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Molecular Basis for Differential Sensitivity of KCNQ and I_{Ks} Channels to the Cognitive Enhancer XE991

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ABSTRACT

Channels formed by coassembly of the KCNQ1 (KvLQT1) subunit and the minK subunit underlie slowly activating cardiac delayed rectifier (I_{Ks}) in the heart, whereas two other members of the KCNQ channel family, KCNQ2 and KCNQ3, coassemble to underlie the M current in the nervous system. Because of their important physiological function, KCNQ channels have potential as drug targets, and an understanding of possible mechanisms that would enable tissue-specific targeting of these channels will be of significant value to drug development. In this study, we examined the role of the minK subunit in determining the response of KCNQ1 channels to blockade by the cognitive enhancer XE991. Coexpression with minK markedly decreased the sensitivity of KCNQ1 to blockade by XE991. When measured at the end of a 500-ms step, XE991 blockade of the KCNQ1+minK current had a K_D value of 11.1 ± 1.8 μM, approximately 14-fold less sensitive than the block of the KCNQ1 current (K_D = 0.78 ± 0.05 μM). In addition, XE991 reduced activation and deactivation time constants and caused a rightward shift in the activation curve of KCNQ1+minK, but affected none of these parameters for KCNQ1 alone. Also, XE991 block of KCNQ1+minK, but not of KCNQ1, was time- and voltage-dependent. We conclude that the presence of minK in the I_{Ks} channel complex gives rise to differential sensitivity of KCNQ and I_{Ks} channels to blockade by XE991. Our results have implications for drug development by demonstrating the important potential role of accessory subunits in determining the pharmacological properties of KCNQ channels.

The KCNQ potassium channels have recently emerged as a family of channels with important physiological functions. Members of the family contribute to several key potassium currents in the heart and nervous system, and mutations in the *KCNQ* genes are closely related to several human genetic diseases. In coassembly with the minK (KCNE1) accessory subunit, the KCNQ1 (KvLQT1) channel subunit underlies the cardiac slowly activating cardiac delayed rectifier (I_{Ks}) current (Barhanin et al., 1996; Sanguinetti et al., 1996), a slowly activating and deactivating delayed-rectifier potassium current that contributes to the repolarization of the cardiac action potential. Mutations in the *KCNQ1* (*KvLQT1*) gene cause delayed cardiac action potential repolarization and a prolonged QT interval in the ECG, resulting in a congenital cardiac disorder known as long QT syndrome that can lead to ventricular arrhythmias and sudden death (Wang et al., 1996). Two new members of the KCNQ family, KCNQ2 and KCNQ3, have been recently identified (Biervert et al., 1998; Charlier et al., 1998; Singh et al., 1998). Unlike KCNQ1, which is predominantly a cardiac channel, these two channels are present exclusively in the nervous system. We have shown previously that the KCNQ2 and KCNQ3 channel subunits coassemble to form heteromultimers that underlie the M current (Wang et al., 1998), a voltage-gated potassium current that plays a critical role in regulating neuronal excitability in the nervous system (Brown, 1988; Yamada et al., 1989; Wang and McKinnon, 1995).

Because of their important physiological functions, KCNQ channels have clear potentials as drug targets. In particular, the potentials of the neuronal KCNQ channels as drug targets are demonstrated by the recent development of a class of chemical compounds represented by linopirdine and the newer analog XE991. These compounds are potent blockers of cloned KCNQ channels (Wang et al., 1998) and the native M current in a variety of neurons (Costa and Brown, 1997; Lamas et al., 1997; Schnee and Brown, 1998; Wang et al., 1998). These compounds have been shown to have cognitive enhancing effects, and they act by increasing the stimulus-evoked release of a number of neurotransmitters in the central nervous system (Aiken et al., 1996; Zaczek et al., 1998). It has been suggested that blockade of the M channel underlies, at least in part, the enhancement of transmitter release by these drugs (Kristufek et al., 1999).

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ABBREVIATIONS: I_{Ks}, slowly activating cardiac delayed rectifier; *G*, conductance; G_{max}, maximum conductance.

However, because the KCNQ channels have generally similar pharmacological profiles, a potential problem in the development of drugs that target neuronal KCNQ channels is that the cardiac KCNQ1 (KvLQT1) channel, which together with the minK subunit underlies the native I_{Ks}, may also be affected. The need for neuronal specificity is underscored by the finding that adverse blockade of I_{Ks} can prolong cardiac action potential duration and cause acquired forms of long QT syndrome (Roden and George, 1996). In this study, we tested the role of the minK accessory subunit in determining the sensitivity of I_{Ks} channels to these cognitive enhancing drugs. Using the blockade by the cognitive enhancer XE991 as a case study, we have shown that incorporation of the minK regulatory subunit in I_{Ks} channels confers a lower sensitivity of the channel complex to XE991, providing a molecular basis for the differential sensitivity of KCNQ and I_{Ks} channels to XE991.

Materials and Methods

Preparation of KCNQ1 and minK cRNA. The human KCNQ1 cDNA (a generous gift from Dr. M. T. Keating, University of Utah; Wang et al., 1996) was linearized with *Eco*RI, and cRNA was transcribed using SP6 RNA polymerase. The minK cDNA (originally obtained from Dr. S. Nakanishi, Kyoto University, Japan; Takumi et al., 1988) was linearized with *Not*I, and cRNA was transcribed using T7 RNA polymerase.

Expression in *Xenopus* Oocytes. Oocytes were prepared from mature female *Xenopus laevis* as described previously (Wang et al., 1997). Frogs were anesthetized in ice water containing 0.1% solution of Tricaine. Defolliculation was performed by incubation for 2 h in 2 mg/ml collagenase (type VIII, Sigma, St. Louis, MO) in Ca^{2+}-free OR2 oocyte medium with gentle agitation. Oocytes were stored in OR3 solution [50% L-15 medium (Life Technologies, Gaithersburg, MD), 1 mM glutamine, 15 mM Na-HEPES (pH 7.6), 0.1 mg/ml gentamicin] at 18°C. Oocytes were injected with 35 ng of KCNQ1 cRNA or a 50:1 ratio of KCNQ1 and minK cRNAs (17.5 and 0.35 ng, respectively) using a microdispenser and a micropipette with tip diameter of 10 to 15 μm. Injected oocytes were incubated at 18°C for 24 to 48 h before analysis.

Oocytes were voltage-clamped using a two-microelectrode voltage clamp (TEV 200, Dagan, Minneapolis, MN). Intracellular electrodes were filled with 3 M KCl and had resistances of 0.5 to 3 MΩ. The standard extracellular recording solution (OR2 solution) contained 85 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Na-HEPES (pH 7.6). Data collection and analysis were performed using pCLAMP software (Axon Instruments, Foster City, CA). XE991 was obtained from DuPont Pharmaceuticals (Wilmington, DE) and dissolved in 0.1 N HCl as a 10-mM stock solution.

Data Analysis. Group data are presented as mean ± S.E. Statistical tests of drug effects were performed using paired, two-tail Student's *t* tests unless otherwise indicated. A *t* value giving $P < .05$ was considered to be significant.

Results

To test the hypothesis that the minK accessory subunit modulates the effect of XE991 on I_{Ks} channels, voltage-clamp recordings were made from *Xenopus* oocytes injected with KCNQ1 cRNA alone or with KCNQ1 and minK cRNAs in a 50:1 weight ratio. The basic biophysical properties of the expressed KCNQ1 and KCNQ1+minK channels were similar to those described previously (Barhanin et al., 1996; Sanguinetti et al., 1996) (Fig. 1A). The KCNQ1 current is a delayed rectifier that activates relatively rapidly and exhibits weak

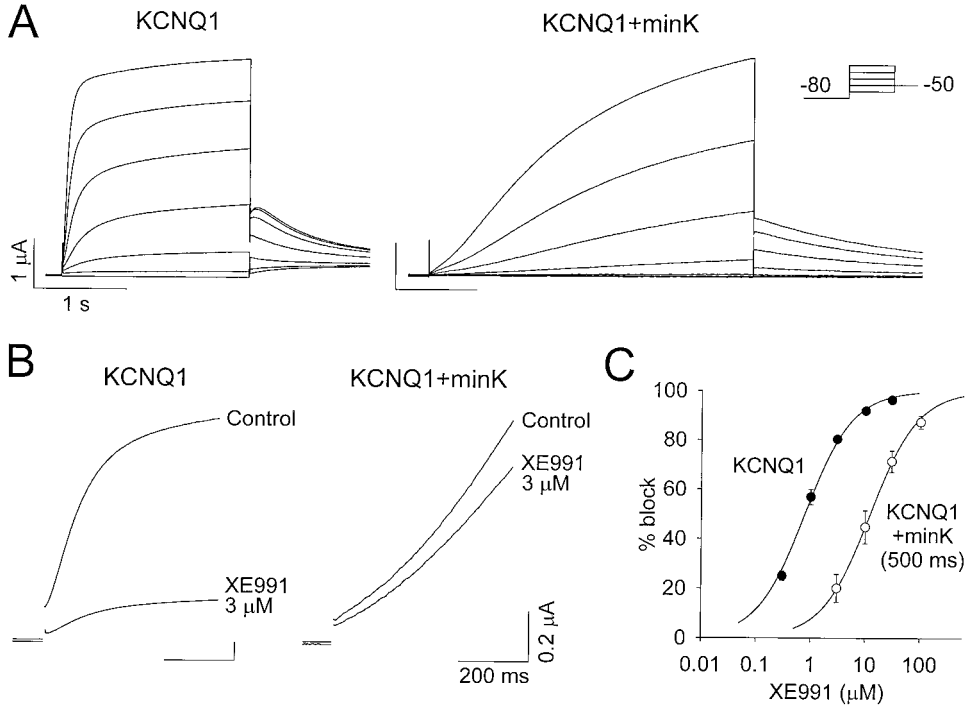

Fig. 1. The KCNQ1 and KCNQ1+minK (I_{Ks}) channels have differential sensitivity to blockade by XE991. A, expression of the KCNQ1 (left) and KCNQ1+minK (right) channels in *Xenopus* oocytes. Recordings show current responses to voltage steps from a holding potential of −80 mV over a range of −60 to +40 mV in 20-mV increments. Tail currents were recorded at −50 mV. B, effect of 3 μM XE991 on the KCNQ1 and KCNQ1+minK currents. Currents were recorded in response to 500-ms voltage steps from a holding potential of −80 to 0 mV. C, dose-response curves for XE991 blockade of KCNQ1 and KCNQ1+minK currents measured at the end of 500-ms depolarizing steps as shown in B. Data points are averages from six and seven oocytes and were fitted with the Hill equation with K_D values of 0.78 and 11.1 μM for blockade of the KCNQ1 and KCNQ1+minK currents, respectively, and Hill coefficient of unity. Error bars ± S.E.M.

rectification at positive voltages caused by rapid inactivation. Deactivation of the current at −50 mV was slow and preceded by an initial phase of recovery from inactivation. Coexpression of minK with KCNQ1 channels resulted in a current with considerably slower activation and deactivation kinetics than those of the KCNQ1 channels alone. Activation of the KCNQ1+minK channel was sigmoidal and did not reach steady state even during prolonged depolarizing voltage steps.

The native I_{Ks} in cardiac myocytes has slow activation kinetics and is activated toward the end of the plateau phase of cardiac action potentials, which are typically several hundred milliseconds in duration in human. For this reason, a relatively short (500 ms) depolarizing step to 0 mV was initially used to test the sensitivity of the KCNQ1 and KCNQ1+minK (I_{Ks}) channels to XE991. Bath application of 3 μM XE991 significantly reduced the KCNQ1 current amplitude (Fig. 1B), consistent with the previously described high sensitivity of KCNQ channels to blockade by XE991 (Wang et al., 1998). In contrast, the effect of the same concentration of XE991 on the KCNQ1+minK current was markedly smaller. When measured at the end of the 500-ms step, XE991 blockade of the KCNQ1+minK current has a K_D value of 11.1 ± 1.8 μM (n = 7), which is greater than 14-fold less sensitive than the block of the KCNQ1 current (K_D = 0.78 ± 0.05 μM, n = 6; Fig. 1C). By comparison, the K_D values for XE991 block of KCNQ2 and KCNQ2+KCNQ3 channels are 0.7 and 0.6 μM, respectively (Wang et al., 1998).

The dose-dependent block of the KCNQ1+minK current by XE991 was characterized further using a 4-s depolarizing step (Fig. 2A). The longer step revealed that the blockade of the current is strongly time-dependent, with the percentage of blockade increasing over time, as shown in Fig. 2B. The time dependence of XE991 blockade of KCNQ1+minK is also reflected in the K_D-step duration relationship (Fig. 2C), in which the K_D value decreases from 13.3 ± 1.9 μM at 300 ms to 8.4 ± 1.3 μM at 1 s to 5.0 ± 0.8 μM at 4 s (n = 7). In marked contrast, the K_D value for block of the KCNQ1 current has little time dependence (0.76 ± 0.06 μM at 200 ms and 0.84 ± 0.08 μM at 2 s; n = 6) and is significantly lower than that of the KCNQ1+minK current at all time points tested (Fig. 2, C and D).

One possibility is that the XE991 sensitivity of the two channels appeared different because KCNQ1+minK activates extremely slowly, and the drug blockade is not at steady state for the time period tested. Indeed, the K_D-time curve for the KCNQ1+minK current shown in Fig. 2C apparently had not reached steady state at 4 s. To test this hypothesis, a prolonged 40-s step was used. At the end of the depolarizing step, the blockade of KCNQ1+minK by XE991 was close to steady state, and the K_D value was estimated to be 3.9 ± 0.4 μM (n = 3; data not shown), not significantly lower than that observed at 4 s. This suggests that the lower XE991 sensitivity of the KCNQ1+minK channel is probably not simply attributable to the slow kinetics of the channel activation and blockade.

The time dependence of the XE991 block of the KCNQ1+minK current is possibly attributable to a change in activation kinetics of the current in the presence of the drug. Application of XE991 significantly increased both the rate of activation and deactivation of the KCNQ1+minK current as shown in Fig. 3A, in which the outward and tail currents in the presence of 3 μM XE991 were normalized to those in control. The effect of XE991 is dose-dependent (Fig. 3B). Activation of KCNQ1+minK can be described by the second power of a single exponential function, and the time constant was 1.28 ± 0.20 s in control and was decreased to 0.94 ± 0.18 s and 0.84 ± 0.15 s in the presence of 3 and 10 μM XE991, respectively ($P < .01$, n = 4). For deactivation of the current, which can be approximated by a single exponential, the time constant was 1.64 ± 0.32 s in control and was decreased to 1.04 ± 0.22 s and 0.84 ± 0.17 s in the presence of 3 and 10 μM XE991, respectively ($P < .05$, n = 4). In contrast, the effect of XE991 on the kinetics of the KCNQ1 current was much smaller (Fig. 3C). Activation of the KCNQ1 current is biphasic, and to simplify data analysis and presentation, the half-rise time for 2-s depolarizing steps was used as a measurement of the activation rate. Application of 1 and 3 μM XE991 did not cause any significant change in the average half-rise time of activation and the time constant of deactivation of the KCNQ1 current ($P > .5$; Fig. 3D).

Association with the minK subunit altered the voltage dependence of the block of the KCNQ1 channel by XE991. Figure 4A shows the K_D values for XE991 block measured at the end of 2-s depolarizing steps to various voltages. The blockade of KCNQ1 current by XE991 is essentially voltage-independent for the voltage range tested, whereas the blockade of the KCNQ1+minK current shows a weak voltage

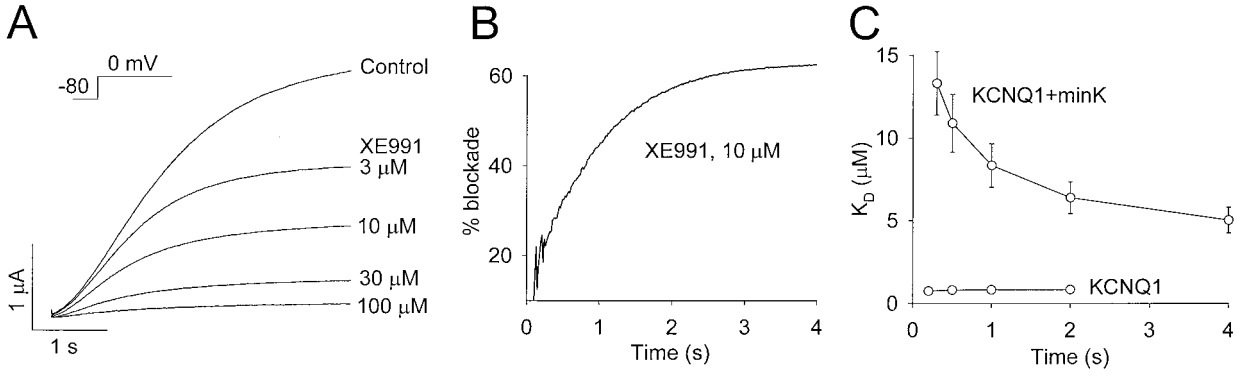

Fig. 2. Comparison of time dependence of XE991 block of KCNQ1 and KCNQ1+minK channels. A, dose-dependent block of the KCNQ1+minK current by XE991. Currents were in response to 4-s voltage steps to 0 mV from a holding potential of −80 mV. B, time dependence of the block of KCNQ1+minK by 10 μM XE991, for the experiment shown in A. Percentage of blockade of the current was calculated for each time point and plotted as a function of time. C. K_D-time relationships for the XE991 blockade of KCNQ1 and KCNQ1+minK currents. Data points are averages from six and seven oocytes for KCNQ1 and KCNQ1+minK currents, respectively. Error bars are ±S.E.M.

dependence, with a lower affinity at positive voltages. We also examined the effect of XE991 on the activation curves of the KCNQ1 and KCNQ1+minK current. Coexpression with minK shifted the average midpoint for the conductance-voltage curve of the KCNQ1 channel to a more positive potential by 18.3 mV and decreased the slope factor by 2 mV (Fig. 4B), similar to previously described results (Sanguinetti et al., 1996). Because of the large difference in KCNQ1 and KCNQ1+minK channels' sensitivity to XE991, a concentration that causes half-blockade was used for each current. Application of 1 μM XE991 had a small but significant effect on the conductance-voltage curve of the KCNQ1 current (midpoint, -18.0 ± 0.6 and -19.9 ± 0.3 mV in control and XE991, respectively; $P < .01$, $n = 5$; Fig. 4B). In contrast, application of 5 μM XE991 shifted the midpoint of the isochronal (4 s) activation curve of the KCNQ1+minK current by 8.6 mV in the positive direction, from 0.3 ± 2.4 mV to 8.9 ± 3.0 mV ($P < .05$, $n = 5$; Fig. 4B). This shift was in the direction opposite to that observed for the KCNQ1 channel.

Ionic currents in cardiac myocytes, including I_{Ks}, are activated repeatedly by the rhythmic activity of the heart. Therefore, the use dependence of drug actions must be investigated in pharmacological studies of cardiac ionic currents and their molecular clones. For this reason, the effect of XE991 was tested on the KCNQ1 and KCNQ1+minK currents activated repetitively at 1 Hz by a 250-ms depolarizing step to 0 mV from a holding potential of -80 mV, a protocol designed to mimic the activation of I_{Ks} in cardiac myocytes. Some accumulation of activation of currents was observed, especially for KCNQ1+minK, which quickly reached steady state. Figure 4C shows four consecutive superimposed traces in control and in the presence of 3 μM XE991 for the KCNQ1 and KCNQ1+minK currents. The effect of XE991 on the two currents was markedly different and was similar to that described in Fig. 1B, suggesting that there is little accumulation of block by XE991 for the KCNQ1+minK channel under these conditions.

Discussion

The native cardiac I_{Ks} channels are formed by the coassembly of the pore-forming KCNQ1 subunits with the minK accessory protein. This study tested the hypothesis that KCNQ and I_{Ks} channels have differential sensitivity to the cognitive enhancer XE991 and has shown that the minK subunit confers the lower sensitivity of I_{Ks} (KCNQ1+minK) to XE991. When studied using a 500-ms depolarizing step, the KCNQ1+minK channel was 14- to 18-fold less sensitive to XE991 blockade than were KCNQ1 and neuronal KCNQ channels (Wang et al., 1998). Other characteristics of XE991 blockade of the KCNQ1+minK channels were also different. XE991 blocks KCNQ1+minK in a voltage- and time-dependent manner, whereas XE991 blockade of KCNQ1 was primarily voltage- and time-independent. The activation and deactivation kinetic properties of the KCNQ1+minK channel, but not those of KCNQ1, were altered significantly in the

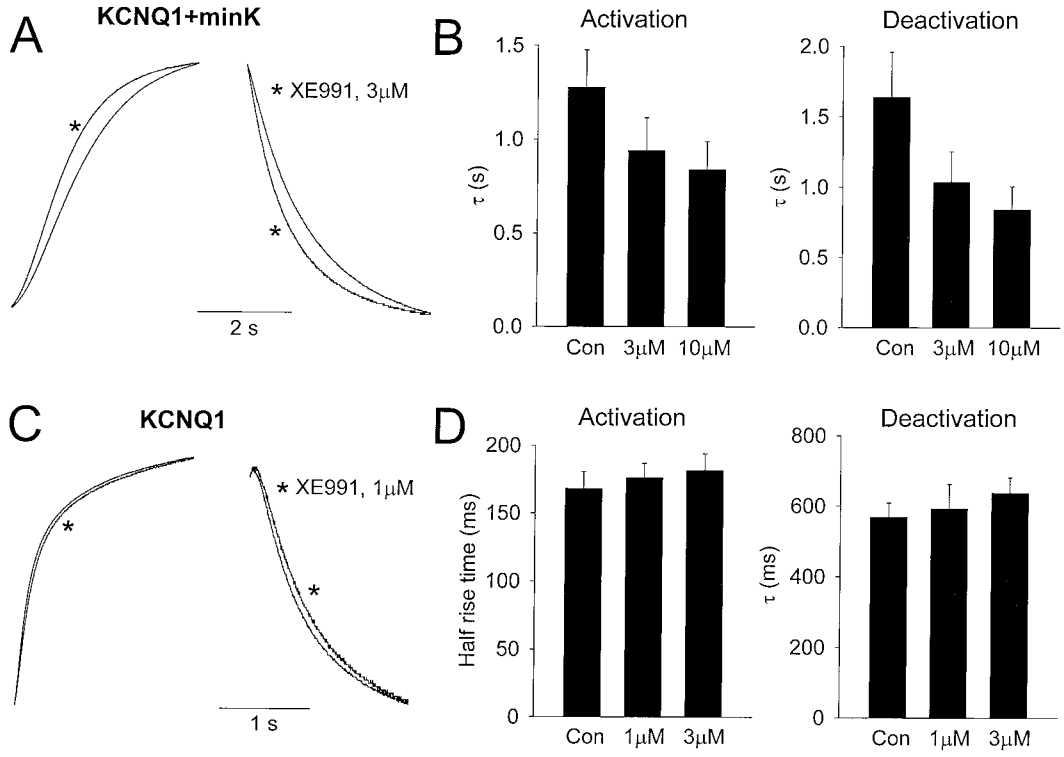

Fig. 3. Effect of XE991 on the gating properties of the KCNQ1 and KCNQ1+minK channels. A, effect of 3 μM XE991 on the activation and deactivation kinetics of the KCNQ1+minK current. Recordings show current responses to voltage steps from -80 to 0 mV. Tail currents were recorded at -60 mV. Activation of the current (left) and tail current (right) in the presence of 3 μM XE991 (*) were normalized to the respective control currents. B, effect of 3 and 10 μM XE991 on the activation and deactivation time constant of the KCNQ1+minK current. Activation in response to a 4-s step to 0 mV was fitted with the second power of a single exponential and deactivation at -60 mV was fitted with a single exponential. C, effect of 1 μM XE991 on the activation (left) and deactivation (right) kinetics of the KCNQ1 current. Protocols are the same as described for A. D, effect of 1 and 3 μM XE991 on the activation half-rise time in response to 2-s depolarizing steps to 0 mV and deactivation time constant of the KCNQ1 current measured at -60 mV. Data points are averages from four oocytes in both B and D. Error bars are $\pm$S.E.M.

presence of XE991. These results suggest that the minK subunit has an important role in regulating the interaction between the I_{Ks} channel complex and XE991. Such an effect of the minK subunit may allow the cardiac I_{Ks} and neuronal KCNQ channels to be differentially affected by XE991. Although no published clinical assessment of XE991 is available, linopirdine, a close analog of XE991, seems to act in a tissue-specific manner. When evaluated in patients, linopirdine enhanced neurotransmitter release while causing no significant adverse changes in cardiac function, including changes in the ECG (Pieniaszek et al., 1995; Rockwood et al., 1997).

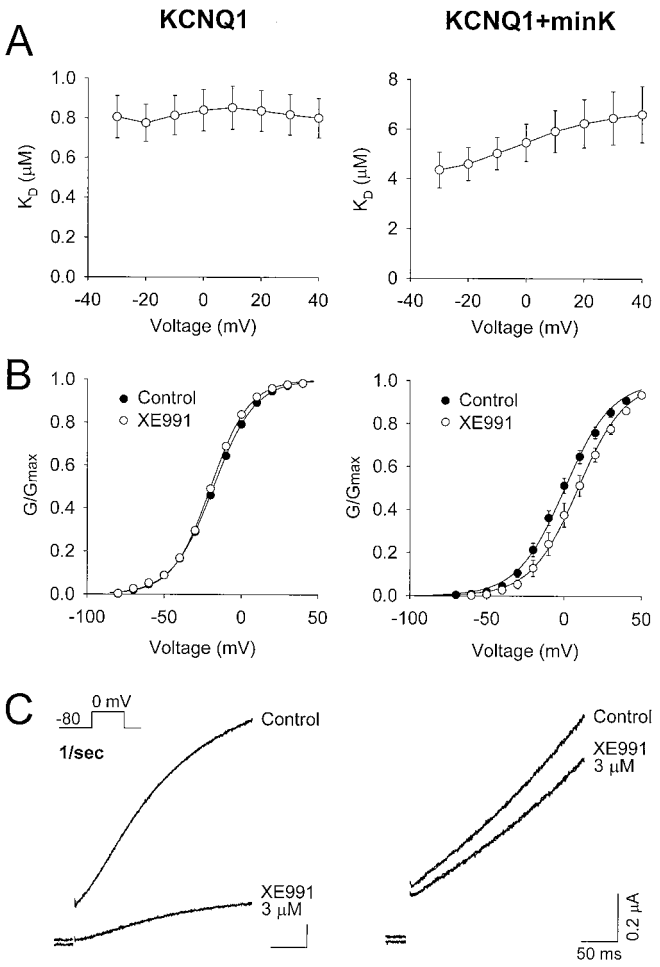

Fig. 4. A, comparison of voltage dependence of XE991 block of KCNQ1 and KCNQ1+minK channels. K_D values for XE991 block were measured at the end of 2-s depolarizing steps to various voltages from a holding potential of −80 mV. Data points are averages from four and seven oocytes for KCNQ1 and KCNQ1+minK, respectively. B, effect of XE991 on the conductance-voltage relationships of KCNQ1 and KCNQ1+minK channels. Tail currents were measured in control and in the presence XE991 at −50 mV after depolarizing steps from −80 mV to various voltages for 2 and 4 s for KCNQ1 and KCNQ1+minK, respectively, and were normalized to the maximum tail current. Data points are averages from five oocytes for both KCNQ1 and KCNQ1+minK and were fitted with the equation: $G/G_{max} = 1/(1 + \exp((V - V_n)/k_n))$, where V_n, the midpoint for activation, was −18.0 and −19.9 mV, and k_n, the slope factor, was −13.4 and −12.5 mV for control and in the presence of 1 μM XE991, respectively, for KCNQ1. V_n was 0.3 and 8.9 mV, and k_n was −15.2 and −15.1 mV for control and in the presence of 5 μM XE991, respectively, for KCNQ1+minK. Error bars are ±S.E.M. C, effect of 3 μM on the KCNQ1 and KCNQ1+minK currents in response to repetitive stimulation. Currents were in response to 250-ms voltage steps from a holding potential of −80 to 0 mV at 1 Hz. Four consecutive superimposed traces in control and in the presence of 3 μM XE991 are shown for the KCNQ1 and KCNQ1+minK channels.

Linopirdine blocks the M channel (KCNQ2+KCNQ3) by a direct interaction with the channel protein rather than through a second messenger-mediated pathway (Costa and Brown, 1997; Lamas et al., 1997). Presumably the closely related compound XE991 blocks the KCNQ1 and KCNQ1+minK by the same mechanism. As a result of the pronounced time dependence of the KCNQ1+minK blockade, the difference in affinity for XE991 of the KCNQ1 and KCNQ1+minK channels is most significant when examined using relatively short steps and decreases over time. The time dependence of the KCNQ1+minK blockade can be simply explained by an open-channel block with a slow on-rate for drug binding. However, such a mechanism is inconsistent with an increased rate of deactivation of the channel in the presence of XE991. Also, there is no cumulative blockade when the current is stimulated repetitively. Although the analysis of the blockade of the KCNQ1+minK is complicated by the extremely slow activation rate and the lack of steady-state activation, we believe that the apparent time dependence of blockade is secondary to the change in channel gating properties. The mechanism by which XE991 alters the gating properties of the KCNQ1+minK channel, but not of KCNQ1, is unknown. It may involve allosteric interactions between XE991 and the KCNQ1 channel at a site that is modified in the presence of minK.

The modulatory effect of minK on I_{Ks} drug sensitivity reported here is not unique for blockade by XE991. It has been shown previously that coexpression of minK subunits with KCNQ1 channels decreases the effect of other drugs, including clofilium and a benzodiazepine, R-L3, on the channel (Yang et al., 1997; Salata et al., 1998). Interestingly, it also has been shown that compared with the KCNQ1 channel, the KCNQ1+minK channel has higher affinity for several antiarrhythmic drugs (Busch et al., 1997). Therefore, association with the minK subunits appears to affect the general pharmacological profile of the KCNQ1 channel, and these intriguing interactions between minK and KCNQ1 subunits may make it possible to develop drugs that can selectively target either the neuronal or cardiac KCNQ channels.

The significance of this study goes beyond the interactions between KCNQ channels with minK. A previous study has shown that the presence of the β-1 accessory subunit alters the block of the cardiac sodium channel by lidocaine (Makielski et al., 1996). Recently, it was suggested that MiRP1, a minK-related peptide with one putative transmembrane domain, coassembles with the HERG potassium channel to form the rapidly activating cardiac delayed rectifier channel I_{Kr}, and it has been shown that coexpression with MiRP1 affects the block of the HERG channel by the methanesulfonanilide E4031 (Abbott et al., 1999). Taken together, these findings and our results have implications for drug development by demonstrating that the pharmacological profile of an ion channel is not only determined by the properties of the pore-forming α-subunits, but also can be influenced by the accessory or regulatory subunits with which the α-subunits coassemble. As a case study, our results also show that the tissue specificity of drugs can be achieved by taking advantage of the presence of accessory channel subunits in addition to targeting channels restricted to a certain tissue type.

References

Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann WH, Timothy KW, Keating MT and Goldstein SAN (1999) MiRP1 forms I-Kr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* **97:**175–187.

Aiken SP, Zaczek R and Brown BS (1996) Pharmacology of the neurotransmitter release enhancer linopirdine (DuP 996), and insights into its mechanism of action, in *Advances in Pharmacology* (August JT, Anders MW, Murad F and Coyle JT eds) vol 35, pp 349–384, Academic Press, San Diego.

Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M and Romey G (1996) KvLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature (Lond)* **384:**78–80.

Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ and Steinlein OK (1998) A potassium channel mutation in neonatal human epilepsy. *Science (Wash DC)* **279:**403–406.

Brown DA. M currents (1988) in *Ion Channels* (Narahashi T ed) pp 55–94, New York, Plenum.

Busch AE, Busch GL, Ford E, Suessbrich H, Lang HJ, Greger R, Kunzelmann K, Attali B and Stuhmer W (1997) The role of the I-sK protein in the specific pharmacological properties of the I-Ks channel complex. *Br J Pharmacol* **122:**187–189.

Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ and Leppert M (1998) A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat Genet* **18:**53–55.

Costa AMN and Brown BS (1997) Inhibition of M-current in cultured rat superior cervical ganglia by linopirdine: Mechanism of action studies. *Neuropharmacology* **36:**1747–1753.

Kristufek D, Koth G, Motejlek A, Schwarz K, Huck S and Boehm S (1999) Modulation of spontaneous and stimulation-evoked transmitter release from rat sympathetic neurons by the cognition enhancer linopirdine: Insights into its mechanisms of action. *J Neurochem* **72:**2083–2091.

Lamas JA, Selyanko AA and Brown DA (1997) Effects of a cognition-enhancer, linopirdine (DuP 996), on M-type potassium currents ($I_{K(M)}$) and some other voltage- and ligand-gated membrane currents in rat sympathetic neurons. *Eur J Neurosci* **9:**605–616.

Makielski JC, Limberis JT, Chang SY, Fan Z and Kyle JW (1996) Coexpression of beta 1 with cardiac sodium channel alpha subunits in oocytes decreases lidocaine block. *Mol Pharmacol* **49:**30–39.

Pieniaszek HJ Jr, Fiske WD, Saxton TD, Kim YS, Garner DM, Xilinas M and Martz R (1995) Single dose pharmacokinetic, safety and tolerance of linopirdine (DuP 996) in healthy young adults and elderly volunteers. *J Clin Pharmacol* **35:**22–30.

Rockwood K, Beattie BL, Eastwood MR, Feldman H, Mohr E, Pryse-Phillips W and Gauthier S (1997) A randomized, controlled trial of linopirdine in the treatment of Alzheimer's disease. *Can J Neurol Sci* **24:**140–145.

Roden DM and George AL (1996) The cardiac ion channels: Relevance to management of arrhythmias. *Annu Rev Med* **47:**135–148.

Salata JJ, Jurkiewicz NK, Wang J, Evans BE, Orme HT and Sanguinetti MC (1998) A novel benzodiazepine that activates cardiac slow delayed rectifier K^+ currents. *Mol Pharmacol* **53:**220–230.

Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL and Keating MT (1996) Coassembly of KvLQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature (Lond)* **384:**80–83.

Schnee ME and Brown BS (1998) Selectivity of linopirdine (DuP 996), a neurotransmitter release enhancer, in blocking voltage-dependent and calcium-activated potassium currents in hippocampal neurons. *J Pharmacol Exp Ther* **286:**709–717.

Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, McHarg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE and Leppert M (1998) A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet* **18:**25–29.

Takumi T, Ohkubu H and Nakanishi S (1988) Cloning of a membrane protein that induces a slow voltage-gated potassium current. *Science (Wash DC)* **242:**1042–1045.

Wang H-S, Dixon JE and McKinnon D (1997) Unexpected and differential effects of Cl^- channel blockers on the Kv4.3 and Kv4.2 K^+ channels: Implication for the study of the I_{to2} current. *Circ Res* **81:**711–718.

Wang H-S and McKinnon D (1995) Potassium currents in rat prevertebral and paravertebral sympathetic neurones: Control of firing properties. *J Physiol (Lond)* **485:**319–335.

Wang H-S, Pan ZM, Shi WM, Brown BS, Wymore RS, Cohen IS, Dixon JE and McKinnon D (1998) KCNQ2 and KCNQ3 potassium channel subunits: Molecular correlates of the M-channel. *Science (Wash DC)* **282:**1890–1893.

Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, deJager T, Schwartz PJ, Towbin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD and Keating MT (1996) Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet* **12:**17–23.

Yamada WM, Koch C and Adams PR (1989) Multiple channels and calcium dynamics in *Methods in Neuronal Modeling* (Koch C and Segev I eds) pp 97–133, Bradford, Cambridge, MA.

Yang W-P, Levesque PC, Little WA, Lee Conder M, Shalaby FY and Blanar MA (1997) KvLQT1, a voltage-gated potassium channel responsible for human cardiac arrhythmias. *Proc Natl Acad Sci USA* **94:**4017–4021.

Zaczek R, Chorvat RJ, Saye JA, Pierdomenico ME, Maciag CM, Logue AR, Fisher BN, Rominger DH and Earl RA (1998) Two new potent neurotransmitter release enhancers, 10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone and 10,10-bis(2-fluoro-4-pyridinylmethyl)-9(10H)-anthracenone: Comparison to linopirdine. *J Pharmacol Exp Ther* **285:**724–730.

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