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mRNA Levels of *ERG*, *KVLQT1* and *minK* in Rabbit Right and Left Ventricles

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Abstract: Our previous study showed that two components of the delayed rectifier potassium current, I_{Kr} and I_{Ks}, were heterogeneously distributed in right (RV) and left (LV) ventricles in rabbit hearts. However, the mechanisms of heterogeneous distribution of these channels were not known. In the present study, we investigated the encoding mRNA levels of I_{Kr} (*ERG*) and I_{Ks} (*KVLQT1* and *minK*) in RV and LV. By using a quantitative real-time PCR method, we found mRNA levels were not significantly different between two ventricles in *ERG* (RV: 1103 ± 218 molecules/10^5 *GAPDH* molecules, LV: 886 ± 155, n=4), *KVLQT1* (RV: 645 ± 113, LV: 509 ± 170, n=4) and *minK* (RV: 209 ± 33, LV: 185 ± 47, n=4). These results suggest that heterogeneous distribution of I_{Kr} and I_{Ks} in RV and LV could not be explained by the mRNA levels in ventricles. Other unknown factor may underlie this phenomenon.

Key words: rabbit, RV and LV, delayed rectifier K^+ current, mRNA levels, regional distribution

I_{Kr} and I_{Ks} are two important repolarizing potassium currents that determine action potential durations (APD).[1)] It had been clarified that I_{Kr} and I_{Ks} heterogeneously distributed in ventricles among epi-, endo- and mid myocardium as well as between base and apex of hearts.[2,3)] Regional differences in I_{Kr} and I_{Ks} may lead to regional disparity in APD.[3)] In our previous study showing biventricular heterogeneity, the amplitude of I_{Kr} density was similar in RV and LV, I_{Ks} density was considerably smaller in RV than in LV.[4)] The mechanism of the heterogeneous distribution of currents is still not known. I_{Kr} is encoded by *ERG*.[5)] I_{Ks} is encoded by *KVLQT1* and *minK*.[6)] Heterogeneous expression of these genes might explain the heterogeneous distribution of I_{Kr} and I_{Ks} between ventricles. To clarify this point, we examined the regional different expression of these three mRNAs in rabbit RV and LV.

Methods

Four Japanese male white rabbits weighing 1.5 ± 0.2 kg were anesthetized with thiamylal sodium after being heparinized. The hearts were quickly excised and washed in a beaker filled with normal Tyrode's solution. When the hearts became clear of blood, subepicardium was divided from the middle region of the RV and LV free wall. Total RNA was extracted with acid guanidinium thiocyanate-phenol-chloroform method. To eliminate the contamination of genomic DNA, total RNA was treated with DNAase (Takara). Single-stranded cDNA synthesis was performed with total RNA using oligo d(T) primer and SuperScript II RNase H reverse transcriptase (Gibco BRL).

The quantity polymerase chain reaction (PCR) as described previously[7)] was carried out to quantify the expression of each gene by a real-time fluorogenic 5′-nuclease PCR assay (Perkin-Elmer ABI Prism 7700). The *GAPDH* gene was used as an endogenous control. The respective primers and TaqMan probes used for *GAPDH*, *ERG*, *KVLQT1* and *minK* are listed in Table 1. Five different molecules of cDNA standards for genes (1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3) were amplified to determine the linear relationship between threshold cycle (Ct) and log starting molecule number of cDNA standards. Slope factors of *GAPDH*, *ERG*, *KVLQT1* and *minK* were –3.723, –3.659, –3.385, and –3.542 cycles/log decade, respectively. Data were expressed as mean ± SEM. Results were compared using Student's t-test for paired data to evaluate statistical significance and differences were considered significant at $P<0.05$.

Results

mRNA levels of *GAPDH*, *ERG*, *KVLQT1* and *minK* in the subepicardium of RV and LV were quantified by real-time PCR. Fig. 1A shows the typical result of the real-time PCR. The starting quantity of each gene was figure out from standard curve.

Fig. 1B shows the mRNA levels of *ERG*, *KVLQT1* and *minK* between RV and LV. The amount of each mRNA was normalized to 10^5 *GAPDH*. Compared each mRNA between RV and LV showed no significant differences of *ERG* (RV:

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Table 1 Sequence of PCR Primers and Sequence-Specific Probes for Rabbit GAPDH, ERG, KVLQT1 and minK

Target Sequence	Accession No.*	Primer	Sequence (5′–3′)	Position	Amplicon Length, bp
GAPDH	L23961	sense	CTTCACCACCATGGAGAAGGC	374–395	231
		antisense	CTCATGACCACGGTGCACGCC	608–629	
		probe	TCATCCACGACCACTTCGGCAT	556–578	
EGR	U87513	sense	GCATCGCCGTGCATTACTTC	1418–1438	101
		antisense	GCTCTGGCTCTGAGGAGTTGATC	1518–1541	
		probe	TGGCTGCCATCCCGTTTGACCT	1463–1485	
KVLQT1	OCU29136	sense	CTCCGTGGTCTTCATCCACC	303–323	101
		antisense	TTCGTGTACCTGCTGAGAAGGA	403–425	
		probe	TCACCACCCTGTACATCGGCTTCCT	335–360	
minK	L41659	sense	GGAAGCGCTCTACATCCTCATG	261–283	101
		antisense	AGAAACTGGAGCACTCGCATG	361–382	
		probe	CATCATGCTGAGCTACATCCGCTCC	315–340	

* In GenBank release 120.0 (December 2001)

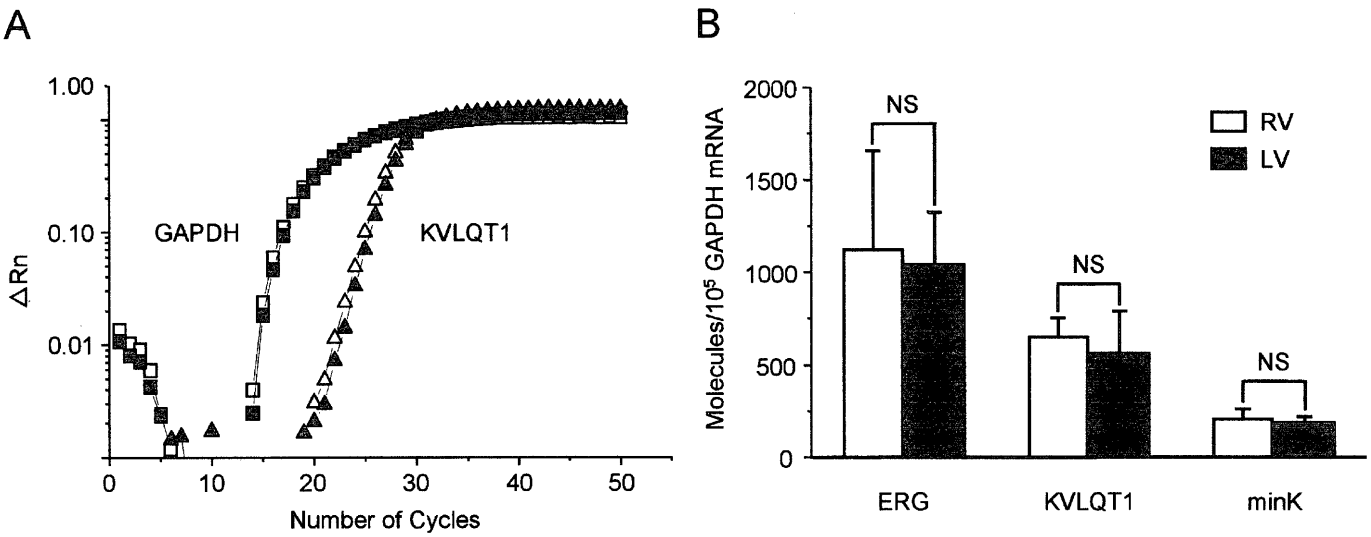

Fig. 1 Comparison of *ERG*, *KVLQT1* and *minK* mRNA levels between RV and LV.
(A) Typical amplification plot of *KVLQT1*. The amount of the PCR product was normalized by dividing the reporter dye by the reference dye to obtain a ratio defined as Rn. ΔRn was the difference between Rn and the baseline signal in each PCR cycle. (B) Comparison of the expression of each mRNA between RV and LV. The amount of each mRNA was normalized to 10^5 GAPDH. Data are means ± SEM. NS mean no significant difference.

1125 ± 532 molecules/10^5 *GAPDH* molecules, LV: 1041 ± 283, n=4), *KVLQT1* (RV: 653 ± 100, LV: 564 ± 225, n=4) and *mink* (RV: 211 ± 52, LV: 190 ± 31, n=4). The amount of *ERG* averaged in ventricles was larger than those of *KVQLT1* and *minK*. The amount of *KVLQT1* was about three times of *minK*. The results indicated that the heterogeneous distribution of I_{Kr} and I_{Ks} were not controlled by the mRNA levels.

Discussion

In the present study, we investigated the mRNA levels of *ERG*, *KVLQT1* and *minK*, which encode I_{Kr} and I_{Ks} channels, in rabbit RV and LV by using a quantitative real-time PCR. Our results indicated that the mRNA levels of these three genes were not significantly different between RV and LV. We have previously shown the heterogeneous distribution of I_{Ks} between ventricles described the smaller I_{Ks} in RV than in LV.[4] Thus, homogenous distribution of mRNA levels of *KVLQT1* and *minK* could not explain the heterogeneous distribution of I_{Ks} between RV and LV. There may be other mechanisms that regulate the density of the current such as protein kinase activity,[8] or post transcription process after mRNA synthesis may be important to produce heterogeneous distribution of ion channels.

Scarce expression of *minK* relative to *KVLQT1* in this study may explain a profile of I_{Ks} in rabbit ventricular myocytes. Our previous study in I_{Ks} of rabbit ventricular myocytes showed smaller current density and more rapid activation compared to that of guinea pig ventricular myocytes. It is well known that *minK* when co-injected with *KVLQT1* increases the amplitude and slows the activation of expressed currents. Our data in mRNA levels of *minK* and *KVLQT1* well coincides with the size and activation kinetics of rabbit ventricles. In accordance with this idea, we expect abundant expression of *minK* relative to *KVLQT1* in guinea pig ventricular myocytes. This point should be clarified in the future experiment.

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