Cloning and Expression of the Human Kv4.3 Potassium Channel

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Cloning and expression of the human Kv4.3 potassium channel. J. Neurophysiol. 81: 1974–1977, 1999. We report on the cloning and expression of hKv4.3, a fast inactivating, transient, A-type potassium channel found in both heart and brain that is 91% homologous to the rat Kv4.3 channel. Two isoforms of hKv4.3 were cloned. One is full length (hKv4.3 long), and the other has a 19 amino acid deletion (hKv4.3 short). RT-PCR shows that the brain contains both forms of the channel RNA, whereas the heart predominantly has the longer version. Both versions of the channel were expressed in Xenopus oocytes, and both contain a significant window or noninactivating current near potentials of −30 to −40 mV. The inactivation curve for hKv4.3 short is shifted 10 mV positive relative to hKv4.3 long. This causes the peak window current for the short version to occur near −30 mV and the peak for the longer version to be at −40 mV. There was little difference in the recovery from inactivation or in the kinetics of inactivation between the two isoforms of the channel.

INTRODUCTION

Proteins of the Shal or Kv4 family of ion channels are now thought to underlie many of the native A-type currents that were recorded from different primary cells (Dixon et al. 1996; Serodio et al. 1994, 1996, 1998). The Kv4 channels are thought to underlie the notch seen in cardiac myocyte action potentials and to play an important role in determining action potential duration (Dixon et al. 1996). In neurons, Kv4 channels and the A currents they may comprise have been shown to play an important role in modulation of firing rate, action potential initiation, and shaping burst pattern (Byrne 1980; Connor and Stevens 1971a,b; Getting 1983; Hille 1982; Llinas 1988; McCormick and Huguenard 1992; Rudy 1988; Thompson and Aldrich 1980). Recently, A currents have also been shown to be localized to dendritic regions in the hippocampus, where they play a complex role in modulating synaptic input and inactivation of these expressed Kv4 channels appeared to be shifted 20 mV positive to that seen in neurons, which would place them out of the subthreshold category. In addition, the recovery from inactivation of the expressed channels was slower than that seen in the native cells. Fortunately, it was discovered that addition of a low molecular weight RNA fraction from rat brain, presumably containing a β subunit for the Kv4 channel, shifts the voltage dependence and speeds the channels rate of recovery from inactivation to that seen in the native cells (Chabala et al. 1993; Serodio et al. 1994, 1996). Further confirmation of the role of Kv4 channels in forming A-type currents comes from antisense hybrid-arrest and dominant-negative experiments in both neurons and cardiac myocytes (Fiset et al. 1997; Johns et al. 1997).

METHODS

Cloning of hKv4.3 long

To obtain hKv4.3 cDNA, oligonucleotides were synthesized based on the published rat sequence (Serodio et al. 1996) (GenBank U42975). Four overlapping partial cDNA fragments were pulled out of a whole heart cDNA library (GIBCO BRL). A 511-bp fragment was used as a probe to rescreen the same library, but the resulting clones still lacked the extreme 5’ and 3’ coding regions. Therefore rapid amplification of cDNA ends (5’ and 3’ RACE) was used to amplify these sequences from a human brain stem cDNA library (Stratagene).

Cloning of hKv4.3 short

To see if a shorter form of hKv4.3 existed in human tissue we synthesized primers that flanked the 57-bp insert in the long form of hKv4.3. These generated products of either 237 or 180 bp, reflecting the presence or absence of the 57-bp insertion (Fig. 2B). To clone the shorter version a second set of flanking primers was synthesized and used to generate two bands (767 and 710 bp). The shorter band was cloned and sequenced to confirm the absence of the 57-bp region. Bgl II and Sac I sites flank this region and were used with the 710-bp product to directionally clone the shorter fragment of hKv4.3 into the corresponding region of full length hKv4.3.

RESULTS

Figure 1A shows the complete amino acid sequence of hKv4.3 long with the insert region highlighted along with an alignment with rat Kv4.3 (Ohya et al. 1997), rat Kv4.2 (Baldwin et al. 1991), and mouse Kv4.1 (Pak et al. 1991). When compared with the previously reported rat clones of Kv4.3 (Dixon et al. 1996; Ohya et al., 1997; Serodio et al. 1996), the two variants of the hKv4.3 clones are 91% homologous at the nucleotide level and differ by only three amino acids (Fig. 1A, arrows). Figure 1B shows a human multitissue northern blot (Clontech) and RT-PCR for both hKv4.3 short and hKv4.3...
Human heart primarily expresses hKv4.3 long, whereas human brain can be seen to contain both forms. Figure 2A shows raw current traces from depolarizing voltage pulses for hKv4.3 long and hKv4.3 short expressed in Xenopus oocytes. The inactivation of the currents during the pulses was well fit by two exponentials and was only weakly voltage dependent, as shown on Fig. 2C. There was no obvious difference in the kinetics of the currents between the two forms. Figure 2B shows the steady-state activation and inactivation curves for both forms of hKv4.3 expressed in Xenopus oocytes. The Boltzman fits of the steady-state inactivation curves for the short and long forms of hKv4.3 reveal that the $V_{1/2}$ of inactivation is shifted 10 mV positive for the hKv4.3 short relative to the hKv4.3 long (see Fig. 2B legend). The overlap region for the steady-state activation and inactivation of both forms of the channel shows a prominent window current with a relative peak amplitude of 16% for hKv4.3 long and 18% for hKv4.3 short compared with the maximal currents seen at +80 mV. This window current could occur at surprisingly positive potentials, as demonstrated in Fig. 3, A and B, which shows two individual oocytes expressing hKv4.3 short or hKv4.3 long. In the two selected cells the peak of the window current occurred at −30 (hKv4.3 long) and −22 mV (hKv4.3 short). Figure 3B shows the partially inactivated current traces for the pulse protocol near the peak of the window current. Figure 3C shows two different oocytes expressing both forms of hKv4.3 and the raw currents resulting from application of a voltage pulse.
Pulse protocol designed to look at the kinetics of the recovery from inactivation. In these two cells there was no difference in this recovery process. Pooled data from experiments on additional eggs gave similar data with population average time constants of 78.8 and 451.6 ms for hKv4.3 long \( (n=5) \) and 78.0 and 686.8 ms for hKv4.3 short \( (n=6) \), with no statistical difference between the two forms as shown in Fig. 3D.

**DISCUSSION**

We cloned the human ortholog of the rat Kv4.3 channel from human heart and brain. There is a 19-amino acid region that is presumably alternatively spliced to form hKv4.3 short. Both forms of hKv4.3 show high homology to the two rat forms that were previously cloned with only a three-amino acid difference. This high degree of amino acid conservation suggests that the Kv4.3 channel is evolutionarily highly conserved. We find from RT-PCR that both the long and short forms of the channel are expressed in the human brain but that only the long form is found in the heart. This is similar to the findings of Ohyae et al. (1997) and Takimoto et al. (1997) for the two alternatively spliced forms of the rat Kv4.3 channel. The electrophysiologic data from the hKv4.3 channel are in accord with those of Serodio et al. (1996) for the short form of rat Kv4.3 in that both steady-state activation and inactivation appear to be shifted positive relative to that seen for A currents from native cells (Serodio et al. 1994, 1996). Serodio et al. (1996) fail to show saturation of the rKv4.3 current at potentials of +50 mV, although co-injection of low molecular weight RNA did shift the activation curve by 20 mV negative, which allowed for complete saturation of the current at +50 mV. Our data show that the saturating conductance for hKv4.3 alone does not occur until potentials of +80 mV (Fig. 2B).

To date no one has characterized the electrophysiological
differences between the two alternatively spliced variants of the Kv4.3 channel. The 19-amino acid insertion contains two adjacent PKC consensus sites and occurs in the carboxy domain after the sixth transmembrane domain. We find that the steady-state inactivation of hKv4.3 short is shifted 10 mV positive relative to hKv4.3 long. We also report on a prominent window current region where steady-state inactivation and activation overlap. Serodio et al. (1996) show a similar region for the short form of rKv4.3 in their study. This window current region may be of particular physiological significance as it would provide for a sustained K⁺ conductance within its voltage range and thus provide a repolarizing influence even for very slow membrane potential events. We did not explore the effect of low molecular weight mRNA on the voltage dependence of this window current, although from Serodio et al. (1996) it would be expected to have the effect of shifting the window current voltages into a subthreshold range.

Hoffman et al. (1997) recently reported on a integrating function for the A current seen on proximal and distal hippocampal neurons. In the hippocampal neurons the A current serves as an attenuator for synaptic potentials and prevents excessive retrograde propagation of action potentials triggered in the axon region. Sheng et al. (1992) and Maletic-Savatic et al. (1995) presented evidence that the molecular correlate for the A current in hippocampal dendrites is likely to be a Kv4 channel type. If this is true the window current region seen in our recordings of hKv4.3 channels may play a particularly significant role in damping synaptic and excitatory membrane potentials within its voltage range. For more positive potentials Kv4.3 would serve as a high-frequency filter, reducing fast membrane transients but allowing slower ones through, as the inactivation kinetics of Kv4.3 would quickly inactivate the channel at these more positive potentials.

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REFERENCES


