Functional Implications of KCNE Subunit Expression for the Kv7.5 (KCNQ5) Channel

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Key Words
Voltage-dependent channels • Regulatory subunits • Skeletal muscle

Abstract
Kv7 (KCNQ) proteins form a family of voltage-gated potassium channels that is comprised of five members, Kv7.1-Kv7.5. While Kv7.1 is crucial in the heart, the Kv7.2, Kv7.3, Kv7.4 and Kv7.5 channels contribute to the M-current in the nervous system. In addition to the brain, Kv7.5 is expressed in skeletal and smooth muscle, where its physiological role is currently under evaluation. Kv7 associations with KCNE accessory subunits (KCNE1-5) enhance channel diversity and their interaction provides mechanisms to respond to a variety of stimuli. KCNE peptides control the surface expression, voltage-dependence, kinetics of gating, unitary conductance, ion selectivity and pharmacology of several channels. KCNE subunits have been primarily studied in the heart; however, their activity in the brain and in many other tissues is being increasingly recognized. Here, we found that Kv7.5 and KCNE subunits are present in myoblasts. Therefore, oligomeric associations may underlie some Kv7.5 functional diversity in skeletal muscle.

An extensive study in Xenopus oocytes and HEK-293 cells demonstrates that KCNE1 and KCNE3, but none of the other KCNE subunits, affect Kv7.5 currents. While KCNE1 slows activation and suppresses inward rectification, KCNE3 drastically inhibits Kv7.5 currents. In addition, KCNE1 increases Kv7.5 currents in HEK cells. Changes in gating and amplitude indicate functional interactions. Our results have physiological relevance since Kv7.5 is abundant in skeletal and smooth muscle and its association with KCNE peptides may fine-tune cellular responses.

Introduction

Potassium channels are one of the most diverse classes of membrane proteins in terms of function and structure. They establish the resting membrane potential and modulate the duration of the action potential in nerves and muscle. They are also involved in the maintenance of vascular smooth muscle tone, cell volume regulation, and leukocyte activation and proliferation, among other physiological responses [1]. More than 80 different genes
have been classified and have been distributed into 4 superfamilies. The voltage-dependent potassium (Kv) superfamily may be further subdivided into seven conserved gene families. These comprise the voltage-dependent channels Kv1-4 (Shaker, Shab, Shaw, Shal-like subunits), the KCNQ channels (Kv7), the silent Kv5, Kv6, Kv8 and Kv9 subunits (modulators), and the eag-like channels (Kv10-12) [2].

The Kv7 family consists of five members (Kv7.1-Kv7.5), encoded by single genes that all give rise to proteins that form slowly activating potassium selective ion channels [2]. The fact that mutations in four genes of these subunits (Kv7.1-Kv7.4) give rise to human genetic disorders highlights the physiological relevance of this family [3, 4]. Kv7.1, mostly expressed in heart, is present in several tissues, including testis and skeletal muscle [5]. Kv7.2 and Kv7.3 channels are confined to the nervous system, where they have been found in various cell types, including hippocampus, cortex and dorsal root ganglia [6]. Kv7.2-Kv7.5 channels have been proposed to constitute the M-current. This current is central in the control of neuronal firing due to its modulation by neurotransmitters and voltage-sensitivity [3]. While Kv7.5 might produce different variants of M-current in different parts of the nervous system, its role in skeletal and smooth muscle, where is expressed, is currently under investigation [7-10]. In this context, we have recently described that Kv7.5 is involved in myoblast proliferation [11].

The interaction of Kv channels with accessory subunits provides a mechanism for channels to respond to a variety of stimuli beyond changes in membrane potential. Kv7 channels associate with KCNE proteins, contributing to the functional diversity of K+ currents. The significance of these interactions is manifest in reports of genetic disorders such as the Long QT Syndrome, linked to KCNE mutations [12, 13]. The KCNE gene family also comprises five known members, KCNE1 to KCNE5 [12, 13]. KCNE regulatory subunits have been primarily studied in association with cardiac Kv7.1. Thus, the Kv7.1-KCNE1 interaction was identified as the molecular basis underlying the cardiac I_M current. However, KCNE activity in the brain and in many other tissues is being increasingly recognized [12, 13]. Several KCNE proteins interact not only with Kv7.1, but also with other Kv7 members. While KCNE1 and KCNE2 have been shown to mainly function in the heart and KCNE3 functions in skeletal muscle, the roles of KCNE4 and KCNE5 are not yet understood [12, 13]. Although Kv7.5 is highly expressed in skeletal muscle, preliminary reports on the Kv7.5-KCNE interaction are contradictory and have argued against any physiological relevance because, at that time, the presence of most KCNEs in skeletal muscle was controversial [14, 15]. However, it is clear today that missense mutations of KCNE3 lead to the dysregulation of muscular excitability, triggering a form of hereditary Familial Periodic Paralysis [16].

In light of this, we aimed to study whether the co-expression of KCNE1-5 proteins can affect the characteristics of Kv7.5. We here describe that all five KCNE proteins are expressed in skeletal muscle, indicating that a physiological interaction with Kv7.5 may occur. However, only KCNE1 and KCNE3 alter the gating of the Kv7.5 channel in Xenopus oocytes and HEK-293 cells. While KCNE1 slows activation and inhibits inward rectification, KCNE3 inhibits the current amplitude and differentially affects activation. Furthermore, KCNE1 increases Kv7.5 currents, when co-expressed in HEK cells. Our results have physiological relevance since Kv7.5 is involved during cell cycle progression in skeletal myoblasts [11], KCNE1 is induced in atrial tumors and germ cell neoplastic growth [5, 17], and KCNE3 is associated with skeletal muscle channelopathies [16].

Materials and Methods

RNA isolation and RT-PCR analysis
Total RNA from rat tissues (brain, heart, and skeletal muscle) and L6E9 cells was isolated using the Tripure reagent (Roche Diagnostics). All animal handling was approved by the ethics committee of the University of Barcelona. RNA was treated with DNasel and PCR controls were performed in the absence of reverse transcriptase. cDNA synthesis was performed with transcriptor reverse transcriptase (Roche) with a random hexanucleotide and oligo dT, according to the manufacturer’s instructions. Once cDNA was synthesized, the PCR conditions used were 94°C for 1 min, 1 min at the optimal annealing temperature (see Table 1 for temperatures and primers), and 72°C for 1 min. These settings were applied for 30 cycles. DNA from 10 µl of the total reaction was separated by gel electrophoresis in a 1.2% agarose 0.5X TBE (50 mM Tris, 50 mM boric acid, 1 mM EDTA, pH 8.0) gel.

Molecular Biology
Human Kv7.5 cDNA in the pTNL vector was kindly provided by T. Jentsch (Leibniz-Institut für Molekulare Pharmacologie and Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany). Human KCNE1 and KCNE2 were obtained from S. de la Luna (Centro de Regulación Genómica-CRG, Barcelona, Spain) and were subcloned between the BamHI and XhoI sites and the BamHI and XbaI sites in the pXOOM vector, respectively. Human KCNE3 and KCNE5 in pXOOM were provided by J. Barhanin (Institut de Pharmacologie Moléculaire...
Channel expression and electrophysiology in Xenopus oocytes

After linearizing the cDNAs, the cRNAs were generated with T7 and SP6 RNA polymerase by using the mMESSAGE mMACHINE kit, according to the manufacturer’s instructions (Ambion). Oocyte preparation was performed as described previously [18]. Stage V or VI oocytes were enzymatically defolliculated with collagenase (Sigma), 1 mg/ml in Ca²⁺-free solution, in a solution containing, in mM, 82.5 NaCl, 2.5 KCl, 1 MgCl₂, and 5 HEPES, pH 7.5. They were then transferred to a Ca²⁺-containing solution, ND96, consisting of, in mM, 96 NaCl, 2 KCl, 1.8 CaCl₂, 1 MgCl₂, and 5 HEPES, pH 7.5. The oocytes were injected with 50 nl of Kv7 or KCNE cRNA solution, containing 5 ng of Kv7.1 or Kv7.5, or 7.5 ng of a 1:0.5 mixture of Kv and KCNE in coexpression experiments. For unknown reasons, higher doses of some KCNEs killed oocytes, as has been previously described [19]. Oocytes were kept in ND96 buffer at 18ºC and used for recording two to four days after injection.

Whole-cell currents were recorded in oocytes at room temperature (22ºC) with a two-electrode voltage clamp using a virtual-ground Geneclamp 500B amplifier (Axon Instruments). Borosilicate electrodes were filled with 3 M KCl and had resistances of 1 MΩ. The oocytes were perfused continuously in Xenopus saline comprising, in mM, 100 NaCl, 2.5 KCl, 1 MgCl₂, 2 MnCl₂, and 5 HEPES, pH 7.5. All chemicals, except where otherwise stated, were from Sigma. Data were acquired at a sampling rate of 1 KHz and filtered at 1-2 kHz.

Voltage - Voltage protocols and current analysis were performed with pCLAMP 8.2 software (Axon Instruments). The voltage activation was determined as follows: oocytes were clamped for 3 s from a holding potential of -50 mV to voltages between -100 to +60 mV in 10 mV steps, and followed by a constant pulse to -20 mV of 1 s duration. The current activated at different voltage test pulses was estimated by measuring the amplitude of corresponding tail currents at -20 mV. For each oocyte, the current (I), was normalized relative to its maximal value. To evaluate the voltage dependence of potassium channels, the mean values of currents (±SE) were plotted versus voltage, and the I/V relationship was fitted to a Boltzmann function of the form:

\[ I = \frac{I_{\text{max}}}{1 + \exp\left(\frac{V_{1/2} - V_m}{\text{slope}}\right)} \]

where \( I_{\text{max}} \) is the maximal current and \( V_{1/2} \) is the membrane potential (\( V_m \)) at which \( I = I_{\text{max}}/2 \). Fits were made by treating \( I_{\text{max}} \), \( V_{1/2} \) and slope as free parameters, and the best values of constants were obtained by applying an iterative procedure of fitting to minimize the least-squares error between the data and the calculated fit point. Data were normalized in Excel and plotted with SigmaPlot. For statistical evaluation, the unpaired Student’s t-test was applied. A p<0.05 was considered significant.

Cell culture, transient transfection and electrophysiology in HEK-293 cells

HEK-293 cells were grown on poly-L-lysine-coated coverslips in DMEM medium containing 10% FBS with 10U/ml penicillin and streptomycin, and 2mM L-glutamine. Transient transfection was performed using Metafectene™Pro (Biontx) at nearly 80% confluence. Kv7.5 and KCNE cDNAs were used at 1:1 ratio. Two to three days after transfection, coverslips were transferred to a special chamber (Harvard Apparatus) mounted on the stage of an inverted microscope (Olympus IX-50) to perform the recordings. Whole cell currents were measured with an Axopatch-1D (Axon Instruments) amplifier using the patch clamp technique. WinWCP Strathclyde Whole Cell Program 3.8.2 (John Dempster, University of Strathclyde, U.K) was used for pulse generation, data acquisition and, subsequent analysis through a 1320A Axon Digitada A/D interface. After achieving the whole-cell configuration, currents were recorded at 2-10 KHz and low-pass filtered at 1-2 kHz.
without leak substraction. Series resistance compensation was always above 70%. All experiments were performed at room temperature. Electrodes were fabricated from borosilicate glass capillaries (Harvard Apparatus) using a P-97 micropipette puller (Sutter Instruments) and fire polished. Pipettes had a resistance of 2-4 MΩ when filled with a solution containing (in mM): 120 KCl, 5.37 CaCl₂, 2 MgCl₂, 10 EGTA, 10 HEPES, 2 ATP (pH 7.2 by KOH). The extracellular solution contained (in mM): 140 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES (pH 7.4 by NaOH). Cells were clamped to a holding potential of -90 mV. To evoke voltage-gated currents, all cells were stimulated with 2000-ms square pulses ranging from -80 to +60 mV in 10 mV steps. Traces were fitted with Sigma Plot 10.0 (SPSS Inc.).

Results and Discussion

**Kv7.5 and KCNE peptides are coexpressed in skeletal muscle**

The complexity of Kv7 channels may be further increased by their associations with KCNE modulatory subunits. Studies in heterologous expression systems have highlighted the wide variety of biophysically and pharmacologically distinct Kv7 channels that carry out diverse functions as key determinants of membrane excitability [16]. Kv7.5 is expressed in the brain, skeletal and vascular smooth muscle [7-10, 14, 15]. While Kv7.5 function has been evaluated in brain, where, in combination with Kv7.3, it contributes to neuronal excitability, its role in skeletal muscle is unknown [14, 15]. However, recent data in vascular smooth muscle identify Kv7.5 as target for vasoconstrictor signal transduction [7-10]. Kv7.5 is inhibited by M1 muscarinic receptor activation and shows pharmacological properties suggesting that it plays a role in generating M-like currents in different parts of the nervous system [20]. In muscle, we have recently described cell-cycle-dependent regulation of Kv7.5. Kv7.5 is induced prior to the S-phase and is involved in cell cycle progression [11]. Since Kv7...
channels are tightly modulated upon association with KCNE subunits, we aimed to analyze how all five KCNE subunits modulate Kv7.5. The cloning of Kv7.5 was simultaneously described by two laboratories and preliminary KCNE coexpression data led to controversy [14, 15]. While Lerche et al. failed to detect evidence for functional interactions with KCNE peptides [14], Schroeder et al. argued against any physiological relevance of KCNE proteins in conjunction with Kv7.5 since KCNE1 is not present in the brain and had not yet been detected in skeletal muscle by northern blot analysis [15]. However, Lazdunski and coworkers found KCNE1 mRNA in mouse skeletal muscle by PCR [21]. Additionally, Figure 1 shows that Kv7.5 and KCNE subunits are present in rat skeletal muscle. Likewise, all five KCNE subunits are coexpressed with Kv7.5 in vascular smooth muscle [9].

Effects of KCNE subunits on Kv7.5 currents

The significance of Kv7.5-KCNE interactions is clear since these proteins are coexpressed in skeletal and smooth muscle and since KCNE peptides affect the gating of Kv channels. KCNE1 functions in the heart and ear, KCNE2 in the heart and stomach, and KCNE3 in the colon, ear, and skeletal muscle [12, 13]. Although roles for KCNE4 and KCNE5 are under investigation, KCNE5 is associated with AMME syndrome (Alport syndrome, mental retardation, midface hypoplasia, and elliptocytosis) [22]. Preliminary Kv7.5-KCNE studies have revealed important discrepancies between laboratories and a full characterization had not been undertaken [14, 15]. Therefore, we wanted to analyze whether Kv7.5 channels can be functionally modulated by KCNE accessory subunits. To that end, we undertook a complete analysis in Xenopus oocytes and HEK-293 cells. Since the modulation of Kv7 channels by KCNE accessory subunits is currently under investigation, we first controlled the effects of our KCNE constructs on Kv7.1 currents. As expected, KCNE subunits modulated Kv7.1 currents as previously described (not shown) [22-29]. Figure 2 illustrates representative K+ current tracings and normalized peak-current densities obtained from Kv7.5-injected oocytes in the presence or the absence of KCNE. The electrophysiological analysis took place two to four days after injection, and no currents were detected in water-injected control oocytes (not shown). Kv7.5-injected oocytes yielded currents that needed several seconds to fully activate upon depolarization (Fig. 2 A). Activation of Kv7.5 ($V_{1/2}$, -46.7±1.6 mV; k, 11.8±0.8 mV) currents was in the range of previous works and generally faster than that of other Kv7 currents. Thus, the half-maximal activation of Kv7.5 was more negative than that of Kv7.2 (-37 mV), Kv7.4 (-11 mV), and Kv7.3/Kv7.5 heterotetramers (-35 mV), but in the range of that of Kv7.2/Kv7.3 (-40 mV) [6, 30-32]. Hyperpolarizing test pulses in Kv7.5-KCNE-injected oocytes failed to indicate relevant change in K+ current amplitude with most KCNEs (Fig. 2B, C, E and F). However, coexpression with KCNE3 significantly suppressed more than 60% of Kv7.5 currents (Fig. 2 D and G). While Schroeder and

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coworkers suggest similar results, Lerche et al. detected no evidence for a functional interaction [14, 15]. However, Schroeder et al. argued against any physiological significance due to the small magnitude of the effects [15]. Our results indicate that a 1:1 ratio (in kilobases) of KCNE3-Kv7.5 cRNA drastically inhibited Kv7.5. Similar inhibitory effects of KCNE3 have been also observed on Kv7.4 currents [31]. KCNE3 and Kv7.4 are coexpressed in the inner and outer hair cells of the inner ear, and a functional interaction may influence electrical excitability and cell survival [31, 33]. In addition, KCNE3 also modulates Kv members from other families. Thus, KCNE3 alters the gating of Kv3.4 and reduces Kv2.1 and Kv3.1 currents in the brain and skeletal muscle [16, 34]. Since KCNE3 turns out to be essential for regulating membrane potential in skeletal muscle [16], our results point to a functional interaction of this subunit with Kv7.5.

Tail current analysis was used to estimate activation parameters. Current-voltage relationships of Kv7.5 currents in the presence or absence of KCNE subunits are shown in Figure 3. Currents were activated at positive depolarizing potentials above -80 mV and displayed a “crossover” phenomenon that was observed at potentials greater than 0 mV and independent of current amplitudes (Fig. 3 A). This inward rectification [14] was suppressed in the presence of KCNE1 (Fig. 3 B). This KCNE1-dependent process is specific since similar results have also been observed with Kv7.1, but not Kv7.4 currents [31, 35]. Normalizing currents against test potentials showed that KCNE1 suppressed the inward rectification more than 70 % (Fig. 3 C and D), a phenomenon that may have physiological relevance. For instance, KCNE1 abundance increases during neoplastic growth in atrial and germinal tumours [5, 17]. To what extent a lack of inward rectification would support a sustained outward K+ flux in proliferating cells is not known, but Kv7.5, which is induced during the G1/S phase of the myoblast cell cycle, may contribute to a transient hyperpolarization in cell cycle progression like other Kv channels [11, 36-38].

No important differences were observed in the half-maximal activation of Kv7.5 in the presence or absence of KCNE (Fig. 3 A, B and Table 2). However, KCNE1 significantly shifted the V1/2 to depolarised values (-42.4 ± 0.9 mV vs. -46.7 ± 1.6 mV, p<0.05) (Table 2). This depolarized shift is again shared with Kv7.1 [25, 26, 35]. In contrast, KCNE1 hyperpolarizes the half point of voltage-dependent activation of Kv7.4 [31]. This modulation of activation by KCNE1 seems specific to Kv7 channels since no significant differences for time constants of activation were found when KCNE1 was coexpressed with hERG, Kv3.1 and Kv3.2 channels [39, 40]. Similar to our findings, Schroeder et al. indicate that KCNE1 slowly activates Kv7.5, whereas Lerche et al. describe no such effects [14, 15]. Our results indicate that only KCNE1 and KCNE3 alter Kv7.5 currents.

Next, we further analyzed the effects of these KCNE subunits on Kv7.5 activation at different voltages. Kv7.5 currents were evoked in the presence or absence of KCNE by 3 s depolarizing pulses at -30 mV (close to the V1/2 of activation), 0 mV (peak current) and +60 mV (inward rectification). While KCNE1 slowed Kv7.5 activation at all voltages, KCNE3 exerts a crossover phenomenon (Fig. 4). KCNE3 accelerated activation at -30 mV and 0 mV, although it slowed the activation at +60 mV. No other KCNE subunits modified Kv7.5 activation (not shown). Similar KCNE1 and KCNE3 differential tuning occurs with other Kv members. KCNE1 slows Kv3.1 and Kv3.2 activation [40]. KCNE3 increases the activation time constants of Kv2.1, Kv3.1 and Kv3.2 [34, 40]. However, while KCNE3 exerts profound effects on Kv3.4 current has no significant effect on either Kv1.4 or Kv4.1 [16, 34, 40]. Since channel activation could be analysed by a sum of three exponential functions [15], traces evoked by 3 s depolarizing pulses at -30 mV, 0 mV and +60 mV were further analyzed (Table 3). For a step to -30mV, the presence of KCNE1 raised τ2 and τ3 rate constants, whereas KCNE3 significantly diminished τ1. A 0 mV step showed similar consequences. Thus, while KCNE1 slowed τ2 and τ3 activation constants, KCNE3 accelerated the activation at τ1 and τ2. At the +60 mV step, KCNE1 increased the activation constants τ2 and τ3.

<table>
<thead>
<tr>
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<th>V1/2 (mV)</th>
<th>Slope (k)</th>
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<tr>
<td>Kv7.5</td>
<td>-46.7 ± 1.6</td>
<td>11.8</td>
</tr>
<tr>
<td>Kv7.5 + KCNE1</td>
<td>-42.2 ± 0.9*</td>
<td>11.3</td>
</tr>
<tr>
<td>Kv7.5 + KCNE2</td>
<td>-46.4 ± 1.2</td>
<td>11.4</td>
</tr>
<tr>
<td>Kv7.5 + KCNE3</td>
<td>-45.9 ± 0.9</td>
<td>11.5</td>
</tr>
<tr>
<td>Kv7.5 + KCNE4</td>
<td>-45.1 ± 1.4</td>
<td>11.5</td>
</tr>
<tr>
<td>Kv7.5 + KCNE5</td>
<td>-44.0 ± 1.2</td>
<td>10.5</td>
</tr>
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Table 2. Activation of Kv7.5 currents in the presence of KCNE subunits. Currents were evoked in Kv7.5-injected oocytes in the presence or absence of KCNE. Voltage for half-maximal activation (V1/2) and slope values were calculated following the pulse protocol described in Figures 2 and 3. Values are the mean ± SEM of 10 to 15 oocytes. *, p<0.05 vs. Kv7.5, Student’s t-test.
Fig. 4. KCNE1 and KCNE3 modulate Kv7.5 activation. Representative current traces from Kv7.5-injected oocytes in the presence or absence of KCNE1 and KCNE3. Normalized current traces of experiments are the same as those used for Figure 2. Currents elicited by depolarizing pulses to -30 mV (left), 0 mV (center) and +60 mV (right) are shown. (A) KCNE1 slows Kv7.5 activation. (B) KCNE3 differentially shapes Kv7.5 activation. While KCNE3 accelerates activation at -30 mV and 0 mV, it slows activation at +60 mV.

<table>
<thead>
<tr>
<th>Subunit</th>
<th>τ1 ± SEM (ms)</th>
<th>τ2 ± SEM (ms)</th>
<th>τ3 ± SEM (ms)</th>
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<tr>
<td>Kv7.5</td>
<td>93.7 ± 7</td>
<td>328 ± 22</td>
<td>1561 ± 104</td>
</tr>
<tr>
<td>+ KCNE1</td>
<td>79.3 ± 8</td>
<td>401 ± 21*</td>
<td>1819 ± 56*</td>
</tr>
<tr>
<td>+ KCNE2</td>
<td>79.4 ± 13</td>
<td>332 ± 12</td>
<td>1565 ± 56</td>
</tr>
<tr>
<td>+ KCNE3</td>
<td>69.4 ± 9*</td>
<td>326 ± 10</td>
<td>1777 ± 81</td>
</tr>
<tr>
<td>+ KCNE4</td>
<td>104.9 ± 14</td>
<td>336 ± 19</td>
<td>1813 ± 115</td>
</tr>
<tr>
<td>+ KCNE5</td>
<td>111.2 ± 14</td>
<td>309 ± 18</td>
<td>1781 ± 99</td>
</tr>
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Table 3. Activation time constants of Kv7.5 in the presence of KCNE subunits. The voltage activation was determined by clamping the oocytes for 3 s from a holding potential of -50 mV to -30 mV, 0 mV and +60 mV, and followed by a constant pulse to -20 mV of 1 s duration. The current activated at different voltage test pulses was estimated by measuring the amplitude of corresponding tail currents at -20 mV. Time constants of activation were fit to the sum of three exponentials as described in the Materials and Methods. Values are the mean ± SEM of 10 to 15 oocytes. *, p<0.05; **, p<0.01; ***, p<0.001 vs. Kv7.5, Student’s t test.

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τ3, and KCNE3 significantly raised the τ3 constant about 35%. In summary, KCNE1 and KCNE3 modulated the activation constants of Kv7.5. While KCNE1 tended to slow time constants, KCNE3 exerted a voltage-dependent differential modulation. This KCNE3-influenced pattern is probably the reason why Kv7.5 macroscopic currents behave similarly in the presence or absence of this subunit and why the half-maximal activation remained constant. Taking all together, our results would further support the discrete findings reported by Jentsch and coworkers [15].

We finally analyzed whether KCNE subunits modulated the deactivation time constant of Kv7.5 currents. Tail currents (see pulse protocol in Fig. 2) from -20 mV back to -50 mV (holding potential) were fitted to a single-exponential function. KCNE subunits exerted no modulation of the apparent deactivation of Kv7.5. Time constants of deactivation (in ms) were 181±9 for Kv7.5 alone and 227±22, 220±23, 216±18, 161±8 and 164±5 in the presence of KCNE1-5, respectively.

The biophysics of most ion channels has been characterized by functional expression in Xenopus oocytes. However, studies reveal discrepancies in channel properties between amphibian and mammalian expression systems suggesting that endogenous oocyte factors may regulate channel gating [13, 23, 29, 41, 42]. In fact, Abbott and co-workers demonstrated that oocytes may express some endogenous KCNE ancillary subunits [43]. Therefore, to further confirm our findings we co-expressed Kv7.5 and KCNE peptides in HEK-293 cells.
oocytes, as previously demonstrated [8, 44], Kv7.5 conducted very low delayed-rectifier K+ currents in mammalian cells (Fig. 5A), While KCNE1 increased the peak current density at +60 mV by 2-fold, KCNE3 inhibited the current by 75% (Fig. 5A and B). This result is in agreement with the effect of KCNE1 on Kv7.1, which is responsible for the cardiac Iks current [25]. In addition, KCNE1 slowed the activation of Kv7.5 currents (Fig 5 C). Thus, τ for activation were 42±3 and 129±2 ms for Kv7.5 and Kv7.5/KCNE1 respectively (p<0.001, n=4-8) Again, the rest of KCNE subunits did not exert any effect. Unlike oocytes [14, 15], Kv7.5 currents in HEK cells showed no inward rectification at positive potentials [8] (Fig 5 D). However, the presence of KCNE1 clearly modulated the I/V curve (Fig 5 D). Overall our results in HEK cells further support those obtained in oocytes. The apparent discrepancy in the magnitude of current density and gating between Xenopus oocytes and the mammalian expression system could be explained by interactions with amphibian endogenous subunits [43]. This deserves further research.

Our results demonstrate that KCNE1 and KCNE3 modulate the gating of Kv7.5. These findings may be of physiological interest since both KCNE peptides and Kv7.5 are present in skeletal and vascular smooth muscle. While KCNE1 slowed activation and suppressed inward rectification, KCNE3 drastically inhibited the peak current amplitude. Unlike Kv7.4, KCNE1 exerts similar effects on Kv7.1 [31]. Skeletal muscle expresses Kv7.1 and Kv7.5, but not Kv7.4. It is tempting to speculate that KCNE1 would specifically partner with skeletal muscle Kv7 isoforms. In fact, proliferation increases Kv7.1 and Kv7.5 expression in myoblasts, and KCNE1 is up-regulated in atrial tumors and germ cell neoplastic growth [5, 11, 17]. Unlike activation or inward rectification, KCNE3 suppressed the Kv7.5 current amplitude. KCNE3 is a predominant skeletal muscle isoform that has been shown to be involved in periodic paralysis [16]. KCNE3 has drastic effects on Kv7.1 and inhibits other channels, such as Kv7.4 and the skeletal muscle isoforms of Kv2.1, Kv3.1 and Kv3.4 [31, 34, 40]. Therefore, this modulation should be taken into account when the role of Kv7.5 in skeletal muscle is contemplated.

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