Molecular and Electrophysiological Differences in the L-Type Ca\(^{2+}\) Channel of the Atrium and Ventricle of Rat Hearts

Seiji Hatano, MD; Takeshi Yamashita, MD; Akiko Sekiguchi, PhD; Yuki Iwasaki, MD*; Kiyoshi Nakazawa, MD**; Koichi Sagara, MD; Hiroyuki Inumia, MD; Tadanori Aizawa, MD; Long-Tai Fu, MD

Background Many pathological conditions induce electrical remodeling, possibly through intracellular Ca\(^{2+}\) overload, but the currently available L-type Ca\(^{2+}\) channel blockers may be detrimental because of their global negative inotropic effects.

Methods and Results To determine whether the L-type Ca\(^{2+}\) channel is identical throughout the heart, the distribution of the mRNAs and proteins comprising the L-type Ca\(^{2+}\) channel and its electrophysiological properties were analyzed in rat atria and ventricles. The mRNA of \(\beta_{2}-1\) (Cacna2d2) was more abundantly expressed in the atrium (−5-fold) than in the ventricle. In contrast, \(\beta_{1C}\) (Cacna1c) (Cav1.2) mRNA was significantly less abundant in the atrium. The level of the \(\beta_{1C}\) (Cacna1c) (Cav1.2) protein was decreased (−0.5-fold) and that of \(\beta_{2}-1\) (Cacna2d1) was increased (−2-fold) in the atrium compared with the ventricle. Although the peak I\(_{\text{Ca,L}}\) density showed no significant differences, voltage dependence of inactivation and activation of the current showed a more depolarized shift in the atrium than in the ventricle.

Conclusion These results indicate that in the rat heart the L-type Ca\(^{2+}\) channel differs between the atrium and ventricle with regard to gene expression and electrophysiological properties.

Key Words: Atrial fibrillation; Atrium and ventricle; L-type Ca\(^{2+}\) channel; Remodeling; Subunit

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The L-type Ca\(^{2+}\) channel expressed in cardiac muscle plays an important role in determining the intracellular Ca\(^{2+}\) and thus the action potential duration of cardiomyocytes. Although Ca\(^{2+}\) overload is a major cause of myocardial cell injury, blocking this channel could be either beneficial or detrimental. In fact, the available L-type Ca\(^{2+}\) channel blockers should be useful for treating several types of cardiac arrhythmias, but might cause negative inotropic effects that lead to pump failure. A T-type Ca\(^{2+}\) channel blocker, mibefradil, has shown potential for preventing tachycardia-induced atrial fibrillation remodeling, although its underlying mechanism of prevention remains unclear. Because the T-type Ca\(^{2+}\) channel blockers are one of the “atrium specific Ca\(^{2+}\) channel blockers”, regional differences in the L-type Ca\(^{2+}\) channel blocker, if any, would open a new paradigm for controlling regional intracellular Ca\(^{2+}\).

The function of the L-type Ca\(^{2+}\) channel is characterized by its main subunit, \(\beta_{1C}\) (Cacna1c) (Cav1.2), and also the auxiliary subunits \(\beta_{2}\) (Cacna2d) and \(\alpha_{2}\) (Cacnb). However, there are few reports investigating regional differences in Ca\(^{2+}\) channel composition. Because previous studies have focused on regional or developmental differences of a single subunit, it would be inappropriate to discuss regional differences of the L-type Ca\(^{2+}\) channel with special reference to the relative expression of the composite subunits. In the present study, to determine whether the composition of the L-type Ca\(^{2+}\) channel is identical throughout the heart, we analyzed both the distribution of the mRNAs and proteins that comprise the L-type Ca\(^{2+}\) channel and its electrophysiological properties in rat atria and ventricles.

Methods

mRNA Analysis and Ribonuclease Protection Assay Hearts were harvested from 10-week-old female Sprague-Dawley (SD) rats. For mRNA analysis, the excised hearts were divided into the sinus-node, the right atrium, the left atrium, the intraventricular septum, the epicardial side of the left ventricular free wall, the endocardial side, the left
ventricular apex and the right ventricle, and then immediately snapfrozen in liquid nitrogen. RNA was extracted by the AGPC (acid guanidinium-phenol chloroform) method.

Digoxigenin-labeled RNA probes were made using a RT-PCR kit (access RT-PCR system, Promega, Madison, WI, USA) with subunit-specific primers (Table 1) and myocardial RNA as a template. The RT-PCR products were subcloned into a PCRII vector (Invitrogen, San Diego, CA, USA), and the digoxigenin (Boehringer Mannheim)-labeled RNA probes were made with the cDNA as a template. All of the prepared RNA probes were partial lengths confirmed not to cross-react with each other. Ribonuclease protection assays were performed using a RPAIII kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions.

**Protein Analysis and Western-Blot Analysis**

For protein analysis, hearts harvested from 10-week-old female SD rats were divided into the atrium and the ventricle. The samples were immediately homogenized while gently suspended in Tris/EDTA (TE) buffer and 1/10 volume of a protease inhibitor cocktail (Halt Protease Inhibitor Cocktail, EDTA free Pierce, Rockford, IL, USA). Tissue lysates were centrifuged for 20 min at 15,000 rpm. Soluble proteins were removed and the precipitate was dissolved in TE buffer containing 10% trichloroacetic acid. After centrifuging for 30 min, the pellet was dissolved in TE buffer containing 25% urea, 4% D-mercaptoethanol, 2% Triton-X and 2.5% LDS, and finally prepared as membranous fraction of the proteins. All subsequent manipulations were performed on ice.

Proteins were separated using SDS (sodium dodecyl sulfate)-PAGE (polyacrylamide gel electrophoresis), electrophoresed on PVDF (polyvinylidene difluoride) membrane, blocked overnight by 5% skim milk TBST (Tris-bufferd saline Tween-20), and thereafter incubated overnight with the specific primary antibodies (Alamone Labs, Jerusalem, Israel). The prepared antibody concentrations were 1:833 for anti-αc and anti-β3, and 1:1,000 for anti-β1-1. Anti-rabbit HRP (horseradish peroxidase)-linked IgG (Cell Signaling, Beverly, MA, USA) was used as the secondary antibody. Protein levels were detected with chemiluminescence reagents (Western Lightning Chemiluminescence Reagent Plus, Perkin Elmer Life Science, Boston, MA, USA) and quantitatively analysed using microcomputer imaging software (ATTO Bioscience & Biotechnology, Tokyo, Japan).

**Patch-Clamp Analysis**

Hearts from 10-week-old female SD rats were removed and immediately retrogradely perfused on a Langendorff apparatus with oxygenated 37°C normal Tyrode solution. Thereafter, oxygenated Ca²⁺-free Tyrode solution was superfused for 10 min followed by 15-min perfusion with 1% collagenase-containing solution. The heart was divided into the atrium and the ventricle, and cells were separated on ice from the tissue soaked in modified KB solution (in mmol/L: KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH₂PO₄ 20, MgCl₂ 3, glucose 10, EGTA 1, HEPES 10 (pH 7.4)).

A small aliquot of isolated cells was set on the stage of an inverted microscope, superfused with normal Tyrode solution at room temperature. Whole-cell patch clamp recording was performed with 2–5 MΩ glass pipettes (Clark Electromedical Instruments, Pangbourne Reading, UK) filled with pipette solution (in mmol/L: CsCl 13, MgCl₂ 6H₂O 0.1, EGTA 1, GTP 0.01, ATP-Mg 0.5, HEPES 1 (pH 7.4)), connected to a patch-clamp amplifier (L/M-EPC 7, List Electronic, Darmstadt-Eberstadt, Germany). Command pulses were generated by a digital-to-analog converter (VB-10B digital data recorder CRC), which was controlled by Pulse + pulsefit software (HEKA Elektronik, Lambrecht, Germany). Recordings were stored on the hard disk of the computer. The cells were superfused with the extracellular solution (in mmol/L: TEA-Cl 126, CsCl 5.4, MgCl₂ 1, CaCl₂ 2, dextrose 10, HEPES 10, ryanodine 2x10⁻³ (pH 7.4)) while recording Icₐ,L. Only clearly striated rod-shaped cells were studied.

**Data Analysis and Statistical Analysis**

The mRNA expression level of each subunit was normalized with that of cyclophilin. Statistical analysis was performed with ANOVA and multiple comparison was done by Bonferoni's modified t-test. The protein levels and the current densities were analyzed using unpaired t-test. Activation and inactivation of the current were fitted to the Boltzmann formula (\(f = \frac{1}{1 + \exp\left(\frac{V + V_{1/2}}{b}\right)}\)), where \(f\) is the steady-state parameter, \(V\) is the membrane voltage potential, and \(V_{1/2}\) is the membrane voltage potential at half steady-state function. Recovery from inactivation was fitted to a biexponential function.

Values of \(p<0.05\) were considered to be significantly different.

**Results**

**Ribonuclease Protection Assay**

The main subunit \(\alpha\c (Cacna1c) (Cav1.2)\) and the auxiliary subunits \(\beta 1\) with splice variants c, d and e, -2 with splice variants I and II (Cacna2d1 and Cacna2d2) and \(\beta 2\) (Cacnb2 and Cacnb3) showed homogeneous mRNA expression throughout the atrial regions investigated (ie, the sinus-node and the right and left atria). The mRNA expressions of these molecules were also homogeneously distributed through the ventricle. However, when we compared the mRNA level of the \(\beta c\) subunit (Cacna1c) (Cav1.2) in the atrium with that in the ventricle, it was significantly less abundant in the atrium, approximately 50% of that in the ventricle. In contrast, the mRNA level of the auxiliary subunit \(\beta 2\) (Cacna2d2) (splice variants I and II) was approximately 5-fold more abundant in the atrium than in the ventricle. The mRNA levels of the other auxiliary subunits \(\beta 1\) (Cacna2d1), \(\beta 3\) (Cacnb2), and \(\beta 4\) (Cacnb3) showed no significant regional differences between the atrium and the ventricle (Fig 1A).

**Western-Blot Analysis**

The \(\alpha\c (Cacna1c) (Cav1.2)\) immunoreactive protein was significantly decreased in the atrium to approximately 50% of the level in the ventricle, which was consistent with the results of its mRNA expression. On the other hand, the \(\beta 1\) (Cacna2d1) immunoreactive protein was approximately 2-fold more abundant in the atrium than in the ventricle. The \(\beta 2\) (Cacnb3) auxiliary subunit showed no significant differences between the atrium and the ventricle (Fig 1B). The protein levels of \(\beta 2\) (Cacna2d2) and \(\beta 4\) (Cacnb2) could not be determined in the present study because their antibodies were not available.

**Patch-Clamp Analysis**

The Icₐ,L was elicited by 240 ms depolarizing pulses to
–40 mV to ~ +60 mV with +10 mV increments from a holding potential of –40 mV, and the ICa,L density was obtained by normalizing the ICa,L with the membrane capacitance. Representative recordings at –20, 0, +10, +20 mV test pulses are shown in Fig 2A, and the mean current density-voltage relations for the atrial and ventricular cells are shown in Fig 2B. Though the peak ICa,L density was slightly smaller in the atrial cells than in the ventricular cells, the difference was not statistically significant. However, in the atrial cells, the potential of the peak ICa,L shifted to a more significantly depolarized level than in the ventricular cells. Figs 2C, D shows the voltage dependence of inactivation and activation of ICa,L. The V1/2 of the voltage dependence of inactivation was –24.4±0.6 mV in the atrium and –29.0±0.1 mV in the ventricle. The V1/2 of the voltage dependence of activation was –7.8±2.0 mV in the atrium, and –15.7±4.9 mV in the ventricle. Accordingly, in the atrial cells, both the voltage dependence of inactivation and activation curves showed a significantly more depolarized shift than in the ventricular cells, although the current density was similar throughout the heart.

**Discussion**

The major findings of the present study are as follows. (1) Rat hearts have inhomogeneous mRNA expression of the subunits encoding the L-type Ca2+ channel. The auxiliary subunit β2δ-2 (Cacna2d2) mRNA is abundantly expressed in the atrium, whereas the main subunit α1C (Cacna1c) (Cav1.2) mRNA is predominantly expressed in the ventricle. The α1C mRNA showed no regional differences in mRNA expression. (B) The protein levels of the main subunit α1C (Cav1.2) (Cacna1c) and auxiliary subunits β2δ-1 (Cacna2d1) and β3 (Cacnb3) in the atrium and ventricle. The α1C (Cav1.2) (Cacna1c) immunoreactive protein was significantly less abundant in the atrium than in the ventricle. In contrast, the β2δ-1 (Cacna2d1) immunoreactive protein level showed an approximately 2-fold increase in the atrium compared with the ventricle. The β3 (Cacnb3) immunoreactive protein level showed no significant differences between the atrium and ventricle. The mean values are shown in the lower panel (n=5, *p<0.05 when the atrium compared with the ventricle). N, negative control; SN, sinus node; RA, right atrium; LA, left atrium; IVS, intraventricular septum; epi, left epicardial ventricle; end, left endocardial ventricle; apex, left ventricular apex; RV, right ventricle.
depolarized shift in atrial cells than in ventricular cells.

In cardiac myocytes, the L-type Ca2+ channel is composed of multiple subunits, including the main subunit \( \alpha_1C \) (Cacna1c) (Cav1.2) and the auxiliary subunits \( \beta_2 \) (Cacna2d) and \( \delta \) (Cacnb). Variations have been reported in the auxiliary subunits, including \( \beta_2\)-1 to 4 (Cacna2d1 to 4) and \( \delta \)-1 to 4 (Cacnb1 to 4). The subunits synergistically control the L-type Ca2+ channel, thereby regulating the excitation-contraction coupling process of the myocytes. Many pathological conditions in several experimental models are reported to have induced reduction in the \( \text{ICa}_{\text{L}} \) density,\(^6,7\) which is ascribed partially to the transcriptional downregulation of the main subunit \( \alpha_1C \) (Cacna1c) (Cav1.2). A typical example is atrial fibrillation, in which the \( \text{ICa}_{\text{L}} \) density decreases progressively during the maintenance of the arrhythmia.\(^8\) In accordance with the reduced \( \text{ICa}_{\text{L}} \), the mRNA level of the main subunit \( \alpha_1C \) (Cacna1c) (Cav1.2) decreases progressively during the maintenance of the arrhythmia.\(^8\) In accordance with the reduced \( \text{ICa}_{\text{L}} \), the mRNA level of the main subunit \( \alpha_1C \) (Cacna1c) (Cav1.2) decreases progressively during the maintenance of the arrhythmia.\(^8\)

A recent study reported inhomogeneous distribution of the L-type Ca2+ channel subunits between the atrium and the ventricle of rat hearts: the mRNA of the main subunit \( \alpha_1C \) (Cacna1c) (Cav1.2) was more abundantly expressed in the ventricle than in the atrium.\(^{10}\) Moreover, the mRNA levels of \( \beta_2\)-2 (Cacna2d2) and \( \delta \)-3 (Cacna2d3) were higher in the atrium than in the ventricle.\(^{11}\) As for the protein expressions, \( \delta \) (Cacnb2) is reported to be more abundant in the ventricle than in the atrium.\(^{12}\) These previous reports clearly demonstrate that the molecular basis of the L-type Ca2+ channel differs between the atrium and the ventricle. Our present study also supports this concept, adding the new finding that the rat atrium is characterized by a lower level of the immunoreactive protein of the main subunit \( \alpha_1C \) (Cacna1c) (Cav1.2) and a higher level of the auxiliary subunit \( \beta_2\)-1 (Cacna2d1). However, surprisingly, our electrophysiological data demonstrated that the peak density of the L-type Ca2+ channel was similar in the atrium and the ventricle, irrespective of the inhomogeneous distribution of the subunits.

The function of the L-type Ca2+ channel should be determined by the composition of the co-expressed subunits. Although the main subunit \( \alpha_1C \) (Cacna1c) (Cav1.2) accounts for the Ca2+ ion channel pore, the voltage sensor, the selectivity filter and the drug binding sites and is alone able to function as the L-type Ca2+ channel, co-expression of the auxiliary subunits modifies the electrophysiological function of the main subunit. The \( \beta_2\) (Cacna2d) auxiliary subunit is known to increase the peak \( \text{ICa}_{\text{L}} \) density and to shift the voltage dependence of inactivation and activation to a more hyperpolarized level.\(^{13,14}\) Also, the peak \( \text{ICa}_{\text{L}} \) density is known to be increased by co-expression of the \( \delta \) (Cacnb) auxiliary subunit\(^{15,16}\) although there have been contro-
versal results reported for \( \beta \) (Cacnb3).17 On the basis of these reports, the present electrophysiological observations would be explained simply by the decreased amount of the main subunit \( \alpha \) (Cacna1c) (Cav1.2) in the atrium being compensated for by the increased auxiliary subunit \( \alpha \) (Cacna2d), leading to similar \( I_{\text{Ca,L}} \) density in the atrium and the ventricle. In this consideration, the voltage dependence of inactivation and activation of \( I_{\text{Ca,L}} \) should show a hyperpolarized shift in the atrium through the increased \( \beta \) (Cacna2d), contradictory to our observation of a depolarized shift. However, that could be attributed to glycosylation of the \( \beta \) (Cacna2d) subunit, which is known to have many glycosylation sites that cause surface charge effects.18

The subunit composition and electrophysiological function of the L-type \( \text{Ca}^{2+} \) channel might differ among animal species. Human hearts are known to express the possibility of differing regional turnover of its main subunit \( \alpha \) in the atrium and the ventricle. In this consideration, the voltage dependence of inactivation and activation of \( I_{\text{Ca,L}} \) within the chambers of the rat heart. J Mol Cell Cardiol 2002; 34: 519 – 532.


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