Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 346 (2006) 958–967
BBRC
www.elsevier.com/locate/ybbrc

KCNE3 is an inhibitory subunit of the Kv4.3 potassium channel

Alicia Lundby, Søren-Peter Olesen *

The Danish National Research Foundation Centre for Cardiac Arrhythmia, Department of Medical Physiology, University of Copenhagen, The Panum Institute, 3 Blegdamsvej, DK-2200 Copenhagen N, Denmark

Received 27 May 2006
Available online 9 June 2006

Abstract

The mammalian Kv4.3 potassium channel is a fast activating and inactivating K^+ channel widely distributed in mammalian tissues. Kv4.3 is the major component of various physiologically important currents ranging from A-type currents in the CNS to the transient outward potassium conductance in the heart (I_{to}). Here we show that the KCNE3 β-subunit has a strong inhibitory effect on current conducted by heterologously expressed Kv4.3 channels. KCNE3 reduces the Kv4.3 current amplitude, and it slows down the channel activation and inactivation as well as the recovery from inactivation. KCNE3 also inhibits currents generated by Kv4.3 in complex with the accessory subunit KChIP2. We find the inhibitory effect of KCNE3 to be specific for Kv4.3 within the Kv4 channel family. Kv4.3 has previously been shown to interact with a number of β-subunits, but none of the described subunit-interactions exert an inhibitory effect on the Kv4.3 current.

Keywords: Voltage-gated potassium channel; I_{to} current; Inhibitory β-subunit; MIRP2; Kv4.3; KCND3; KCNE3

Voltage-gated potassium (Kv) channels are key attenuating control elements of cellular excitability. Kv channels are found in almost all cell types, and their importance in cellular physiology is evidenced by the number of diseases caused by their dysfunction; CNS disorders, cardiac arrhythmias, and epilepsy [1]. Kv channels comprise a large family of proteins; thus far twelve Kv channel α-subunit families (Kv1–12) have been identified in mammals [2]. Within the Kv4 subfamily three α-subunits (Kv4.1–4.3) have been described. These are all fast activating and inactivating channels, and they recover from inactivation faster than any other Kv channels characterized. Kv4 channels are mainly expressed in the brain and the heart [3–7].

Although not always necessary, many K^+ channels have associated subunits. Members of different families of β-subunits have been reported to interact with Kv4.3, namely KCNE1-2, Kvβ1-3, KChIP2, and DPPX-Y [8–12].

Currents recorded from native cells have in several cases been found to be similar to currents conducted by complexes of K^+ channel α-subunits and KCNE β-subunits. Thus, I_{Ks}-like currents can be generated by KCNQ1 + KCNE1 [13], I_{Kr}-like currents by hERG + KCNE2 [14], and A-type currents in skeletal muscle by Kv3.4 + KCNE3 [15]. The KCNE family consists of five members (KCNE1–5) that are all found to modulate the KCNQ1 channel *in vitro* [13,16–19]. KCNE1–3 have additionally been shown to alter the currents of other KCNQ channels, ERG, HCN, and members of the Kv1-4 channel families [17,20–23].

Previously, no inhibitory effect of any β-subunit on the Kv4.3 current has been reported. In the present study, we demonstrate a drastic inhibition of Kv4.3 currents by the KCNE3 subunit. The inhibition is observed on heterologously expressed channels in *Xenopus laevis* oocytes as well as in HEK-293 cells. The inhibition is also observed for Kv4.3 channels in complex with the accessory subunit KChIP2. We find the inhibitory effect of KCNE3 to be specific for Kv4.3 within the Kv4 channel family as KCNE3 does not affect currents carried by Kv4.1 or Kv4.2. Given that Kv4.3 and KCNE3 are both found in human heart and brain [15,20,24], the interaction described in this study might have interesting physiological relevance.

* Corresponding author. Fax: +45 35327449.
E-mail address: spo@mfi.ku.dk (S.-P. Olesen).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.06.004

Materials and methods

Molecular biology

cDNAs coding for hKv4.1 (AF166003) and hKv4.2 (NM012281) were a kind gift from D. Isbrandt and cDNA coding for hKv4.3 (short isoform, NM172198) was kindly provided by O. Pongs. cDNAs encoding hKv4.3, hKCNE3 (AF302494), and hKChIP2.2 (AF199598) were subcloned into the expression vector pXOOM and cDNAs encoding hKv4.1 and hKv4.2 were subcloned into the expression vector pGEM. After linearization cDNA was purified by High Pure PCR Product Purification Kit (Roche A/S Diagnostics, Hvidovre, Denmark). Synthesis of capped cRNA was performed by *in vitro* transcription using the mMESSAGE mMACHINE T7 kit (Ambion, Austin, TX). cRNA was extracted and dissolved using the MegaClear kit (Ambion, Austin, TX) to a final concentration between 0.2 and 1 μg/μl. The concentration was determined photometrically. The integrity of the transcripts was confirmed by agarose gel electrophoresis and cRNA was stored at −80 °C until injection.

Transient expression in X. laevis oocytes and HEK-293 cells

Xenopus oocytes. Female *X. laevis* frogs were anaesthetized for 15–20 min in 2 g/L Tricain (3-aminobenzoic acid ethyl ester, Sigma A-5040) before an ovarian lobe was removed from the abdominal cavity through a small insertion. Oocytes were defolliculated enzymatically by incubation in 1% collagenase (Boehringer Mannheim, 1088831) and 0.1% trypsin inhibitor (Sigma T-2011) in Kulori medium (90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM Hepes, pH 7.4) for 1 h followed by five washes in Kulori containing 0.1% BSA (Sigma A-6003), and incubation for 1 h in a hypertonic phosphate buffer (100 mM K_2HPO_4, pH 6.5). Subsequently, stage V and VI oocytes were selected, and kept in Kulori medium for 24 h at 19 °C before injection of 50 nl cRNA. cRNA was injected using a Nanoject microinjector (Drummond Scientific, Broomall, PA). Oocytes were kept at 19 °C in Kulori medium for 1–4 days before measurements were performed.

HEK-293 cells. HEK-293 cells were cultured in DMEM (Life Technologies, Carlsbad, CA) supplemented with 10% FCS (Life Technologies, Carlsbad, CA) and grown in T25 flasks (Nunc) at 37 °C in 5% CO_2. The day of transfection 50% of the T25 flask surface was covered by cells; cells were transfected with 0.5 μg of each DNA sample using Lipofectamine (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. In all cases GFP was cotransfected as a reporter. Forty-eight hours post transfection the cells were trypsinized and transferred to coverslips for electrophysiological experiments.

Electrophysiology

Two-electrode voltage-clamp experiments. Currents were recorded using a two-electrode voltage-clamp amplifier (CA-1B, Dagan, Minneapolis, MN). Electrodes were pulled from borosilicate glass capillaries on a horizontal patch electrode puller and had a tip resistance between 0.3 and 2.0 MΩ when filled with 1 M KCl. During experiments oocytes were placed in a small chamber (volume: 200 μl) connected to a continuous flow system, and channel activity was measured in Kulori medium. All experiments were performed at room temperature 48 h after injection unless indicated otherwise.

Patch-clamp experiments. All experiments were performed in the whole-cell configuration. Currents were recorded using an EPC-9 amplifier (HEKA electronics, Germany), data were sampled with Pulse software (HEKA electronics, Germany), and analyzed with IGOR software (Wavemetrics, Lake Oswego, OR). R_s was compensated 80% and did not exceed 7 MΩ. Electrodes were pulled from borosilicate glass capillaries on a horizontal patch electrode puller and had a tip resistance between 1.8 and 2.4 MΩ when filled with intracellular Ringer solution (110 mM KCl, 31/10 mM KOH/EDTA, 5.17 mM $CaCl_2$, 1.42 mM $MgCl_2$, and 10 mM Hepes, pH 7.2). During recordings transfected HEK-293 cells were exposed to a continuous flow of extracellular NaCl Ringer solution (140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes, pH 7.4). All experiments were performed at room temperature.

Analysis of electrophysiological data

Data analysis was performed using IGOR software. All values are shown as means ± SEM. Inactivation time-constants as well as the time-constant for recovery from inactivation were found by fitting the data to a single exponential, i.e., $I(t) = y_o + A \times \exp(t/\tau)$. The difference between the peak current amplitude and the current at the end of a test pulses in absolute current values is referred to as the transient outward current. Current–voltage relations were obtained by plotting the transient outward current as a function of the test potential. The inactivation as well as the activation kinetics were analyzed by fitting the current–voltage relations to a two-state Boltzmann distribution of the form $I(V) = 1/(1 + \exp((V_{1/2}\text{-}V)/a))$, where $V_{1/2}$ is the potential for half-maximal inactivation or activation, respectively, and a is the slope factor. Data shown for inactivation time-constants, time to peak and mean current levels are found from current traces recorded at +60 mV. *n* indicates the number of independent experiments. Comparison of the biophysical properties in the presence and absence of accessory subunits was performed using an unpaired *t*-test for cases where two groups were compared and using a one-way ANOVA followed by Dunnet's multiple comparison tests when more than two groups were compared. Data were considered significant at $P < 0.05$.

Results

KCNE3 inhibits the Kv4.3 current and affects its gating properties

To investigate if KCNE3 β-subunits affect currents carried by Kv4.3 potassium channels, we expressed Kv4.3 alone or together with the KCNE3 β-subunit in *X. laevis* oocytes and performed two-electrode voltage-clamp recordings. Fig. 1A shows representative current traces of Kv4.3 recorded from oocytes subjected to a standard activation protocol. Oocytes were held at −80 mV before recording currents for 2 s at potentials ranging from −140 mV to +100 mV in 20 mV increments. Tail currents were measured by 1.5 s pulses to +40 mV. Oocytes expressing Kv4.3 channels showed fast activating and inactivating voltage-dependent currents at potentials positive to −40 mV. The steady-state activation curve obtained by plotting the normalized transient outward current as a function of clamp potential revealed half-maximal activation of 24 ± 2 mV ($n = 10$, Fig. 1). Likewise, the steady-state inactivation curve derived from the tail currents revealed a half-maximal inactivation of −61 ± 2 mV ($n = 10$).

Oocytes co-expressing Kv4.3 and KCNE3 were subject to a slightly modified clamp protocol. In this case, we only tested the Kv4.3 + KCNE3 channel's response between −100 mV and +60 mV (Fig. 2A). Oocytes co-expressing Kv4.3 and KCNE3 were not clamped at potentials negative to −100 mV and positive to +60 mV, because we observed slowly activating endogenous currents in the oocytes at these potentials of considerable magnitude compared to the small magnitude of Kv4.3 + KCNE3 currents. For oocytes expressing Kv4.3 alone this was not a consideration since the Kv4.3 current amplitudes were 10-fold larg-