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Regional Differences in Expression of L-type Ca^{2+} Channel α_1 Subunits in Mouse Heart

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Abstract: L-type Ca^{2+} channels (LTCCs) play essential roles in excitation and contraction. Four different α_1 subunits (α_{1S}, α_{1C}, α_{1D} and α_{1F}) have been recoginized for the pore forming subunit of LTCC. We investigated mRNA expression of the four subunits in adult mouse heart (the left ventricle, the right appendage and the SA node region) using RT-PCR. α_{1C} mRNA was expressed in all examined regions. α_{1D} mRNA was detected in sinoatrial (SA) node as well as in right atrium, but not in the ventricle. Expressions of α_{1S} and α_{1F} mRNAs were below the level of detection at any sites. This observation suggests differential expression and different roles of LTCCs composed of α_{1C} and α_{1D} subunits in the mammalian hearts.

Key words: α_{1C}, α_{1D}, Ca channel, mouse, sinus node

L-type Ca^{2+} channels (LTCCs) play essential roles in excitation and contraction of the heart.[1] In the sinus node, L-type Ca^{2+} channel is an important ion channel contributing to pacemaker activity.[2] For the pore forming (α_1) subunit of LTCCs, four distinct gene subtypes have been identified; α_{1C} (the cardiac $Ca_v1.2$), α_{1D} (the neuro-endocrine $Ca_v1.3$) α_{1S} (the skeletal muscle $Ca_v1.1$) and α_{1F} (the retinal $Ca_v1.4$).[3] LTCCs composed of α_{1C} were shown to be expressed most abundantly in cardiac muscle.[4] Recently it has been reported that α_{1D} gene knockout mice are deaf due to the complete absence of L-type Ca^{2+} current in cochlear inner hair cells and exhibit bradycardia and arrhythmias resulting from SA node dysfunction.[5] However, it remains unclear which subunit genes are expressed in the sinus node.

In the SA node of mammalian hearts, unlike other sites, both α_{1C} and α_{1D} subunits might be expressed to form LTCCs of different electrophysiological properties. To shed light on this issue, we studied expressions of α_{1C}, α_{1D}, α_{1S} and α_{1F} mRNAs in mouse SA node, right atrial appendage and ventricle using RT-PCR method.

Material and Methods

10-week-old ICR mice were used in this study. After cervical dislocation, the whole heart was excised. The right atrium was opened with incision of the anterior wall from the inferior vena cava to the superior vena cava and the intercaval region including a whole SA node was excised. The right atrial appendage and the SA node region medial to the crista terminalis were sampled separately. Total RNA was extracted from the left ventricle, the right appendage and the SA node region using AGPC (Acid Guanidium Thiocyanate-Phenol-Chloroform) method by Chomczynski and Sacchi.[6] Single-stranded cDNA was synthesized by an oligo d(T) primer and SuperScript-II RNase H – reverse transcriptase (Invitrogen Corp., Carlsbad, CA, USA). An aliquot of 150 ng cDNA was added to each PCR tube. The specific primers for α_{1C}, α_{1D}, α_{1S} and α_{1F} genes were designed as shown in Table 1. PCR was performed in a 50 μl reaction mixture in conditions of 95°C for 10 min (1 cycle), 95°C for 15 sec, and 60°C for 1 min (35 cycles). PCR products were subjected to 2% agarose gel electrophoresis. GAPDH mRNA was used to control equal delivery of the samples to RT-PCR.

Results and Discussion

In the ventricle, α_{1C} mRNA was expressed but mRNAs of other three genes (α_{1D}, α_{1S} and α_{1F}) were below the level of detection. In the right atrial appendage and the sinus node region, both α_{1C} and α_{1D} subunits were recognized, although α_{1D} mRNA was less abundant than α_{1C} mRNA. Expressions of α_{1S} and α_{1F} subunit mRNAs were undetectable.

In our previous study it has been shown that mRNAs for α_{1C} and α_{1D} are expressed in cardiac ventricles as well as in the SA node of mouse hearts at the early embryonic stage,[7] when both the SA node and the ventricles possess regular pacing activity. In the present study, we showed α_{1D} expression become undetectable in the adult mouse ventricle when it lost its pacemaking activity as a normal developmental process.

LTCCs composed of α_{1D} subunit expressed heteroge-

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Table 1 Sequence of PCR primers for α1C, α1D, α1S, α1F and GAPDH

Target sequence	Accesion No.	Primer	Sequence (5'→3')	Position	Amplicon length (bp)
α1C	L01776	sense	caacctggaacgactggagtatct	1637–1660	157
		antisense	actaaaaagccctacaaccacg	1772–1793	
α1D	M57975	sense	ccatggacattctgaacatggtc	266–288	107
		antisense	cgtgttccaggcgtcactaaaatac	348–372	
α1S	L06234	sense	gagcttgtatgaaatcgaaggct	1304–1326	101
		antisense	tggaagtgccatgacctagtga	1383–1404	
α1F	NM019582	sense	ttccatcatgaaggcgctt	732–750	101
		antisense	actcgagctattcctcggac	813–832	
GAPDH	M32599	sense	cttcaccaccatggagaaggc	343–363	238
		antisense	ctcatgaccacagtccatgcc	560–580	

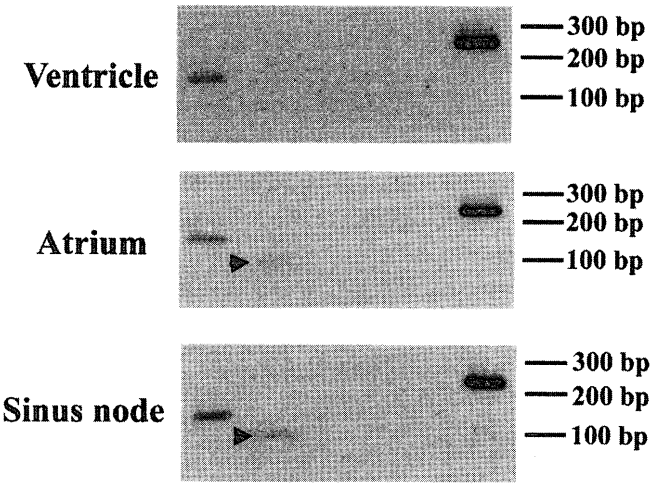

Fig. 1 Expression of α_{1C}, α_{1D}, α_{1S} and α_{1F} mRNAs in adult mouse cardiac heart.
RT-PCR was performed by using specific primers for α_{1C}, α_{1D}, α_{1S} and α_{1F} genes encoding for L-type Ca^{2+} channel α_1 subunits. Upper, middle and lower panel show PCR amplifications in ventricle, right atrial appendage, and sinus node respectively. GAPDH gene was used as an endogenous control. Arrowhead indicates PCR product of α_{1D} gene.

neously were shown to have more negative activation threshold and slower inactivation than those composed of α_{1C} subunit.[8] Such characteristic might favor the diastolic depolarization of SA node cells and immature ventricular cells at early embryonic stage.

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