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Functional modulation of the transient outward current I_{to} by KCNE β-subunits and regional distribution in human non-failing and failing hearts

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Abstract

Objectives: The function of Kv4.3 (KCND3) channels, which underlie the transient outward current I_{to} in human heart, can be modulated by several accessory subunits such as KChIP2 and KCNE1–KCNE5. Here we aimed to determine the regional expression of Kv4.3, KChIP2, and KCNE mRNAs in non-failing and failing human hearts and to investigate the functional consequences of subunit coexpression in heterologous expression systems.

Methods: We quantified mRNA levels for two Kv4.3 isoforms, Kv4.3-S and Kv4.3-L, and for KChIP2 as well as KCNE1–KCNE5 with real-time RT-PCR. We also studied the effects of KCNEs on Kv4.3+KChIP2 current characteristics in CHO cells with the whole-cell voltage-clamp method.

Results: In non-failing hearts, low expression was found for KCNE1, KCNE3, and KCNE5, three times higher expression for KCNE2, and 60 times higher for KCNE4. Transmural gradients were detected only for KChIP2 in left and right ventricles. Compared to non-failing tissue, failing hearts showed higher expression of Kv4.3-L and KCNE1 and lower of Kv4.3-S, KChIP2, KCNE4, and KCNE5. In CHO cells, Kv4.3+KChIP2 currents were differentially modified by co-expressed KCNEs: time constants of inactivation were shorter with KCNE1 and KCNE3-5 while time-to-peak was decreased, and $V_{0.5}$ of steady-state inactivation was shifted to more negative potentials by all KCNE subunits. Importantly, KCNE2 induced a unique and prominent 'overshoot' of peak current during recovery from inactivation similar to that described for human I_{to} while other KCNE subunits induced little (KCNE4,5) or no overshoot.

Conclusions: All KCNEs are expressed in the human heart at the transcript level. Compared to I_{to} in native human myocytes, none of the combination of KChIP2 and KCNE produced an ideal congruency in current characteristics, suggesting that additional factors contribute to the regulation of the native I_{to} channel.

Keywords: Gene expression; Heart failure; Ventricular function; K-channel; Membrane currents

1. Introduction

The transient outward current (I_{to}) determines the early repolarization phase of cardiac action potential and thus contributes to the determination of the repolarization phase as well as excitation–contraction coupling via modulating Ca^{2+} and other K^+ currents [1,24]. In human ventricle Kv4.3

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(KCND3) is the main pore-forming α-subunit of I_{to} [11], and short and long splice variants (Kv4.3-S and Kv4.3-L) were detected [10,14]. The electrophysiological properties of I_{to} are modulated by several β-subunits [9] among them KChIP2 (K^+-channel interacting protein) is the most thoroughly investigated. KChIP2 increases peak I_{to} current density by promoting trafficking of Kv4.3 to the cell membrane [2], slows inactivation, and accelerates recovery from inactivation [8,23].

KCNE1 (minK) is the major accessory subunit of the KvLQT1 (KCNQ1) channel forming the slow delayed rectifier current I_{Ks} [5]. KCNE2 encoding for MinK-related peptide 1 (MiRP1) is also expressed in human myocardium [12] and associates with the cardiac Kv4.3 protein [1]. Kv4.3 has been shown to be modulated by KCNE1 and KCNE2 in heterologous systems [9]. However, the kinetic properties of native I_{to} in human ventricular myocytes can not be properly explained by Kv4.3 interaction with KChIP2, KCNE2 or KCNE1 [9]. Other accessory β-subunits with accelerating effects on the I_{to} current kinetics may contribute to the characteristics of I_{to}. One potential candidate is the dipeptidyl-aminopeptidase-like protein (DPP6), which has been recently identified in neuronal and heart tissue and can substantially accelerate the inactivation of transient K^+ currents [19,21]. Furthermore, KCNE3, KCNE4 and KCNE5 exhibit pronounced and distinct effects on important potassium channel β-subunits such as KCNQ1 and HERG [3,17]. It is conceivable that these promiscuous KCNE proteins also interact with Kv4.3 and influence expression and kinetics of I_{to}.

Native I_{to} exhibits a characteristic transmural gradient in ventricles, with larger I_{to} density in epicardium than in endocardium [18,27]. In human and dog hearts, this I_{to} gradient is believed to be determined by differential KChIP2 expression [22], although there is no consensus on this point [8]. In failing myocardium I_{to} is reduced in epicardial as well as endocardial tissue layers [13,18,27], and this is correlated with a reduction of Kv4.3 mRNA [13]. Little is known about the influence of heart failure on the expression of KChIP2, or other putative I_{to} β-subunits.

The aim of the present investigation was to quantify the mRNA levels of I_{to} α-subunit isoforms, Kv4.3-L and Kv4.3-S, and putative accessory β-subunits KCNE1–KCNE5 and KChIP2 in human non-failing and failing ventricles using the real-time RT-PCR technique. Our main finding is that the expression of α- and β-subunits was differentially regulated in failing hearts. In functional expression experiments in CHO cells we found distinct patterns of modulation of Kv4.3 +KChIP2 gating kinetics by KCNE subunits, which suggest that in addition to Kv4.3+KChIP2 association with KCNE2 and other still not identified subunits or regulators are required to mimic the native I_{to} function in human heart.

2. Methods

2.1. Tissue

Tissue samples were collected from explanted hearts of NYHA IV patients (four male, one female) with DCM with written consents. Medication included digitoxin, metoprolol and torasemide. Healthy tissue was derived from five donor hearts (two male, three female). Biopsies were excised from the central region of the anterior wall of left (LV) and right (RV) ventricles, were separated into subepi-(epicardial) and subendocardial (endocardial) layers and immediately stored in liquid nitrogen. The study conformed with the Declaration of Helsinki.

2.2. Molecular biology

Total RNA was isolated using the LiCl-method [25]. Quantitative real-time RT-PCR was performed as described

Table 1
Primers and conditions for PCR

Gene	Acc.-No.	Primer Sequence (5′-3′)	Position [bp]	Size [bp]	T_A [°C]	$MgCl_2$ [mM]
Kv4.3-L	AF205857	S:TCC ACC ATC AAG AAC CAC G	1507–1525	133	58	2.5
		A:AGC AGG TGG TAG TGA GGC C	1621–1639			
Kv4.3-S	AF205856	S:GGA AAA AAC CAC TAA CCA CGA GT	1372–1390	211	63	6.0
		A:AGC AGG TGG TAG TGA GGC C A GT	1564–1582			
KChIP2	AF199598	S:ATG CTT GAC ATC ATG AAG TCC GT	547–572	162	58	2.5
		A:TTG ACA AGA CTC AAT GAA TTC GT	687–708			
KCNE1	BC036452	S:CAC ACA ATC ATC AGG TGA GCC GAG	83–106	209	60	2.5
		A.ATG TTG CCA CCC TGC TGA ACT GTC	268–291			
KCNE2	AF302095	S:CAC ACA CTG CAT AGC AGG AGT GTC	115–134	229	58	3.0
		A:AGG ATG GCC ACG ATG ATG AAT GTC	324–343			
KCNE3	NM005472	S:GTC TGA GCT TCT ACC GAG TCT TCC	147–170	363	54	4.0
		A:CTC GTG TTA GAT CAT AGA CAC ACG G	485–509			
KCNE4	NM080671	S:AAG AGG CGG GAG AAG AAG TCC ACG G	270–289	337	54	4.0
		A:CCC TGA TGC TGA ACA TGC TCC ACG G	385–403			
KCNE5	NM012282	S:CCC CTA CCC CGC ACA TCC TCC ACG G	790–806	107	58	3.0
		A:TTG GAC GTG TTG GAT TCA GTT CCG G	880–896			
T7	A32834	S:TAA TAC GAC TCA CTA TAG GGC GGC CGC GG	12–40		58	2.5

The table specifies sense (S) and antisense (A) primers and reaction conditions used for RT-PCR of α- and β-subunits and cRNA standard generation. T_A: annealing temperature.

in detail recently [21]. Primer pairs specific for human Kv4.3-L and Kv4.3-S, KChIP2 and KCNE1–KCNE4 were intron-spanning. KCNE5 primers were modified from published sequences [16] (Table 1).

2.3. CHO expression system

Chinese hamster ovary (CHO) cell lines stably transfected with hKv4.3-L or hKv4.3/hKChIP2 were donated from Sanofi–Aventis (Frankfurt, Germany) and cultured as described in Ref. [21]. KCNE1-3 and KCNE5 cDNAs were obtained from human ventricle mRNA and cloned in pIRES2-EGFP (BD Biosciences, Heidelberg, Germany). pXOOM-KCNE4 was donated from Dr. M. Grunnet (Copenhagen, Denmark). Both pIRES and pXOOM plasmids drive cDNA expression under the control of CMV promoters and use EGFP as a transfection marker. KCNE1-5 plasmids were transfected using 2.5 µl of Roti-Fect® transfection reagent (Carl Roth, Karlsruhe, Germany) and a total amount of 0.5 µg plasmid each.

2.4. Electrophysiological experiments

Whole-cell patch-clamp experiments were performed using a HEKA-EPC8 amplifier (HEKA Elektronik, Lambrecht, Germany). Bath solution contained (in mM): NaCl 150, KCl 5.4, $MgCl_2$ 2, $CaCl_2$ 1.8, HEPES 10, glucose 11, pH 7.4. Pipette solution contained (in mM): KCl 40, potassium aspartate 80, NaCl 8, $CaCl_2$ 2, MgATP 5, EGTA 5, GTP 0.1, HEPES 10, pH 7.4 adjusted with KOH. Pipette tip resistance was between 2.5 and 3.5 MΩ when filled with pipette solution.

The holding potential was −80 mV. Currents were measured with clamp steps between −60 and +60 mV. Series resistance was compensated up to 85%. Clamp pulse generation, data collection and analysis were performed with ISO2 software (MFK, Niedernhausen, Germany). Data were not corrected for junction potentials which was calculated with 11 mV for the electrode solution with JPCalc software (P.H. Barry, Sydney, Australia). All experiments were performed at room temperature (22 °C).

2.5. Statistics

Results are given as means±S.E.M. Statistical analysis was performed with Student's *t*-test or one-way ANOVA with Bonferroni`s post hoc test (GraphPad Prism software, V4.1; San Diego, CA). Differences were considered to be significant if $P<0.05$.

3. Results

3.1. Differential remodeling of Kv4.3 isoforms and KChIP2 expression in human failing heart

The two isoforms, Kv4.3-S and Kv4.3-L, were homogeneously expressed with similar mRNA levels in all regions of non-failing hearts. In failing heart, their expression was differentially regulated. While Kv4.3-L was upregulated, Kv4.3-S was markedly downregulated, so that the long isoform clearly dominated (Fig. 1A, Table 2). KChIP2 expression showed a steep transmural gradient in non-failing hearts in both left and right ventricles. In failing hearts KChIP2 expression was markedly reduced in epicardial

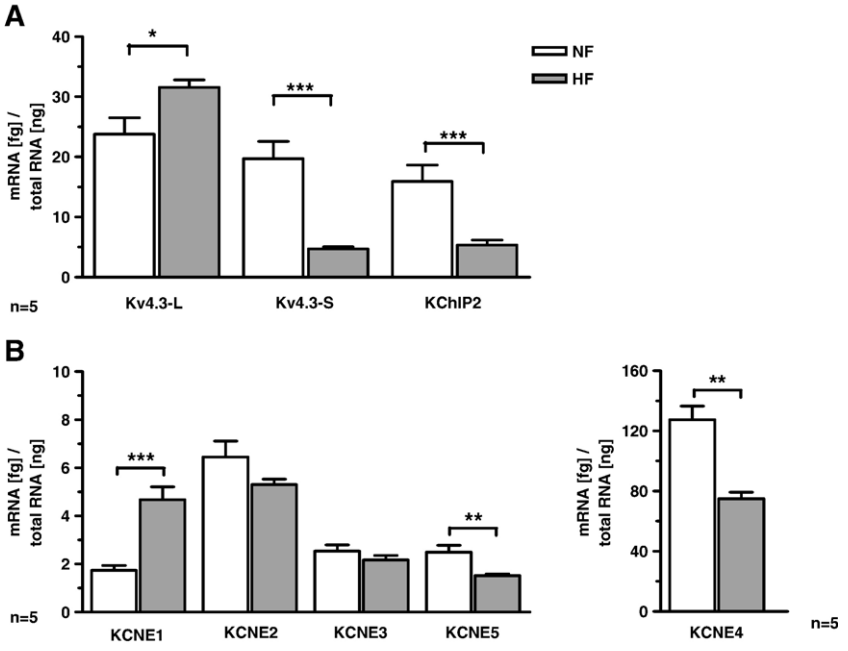

Fig. 1. mRNA expression of Kv4.3, KChIP2 and KCNE in human non-failing (NF) and failing hearts (HF). (A) Kv4.3-L, Kv4.3-S and KChIP2 mRNA expression. (B) KCNE1–KCNE5 mRNA. Mean values±S.E.M. of mRNA ([fg] of total isolated RNA [ng]) (*$P<0.05$, **$P<0.01$, ***$P<0.001$), $n=5$.

Table 2
Summary of quantitative mRNA expression data in all samples from 5 non-failing and 5 failing hearts

	Non-Failing Hearts (n=5)					Failing Hearts (n=5)				
	Right ventricle		Left ventricle		NF	Right ventricle		Left ventricle		HF
	EPI	ENDO	EPI	ENDO	Pooled	EPI	ENDO	EPI	ENDO	Pooled
Kv4.3-S	17.2±7.6	18.7±4.1	21.3±6.8	21.7±5.5	19.7±2.9	4.4±0.9	4.5±0.5	4.2±0.4	5.7±0.9	4.7±0.4
Kv4.3-L	22.0±5.7	26.1±5.7	21.9±5.1	25.2±6.5	23.8±2.7	31.4±2.2	33.3±4.5	31.4±0.5	30.1±1.8	31.6±1.3
KChIP2	21.9±5.4	10.6±4.2	25.2±4.2	6.0±4.2	15.9±2.8	6.9±1.5	4.3±0.6	8.1±2.2	2.2±0.4	5.4±0.8
KCNE1	1.7±0,6	1.5±0.3	1.4± 0.3	2.4±0.5	1.7±0.2	4.4±1.2	4.9±1.4	4.6±0.9	4.8±1.3	4.7±0.5
KCNE2	8.0±1.2	7.2±1.5	4.0±0.6	6.6±1.4	6.5±0.7	5.3±0.1	5.3±4.5	5.4±0.8	5.3±0.4	5.3±0.3
KCNE3	2.2±0.6	3.0±0.5	2.5±0.7	2.4±0.3	2.5±0.3	2.0±0.3	2.5±0.4	1.8±0.3	2.3±0.4	2.2±0.2
KCNE4	114.4±9.6	159.0±19.2	127.9±24.0	109.1±13.7	127.6±8.9	73.2±11.6	69.6±2.0	78.6±6.7	78.9±11.3	75.1±4.1
KCNE5	2.4±0.4	3.4±0.9	2.1±0.2	2.1±0.3	2.5±0.3	1.7±0.2	1.5±0.1	1.4±0.2	1.4±0.1	1.5±0.1

Values are given in fg/ng of total RNA. Mean values±S.E.M. include double or triple (KCNE2) determination for each sample.

tissue of both ventricles. The reduction in endocardial layers of both left and right ventricles was less, although differences between pooled data were significant. The transmural gradient of KChIP2 was preserved in failing hearts although to a reduced level (Fig. 1A, 2A, Table 2).

3.2. Expression pattern and remodeling of KCNE1–KCNE5 in heart failure

In specimens from non-failing hearts the mRNA expression of KCNE subunits was substantially smaller by a factor of 5 to 10 compared to the α-subunit Kv4.3 and KChIP2, with the exception of KCNE4, the expression of which was 6 times higher than for Kv4.3 (Fig. 1, Table 2). The order of expression level was KCNE4>>Kv4.3-L ~ Kv4.3-S>KCNE2> KCNE1 ~ KCNE3 ~ KCNE5. All KCNE β-subunits, with the exception of KCNE2, were homogeneously expressed between the two ventricles of non-failing human hearts. For KCNE2, we detected a significantly larger expression in epicardial specimen of the right compared to the left ventricle (Fig. 2B, Table 2). Unlike expression of KChIP2, KCNE2 did not exhibit an expression gradient between epi- and endocardium in right ventricle. In left ventricle KCNE2 expression tended to be smaller in epicardial than endocardial tissue thus exhibiting a transmural gradient opposite in direction to that of KChIP2.

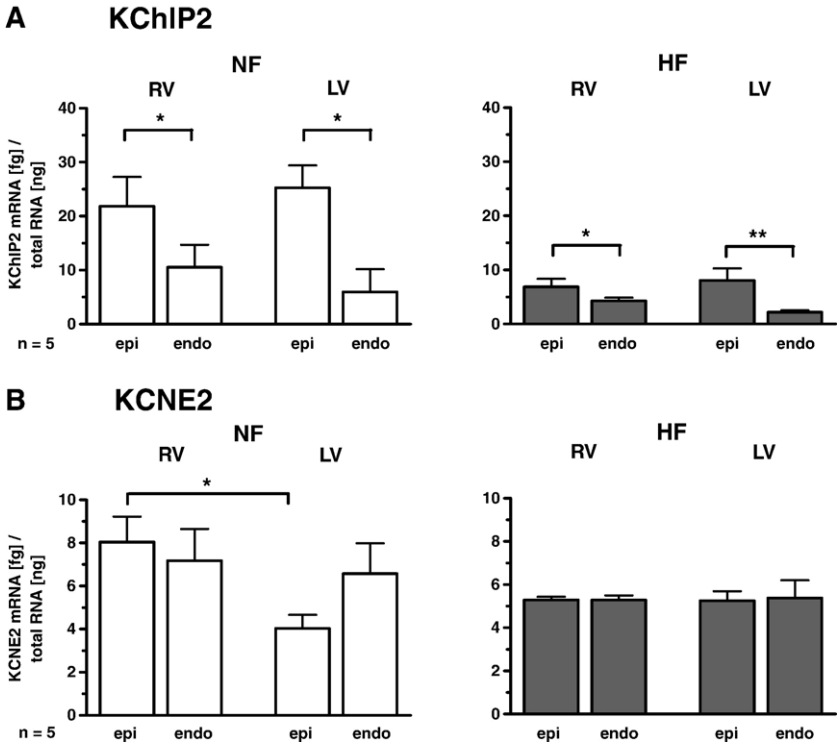

Fig. 2. Regional expression of KChIP2 and KCNE2 mRNA in human heart. KChIP2 and KCNE2 mRNA was determined in subendocardial (endo) and subepicardial (epi) layers of RV and LV in non-failing (NF) and failing (HF) hearts (n=5). (A) KChIP2 expression in epi and endo layers of RV and LV in NF and HF. (B) KCNE2 mRNA in epi and endo of RV and LV in NF and HF. Mean values±S.E.M.

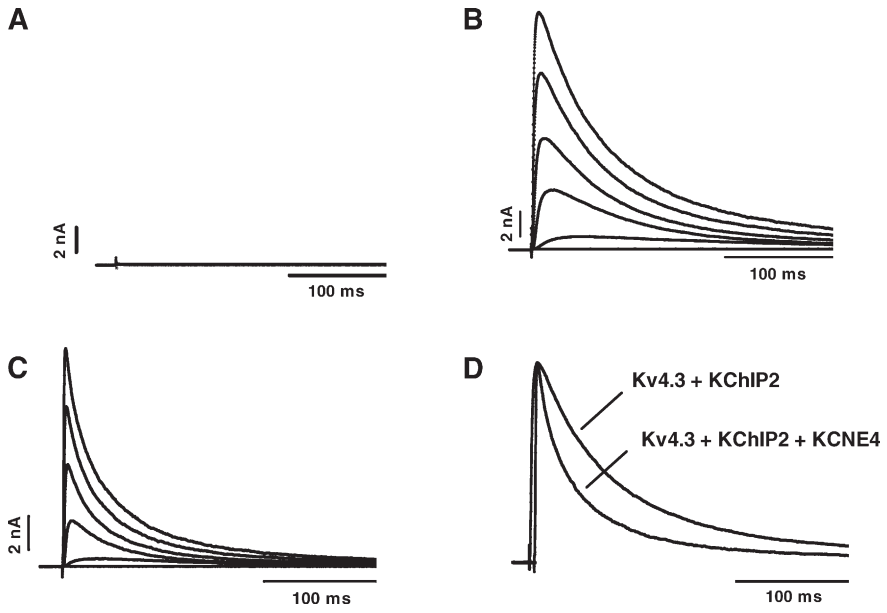

Fig. 3. Effects of KChIP2 and KCNE4 on Kv4.3 currents. Current tracings (initial 300 ms) elicited by test steps (1000 ms) from −80 mV to −40, −20, 0,+20,+40 and +60 mV in CHO cells stably expressing (A) Kv4.3-L+KCNE4, (B) Kv4.3-L+KChIP2 (C) Kv4.3-L+KChIP2+KCNE4, (D) normalized currents at +50 mV to compare rate of inactivation.

In samples from failing hearts the expression levels of KCNEs were differentially altered. While expression of KCNE1 was significantly larger, that of KCNE4 and KCNE5 was lower compared to non-failing hearts. For KCNE2 and KCNE3, the mRNA levels were not different in non-failing and failing hearts (Fig. 1B, Table 2).

3.3. Effects of KCNE1–KCNE5 on Kv4.3+KChIP2 stably expressed in CHO cells

3.3.1. Current density and current-voltage relations

None of the 5 KCNE subunits was able to produce membrane currents when co-expressed with Kv4.3-L alone (Fig. 3A). Only in combination with KChIP2, expression of Kv4.3-L led to prominent transient outward currents (Fig. 3 B). KChIP2 is an obligatory accessory subunit of native I_{to} channels in the heart [15]. Therefore, Kv4.3-L+KChIP2 stably expressed in CHO cells served as control, and KCNE subunits were transiently co-expressed to test their effects on the I_{to} kinetics. Kv4.3-L+KChIP2 had an average peak outward current density of 303±54 pA/pF (+50 mV, n=34). In the presence of KCNE subunits, current densities were not significantly altered (Table 3).

3.3.2. Time course of activation and inactivation

All KCNE subunits accelerated the time course of activation, as indicated by the reduction in time-to-peak current by about 50% (Fig. 4A). The smallest effect was detected with the combined expression of KCNE2 plus KCNE4. The time course of I_{to} inactivation, which could be best described by a two-exponential function, was also affected by KCNEs. KCNE3, KCNE4 and KCNE5 significantly reduced the fast time constant of inactivation. KCNE4 and KCNE5 also markedly reduced the slow time

Table 3
Electrophysiological parameters of I_{to} at room temperature in CHO cells and human ventricular myocytes

I_{to} channel subunits	I_m (pA/pF) +50 mV	n	Activation TtP (ms)	n	Inactivation τ (ms)	n	SS-Activ. $V_{0,5}$ (mV)	n	SS-Inactiv. $V_{0,5}$ (mV)	n	Recovery τ_{fast} (ms)	n	Overshoot amplitude (H.P.-80 mV)	n*
Kv4.3/KChIP2	303±54	34	7.3±0.4	12	56±3	21	6±2	34	−26±3	30	53±7	26	0.09±0.03	20
Kv4.3/KChIP2/KCNE1	310±42	12	4.4±0.4	11	45±5	15	−1±2	12	−39±2	12	65±10	9	0	0
Kv4.3/KChIP2/KCNE2	199±49	16	3.6±0.2	10	60±6	19	7±4	16	−36±1	18	90±12	17	0.27±0.05	11
Kv4.3/KChIP2/KCNE3	234±46	17	4.0±0.3	12	33±3	21	8±3	16	−43±2	19	89±13	13	0.09	2
Kv4.3/KChIP2/KCNE4	311±44	18	2.8±0.2	11	21±2	17	11±2	18	−34±3	14	38±4	11	0.12±0.02	8
Kv4.3/KChIP2/KCNE5	279±65	16	2.9±0.2	11	32±2	17	4±3	14	−41±3	15	61±10	10	0.15±0.03	9
Kv4.3/KChIP2/KCNE2/KCNE4	225±71	12	5.5±0.5	12	45±6	12	9±2	11	−29±2	11	98±19	10	0.23±0.07	8
Human vent. myocytes [27]					54±3	5	2±3	4	−46±1	5	24±3	6	0.31±0.07	6

The highlighted values show the closest congruency with data from human. Overshoot is expressed as amplitude of descending term of function for data fit. n* number of cells producing "overshoot".

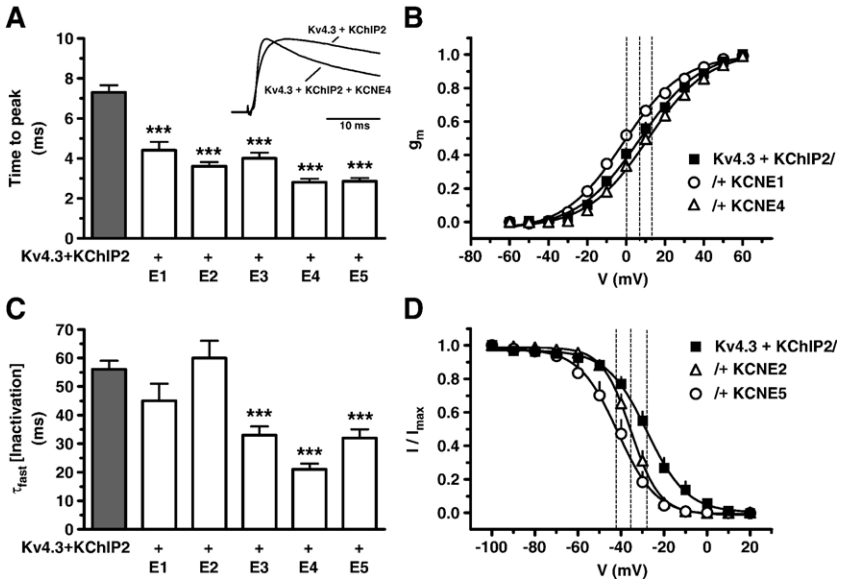

Fig. 4. Effects of KCNE1–KCNE5 (E1–E5) on Kv4.3+KChIP2 current activation and inactivation. (A) Time course of activation is characterized by time to reach peak current (ms). Inset: tracings of current activation for Kv4.3+KChip2 and Kv4.3+KChip2+KCNE4 at +50 mV. (B) Normalized steady-state activation (g_m) curves for I_{to} calculated from I/V-curves assuming an E_{rev} of −60 mV. (C) Fast time constant of inactivation determined from fitting biexponential function to individual current traces at +50 mV; (D) Normalized steady-state inactivation (I/I_{max}) curves for I_{to}. In panels (B) and (D), mean data were fitted by Boltzmann functions to estimate half maximum voltage ($V_{0.5}$, dotted lines). Compare Table 3.

constant of inactivation (data not shown). Therefore, co-expression of KCNE4 and KCNE5 resulted in a pronounced acceleration of the inactivation time-course (shown for KCNE4 in Fig. 3 C,D; mean data in Fig. 4C, Table 3).

3.3.3. Voltage-dependence of activation and inactivation

The activation curves were calculated from current-voltage relations assuming a constant reversal potential of −60 mV [18]. The voltage-dependence of I_{to} activation was not markedly altered by co-expression of KCNEs (Fig. 4B, Table 3). KCNE β-subunits had, however, a more pronounced impact on the voltage-dependence of I_{to} current inactivation. All KCNE β-subunits shifted $V_{0.5}$ of steady-state inactivation to more negative membrane potentials (Fig. 4D, Table 3).

3.3.4. Recovery from inactivation

The time course of recovery from inactivation was determined with a double-pulse protocol starting at −80 mV to

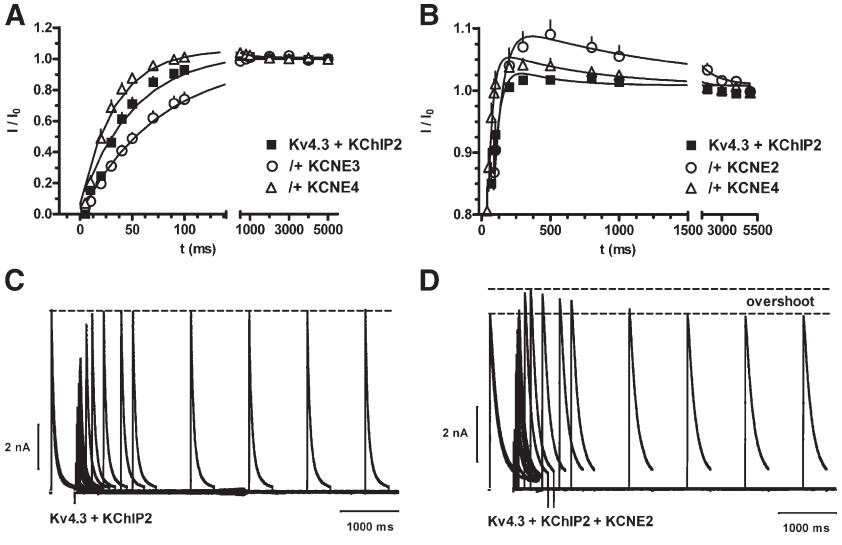

Fig. 5. Effects of KCNEs on the time course of Kv4.3+KChIP2 current recovery from inactivation. (A) Time course of recovery from inactivation of I_{to}, test pulse +50 mV, recovery potential −80 mV. Curve fitting to a biexponential function [$Y=A*(1-\exp(-t/\tau_{fast}))+B*\exp(-t/\tau_{slow})+C$] yielded τ_{fast}-values of 53±7 ms for Kv4.3+KChIP2 ($n=26$), 89±13 ms for Kv4.3-L+KChIP2+KCNE3 ($n=13$), 38±4 ms for Kv4.3-L+KChIP2+KCNE4 ($n=11$). Expanded time scale to focus on fast time course of recovery. (B) Same data as in (A) compressed time scale focussing on "overshoot" phenomenon. Complete data in Table 3. (C) Original current traces of Kv4.3-L+KChIP2 and (D) Kv4.3-L+KChIP2+KCNE2 during recovery from inactivation.

+50 mV including a recovery interval at −80 mV of 5 to 5000 ms. KCNE co-expression had differential impacts on the kinetics of recovery from inactivation. KCNE1, KCNE2, KCNE3 and KCNE2 plus KCNE4 slowed the time course of recovery. KCNE4 alone, however, accelerated recovery from inactivation (Fig. 5, Table 3). In addition KCNE2, KCNE4 and KCNE5 produced a so-called "overshoot" in I_{to} current amplitude, a phenomenon described for native I_{to} in human ventricular myocytes [27]. The "overshoot" implies that with recovery intervals up to 1000 ms peak amplitude of I_{to} was transiently larger than during the reference pulse, with a maximum at the recovery interval of 200 ms. The amplitude of the "overshoot" was largest with KCNE2 and amounted to about 10% for the reference amplitude. Control cells (Kv4.3 +KChIP2) and cells co-expressing KCNE1 and KCNE3 did not manifest an "overshoot" during recovery from inactivation (Fig. 5, Table 3).

3.3.5. Co-expression of the two KCNE subunits KCNE2 plus KCNE4

Since co-expression of individual KCNE subunits produced different current characteristics of I_{to}, we tested whether a combined expression of KCNE2 (inducing the largest I_{to} overshoot) and KCNE4 (having the largest expression among KCNE subunits) led to a better recapitulation of native I_{to} kinetics. With KCNE2 plus KCNE4 the overshoot was preserved, $V_{0.5}$ of current inactivation, however, was rather positive and time constant of recovery was slowed. Thus, the effects of KCNE2 dominated those of KCNE4.

4. Discussion

4.1. Kv4.3 and KChIP2 as two major components of I_{to} channels in human heart

In human heart, Kv4.3 is the major ion conducting α-subunit underlying the transient outward current. Previous studies reported a long (Kv4.3-L) and a short isoform (Kv4.3-S) [15], whose electrophysiological characteristics are identical [10]. Kv4.3-L possesses 2 additional PKC phosphorylation sites and represents the isoform which is susceptible to PKC-dependent reduction in current amplitude via α-adrenoceptor stimulation [20].

In the present study we separately examined the expression levels of Kv4.3-L and Kv4.3-S in failing and non-failing hearts, but since we did not have access to a plasmid for Kv4.3-S, functional aspects of the channel in the expression system could only be investigated with Kv4.3-L. This approach can be justified if the identity of Kv4.3-L and Kv4.3-S with respect to the proposed binding site for KChIP2 at the N-terminal domain is taken into account. The 2 isoforms also exhibit homology in their transmembrane and extracellular domains where KCNE2 and other KCNE subunits are supposed to bind (Tseng, unpublished observations). Therefore, the interactions with KChIP2 and KCNE subunits are likely to be similar for both Kv4.3 isoforms.

In non-failing hearts, Kv4.3-L and Kv4.3-S are expressed in similar mRNA quantities with no transmural gradient. In failing hearts, Kv4.3-L is up-regulated and Kv4.3-S is down-regulated, leading to a predominant expression of Kv4.3-L. Nevertheless, the sum of Kv4.3-L and Kv4.3-S is reduced in failing compared to non-failing hearts, which confirms the well known heart failure-associated down-regulation of global Kv4.3 and I_{to} amplitude [13]. Furthermore, high plasma concentrations of noradrenaline in chronic heart failure can stimulate cardiac α-adrenoceptors and activate PKC. The predominant expression of Kv4.3-L which is sensitive to PKC phosphorylation (see above) may contribute to reduced I_{to} function.

KChIP2 is required for proper trafficking of Kv4.3 channels to the cell membrane [2,4,15]. In various expression systems, i.e. Xenopus oocytes and HEK-293 cells, co-expression of Kv4.3 with KChIP2 enhances I_{to} current density, slows current inactivation and accelerates recovery from inactivation [8,28]. We could not study an effect of KChIP2 on I_{to} kinetics because unlike the other expression systems, CHO cells did not exhibit I_{to} when stably transfected with Kv4.3 only. In any case, co-expression of Kv4.3 +KChIP2 in CHO cells yields current with a time course of inactivation similar to native cardiac I_{to}, but markedly slower recovery from inactivation and steady-state inactivation in a more positive potential range. These findings suggest that KChIP2 may not be the only accessory subunit of native I_{to} in cardiomyocytes [12], although we cannot exclude that other regulatory processes may also be involved.

We did not distinguish between various KChIP2 isoforms [7], because there is no consensus as to which ones are present in the heart. Nevertheless, we confirm the differential epi-/endocardial expression of KChIP2, underlying the steep transmural gradient in I_{to} [22,23]. In heart failure, KChIP2 is substantially down-regulated which can further contribute to the reduced amplitude of I_{to} [13].

4.2. Role of KCNE subunits in native I_{to}?

In heterologous expression systems, several putative accessory proteins such as KCNEs, DPP6, KChAPs, Kvβ, and even the sodium channel β-subunit NaChβ1 [9] have been found to modulate the properties of Kv4.3 channels. Here we have focused on the KCNE-protein family and all KCNE subunits were detected at the mRNA level in human heart. At the protein level, only KCNE2 has been demonstrated in human heart [12].

Depending on the expression system, there have been conflicting reports as to whether and how KCNE1 can modulate Kv4.3 or the related Kv4.2 channels. In oocytes, KCNE1 did not affect Kv4.2 gating kinetics [28]. In HEK293 cells KCNE1 slowed all kinetic parameters of Kv4.3 and increased current amplitude [9]. A similar regulatory role of KCNE1 in human hearts seems unlikely, since KCNE1 up-regulation in heart failure is accompanied by a reduction in I_{to} amplitude instead of the expected

increase. Kinetic parameters of I_{to} in CHO cells co-expressing the standard combination of Kv4.3+KChIP2 with KCNE1 reveal better congruency with native I_{to} for time course of inactivation and steady-state inactivation but recovery from inactivation is slowed and an "overshoot" is absent.

KCNE2 is a promiscuous β-subunit and can interact with several K^+ channels (HERG, KCNQ1, KCNQ4, Kv3.4) in heterologous expression systems [17]. When co-expressed with Kv4.3 in Xenopus oocytes in the absence of KChIP2, KCNE2 significantly slows the time course of I_{to} activation and inactivation and shifts the voltage dependence of activation to positive membrane potentials [28]. However, in our CHO cell expression system KCNE2 appears to have opposite effects. We found that KCNE2 is exceptional because its co-expression can best reproduce the unique "overshoot" phenomenon during recovery from inactivation of human left ventricular epicardial I_{to} [27], while other KCNE subunits were either ineffective or induced only a small "overshoot" (Table 3). Therefore we suggest that KCNE2 could be an important component of the native I_{to} channel complex at least in human epicardial cardiomyocytes [28].

Expression level of KCNE3 in cardiac tissue is low and may therefore not have a major effect on I_{to} kinetics in vivo. KCNE3 induced, however, the largest shift in I_{to} steady-state inactivation to negative potentials close to the value reported for native myocytes. All other kinetic parameters were less similar to native I_{to} compared to Kv4.3+KChIP2 alone. In addition, KCNE3 did not abolish outward rectification of I_{to} as described for KCNQ1 [26].

KCNE4 is the most abundant of all KCNE subunits and even more abundant than the α-subunit Kv4.3 in human hearts. The former finding confirms recently published data [6,16] although the absolute amount of mRNA for KCNE4 was lower than in our study. This apparent discrepancy is probably due to different primers and general PCR conditions which preclude direct comparison of quantitative expression data from different groups. Because of its exceptionally high expression level KCNE4 is likely to regulate the native I_{to}. In our experiments KCNE4 accelerated Kv4.3+KChIP2 current inactivation kinetics, shifted voltage dependence of steady-state inactivation to more negative membrane potentials and accelerated recovery from inactivation. Therefore, the properties of the heterologous I_{to} with KCNE4 as an additional subunit more closely resemble native I_{to}.

Expression of KCNE5 is also low and we suggest that KCNE5 does not have a prominent role in I_{to} kinetics. Nevertheless, KCNE5 also yields a rather narrow fit to the kinetics of native I_{to} including a small "overshoot" in recovery from inactivation.

When co-expressing both KCNE2 and KCNE4 with Kv4.3+KChIP2, the overshoot was still preserved and inactivation of I_{to} remained accelerated. On the other hand, the time course of inactivation is slowed and steady-state inactivation is shifted to positive values. Therefore, even the combination of the 2 KCNE subunits with the standard channel did not provide a perfect match with native I_{to}.

In failing hearts, the expression of KCNE subunits was differentially regulated: Expression of KCNE1 was roughly doubled, that of KCNE2 and KCNE3 remained unchanged, and expression of KCNE4 and KCNE5 was reduced. However, despite the failure-associated reduction in absolute mRNA level, KCNE4 remained the largest of the KCNE subunits in failing hearts.

4.3. Study limitations

Tissues samples originated from a heterogeneous patient group. In addition tissue from failing hearts was exposed to chronic drug therapy. Therefore it cannot be excluded that part of the differences originate from drug exposure or are based on differences in the disease state. mRNA content though determined quantitatively does not necessarily correlate with the respective protein levels. Since protein expression has been reported only for KCNE2, it is not known whether the native KCNE protein levels are sufficiently high to have an impact on I_{to} regulation.

The attempt to identify the composition of the native channel complex by co-expression of putative α- and β-subunits can only provide incomplete information because neither the stoichiometry of expressed subunits nor the effectiveness of the interaction can be controlled. The similarity in current characteristics compared to native currents is an indirect indication of subunit interaction and has to be confirmed by other means such as immunoprecipitation or fluorescence resonance energy transfer between tagged subunits.

4.4. Conclusion

In the failing heart the changes in the mRNA expression levels of the α-subunit of I_{to} and its putative accessory β-subunits show substantial up-and down-regulation. Assuming that these changes are translated into membrane protein expression they may contribute to alterations in cardiac electrophysiology and to risk for arrhythmias of the diseased heart. KCNEs do not substitute for KChIP2 in promoting channel trafficking. The heterologous co-expression of KCNE proteins in CHO cell support the functional interaction of KCNE β-subunits with Kv4.3 in native myocytes, with KCNE2 and possibly KCNE4 being the likely candidates.

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