 55: 583–6.
4. Marrion NV. Control of M-current. Annu Rev Physiol 1997; 59: 483–504.
5. Loussouarn G, Park KH, Bellocq C, Baro I, Charpentier F, Escande D. Phosphatidylinositol-4,5-bisphosphate, PI(2), controls KCNQ1/KCNEL voltage-gated potassium channels: a functional homology between voltage-gated and inward rectifier K+ channels. EMBO J 2003; 22:
Zhang H, Craciun LC, Mirshahi T, Rohacs T, Lopes CM, Jin T, et al. PIP2 activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. Neuron 2003; 37: 963–75.

Hoshi N, Zhang JS, Omaki M, Takeuchi T, Yokoyama S, Wanaverbecq N, et al. AKAP150 signaling complex promotes suppression of the M-current by muscarinic agonists. Nat Neurosci 2003; 6: 564–571.

Zhang H, Craciun LC, Mirshahi T, Rohacs T, Lopes CM, Jin T, et al. PIP2 activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. Neuron 2003; 37: 963–75.

Suh BC, Hille B. Does diacylglycerol regulate KCNQ channels? Pflugers Archive Eur J Physiol 2006; 453: 293–301.

Nakajo K, Kubo Y. Protein kinase C shifts the voltage dependence of KCNQ/M channels expressed in Xenopus oocytes. J Physiol 2005; 569: 59–74.

Delmas P, Brown DA. Pathways modulating neural KCNQ/M (Kv7) potassium channels. Nat Rev Neurosci 2005; 6: 850–62.

Su CC, Li SY, Yang JJ, Su MC, Lin MJ. Studies of the effect of ionomycin on the KCNQ4 channel expressed in Xenopus oocytes. Biochem Biophys Res Commun 2006; 348: 295–300.

Gamper N, Stockland JD, Shapiro MS. Subunit-specific modulation of KCNQ potassium channels by Src tyrosine kinase. J Neurosci 2003; 23: 84–95.

Chambard JM, Ashmore JF. Regulation of the voltage-gated potassium channel KCNQ4 in the auditory pathway. Pflug Arch Eur J Physiol 2005; 450: 34–44.

Surti TS, Huang L, Jan YN, Jan LY, Cooper EC. Identification by mass spectrometry and functional characterization of two phosphorylation sites of KCNQ2/KCNQ3 channels. Proc Natl Acad Sci USA 2005; 102: 17828–33.

Schwake M, Jentsch TJ, Friedrich T. A carboxy-terminal domain determines the subunit specificity of KCNQ K+ channel assembly. EMBO Rep 2003; 4: 76–81.

Howard RJ, Clark KA, Holton JM, Minor DL Jr. Structural insight into KCNQ (Kv7) channel assembly and channelopathy. Neuron 2007; 53: 663–75.

Walker DH, DePaoli-Roach AA, Maller JL. Multiple roles for protein phosphatase 1 in regulating the Xenopus early embryonic cell cycle. Mol Biol Cell 1992; 3: 687–98.

Lee TH. The role of protein phosphatase type-2A in the Xenopus cell cycle: initiation of the G2/M transition. Semin Cancer Biol 1995; 6: 203–9.

Janssens V, Goris J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signaling. Biochem J 2001; 353: 417–39.