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Acceleration of K^+ channel inactivation by MEK inhibitor U0126

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Yuan, Li-Lian, Xixi Chen, Kumud Kunjilwar, Paul Pfaffinger, and Daniel Johnston. Acceleration of K^+ channel inactivation by MEK inhibitor U0126. *Am J Physiol Cell Physiol* 290: C165–C171, 2006. First published August 31, 2005; doi:10.1152/ajpcell.00206.2005.—Voltage-dependent (Kv)4.2-encoded A-type K^+ channels play an important role in controlling neuronal excitability and are subject to modulation by various protein kinases, including ERK. In studies of ERK modulation, the organic compound U0126 is often used to suppress the activity of MEK, which is a kinase immediately upstream from ERK. We have observed that the inactivation time constant of heterologously expressed Kv4.2 channels was accelerated by U0126 at 1–20 μM. This effect, however, was not Kv4 family specific, because U0126 also converted noninactivating K^+ currents mediated by Kv1.1 subunits into transient ones. To determine whether U0126 exerted these effects through kinase inhibition, we tested U0125, a derivative of U0126 that is less potent in MEK inhibition. At the same concentrations, U0125 had effects similar to those of U0126 on channel inactivation. Finally, we expressed a mutant form of Kv4.2 in which three identified ERK phosphorylation sites (T602, T607, and S616) were replaced with alanines. The inactivation of K^+ currents mediated by this mutant was still accelerated by U0126. Our data favor the conclusion that the increase in the rate of channel inactivation by U0126 is likely to be independent of protein kinase inhibition and instead represents a direct action on channel gating.

voltage-gated potassium channel; kinase; gating

VOLTAGE-DEPENDENT K^+ CHANNELS are critical regulators of membrane excitability. Neurons express various types of K^+ channels with different voltage dependencies and different time-dependent inactivation kinetics. Among the most distinguishable is the A-type K^+ current, which activates and inactivates rapidly upon membrane depolarization.

The Kv4.2 channel gene encodes a channel-forming α-subunit that exhibits rapid activation and inactivation when exogenously expressed and is probably the major contributor to A-type K^+ current found in many types of neurons (5). Biochemically, Kv4.2 subunits are directly phosphorylated by PKA, PKC, CaMKII, and MAPK-ERK (1, 3, 21). Phosphorylation of Kv4.2 subunits affects certain aspects of channel behavior, including surface expression and voltage-dependent kinetics (12, 21, 25). The phosphorylation of Kv4.2 by ERK is especially interesting because ERK appears to mediate downstream effects of both PKA and PKC in kinase modulation of both hippocampal A-type K^+ channels and Kv4.2 subunits (25).

The study of the ERK signaling pathway, however, is limited by the lack of specific pharmacological agents directly targeting ERK. Nevertheless, manipulation of ERK activity can be achieved by using organic compounds to inhibit MEK, a kinase immediately upstream from ERK. U0126 and PD-98059 are two MEK inhibitors proved to be specific among kinases (7, 10) and are thus valuable pharmacological tools for studying the ERK signaling pathway. Specifically, it was reported previously that 10 μM U0126 treatment completely blocked intracellular ERK phosphorylation in cultured COS cells (10). Using these pharmacological tools, it has been demonstrated in various preparations and cell types that the ERK signaling pathway plays important roles in modulating K^+ channels (13, 14, 25), as well as the induction and expression of synaptic plasticity (8, 9, 17, 22, 23, 24). The concentration of U0126 used in these studies involving brain slices has typically been 20 μM.

Accumulating evidence, however, also suggests that some protein kinase inhibitors interact directly with voltage-gated ion channels in ways separate from kinase inhibition. For example, the tyrosine kinase inhibitor AG-1478 has been shown to block Kv1.5-mediated K^+ currents, possibly acting as an open channel blocker (6). Similarly, calphostin directly inhibits muscarinic K^+ channels (15), whereas staurosporine can directly inhibit L-type Ca^{2+} channels (11). It is therefore important to investigate possible nonspecific effects associated with a kinase inhibitor.

In this study, we investigated MAPK-ERK regulation of Kv4.2 subunits by using the MEK inhibitor U0126. We found that U0126 significantly accelerated the inactivation of Kv4.2-mediated A-type K^+ currents. The effect had rapid onset, was fully reversible, and did not depend on the modulation of protein phosphorylation.

METHODS

Transfection and cell culture. Chinese hamster ovary (CHO-K1) cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% FBS, 40 μg/ml L-proline, 100 U/ml penicillin, and 100 U/ml streptomycin. CHO cells were transiently transfected with rat full-length Kv4.2 or triple ERK phosphorylation site mutant (triple A mutant) using LipofectAMINE reagent (Invitrogen, Carlsbad, CA). Enhanced green fluorescence protein (EGFP) was cotransfected to help identify transfected cells. To increase surface expression of functional Kv4.2 channels, cells were also cotransfected with K^+ channel-interacting protein 3 (KChIP3). Construct of the triple ERK site mutant of Kv4.2 was a gift from Dr. David Sweatt (Baylor College of Medicine). CHO cells stably expressing rat Kv1.1 also were used in this study.

Electrophysiological recordings. A Zeiss Axioskop fitted with a ×40 magnification water-immersion objective and differential interference contrast was used to view cultured cells. Transfected cells expressing EGFP were identified under the fluorescence microscope. Whole cell voltage-clamp and outside-out patch-clamp experiments were performed in CHO cells using an Axopatch 200 amplifier. All experiments were performed at room temperature. The bath solution

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contained (in mM) 134 NaCl, 2 KCl, 5.6 NaOH, 1.8 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, with pH 7.4. The pipette solution contained (in mM) 140 KCl, 1 $MgCl_2$, 1 EGTA, 0.133 $CaCl_2$ (free Ca^{2+} concentration ~10 nM), and HEPES 10, pH 7.4. The K^+ current reversal potential measured under this condition was approximately −85 mV. The liquid junctional potential between the pipette solution and the bath solution was measured as −7 mV and was not corrected. Pipettes had resistances between 2 and 5 MΩ. Whole cell capacitance and series resistances were compensated to >80%.

Data acquisition and analysis. Pulse generation and data acquisition were controlled with custom software written in Igor Pro (Wavemetrics). Current records were analog filtered at 2 kHz and digitized at 10 kHz. Linear leak and capacitive currents were digitally subtracted using null traces or scaled traces with smaller voltage steps. Steady-state activation and inactivation curves were constructed and fitted as described previously (12). Current inactivation and recovery from inactivation kinetics were determined by single or double exponential curve fitting. Statistical significance was set at $P < 0.05$ and was determined using two-sample *t*-tests. Error bars represent SE.

Drugs. U0126 (Promega, Madison, WI), U0125, and PD-98059 (Calbiochem, CA) were dissolved in DMSO to 10 mM, kept frozen until use, and then diluted to the appropriate concentrations.

RESULTS

Reversible and direct effect on Kv4.2 subunit-mediated currents by U0126. We expressed A-type K^+ currents in heterologous CHO cells by coexpression of Kv4.2 with KChIP3 and tested for functional effects of the drug U0126. Bath application of 20 μM U0126 rapidly (within 1–2 min at 1.5–2 ml/min flow rate) decreased current amplitude and increased channel inactivation (Fig. 1*A*). These effects were readily reversible. Before U0126 application, the decay phase of Kv4.2-encoded K^+ currents could be fit with a single exponential with a time constant (τ) that became slower as a function of membrane voltage depolarization under control conditions (Fig. 1*C* and Table 1). In the presence of U0126, however, the decay phase of the K^+ currents became accelerated and were best fit with two exponentials. The accelerating effect of U0126 on channel inactivation was also voltage dependent; however, in this case, both the fast and slow τ values became faster with depolarization (Fig. 1, *C* and *D*, and Table 1).

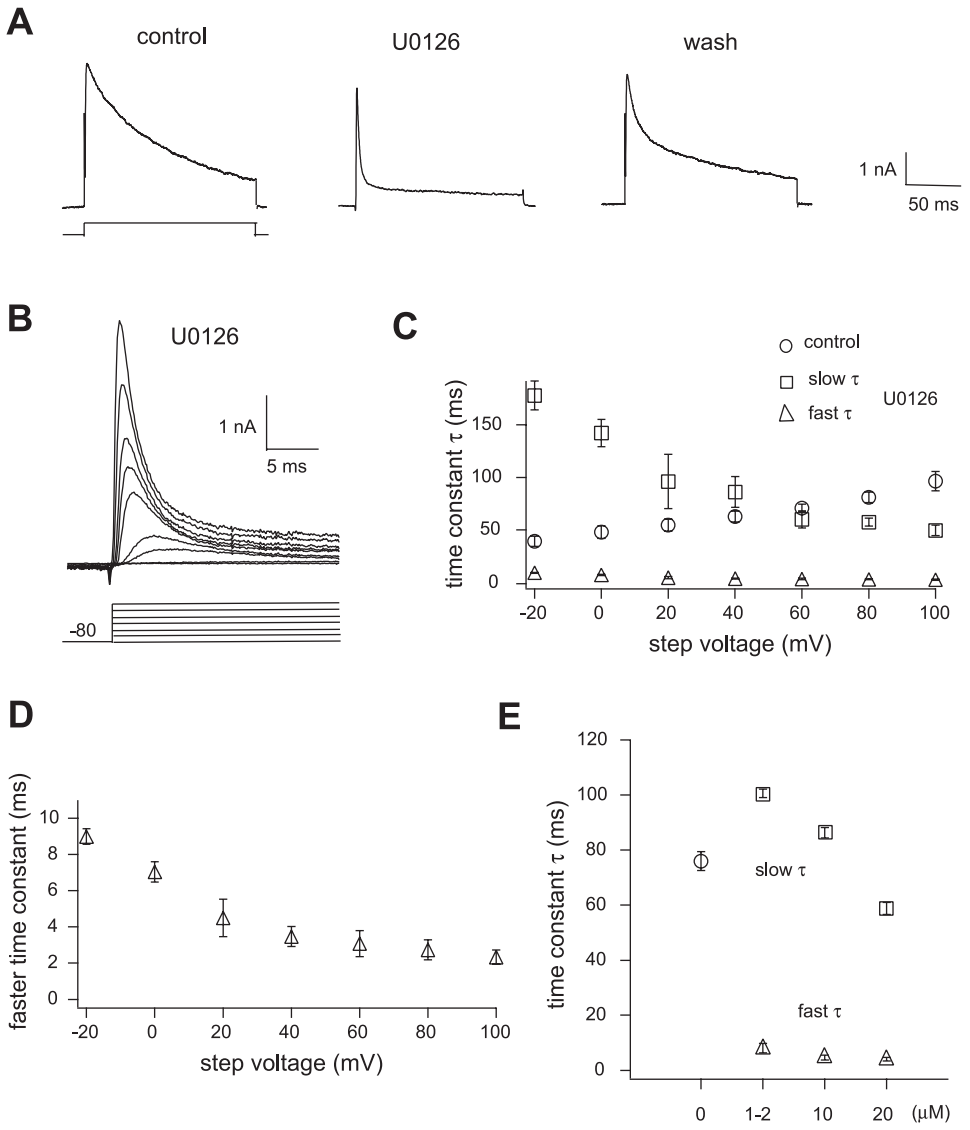

Fig. 1. Effects of U0126 on voltage-dependent K^+ (Kv)4.2-encoded channels were reversible as well as concentration and voltage dependent. *A*: application of 20 μM U0126 changed the kinetics of inactivation of K^+ currents mediated by Kv4.2 subunits coexpressed with K^+ channel-interacting protein 3 (KChIP3). The accelerating effect on channel inactivation occurred within 1–2 min and was readily reversible with washout. *B*: representative K^+ current traces in the presence of 20 μM U0126 in response to a family of voltage steps from −80 mV to +100 mV. *C*: current inactivation under control condition was fit with a single exponential function, and the time constant (τ) increased as a function of voltage (○). In the presence of U0126, current inactivation was best fit with a double exponential function. Both fast τ (△) and slow τ (□) decreased as a function of voltage. The voltage dependence of the fast τ can be visualized better in *D*. *E*: U0126 increased channel inactivation in a concentration-dependent manner.

Table 1. *Current decay kinetics at various voltages*

V_c, mV	−40	−20	0	+20	+40	+60	+80
Control	39.1±4.8	47.8±6.2	54.5±5.7	62.6±5.3	70.7±3.8	80.9±4.9	96.3±9.3
U0126, τ_{fast}	177.9±13.7	142.2±13.1	96.0±25.9	86.0±14.7	60.0±8.1	57.2±3.7	49.4±5.0
U0126, τ_{slow}	9.0±0.4	7.0±0.6	4.5±1.0	3.5±0.5	3.1±0.7	2.7±0.5	2.3±0.4

Values are means ± SE. V_c, command voltage. Inactivation time constants (τ; ms) of voltage-dependent K^+ (Kv4.2)-mediated currents at various voltages in control condition and in presence of 20 μM U0126.

U0126 exerted its effects on channel inactivation in a concentration-dependent manner. At the concentrations tested (~1–2, 10, and 20 μM), both slow τ (100.6 ± 1.5 ms, 86.2 ± 1.9 ms, and 58.8 ± 2.2 ms) and fast τ (8.1 ± 1.9 ms, 4.7 ± 0.9 ms, and 4 ± 0.7 ms) accelerated as a function of U0126 concentration at the holding potential (V_h) of −80 mV and command voltage (V_c) of +100 mV (Fig. 1*E*). To exclude the possibility that the coexpressed KChIP3 mediated this effect, we conducted similar experiments on CHO cells expressing Kv4.2 subunits alone. Although currents mediated by Kv4.2 alone were much smaller in amplitude and faster in inactivation kinetics (2), U0126 accelerated inactivation in a manner similar to that of currents mediated by Kv4.2 with KChIP3. For example, inactivation of currents mediated by Kv4.2 subunits alone can be fitted with a single exponential with τ = 20.7 ± 3.0 ms, while the presence of 20 μM U0126 accelerated τ to 2.7 ± 1.0 ms (V_c = ~60–80 ms; n = 4).

We tested whether modulation with another commonly used MEK inhibitor could mimic these effects on current decay. As previously reported, PD-98059 at 25 μM shows effects on inhibiting MEK activity similar to those of 20 μM U0126. We found that 25 μM PD-98059 had no significant effect on the inactivation of Kv4.2/KChIP3-encoded K^+ currents (control, 86.7 ± 5.3 ms, n = 7, vs. PD-98059, 73.9 ± 5.8 ms, n = 4; P = 0.15).

U0126 exerted its effect on Kv4.2 channel inactivation independently of protein kinase inhibition. The quick onset and rapid washout of the effect of U0126 on channel inactivation and the lack of effect of 25 μM PD-98059 suggested that U0126 may have exerted this effect independently of protein kinase inhibition. We tested this hypothesis in several ways.

We first tested whether the effect persisted in the absence of GTP/ATP, substrates that are necessary for protein phosphorylation. We pulled outside-out patches from CHO cells expressing Kv4.2 and KChIP3 without including ATP/GTP in the pipette solution. The decay phase of currents recorded under this condition was fitted with a single exponential with τ of 64.1 ± 1.5 ms (V_c = +80 mV; n = 5). Consistently, bath application of 10 μM U0126 significantly accelerated the decay kinetics, which were best fit with a double exponential (τ_{slow} = 54.8 ± 4.7 ms, τ_{fast} = 6.0 ± 1.6 ms, V_c = +80 mV; n = 5).

We then tested the U0126 derivative U0125 that structurally differs from U0126 by only two amine groups, while its potency for MEK inhibition is ~10-fold less than U0126 (10). Application of U0125 also produced a dose-dependent acceleration of channel inactivation (Fig. 2*A*). To compare the accelerating effect on channel inactivation produced by 20 μM U0126 and U0125, we measured channel half-inactivation time, or the time needed for current to decay to one-half of the peak amplitude. As shown in Fig. 2*B*, half-inactivation times in the presence of 20 μM U0126 and U0125 were 5.5 ± 1.0 ms (n = 7) and 8.1 ± 2.5 ms (n = 4), respectively, at V_c of +100 mV. Both were significantly different from that of control (60.4 ± 5.4 ms, n = 7; $P < 0.005$).

The third approach involved an ERK site mutant of Kv4.2. Three ERK phosphorylation sites, T602, T607, and S616, have been identified in Kv4.2 (1). Mutation of these threonine and serine residues into alanines should prevent any MEK phosphorylation of Kv4.2 subunits at these sites. We expressed the triple ERK site mutant (triple A mutant) with KChIP3 in CHO cells. There were no significant differences in current kinetics between the mutant and wild-type channels (decay τ: wild type, 81.3 ± 2.6 ms, n = 11; ERK mutant 79.1 ± 4.7 ms, n = 7, V_h = −80 mV, V_c = +80 mV). Despite the removal of all known ERK phosphorylation sites on the channel, application of 10 μM U0126 still resulted in a faster rate of current inactivation (Fig. 2, *C* and *D*), suggesting that U0126 was acting through a phosphorylation-independent mechanism (ERK mutant with 10 μM U0126; τ_{slow} = 81.8 ± 6.5 ms, τ_{fast} = 4.4 ± 2.1 ms; n = 4).

Site of action of U0126. The results obtained from the above experiments do not support a mechanism whereby U0126 accelerated channel inactivation through inhibition of protein phosphorylation. To test further whether U0126 was acting through an intracellular signaling pathway, 20 μM U0126 was included in the pipette solution that recorded Kv4.2-mediated K^+ current in CHO cells expressing Kv4.2 and KChIP. After breakin and establishment of a whole cell configuration, the magnitude and kinetics of Kv4.2-mediated A-type currents were monitored and found to be unaltered compared with control experiments (percentage change of inactivation τ = 7.6 ± 2.5%, n = 4, measured within 10–15 min). Subsequent external application of 20 μM U0126, however, promptly changed the amplitude and kinetics of Kv4.2 currents (n = 3). The results from exponential fitting of the current decay phases before and after U0126 in these experiments are summarized in Table 1. These results suggest that U0126 exerts its effect on Kv4.2 subunits at a site external to the channel. We have observed a similar phenomenon in cultured hippocampal neurons (Yuan LL and Johnston D, unpublished data) in which application of 20 μM U0126 in pipette solution appeared not to change the amplitude or kinetics of native K^+ currents in these neurons (n = 4).

U0126 exerted similar effects on other Kv subunits. Because U0126 appeared to interact with Kv4.2 subunits directly from outside the plasma membrane, we tested whether this interaction was specific for Kv4.2 subunits by expressing Kv4.3 in CHO cells (without KChIP3). We found that in the absence of KChIP3, Kv4.3 subunits in CHO cells produced A-type K^+ currents with comparable amplitude and kinetics similar to those of Kv4.2/KChIP3. Application of 10 μM U0126 to cells

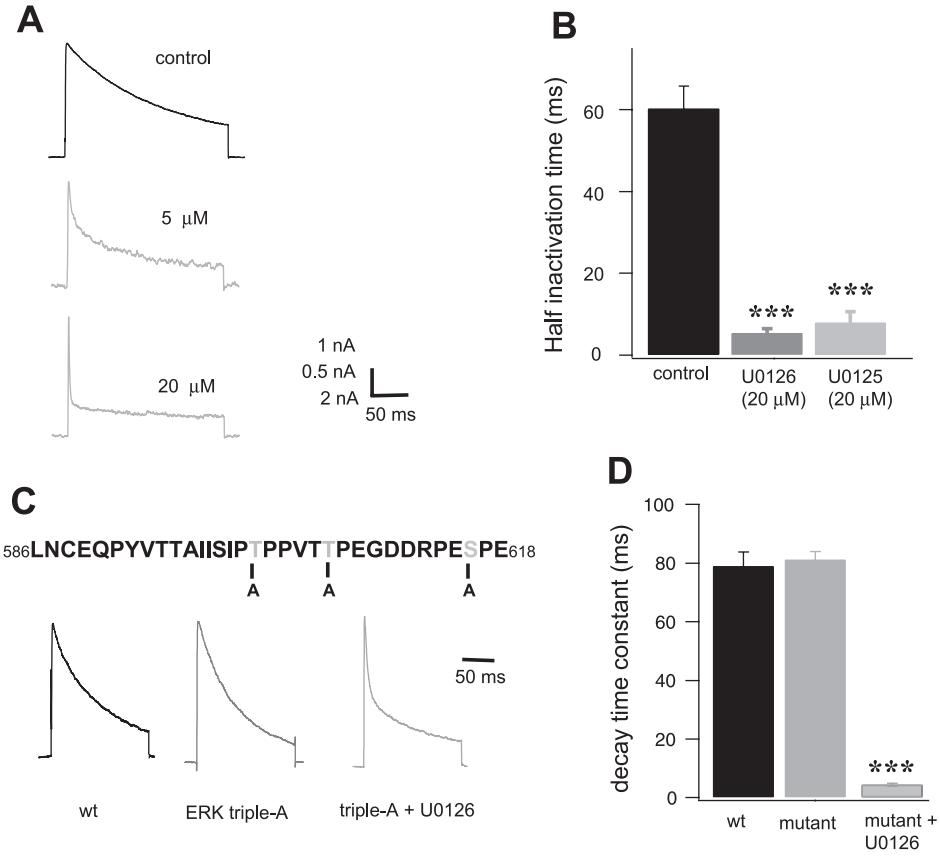

Fig. 2. Effects of U0126 on Kv4.2-encoded channels were not dependent on protein kinase phosphorylation. *A*: U0125 at 5 and 10 μM increased the rate of channel inactivation. *B*: summarized half-inactivation time of K^+ currents in Chinese hamster ovary (CHO-K1) cells mediated by Kv4.2 + KChIP3 [holding potential (V_h) = −80 mV, command voltage (V_c) = +100 mV]. Bath application of U0126 and U0125 at 20 μM decreased the half-inactivation time significantly. ***$P < 0.005$. *C*: three identified ERK phosphorylation sites of Kv4.2 (T602, T607, and S616) were mutated into alanines without triple A mutant. In response to a voltage step from −80 mV to +40 mV, K^+ currents recorded from CHO cells expressing the triple A mutant showed kinetics similar to those of wild-type Kv4.2 subunits. U0126 (10 μM) increased the inactivation rate of currents mediated by the triple A mutant of Kv4.2 subunits. *D*: summarized data. ***$P < 0.005$.

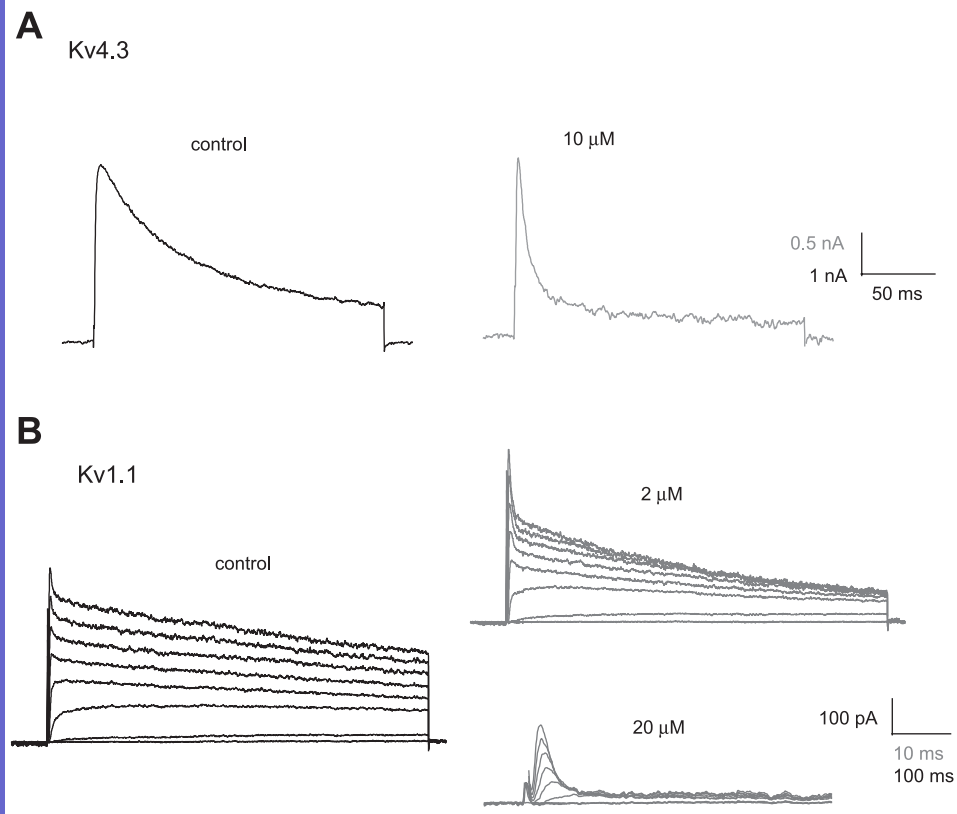

Fig. 3. U0126 exhibited similar effects on other Kv4 and non-Kv4 subunits. *A*: Kv4.3 expression produced fast-activating and fast-inactivating K^+ currents in CHO cells in response to a voltage step from −80 mV to +80 mV. U0126 suppressed the current amplitude and increased the rate of inactivation to an extent similar to that of Kv4.2. *B*: Kv1.1 subunits encoded noninactivating K^+ currents in the absence of its β-subunits. In response to a family of voltage steps from −80 mV to +80 mV with 20-mV increments, the noninactivating currents were converted to fast-inactivating currents by 20 μM U0126.

expressing Kv4.3 subunits accelerated the channel inactivation τ from 57.9 ± 0.4 ms to 10.4 ± 0.4 ms (V_c = +80 mV), which is similar to that observed with Kv4.2 subunits (Fig. 3*A*).

We also extended our investigation to another non-Kv4 subunit, Kv1.1. Kv1.1 was stably expressed in CHO cells. In the absence of other auxiliary subunits, such as appropriate β-subunits, the currents mediated by Kv1.1 represented typical noninactivating K^+ currents. U0126 changed the current kinetics dramatically in a concentration-dependent manner. For example, 20 μM U0126 significantly decreased the peak current amplitude and, more strikingly, converted these noninactivating currents into fast-inactivating or transient ones (Fig. 3*B*). Inactivation kinetics of those transient currents can be fit with single exponentials with τ on the order of several milliseconds (τ = 3.2 ± 0.2 ms, V_c = 80 mV; n = 3).

Mechanisms of U0126 blocking. The increased rate of current inactivation produced by the application of U0126 could result from channel open state blocking, modulation of gating mechanisms, or both. The voltage dependence of acceleration of current decay by U0126 that we observed (Fig. 1, *C* and *D*, and Table 1) suggested that this ERK inhibitor might work as an open channel blocker. To explore these different possibilities further, we chose to measure the low-voltage inactivation kinetic properties of Kv4.2-encoded channel kinetics in the absence and presence of U0126. Most studies on Kv4.2 channels (4) suggest that at voltage near the one-half steady-state inactivation voltage ($V_{1/2}$) the closed-state inactivation process dominates channels inactivation. Consequently, modulation of closed-state inactivation by U0126 should shift $V_{1/2}$ and change the kinetics of entry into closed-state inactivation at low voltages. We first verified that U0126 did not produce any significant effects on channel steady-state activation properties. As expected, steady-state activation of Kv4.2-mediated currents were not significantly changed by 10 μM U0126 ($V_{1/2}$ before and after drug addition were 13.5 ± 4.1 mV and 17.4 ± 4.4 mV, respectively; $P > 0.05$). $V_{1/2}$ of steady-state inactivation curve, however, was shifted in a hyperpolarizing direction by 7.2 mV (Fig. 4*A*) from −49.0 ± 2.1 mV in control to −56.2 ± 3.1 mV in U0126 (n = 5; $P < 0.005$, paired t-test). To assess kinetics of entry into closed-state inactivation, we used a protocol by which Kv4.2 channels were inactivated by a prepulse to −50 mV (Fig. 4*A*, dashed line), followed by a test pulse to +40 mV (Fig. 4*B*). The difference between currents measured using this protocol (*protocol 1*) and the protocol without a prepulse (*protocol 2*) (Fig. 4*B*) represents the portion of Kv4.2 channels that went directly into closed-state inactivation. As shown in Fig. 4, *C* and *D*, the ratio of *current 1* to *current 2* was reduced from 53.2 ± 9.2% to 32.0 ± 9.7% ($P <$ 0.05) by the application of U0126. In other words, the percentage of channels directly entering into closed-state inactivation was higher in the presence of U0126 than that in control experiments. These results suggest that U0126, an ERK inhibitor, sped up the entry of Kv4.2 channels from the closed state directly into closed-state inactivation without opening.

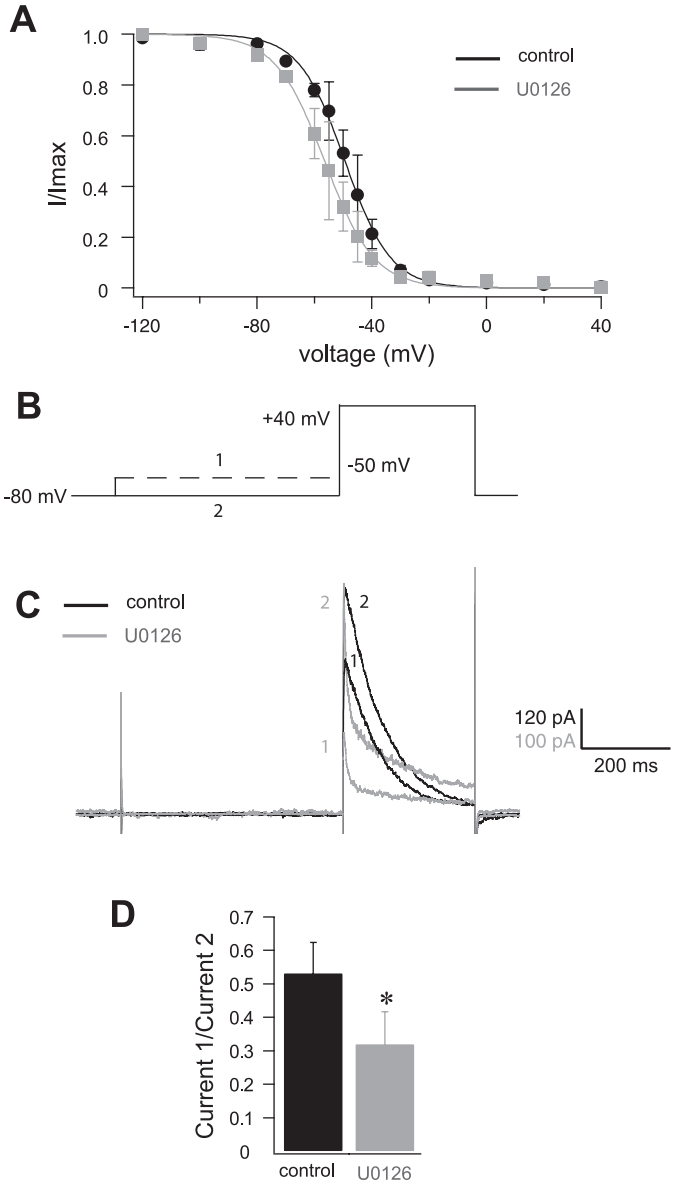

Fig. 4. U0126 modulated the inactivation gating mechanism of Kv4.2 channels. *A*: U0126 (10 μM) shifted the steady-state inactivation curve to a hyperpolarizing direction. *B*: voltage protocols for assessing the kinetics of entry into closed-state inactivation. *C*: representative current traces induced by the protocols described before and after 10 μM U0126 application. A prestep to −50 mV did not activate Kv4.2 channels, but the current after the +40-mV step (*current 1*) was significantly reduced compared with that induced by the +40-mV step alone (*current 2*). The ratio of *current 1* to *current 2* reflected the percentage of channels entering into closed-state inactivation. Traces are scaled to *current 2* in control to show the difference in amplitude ratio better. *D*: application of U0126 caused a significant reduction in ratio of *current 1* to *current 2* (*$P < 0.05$), suggesting a larger percentage of channels entering into closed-state inactivation in the presence of U0126.

DISCUSSION

We have observed that U0126, a widely used MEK inhibitor, suppressed Kv4.2-mediated K^+ currents and accelerated current decay. The decrease in current amplitude and acceleration of current decay by U0126 could be a result of a combination of open channel blocking and channel-gating modulation. These effects of U0126 did not seem to be specific to Kv4 subunits. Although we cannot completely exclude the possibility that this effect of U0126 occurred through kinase inhibition, the results strongly support the conclusion that the inhibition of K^+ currents by U0126 occurs through direct interaction between the drug molecule and the channels.

First, the effect took place rapidly, within 1 min of U0126 application, and reversed within a few minutes of washout.

Although one cannot reach a conclusion solely on the basis of this observation without further testing for any involvement of protein kinases, the quick onset and offset of the drug effect seem to favor U0126's accelerating the inactivation process by interacting directly with channel subunits. Second, U0125, a 10-fold less potent analog of U0126 in MEK inhibition, showed an effect on inactivation similar to that of U0126 at the same concentrations. Third, U0126 showed similar effects on outside-out patches without ATP/GTP supplied. Fourth, we observed that greater inhibition occurred at higher voltage steps, suggesting that U0126 binding of channel subunits modulates a voltage-dependent process. This process is most likely closed-state inactivation. Fifth, we found membrane polarity to the effects of U0126. If U0126 had to cross the membrane to exert its effect on kinase inhibition, then inclusion of U0126 in recording pipette solution should have mimicked the effect of the bath application; however, this was not the case.

Finally, if the channel-inhibiting effects of U0126 were dependent on protein kinase phosphorylation, we would have expected that U0126 would not have less effect on a triple mutant of Kv4.2 2 in which three identified phosphorylation sites were mutated to alanine residues. Instead, U0126 had effects on this triple mutant that were similar to the effects it had on wild-type Kv4.2 channels. The effects of U0126 on current amplitude and channel inactivation kinetics also were not specific to Kv4.2-encoded K^+ channels. The specificity of U0126's direct interaction with K^+ channels was tested on two representative subunits, Kv4.3 and Kv1.1. Kv4.3 is a Kv4 subfamily member that possesses potential MAPK phosphorylation consensus similar to that of Kv4.2 subunits and also underlies transient K^+ currents. In contrast, Kv1.1 shows less sequence conservation with Kv4.2, has no potential MAPK phosphorylation consensus, and encodes noninactivating K^+ currents when expressed without appropriate β-subunits. Both Kv4.2 and Kv1.1 behaved similarly to Kv4.2 subunits in the presence of U0126, which suggests that the effects of U0126 reported herein were not specific to Kv4.2 subunits and not dependent on the ERK phosphorylation sites.

Apart from the unexpected effects of U0126 on Kv4.2 reported herein, an important question is whether ERK phosphorylation alters Kv4.2 channel kinetics. This hypothesis has been tested recently using a different approach (Schrader L, Nadin B, Birnbaum S, Bui D, Anderson A, and Sweatt J, unpublished observations). Schrader and colleagues have mutated the three phospho-sites of Kv4.2 subunits to aspartate to mimic phosphorylation. They found that only the T607D mutation mimicked the expected kinetic changes elicited by ERK/MAPK activation, which is a rightward shift of the activation curve and an overall reduction in current amplitude compared with the wild type. This effect is dependent on the ancillary KChIP subunits. Contrary to expectations, the S616D mutation resulted in an opposite effect: a leftward shift of the activation curve. These results suggest that ERK modulation of Kv4.2 channel kinetics is complex and that the overall effect of modulation may well depend on ancillary subunits.

Finally, it is interesting to note that although the effects of U0126 in suppressing Kv current amplitude and accelerating inactivation are dramatic, we previously performed similar experiments on the native dendritic A-type K^+ currents of hippocampal CA1 pyramidal neurons without noting such effects (23). Application of 20 μM U0126 to the native channel had no effect, regardless of whether it was applied to the outside of the neurons or included in the whole cell patch pipette, despite the fact that these native channels are likely from Kv4.2 subunits (or possibly Kv4.3 subunits) that are clearly sensitive to U0126 (16, 19, 20). One significant difference from native channels is that they inactivate much faster than the channels we recorded in the present study. Thus it is possible that some native modulatory protein or other cofactor missing from our heterologous expression system was acting on the site modulated by U0126.

In conclusion, we observed dramatic and unexpected effects of U0126 on Kv channels. Our results favor U0126's exerting these effects on Kv channels not through kinase-dependent mechanisms but instead through direct action on gating mechanisms (18). The data reported herein suggest that caution should be exercised when interpreting experimental results using U0126.

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