Communication

Molecular Mechanism and Functional Significance of the MinK Control of the KvLQT1 Channel Activity*

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The very slowly activating delayed rectifier K⁺ channel IKᵣ is essential for controlling the repolarization phase of cardiac action potentials and K⁺ homeostasis in the inner ear. The IKᵣ channel is formed via the assembly of two transmembrane proteins, KvLQT1 and MinK. Mutations in KvLQT1 and MinK are associated with a long QT syndrome that causes syncope and sudden death and also with deafness. Here, we show a new mode of association between ion channel forming subunits in that the cytoplasmic C-terminal end of MinK interacts directly with the pore region of KvLQT1. This interaction reduces KvLQT1 channel conductance from 7.6 to 0.58 picosiemens. However, because MinK also reveals a large number of previously silent KvLQT1 channels (× 60), the overall effect is a large increase (× 4) in the macroscopic K⁺ current. Conformational changes associated with the KvLQT1/MinK association create very slow and complex activation kinetics without much alteration in the deactivation process. Changes induced by MinK have an essential regulatory role in the development of this K⁺ channel activity upon repetitive electrical stimulation with a particular interest in tachycardia.

Delayed K⁺ rectifier channels initiate the repolarization that terminates the plateau phase of the action potential. The delayed rectifier K⁺ current is the addition of two components, a rapidly activating one, which is called IKᵣ, and a very slowly activating current called IKᵣ(1). Cardiac arrhythmias, based on abnormal repolarization, are visualized as a prolonged QT interval on an electrocardiogram. Congenital long QT (LQT)1 is an inherited disease characterized by prolonged ventricular repolarization that causes syncope and sudden death due to ventricular arrhythmia (2). The LQT syndrome is genetically heterogeneous with at least four chromosomal loci (LQT1 to LQT4) implicated in the disease. One of them, the LQT2 locus, corresponds to mutations in the HERG gene that encodes the rapidly activating delayed rectifier K⁺ channel generating IKᵣ. Another one, the LQT1 locus, encodes a K⁺ channel protein, KvLQT1, that associates with another small transmembrane protein known as MinK, to generate the slowly activating K⁺ channel IKᵣ. Expression of IKᵣ is not limited to the heart. KvLQT1 as well as MinK are also expressed in many other organs such as kidney and the stria vascularis of the inner ear (5). Some human mutations of the KvLQT1 gene lead to the Jervell-Lange-Nielsen syndrome (6). Patients suffering from this syndrome not only exhibit a long QT wave interval but also profound deafness from birth. On the other hand, mice carrying a null mutation on the MinK gene also display profound inner ear dysfunction associated with drastically altered K⁺ secretion into the endolymph of the inner ear leading to hair cell degeneration (7). Thus, the KvLQT1/MinK assembly forms a K⁺ channel that has a key electrogenic role in ventricular repolarization and a key secretory role in the control of endolymph homeostasis associated with normal hearing.

KvLQT1 has the classical structure of a K⁺ channel protein with six transmembrane regions and one pore structure that is known to confer K⁺ permeability (8), whereas MinK is a small protein (129 amino acids in the mouse) with a single transmembrane domain (9, 10), which serves as an essential modulator of the KvLQT1 subunit (3, 4, 11). This paper shows that MinK has unique properties of interaction with KvLQT1. This peculiar mode of interaction confers functional properties to the IKᵣ channel that probably have very important physiopathological implications.

EXPERIMENTAL PROCEDURES

Electrophysiology—Transfection of COS cells has been previously described (3). The whole cell, cell-attached, and outside-out configurations of the patch-clamp technique were used (12). The external solution at pH 7.4 contained (in mM): 140 NaCl, 5 KCl, 1 CaCl₂, 2 MgCl₂, 10 HEPES/NaOH. Pipette solutions contained either the external medium (cell-attached) or an internal solution at pH 7.3 with (in mM): 140 KCl, 2 MgCl₂, 10 HEPES/KOH, 2 EGTA.

The mean current-variance analysis on COS cells expressing KvLQT1/MinK channels was performed using the Biopatch software (BioLogic, Grenoble, France). Currents were sampled at 1 kHz and low pass filtered at 150 Hz. Methods of cRNA injection into Xenopus oocytes and electrophysiological recordings have been described (10).

Yeast Two-hybrid Interaction Assay—The fragments encompassing part of the N-terminal domain of human MinK (MinKN, aa 11–38) and the entire MinK C terminus (MinKC, aa 67–129) were amplified by polymerase chain reaction using the Vent DNA polymerase (Biolabs) and subcloned in fusion with the GAL4 DNA-binding domain of the yeast vector pAS2 (CLONTECH) into the Smal/Not cloning sites, respectively. The domains of KvLQT1 spanning the entire N terminus (KvLQT1N, aa 1–64), the pore (KvLQT1P, aa 218–259) and the entire C terminus (KvLQT1C, aa 290–604) were amplified by

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polymerase chain reaction and subcloned in fusion with the GAL4 activation domain of the yeast vector pGAD 424 (CLONTECH) into the EcoRl/BamHI cloning sites. All constructs were verified by sequencing.

The two types of hybrid plasmids were transformed into the yeast strain CG-1945 using the lithium acetate method, and transformants were grown on synthetic medium lacking Leu, Trp, and His in the presence of 5 mM 3-aminotriazole to inhibit basal levels of HIS3 expression. Primary HIS+ transformants were then tested for β-galactosidase reporter gene activity using both a filter and a liquid assay.

RESULTS AND DISCUSSION

Although the activation kinetics and level of K+ channel expression of KvLQT1 and KvLQT1/MinK are very different, the deactivation kinetics are comparable with both potentials relative to the resting potential (RP). B, amplitude histogram of unitary currents at +60 mV/RP. C, current-voltage relationship (mean values from three patches). D, a, KvLQT1/MinK channel, outside-out patch, representative K+ current response to step depolarization to +40 mV. b, variance-mean current plot and fit with the parabolic function: $\sigma^2 = i(t) - \bar{i}(t)^2/N$, where $\sigma^2$ = variance of the current, $i$ = unitary current, and $N$ = number of functional channels ($n = 90, \bar{i} = 0.076 \text{pA}$). E, current-voltage relationship for the unitary currents. f, mean from 30 cell-attached patches; ● and ■, from patches shown in D and F, respectively. F, a, cell-attached patch with detectable slowly activating K+ currents in response to a step depolarization to +100 mV relative to RP followed by a return to +40 mV. b, noise analysis. c, amplitude histogram from the same data.

Simulation—Kinetic schemes of Fig. 4 were simulated using “Mathematica” Software (Wolfram Research).

Although the activation kinetics and level of K+ channel expression of KvLQT1 and KvLQT1/MinK are very different, the deactivation kinetics are comparable with both potentials relative to the resting potential (RP). The outward current elicited by consecutive depolarizing pulses of the cardiac action potential (300 ms), a 100% enhancement of the cardiac action potentials preventing the myocardium from premature excitation (13, 14). A dysfunction of Ikr in patients with inherited LQTS syndrome linked to the LQT1 locus will decrease or suppress this large K+ current, which develops at high stimulation frequencies i.e. with tachycardia. It can be pre-
dicted that hearts from these patients would be unable to protect themselves against arrhythmias when sympathetic activity increases with emotional and/or physical stress that precipitates life-threatening events. It makes sense that the most efficient pharmacological strategy in these patients is to prevent tachycardia with β-blockers (15).

At the unitary channel level, the effects of the association MinK-KvLQT1 are also spectacular. COS cells transfected with KvLQT1 only express a small number of channels (1 or 2) in each active patch (n = 10) with a single channel conductance 7.6 ± 0.7 pS (Fig. 2, A–C). Single channel currents cannot be easily detected in most patches (n = 40) from COS cells expressing KvLQT1/MinK. In these patches, the recorded current mimics the global current (Fig. 2D, a). Therefore, variance analysis was used to estimate both the number of active channels and the value of the unitary current. Fig. 2D (b) illustrates this method applied to an outside-out patch. Patches typically contained 50–100 channels with a unitary conductance of 0.58 ± 0.14 pS (n = 25) (Fig. 2, D and E). Because the variance-mean current analysis hypothesizes that current fluctuations are directly related to channels flickering between closed and open states, it is not surprising that the parabola fits indicate the existence of numerous channels of small conductance. Furthermore, Fig. 2 (E and F) provide convergent results in favor of the validity of the parabola fits: (i) the single channel current-voltage curve intersects the voltage axis at the K⁺ equilibrium potential (∼ −80 mV) (Fig. 2E), and the calculated number of channels is near constant for the same patch. (ii) A single channel analysis has also been carried out, in rare patches displaying “visible” single channel activities. Elementary conductances and number of channels calculated by the two methods, i.e. variance analysis and amplitude histograms, have given similar results (0.6 pS, two channels). In the Xenopus oocyte, noise analysis on KvLQT1/MinK containing macropatch yields the same unitary conductance (0.52 ± 0.2 pS, n = 20) with a mean number of active channels of about 1000 (not shown). These results are in agreement with those obtained from cardiac patches, suggesting that I_K is due to the activity of a high density very small K⁺ conductance channel (16, 17).

The large increase in K⁺ current following association of MinK with KvLQT1 results from the melange of two factors: a large reduction in the unitary channel conductance overcompensated by a larger increase in the number of functional channels. The 4-fold increase of the current density in COS cells expressing KvLQT1/MinK (43.1 ± 4.6 pA/pF, n = 31 at +30 mV) as compared with cells expressing KvLQT1 alone (10.2 ± 1.3 pA/pF, n = 29) reflects a 60-fold increase of the number of functional K⁺ channels. Most KvLQT1 channels are “nonfunctional” or in a “silent” state with a low probability of opening. The association of MinK converts them into small conductance channels but with a high open state probability.

A series of experiments (Fig. 3) demonstrates the interaction of the MinK C-terminal domain, known to be an essential element for the KvLQT1/MinK expression (3, 11), with structural elements within or close to the KvLQT1 pore. First, the yeast two-hybrid assay shows that the MinK C terminus interacts strongly with the pore of KvLQT1 (Fig. 3A). It does not interact significantly with either the entire N terminus or the entire C terminus of the KvLQT1 channel protein. The N terminus of MinK fails to interact with any hydrophilic domain (N-terminal, C-terminal, or pore) of KvLQT1 (not shown). Second, affinity chromatography of MinK to the various domains of the KvLQT1 protein was performed. The whole MinK protein was produced in Sf9 insect cells infected with recombinant baculovirus (18). Detergent cell extracts were incubated with the different domains of KvLQT1 produced as glutathione-S-
transferase fusion proteins in E. coli and bound to glutathione-Sepharose beads. The retained proteins were resolved by SDS-polyacrylamide gel electrophoresis and Western blotting using polyclonal anti-MinK antibodies (18). Again, the results show that only the pore region of KvLQT1 specifically associates with MinK (Fig. 3B). The two-hybrid and the affinity chromatography assays have been used in several studies designed to map domains mediating protein-protein interactions in K⁺ channels (19, 20). However, interactions involving other domains of MinK or KvLQT1 may exist that have not been identified in the present study.

Both electrophysiological and biochemical data have been used to propose a minimal model that could help explain how the KvLQT1/MinK association leads to drastic changes in properties of the KvLQT1 channel such as activation kinetics and unitary conductance while preserving K⁺ selectivity and deactivation kinetics (Fig. 4). MinK first binds to the outer shell of the KvLQT1 channel, probably via its transmembrane domain. This step provides a closer positioning of the C-terminal domain of MinK to the pore of KvLQT1. Once the KvLQT1 channel reaches the open conformation (O), the C-terminal domain of MinK enters and binds to the pore (OMK). This leads to a total occlusion that is later transformed into a partial occlusion resulting in a narrower pore (OMK') that creates an additional barrier to K⁺ mobility and drastically reduces the unitary conductance. The total pore occlusion produced by MinK before relaxation to a partial occlusion is supported by the fact that the normal 7.6 pS KvLQT1 conductance was never recorded with the KvLQT1/MinK channel before observing the small conductance behavior. The slow kinetics of activation reflect the conformational change (OMK → OMK') leading to the partial opening of the pore. The difficulty for the channel to close when occupied by the C-terminal end of MinK, the “foot-in-the-door” process (21), leads to the accumulation of open channels (OMK) and to an increase in the number of functional channels. Actually, long open times (>1 s) are observed in the few patches with detectable unitary currents (Fig. 2F, a). Assigning an arbitrary value of 1 to the rate constant of the transition between the closed states C0 and C1, one can then set all the other rate constants (Fig. 4) for a quantitative treatment of the model. A computer simulation provides a satisfactory fit of the key current properties of KvLQT1/MinK, i.e. the slow activation kinetics, the higher level of K⁺ channel expression, the unchanged rates of deactivation and the frequency-dependent accumulation as in Fig. 1E. An extension of the model is presented in Fig. 4E in which a tetramer of KvLQT1 can bind from 1 to 4 MinK subunits depending on the concentration of MinK in the membrane. This model explains (i) the complex kinetic behavior of the slow KvLQT1/MinK channel after a long-lasting depolarization (compare Fig. 4, G and H) and (ii) previous results demonstrating that the kinetics of activation of the slow K⁺ channel formed in the Xenopus oocyte depends on the levels of the MinK protein present in the membrane (22, 23). Thus, MinK appears to be a regulatory protein that finely tunes the KvLQT1 channel activity in a concentration-dependent manner. This property is supported by results shown in Fig. 5. The rapidly activating KvLQT1 currents can be first expressed in Xenopus oocytes injected with KvLQT1 cRNA alone and then converted into slowly activating ones following expression of MinK 24 h later. This modification of pre-existing channels by the association of the non-pore-forming MinK subunit clearly contrasts with the situation described for classical K⁺ channel β-subunits (24) where the association with a subunits only occurs during the translation process in the endoplasmic reticulum (25, 26).

The effect of a change in the MinK concentration on KvLQT1/MinK kinetics is of a particular physiological interest because the level of expression of MinK is known to be altered during development (10) and by hormones such as oestrogens (27). The latter process being a potential explanation for sex differences in cardiac LQT and vulnerabilities to “torsades de pointes” (28).

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