Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 363 (2007) 133–139
BBRC
www.elsevier.com/locate/ybbrc

The KCNE1 beta-subunit exerts a transient effect on the KCNQ1 K^+ channel

Asser Nyander Poulsen *, Dan Arne Klaerke

Department of Physiology and Biochemistry, IBHV, Faculty of Life Sciences, University of Copenhagen, Groennegaardsvej 7, 1870 Frederiksberg C, Denmark

Received 20 August 2007
Available online 31 August 2007

Abstract

The KCNE1 beta-subunit is a modulatory one-trans-membrane segment accessory protein that alters KCNQ1 K^+ channel current characteristics, though it is not required for channel expression. The KCNE1 and KCNQ1 interaction was investigated by looking for effects of expression time on channel currents in *Xenopus laevis* oocytes. We found that long-time expression of KCNQ1+KCNE1 (2–14 days) resulted in gradual changes in current characteristics resembling a disappearance of KCNE1 from the oocyte plasma membrane. Towards the end of the expression period the current of oocytes expressing KCNQ1+KCNE1 was indistinguishable from those expressing KCNQ1 alone. No time dependent effect was seen in oocytes expressing KCNQ1 alone or a concatamer of KCNQ1 and KCNE1. Brefeldin A was tested, showing that measured current was independent of exocytosis (decreased capacitance) thus eliminating a continuous displacement-explanation. Based on the functional data, we suggest that the interaction between KNCE1 and KCNQ1 may be reversible and transient in a "Kiss & Go" manner, supporting a physiological role for KCNE1 as a dynamic regulatory molecule.

Keywords: KCNQ1; KCNE1; Assembly; Plasma membrane; MinK; KvLQT; Long QT; Concatamer; Capacitance; Brefeldin A

The beta-subunits of the KCNE gene family (KCNE1-5) are small (<200 a.a.) single-spanning membrane proteins that alter channel activation kinetics, voltage dependence and macroscopic current, when co-expressed with a variety of K^+ channels of the K_v type including the KCNQ1 channel [1,2].

KCNE1, also known as minK or I_{sK}, slows KCNQ1 channel activation, changes the shape of the voltage–current curve and increases the current of KCNQ1 when co-expressed in *Xenopus* oocytes or in mammalian cell lines [3,4] and the KCNQ1/KCNE1 complex has been identified as the molecular entity responsible for the cardiac slow delayed rectifier current (I_{Ks}) [3,4] and a requirement for endolymph secretion in the inner ear [5].

At the molecular level the intracellular C-terminal [6] as well as a three-amino acid stretch (activation triplet) of the trans-membrane segment of KCNE1 [7,8] are proposed to interact with the pore region of KCNQ1. Although these results could indicate a tight coupling between alpha- and beta-subunits, extensive experimental work employing fusion constructs has suggested that channel complexes with a fixed 2:4 or 4:4 (KCNE:KCNQ1) stoichiometry, resembling certain native heteromeric KCNE+KCNQ1 channel characteristics, may be further modulated by interaction with additional "free" KCNE subunits [9,10]. Thus a variety of stoichiometrical arrangements seem to be possible for assembly of functional channels.

It is disputed whether the assembly of subunits takes place in the endoplasmic reticulum (ER) only or if it can also happen once the proteins have been delivered to the plasma membrane. The latter assumption is supported by experiments showing that delayed (+24–72 h) expression of KCNE results in modulation of KCNQ1 already being expressed [1,6,11]. This apparently takes place with no profound change in cellular KCNQ1 distribution or surface

* Corresponding author. Fax: +45 3533 2525.
E-mail address: aspo@life.ku.dk (A.N. Poulsen).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.08.146

expression level [1] suggesting that KCNE interacts with KCNQ1 in the plasma membrane. However, other results show that KCNE are retained in ER if KCNQ1 are not present in the synthesis pathway at the same time [12,13] questioning the possibility that KCNE can reach the plasma membrane by itself. Regardless this current issue, the sudden changes in KCNQ1 currents induced by KCNE expression, the diverse modulation exerted by different KCNE types and the overlapping expression patterns observed in different tissues [14], have assigned a physiological role of KCNEs as ion channel regulators.

We have studied the dynamics of expression of KCNQ1, KCNE1 and a fusion construct in *Xenopus* oocytes by observing expressed ion channels over a long period (up to 14 days) and have revealed a striking feature of heteromeric channels formed by KCNQ1 and KCNE1; that the modulatory effect of KCNE1 fades away over time and leaves behind apparent homomeric KCNQ1 channels comparable to KCNQ1 expressed without KCNE1. The apparently transient behavior of KCNE1 supports the role for KCNEs as dynamic regulating molecules.

Methods

Expression in Xenopus laevis oocytes. Preparation of oocytes from *X. laevis* was performed as previously described [15]. In brief, ovarian lobes were excised from the abdominal cavity of anaesthetized frogs (0.2% 3-aminobenzoate methanesulfonate). The procedure was done in accordance with national legislation. Explants were digested with collegenase and incubated with hypertonic phosphate buffer to clear the follicle cell layer from oocytes. Oocytes were kept at 19 °C in Kulori medium (90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM Hepes, pH 7.4) that was replaced daily.

Stage V or VI oocytes were sorted out and injected with cRNA solution (50 nl, approximately 1 μg/μl) using a micro-injector (Drummond Nanoject). In experiments with combined expression, cRNA solutions were mixed 1:1 before injection.

Sequences of hKCNQ1 or hKCNE1 were cloned into pXOOM vector [16] and hKCNE1-hKCNQ1 concatamer into MK24-pcDNA3.1 vector and linearized with XbaI, NheI or HindIII, respectively (New England Biolabs). Synthetic cRNA was prepared by *in vitro* transcription (mCAP RNA Capping Kit from Stratagene) from linearized DNA templates. cRNA was extracted by phenol/chloroform, ethanol precipitated and dissolved in RNase-free TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5). cRNA yield was evaluated by measurements of optical density (A_{260}/A_{280}: 1.8-2) and by agarose gel electrophoresis. cRNA was stored at −80 °C until use.

The KNCE1 and KCNQ1 concatamer is a fusion construct with hKCNE1 placed N-terminally of hKCNQ1 with no added linker sequence (Fig. 1). It was kindly provided by Jenny Rao from Columbia University.

Chemicals. Chemicals except from enzymes and kits were obtained from Sigma-Aldrich. Brefeldin A was dissolved in ethanol.

Electrophysiology. Oocyte membrane ion currents were measured by two-electrode voltage clamp using an Oocyte Clamp amplifier from Warner Instruments Corp. (OC-725 B) and a PC-interface (iWorx 118). Data were sampled at 100 Hz with LabScribe software (CB Sciences Inc.). Electrodes were pulled from capillary glass (TW 120-3, World Precision Instruments Inc.) on a programmable micropipette puller (P-97, Sutter Instruments CO.). Electrodes were filled with 1 M KCl and electrode resistance ranged from 0.5 to 1.5 MΩ. Experiments were done at room temperature (19–21 °C) in an air-conditioned lab, with continuous perfusion of Kulori medium. Membrane potential was checked prior to voltage clamping as indicator of electrode-membrane sealing and oocyte condition. Oocytes used for measurements had membrane potentials from −25 to −80 mV. The voltage clamping protocol consisted of depolarizing steps (4 s) from a holding potential of −80 to +60 mV with 20 mV increments (inter-step length 20 s.) (Fig. 1C).

For measurements of membrane capacitance, data was sampled at 10 kHz while the membrane potential was stepped for 100 ms with small increments from −80 to −40 mV with a −80 mV holding potential.

Data analysis. Ohmic "leak" current was subtracted from recorded currents by omitting the first 40 ms of the current traces, which includes the transient capacitance-peak. Leak currents were typically below 0.2 μA. Presented maximal currents (I_{+60}) were obtained from the last depolarizing step to +60 mV (Fig. 1C). The slow activation of KCNQ1 channels allowed this kind of leak correction.

For analysis of activation kinetics, the leak-corrected current trace of the −80 to +60 mV depolarization step was normalized and fitted to a double exponential function of the form $I(t) = I_f \cdot (1 - e^{-t/\tau_f}) + I_s \cdot (1 - e^{-t/\tau_s})$ yielding a fast and a slow activation time constants (τ_{fast}, τ_{slow}) and current components (I_f, I_s, not shown). Non-linear regression was performed with the least squares method in Microsoft Excel® with use of the add-in function Solver [17]. Carefully selected starting parameters were chosen for the fitting procedure to avoid local minima during numerical root-finding and to ensure that reasonable values for current components were found ($I_f < 1$, $I_s < 2$). Fitting parameters were accepted if $R^2 > 0.98$ (coefficient of determination).

For voltage dependence analysis, the leak-corrected current steps were normalized to maximal current (I_{+60}) and plotted against voltage. Leak-corrected currents were used as this increased the sensitivity to changes in voltage dependence. For comparisons of voltage dependence, normalized current during the −80 to 0 mV step (I_0/I_{+60}) was arbitrarily chosen as the representative variable.

Membrane capacitance was obtained from the area under the curve during the transient capacitive current-peak, with subtraction of the ohmic "leak" current.

Statistics. All data are presented as means ± SEM. Statistic tests were performed with GraphPad Instat®. For comparison of pairs of means, unpaired *t*-test was used after testing for normality and equal standard deviations. In case of different standard deviations, an alternative *t*-test (Welch *t*-test) was used. Comparisons of more than two means were done with one-way Anova, or with nonparametric Anova (Kruskal–Wallis Test) in cases where standard deviations were significantly different among groups (Bartlett's test for equal standard deviation) or individual groups were not normal distributed (Kolmogorov & Smirnov test). For comparison of groups against a control group, Dunnett's post test was used, and for multiple comparisons Bonferroni post test was used. A *p*-value of less than 0.05 was considered significant.

Results

Expression time and maximal current. KCNE1 transiently increases KCNQ1 current

When expressing homomeric KCNQ1 channels, the maximal current measured at +60 mV (I_{+60}) slowly increased from approximately 1–4 μA from day 2 to day 14 (Fig. 1A). The current of the concatamer initially (3 days after injection) showed a level similar to that of KCNQ1 expressing oocytes (approximately 1.5 μA), but, in contrast, decreased through-out the period ending at approximately 0.5 μA (14 days after injection, see Fig. 1A).

If KCNE1 was co-expressed with KCNQ1 (simultaneous RNA injection), the maximal current increased to a higher level (approximately 5 μA) 5–10 days after injection in accordance with previous reports [3,4], showing that KCNE1 increases KCNQ1 channel expression in the

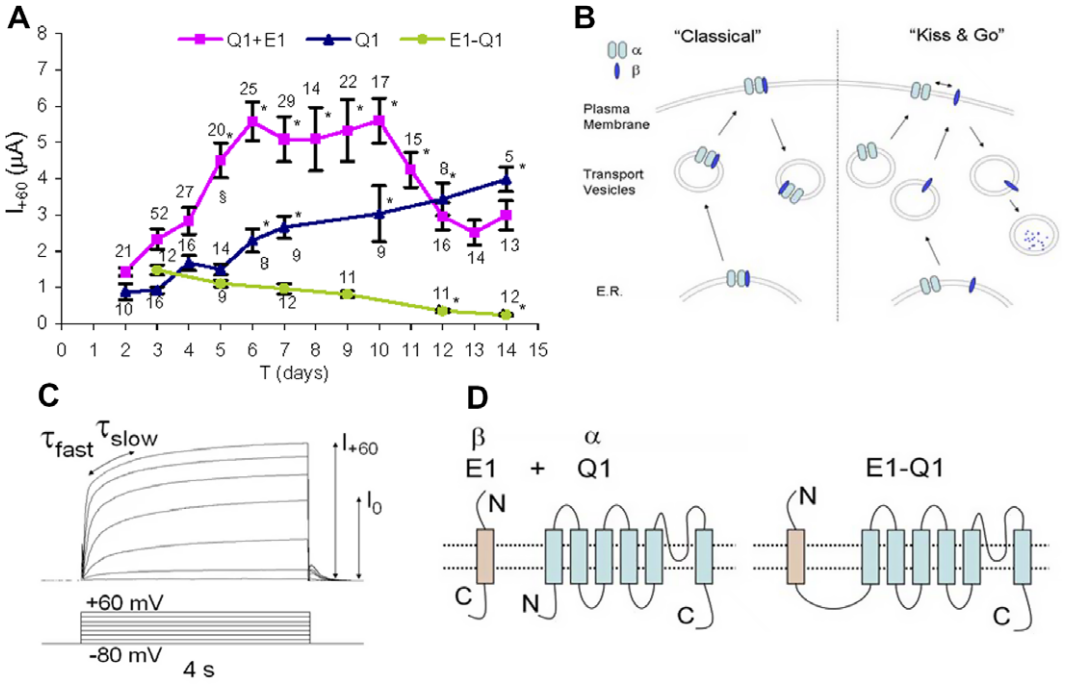

Fig. 1. (A) Maximal currents (I_{+60}) in oocytes measured 2–14 days after injection of cRNA. Oocytes injected with KCNQ1 (Q1), KCNQ1+KCNE1 (Q1+E1) or concatamer KCNE1-KCNQ1 (E1-Q1). Means ± SEM. Numbers in diagram indicate n. $^{*}p < 0.05$ vs. first point in series. $^{\S}p < 0.01$ Q1 vs. Q1+E1. (B) Two different models describing the interaction between ion-channel α and β-subunits. In the "Classical" model, the α and β-subunit complex is assembled during synthesis and persists throughout the lifetime of the complex. In the "Kiss & Go" model, subunits are synthesised separately and are able to associate later in a reversible manner. (C) Voltage stepping protocol and parameters obtained from current traces. (D) Topology of the KCNQ1 α (Q1) and KNCE1 β-subunit (E1), and a KCNE1-KCNQ1 concatamer (E1-Q1).

plasma membrane and/or individual KCNQ1 channel currents. The enhancing effect of KCNE1 on KCNQ1 current may be even greater than shown by the experiment as oocytes co-expressing KCNQ1 and KCNE1 were injected with only half the amount of KCNQ1-RNA injected into oocytes expressing only KCNQ1. The current of KCNQ1+KCNE1 subsequently reverted around 10 days after injection to a level around 3 μA that was similar to the level in KCNQ1 expressing oocytes (Fig. 1A). These observations suggest a loss of impact of KCNE1 after a certain time period; thus the modulation by KCNE1 may be considered transient.

Expression time and voltage dependence. Gradual change in KCNQ1+KCNE1 expressing oocytes

As shown in Fig. 2, the current–voltage curve in oocytes expressing KCNQ1 channels showed a sigmoidal appearance (Fig. 2A, Q1) with a normalized current at 0 mV (I_0/I_{+60}) of 70–80%. The current–voltage relation of the concatamer had a non-sigmoidal appearance (Fig. 2A, E1-Q1) with activation at higher potentials (I_0/I_{+60} of 10–20%). While the voltage dependent activation of KCNQ1 and the concatamer remained constant over time, it showed a time-dependent change throughout the 14 days expression period for KCNQ1+KCNE1 (Fig. 2B). The current–voltage curve gradually changed from appearing similar to that of the concatamer (non-sigmoidal), to an appearance more similar to that of KCNQ1 (sigmoidal) (Fig. 2A, Q1+E1). The I_0/I_{+60} of KCNQ1+KCNE1 changed from approximately 20% at day 2–70% at day 13 and 14. The endpoint for I_0/I_{+60} of KCNQ1+KCNE1 was not significantly different from that of KCNQ1 alone. As also indicated by measurements of the maximal current (see above), this change in voltage-dependent activation of KCNQ1+KCNE1 suggests that the modulatory effect of KCNE1 gradually disappears.

Expression time and activation kinetics

To further establish the apparent time-dependent loss of KNCE1 influence on KCNQ1, the activation kinetics were analyzed by fitting the activation curves to a double-exponential function (see Methods). As expected, the characteristics of KCNQ1 channels and the concatamer were unaffected by expression-time as the shape of the current traces (Fig. 3A) and the activation time constants τ_{fast} and τ_{slow} remained unaltered over time (Fig. 3B) while the characteristics of KCNQ1+KCNE1 channels changed during the first 7 days of expression. The activation became faster by time with current traces becoming steeper (Fig. 3A, Q1+E1). The activation time constants were decreased, starting from a level close to that of the KCNE1-KCNQ1 concatamer and ending at level not significantly different from that of KCNQ1 after day 7

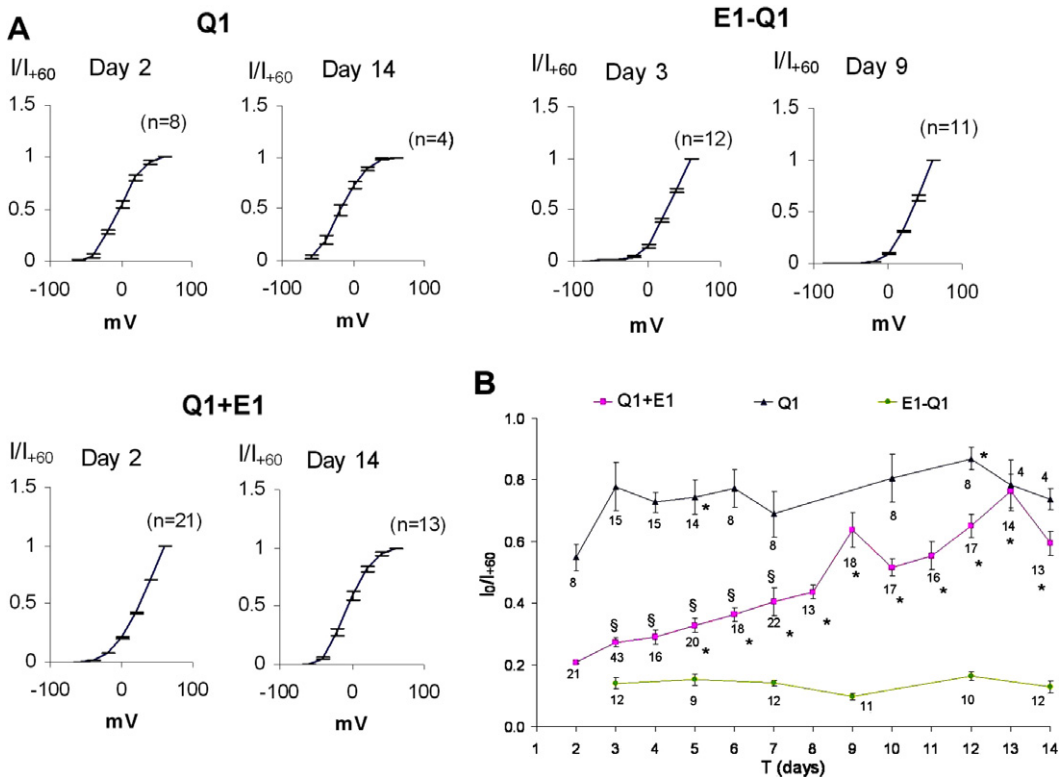

Fig. 2. Effect of expression time on voltage dependence. (A) Normalized voltage–current relations (I/I_{+60}) in oocytes expressing KCNQ1 (Q1), KCNQ1+KCNE1 (Q1+E1) or KCNE1-KCNQ1 concatamer (E1-Q1) at different times. Means ± SEM. (B) Summary showing normalized current at 0 mV (I_0/I_{+60}) in oocytes expressing KCNQ1 (Q1), KCNQ1+KCNE1 (Q1+E1) or KCNE1-KCNQ1 concatamer (E1-Q1). Means ± SEM. Numbers in diagram indicate n. $^{*}p < 0.05$ vs. first point in series. $^{\S}p < 0.001$ Q1 vs. Q1+E1.

(Fig. 3B). Thus, also the kinetic analysis indicates a time dependent loss of KCNE1.

Effect of brefeldin A on KCNQ1+KCNE1 expression

As the apparent gradual "loss" of KCNE1 subunits could be due to a continuous delivery of unpaired KCNQ1 alpha-subunits to the plasma membrane and removal of "old" channels from the membrane, we tested if the expression level and current characteristics were dependent on the exocytotic pathway. For this we used the fungal drug brefeldin A (BFA) that is known to inhibit anterograde vesicle transport from the endoplasmic reticulum to the Golgi apparatus [18] and thus inhibits delivery of newly synthesized protein to the plasma membrane.

In oocytes expressing heteromeric KCNQ1+KCNE1 channels currents were measured at day 3 in two groups of oocytes to obtain current characteristics (I_{+60}, I_0/I_{+60}, τ_{fast} and τ_{slow}) as well as membrane capacitance (C). At day 4, one group was incubated for 6 h with 10 μM BFA while a control group was left untreated. Afterwards, current measurements were repeated for both groups.

BFA significantly reduced the membrane capacitance from 199.8 ± 2.8 to 182.8 ± 3.1 nF (Fig. 4A), while there was no apparent difference between the BFA-treated and control group with regard to the maximal current measurements (I_{+60}), voltage dependence (I_0/I_{+60}) or activation kinetics (τ_{fast}, τ_{slow}) from day 3 to day 4 (Fig. 4B–E). The change in kinetic parameters from day 3 to day 4 was in congruence with that seen in the long-time experiment (compare Fig. 4 with Figs. 1A, 2B, and 3B) indicating that repeated measuring have little effect on the oocytes. In conclusion, the expression of KCNQ1+KCNE1 at this time must be independent of exocytosis as BFA had no apparent effect on current characteristics in particular maximal current, despite a clear effect of BFA on capacitance reflecting a decrease in plasma membrane surface area.

BFA caused a similar capacitance decrease in non-expressing control oocytes while repeated measurement procedure or treatment with the carrier for BFA (0.1% ethanol) had no effect on capacitance (*data not shown*).

Discussion

The heteromeric KCNQ1+KCNE1 channels "become" homomeric KCNQ1 channels

We found that the properties of the heteromeric KCNQ1+KCNE1 channels (voltage dependence and activation kinetics) were affected by oocyte expression time and changed from being most similar to those of the concatamer channel where the alpha- and beta-subunits are linked together, to being indistinguishable from those of the homomeric KCNQ1 channels. The course of the maximal current

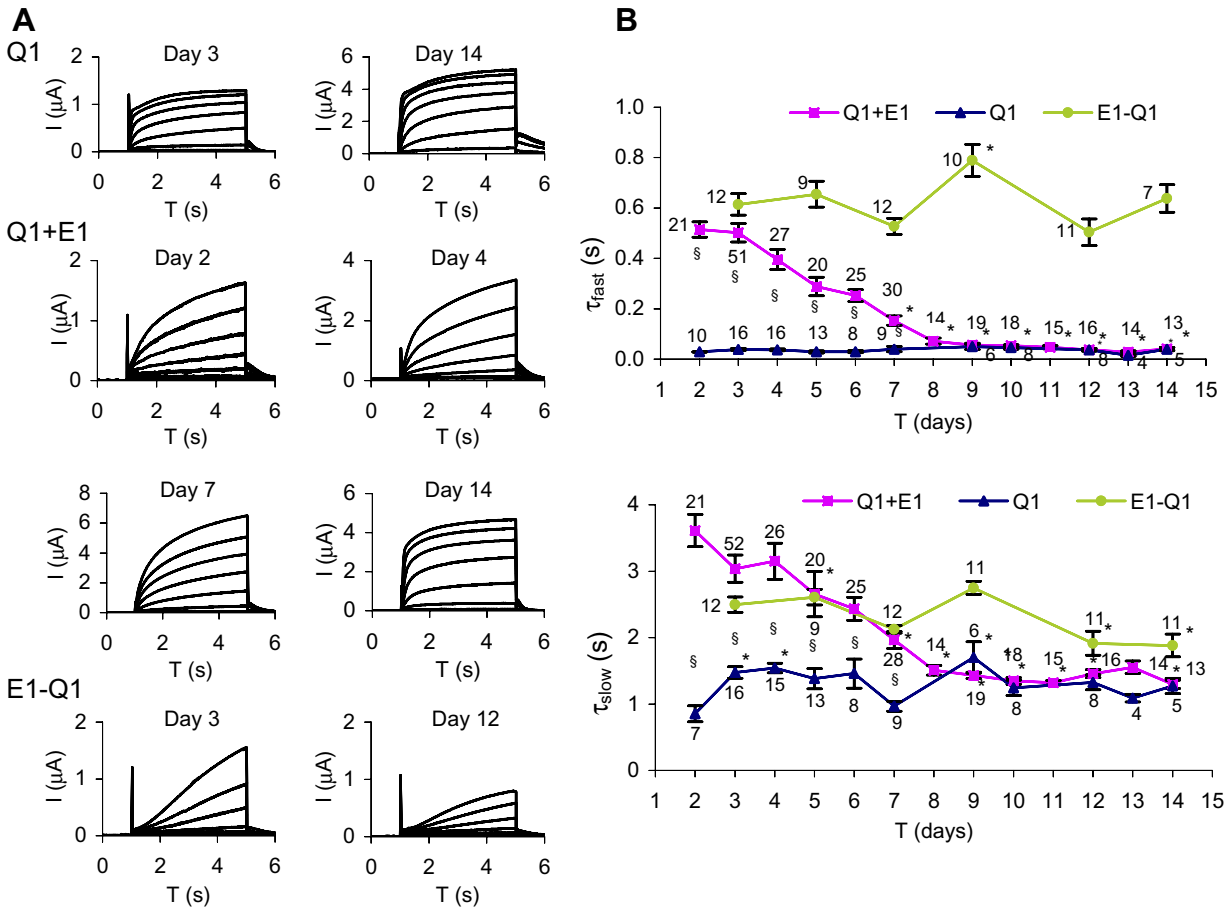

Fig. 3. Effect of expression time on activation kinetics. (A) Examples of current traces from oocytes expressing KCNQ1 (Q1), KCNQ1+KCNE1 (Q1+E1), or KCNE1-KCNQ1 concatamer (E1-Q1). (B) Summary of the effect of expression time on activation time constants τ_{fast} and τ_{slow}. KCNQ1 (Q1), KCNQ1+KCNE1 (Q1+E1) or KCNE1-KCNQ1 concatamer (E1-Q1). Means ± SEM. Numbers in diagram indicate n. $^{*}p < 0.05$ vs. first point in series. $^{\S}p < 0.05$ Q1 vs. Q1+E1.

(I_{+60}) for KCNQ1+KCNE1 expression, which shows a peak after 6–10 days followed by a decline, also suggests that the KCNE1 subunit loses it's influence on the alpha-subunit of the KCNQ1 channels as KCNE1 is known to increase the whole cell KCNQ1 current. This seems to suggest that functional heteromeric KCNQ1+KCNE1 channels gradually changes into or is being replaced by homomeric KCNQ1 channels by time. The change in properties does not seem to be caused by changes in the conditions of the expressing oocytes (e.g. endogenous expression levels) as the current characteristics except maximal current, remained constant over time for homomeric KCNQ1 and the concatamer KCNE1-KCNQ1 channels.

The fact that the current-level of KCNQ1+KCNE1 at the end of the period is similar to the current-level of KCNQ1 (Fig. 1A) indicates that the transition is not caused by selective removal of heteromeric KCNQ1 channels containing KCNE1, as this would result in a smaller residual current as co-injected oocytes must be expected have much fewer unpaired homomeric channels.

Excluding selective removal of heteromers as a cause leaves two explanations for the apparent disappearance of functional heteromeric channels: (1) if protein synthesis and membrane delivery continues throughout the measurement period, and if the transcript for KCNQ1 has a longer life-time than that of KCNE1, then homomeric channels could replace heteromeric channels by time or (2) the KCNE1 beta-subunits could be destined to disassociate from the alpha subunit in a reversible manner leaving still functional homomeric alpha-subunits in the plasma membrane.

The experiments employing BFA, which is known to inhibit exocytosis and thus eliminate the delivery of newly synthesized proteins to the plasma membrane [18], showed that channel expression apparently is independent of membrane delivery, as the current levels were unaffected by BFA despite a significant effect on membrane capacitance. Thus KCNQ1+KCNE1 channels appear to have a particular low turn-over rate (recycling) in oocytes, in contrast to at least some other ion channels, such as the epithelial sodium channels (ENaC) [19] or the $K_v1.5$ channels [20]. This makes it unlikely that heteromeric channels are being replaced by newly synthesized homomeric channels and favors the second explanation suggested above, namely a

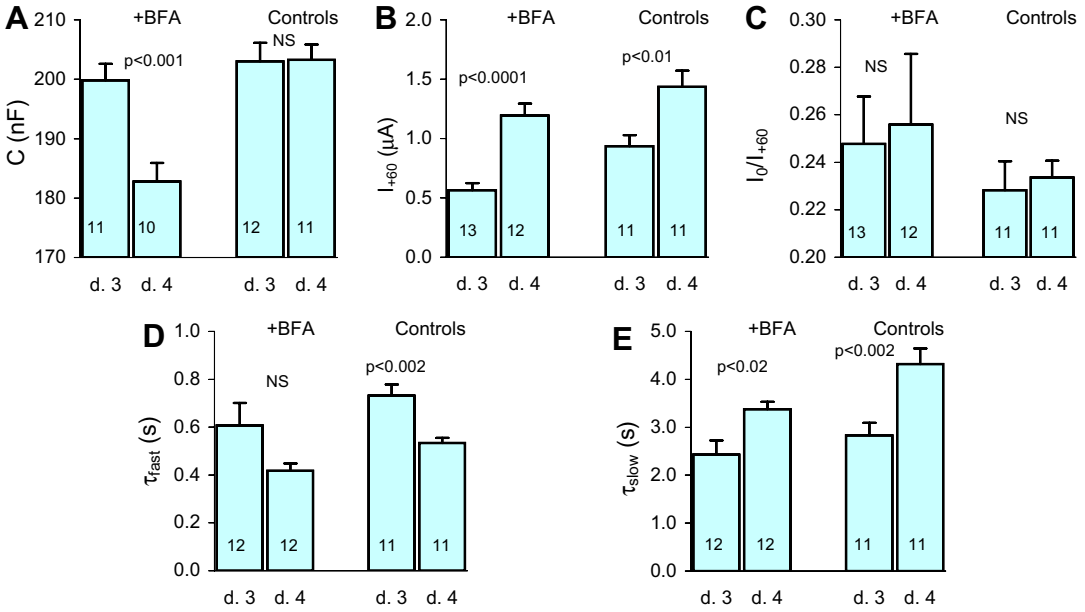

Fig. 4. Effect of brefeldin A (10 μM) on current characteristics, in oocytes expressing KCNQ1+KCNE1 at day 3 and 4 after RNA injection. Parameters obtained from oocytes before (d. 3) and after (d. 4) drug treatment are shown to the left (+BFA). For comparison, results from untreated oocytes are shown to the right (Controls). Means ± SEM. Numbers in columns indicate n. (A) Membrane capacitance. (B) Maximal current. (C) Voltage dependence parameter: Normalized current at 0 mV. (D & E) Activation time constants.

reversible disassociation of the beta-subunit from the alpha-subunit. There are examples of other membrane proteins that are selectively removed by specific ubiquitin–protein ligases [21–23]. The observed decrease in current over time (I_{+60}, Fig. 2) of the KCNE1-KCNQ1 concatamer, could rely on concomitant removal of KCNQ1 being linked to KCNE1.

Physiological role of KCNE beta-subunits

KCNE beta-subunits are differentially expressed in various tissues with several cases of overlapping expression [11,14]. Obviously, the expression profile reflects cell phenotypes, but it may also change under hormonal or pathological influence [11,24–26]. The KCNE's also seem to show a dominant-recessive hierarchal behavior [10,11]. Further, delayed introduction of KCNE into oocytes or mammalian cells already expressing KCNQ1 channels have been shown to result in current characteristic modulation [1,11]. Together these findings suggest that KNCE beta-subunit expression is regulated not only during development, but also within the life-time of cells to meet physiological requirements.

From our findings employing *Xenopus laevis* oocytes as a model system it appears that the beta-subunit (KCNE1) exerts a transient and reversible modulation of the alpha-subunit (KCNQ1). Combined with the findings that beta-subunits are able to interact with already expressed alpha-subunits [1,11], a regulatory paradigm is favored in which e.g. hormones can increase/decrease the expression of beta-subunits and thereby induce a transient and reversible modulation of ion-channels. Thus the interaction between at least some ion channel alpha and beta-subunits seems to resemble a Kiss & Go relationship rather than a life-long partnership (Fig. 1B, "Classical" vs. "Kiss & Go"). Given that fact that the expression of a number of beta-subunits of ion channels is hormone dependent, the regulatory role of beta-subunits may be much more dynamic that previously thought. Studies of the dynamics of protein expression in oocytes including localization and surface expression are obvious future tasks in order to reveal the molecular mechanisms responsible for the observed transient behavior of KCNE1.

Acknowledgments

This work was supported by The Danish Medical Research Council, The Danish Natural Sciences Research Council, Novo Nordisk Fonden, Augustinus Fonden and the Scandinavian Physiological Society.

References

[1] M. Grunnet, T. Jespersen, H.B. Rasmussen, T. Ljungstrom, N.K. Jorgensen, S.P. Olesen, D.A. Klaerke, KCNE4 is an inhibitory subunit to the KCNQ1 channel, J. Physiol. 542 (2002) 119–130.

[2] Z.A. McCrossan, G.W. Abbott, The MinK-related peptides, Neuropharmacology 47 (2004) 787–821.

[3] M.C. Sanguinetti, M.E. Curran, A. Zou, J. Shen, P.S. Spector, D.L. Atkinson, M.T. Keating, Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel, Nature 384 (1996) 80–83.

[4] J. Barhanin, F. Lesage, E. Guillemare, M. Fink, M. Lazdunski, G. Romey, K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current, Nature 384 (1996) 78–80.

[5] D.E. Vetter, J.R. Mann, P. Wangemann, J. Liu, K.J. McLaughlin, F. Lesage, D.C. Marcus, M. Lazdunski, S.F. Heinemann, J. Barhanin, Inner ear defects induced by null mutation of the isk gene, Neuron 17 (1996) 1251–1264.
[6] G. Romey, B. Attali, C. Chouabe, I. Abitbol, E. Guillemare, J. Barhanin, M. Lazdunski, Molecular mechanism and functional significance of the MinK control of the KvLQT1 channel activity, J. Biol. Chem. 272 (1997) 16713–16716.
[7] Y.F. Melman, A. Domenech, L.S. de la, T.V. McDonald, Structural determinants of KvLQT1 control by the KCNE family of proteins, J. Biol. Chem. 276 (2001) 6439–6444.
[8] G. Panaghie, K.K. Tai, G.W. Abbott, Interaction of KCNE subunits with the KCNQ1 K^+ channel pore, J. Physiol 570 (2006) 455–467.
[9] W. Wang, J. Xia, R.S. Kass, MinK-KvLQT1 fusion proteins, evidence for multiple stoichiometries of the assembled IsK channel, J. Biol. Chem. 273 (1998) 34069–34074.
[10] F. Toyoda, H. Ueyama, W.G. Ding, H. Matsuura, Modulation of functional properties of KCNQ1 channel by association of KCNE1 and KCNE2, Biochem. Biophys. Res. Commun. 344 (2006) 814–820.
[11] A.L. Lundquist, L.J. Manderfield, C.G. Vanoye, C.S. Rogers, B.S. Donahue, P.A. Chang, D.C. Drinkwater, K.T. Murray, A.L. George Jr, Expression of multiple KCNE genes in human heart may enable variable modulation of I(Ks), J. Mol. Cell Cardiol. 38 (2005) 277–287.
[12] A. Krumerman, X. Gao, J.S. Bian, Y.F. Melman, A. Kagan, T.V. McDonald, An LQT mutant minK alters KvLQT1 trafficking, Am. J. Physiol. Cell Physiol. 286 (2004) C1453–C1463.
[13] K.D. Chandrasekhar, T. Bas, W.R. Kobertz, KCNE1 subunits require co-assembly with K^+ channels for efficient trafficking and cell surface expression, J. Biol. Chem. 281 (2006) 40015–40023.
[14] A.L. Lundquist, C.L. Turner, L.Y. Ballester, A.L. George Jr, Expression and transcriptional control of human KCNE genes, Genomics 87 (2006) 119–128.
[15] M. Grunnet, B.S. Jensen, S.P. Olesen, D.A. Klaerke, Apamin interacts with all subtypes of cloned small-conductance Ca^{2+}-activated K^+ channels, Pflugers Arch. 441 (2001) 544–550.
[16] T. Jespersen, M. Grunnet, K. Angelo, D.A. Klaerke, S.P. Olesen, Dual-function vector for protein expression in both mammalian cells and *Xenopus laevis* oocytes, Biotechniques 32 (2002) 536–538, 540.
[17] A.M. Brown, A step-by-step guide to non-linear regression analysis of experimental data using a Microsoft Excel spreadsheet; Comput, Methods Programs Biomed. 65 (2001) 191–200.
[18] R.D. Klausner, J.G. Donaldson, J. Lippincott-Schwartz, Brefeldin A: insights into the control of membrane traffic and organelle structure, J. Cell Biol. 116 (1992) 1071–1080.
[19] M.D. Carattino, W.G. Hill, T.R. Kleyman, Arachidonic acid regulates surface expression of epithelial sodium channels, J. Biol. Chem. 278 (2003) 36202–36213.
[20] D.P. McEwen, S.M. Schumacher, Q. Li, M.D. Benson, J.A. Iniguez-Lluhi, K.M. Van Genderen, J.R. Martens, RAB-GTPase dependent endocytic recycling of Kv1.5 in atrial myocytes, J. Biol. Chem. (2007).
[21] S.Y. Flores, C. Debonneville, O. Staub, The role of Nedd4/Nedd4-like dependant ubiquitylation in epithelial transport processes, Pflugers Arch. 446 (2003) 334–338.
[22] A. Traweger, D. Fang, Y.C. Liu, W. Stelzhammer, I.A. Krizbai, F. Fresser, H.C. Bauer, H. Bauer, The tight junction-specific protein occludin is a functional target of the E3 ubiquitin-protein ligase itch, J. Biol. Chem. 277 (2002) 10201–10208.
[23] M. Schwake, T. Friedrich, T.J. Jentsch, An internalization signal in ClC-5, an endosomal Cl-channel mutated in dent's disease, J. Biol. Chem. 276 (2001) 12049–12054.
[24] A. Felipe, T.J. Knittle, K.L. Doyle, D.J. Snyders, M.M. Tamkun, Differential expression of Isk mRNAs in mouse tissue during development and pregnancy, Am. J. Physiol. 267 (1994) C700–C705.
[25] M.D. Drici, T.R. Burklow, V. Haridasse, R.I. Glazer, R.L. Woosley, Sex hormones prolong the QT interval and downregulate potassium channel expression in the rabbit heart, Circulation 94 (1996) 1471–1474.
[26] E. Watanabe, K. Yasui, K. Kamiya, T. Yamaguchi, I. Sakuma, H. Honjo, Y. Ozaki, S. Morimoto, H. Hishida, I. Kodama, Upregulation of KCNE1 induces QT interval prolongation in patients with chronic heart failure, Circ. J. 71 (2007) 471–478.