Molecular and Electrical Remodeling of L- and T-Type Ca²⁺ Channels in Rat Right Atrium With Monocrotaline-Induced Pulmonary Hypertension

Takashi Koyama, MD; Kyoichi Ono, MD*; Hiroyuki Watanabe, MD; Takayoshi Ohba, MD*; Manabu Murakami, MD*; Kenji Iino, MD; Hiroshi Ito, MD

Background Atrial arrhythmia is often encountered in chronic pulmonary disease with pulmonary hypertension (PH), but few studies have investigated the electrical remodeling of atrial Ca²⁺ channels under PH.

Methods and Results Wistar rats were injected with monocrotaline (MCT), resulting in PH with right atrial and ventricular hypertrophy. The L-type Ca²⁺ channel current density was significantly decreased in right atrial cells of MCT-treated rats, accompanied by a significant reduction in mRNA expression of the CaV1.2 (α₁c) subunit and accessory β₂ subunit. Conversely, the low voltage-activated Ca²⁺ current was more marked in the right atrial cells of MCT-treated rats than in those of control rats. The current–voltage relationship and the time course of inactivation closely resembled those of T-type Ca²⁺ channels, although the current was only slightly inhibited by 10–100 μmol/L Ni²⁺. No significant differences were observed in the mRNA expression levels of CaV3.1 (α₁d) and CaV3.2 (α₁H) or the protein level of the CaV3.1 subunit. In left atrial cells, the electrophysiological molecular properties of Ca²⁺ channels were unaffected by MCT treatment.

Conclusions PH causes right atrial hypertrophy, associated with alteration of the electrophysiological molecular properties of Ca²⁺ channels. (Circ J 2009; 73: 256–263)

Key Words: Atrial hypertrophy; Electrical remodeling; Ca channel; Monocrotaline; Pulmonary hypertension

It is well known that fibrillating or hemodynamically overloaded atria are subject to cellular electrical remodeling, which is characterized by shortening of the action potential and often contributes to the occurrence and persistence of atrial fibrillation (AF). AF and chronic obstructive pulmonary disease (COPD) frequently coexist and complicate treatment of both clinical conditions. COPD patients are susceptible to secondary pulmonary hypertension (PH), and the resulting hemodynamic changes, and in addition hypoxia, hypercapnia, acid–base disorders, disturbed sympathovagal balance, and medication can lead to the development of AF. Recent studies demonstrated that the densities of the L-type calcium (Ca²⁺) current (Ical), transient outward current (Iₒ), and voltage-dependent Na⁺ current (INa) are decreased in experimental animal models of atrial tachycardia-related AF. In contrast to tachycardia-induced ionic remodeling, several clinical and experimental studies have indicated a decrease, no change, or even an increase in the Ical density in chronic heart failure (CHF) depending on the experimental conditions. Nonetheless, it is generally agreed that a possible change in Ical is a key factor in electrical remodeling of cardiac myocytes in CHF and/or hypertrophy. In addition, the T-type Ca²⁺ channel is involved in electrical remodeling during CHF or hypertrophy. The T-type Ca²⁺ channel is normally expressed in embryonic and neonatal myocytes, and in the conducting system of the adult heart, and in rats it is re-expressed in pathologic conditions such as ventricular hypertrophy and post-myocardial infarction. In the present study, the electrical properties of Ical and Ical were characterized in rat right atrial cells with monocrotaline (MCT)-induced PH, and the possible causes are discussed in relation to changes in the expression and function of Ca²⁺ channel subunits.

Methods

Animals

The Animal Ethics Committee of Akita University School of Medicine, Japan, approved the study protocol. Four-week-old Wistar rats (CLEA Japan, Tokyo, Japan), weighing 140–150 g each, were treated with 500 mg MCT (Crotaline; Sigma, St Louis, MO, USA) to produce PH. A single dose of MCT 60 mg/kg was injected subcutaneously in the interscapular region.

Measurement of Hemodynamic Parameters and Assessment of Right Ventricular (RV) Hypertrophy

RV pressure and heart rate were measured, using a pressure transducer connected to a monitor (DynaScope 5100E; Fukuda Denshi, Tokyo, Japan). Right atrial (RA) and RV hypertrophy were evaluated by measuring the ratio of the RA weight to body weight (BW), the ratio of the RV free wall weight to BW, and the ratio of the RV weight to the left ventricular free wall plus septum (LV + IVS) weight.
Table 1  Oligonucleotide Sequences of Primers Used for RT-PCR

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<th>Primer (a)</th>
<th>Sense</th>
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<td>5'-GATGACAGACGCTAGGGCTATGAG-3'</td>
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<td>5'-GGCTCTGTTGTGTGCTACTATG-3'</td>
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Table 2  Effect of MCT-Treatment on Rat Hemodynamic and Physiologic Profiles

<table>
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<th>Control</th>
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<tr>
<td>Heart rate (beats/min)</td>
<td>426.5±11.5</td>
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<td>n</td>
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</tr>
<tr>
<td>Body weight (g)</td>
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<tr>
<td>RV systolic pressure (mmHg)</td>
<td>21.2±1.5</td>
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<tr>
<td>RV/BW (g/kg)</td>
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<td>RA/BW (g/kg)</td>
<td>0.27±0.05</td>
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<tr>
<td>RV/LV + IVS (g/g)</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>Cm (pF)</td>
<td>62.7±6.2</td>
</tr>
<tr>
<td>No. cells</td>
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</tr>
<tr>
<td>No. hearts</td>
<td>9</td>
</tr>
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<td>ECG findings</td>
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<tr>
<td>P voltage (mV)</td>
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</tr>
<tr>
<td>P duration (ms)</td>
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<tr>
<td>QT interval (ms)</td>
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<tr>
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<td>Axis (-)</td>
<td>41.25±2.10</td>
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<tr>
<td>n</td>
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</table>

MCT, monocrotaline; RV, right ventricle; BW, body weight; RA, right atrium; LV, left ventricle; IVS, interventricular septum; Cm, cell capacitance. Values are means±SE. *P<0.05.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was isolated from the RA, and reverse transcription was performed using standard methods and 50 cells collected from control and MCT-treated rats. For the RT-PCR, 2.0 µl of template was used. The sequences for the rat voltage-gated Ca²⁺ channel Cav1.2 subunit, Cav1.3 subunit, Cav3.1 subunit, Cav3.2, β2 subunit, and β3 subunit were amplified using subunit-specific primers (Table 1).

Western Blot Analysis

Partially purified cardiac membranes from the atria were prepared and suspended in 50 mmol/L Tris-HCl buffer (pH 7.4), as described previously. The protein concentrations were determined by the Bradford assay. Aliquots of homogenate (100 µg) from each rat were resolved by 6% SDS-polyacrylamide gel electrophoresis. Commercial polyclonal antibodies specific for CaV1.2 (α1C) and CaV3.1 (α1G) (Alomone, Jerusalem, Israel), and anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used for immunodetection by the manufacturer’s protocol. Densitometry was performed with a CCD camera (KODAK Image Station 200R, Kodak, Tokyo, Japan). For quantification, the protein expression levels were normalized to the GAPDH levels.

Electrocardiography (ECG)

ECGs were recorded before the isolation of RA cells from MCT- and saline-treated rats. Standard limb leads (I, II, III, aVR, aVL, aVF) and the V1 lead were recorded (Nihon Koden, Tokyo, Japan). The P wave was evaluated using II leads, and QT and QTc intervals were measured from the longest QT interval in limb leads or the precordial V1 lead. Electrical axis was acquired by using the I, II, and III leads.

RA Cell Isolation

After coronary perfusion with 50 ml of Ca²⁺-free Tyrode solution, the perfusate was switched to a Ca²⁺-free Tyrode solution that contained 0.02% collagenase (Wako, Osaka, Japan), and the heart tissue was digested for approximately 30 min. The heart was rinsed with a high-K⁺, low-CI⁻ storage solution (KB solution). The RA was dissected from the digested heart, the cells were kept in KB storage solution at 4°C and studied within 6 h of isolation.

Solutions for Electrophysiological Recording

The composition of normal Tyrode solution was (in mmol/L): NaCl 136, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.53, NaH₂PO₄ 0.33, HEPES 5.0, and glucose 5.5 (pH 7.4 with NaOH). The composition of KB solution was (in mmol/L): KOH 70, KCl 30, L-glutamic acid 70, taurine 20, KH₂PO₄ 10, MgCl₂ 1.0, glucose 1.0, EGTA 0.3, and HEPES 10 (pH 7.4 with KOH). The pipette solution was composed of (in mmol/L): CsOH 130, TEA-Cl 30, Mg⁴⁺ 5, EGTA 10, HEPES 5.0, and Na₂GTP 0.1 (pH 7.2 with aspartic acid). Ca²⁺-Tyrode solution, which was prepared by replacing the KCl in normal Tyrode solution with equimolar CsCl, was used for measuring whole-cell I_CaL. The Na⁺/K⁺-free solution contained (in mmol/L): Tris/HC1 140, CaCl₂ 1.8, MgCl₂ 0.5, HEPES 5.0, glucose 5.5, and tetrodotoxin (TTX) 0.01 (pH 7.4 with Tris base). The Na⁺/K⁺/Ca²⁺+-free solution was prepared by replacing external CaCl₂ with equimolar MgCl₂.

Electrophysiological Recording

The whole-cell voltage clamp method was used to record membrane currents. Patch pipettes were pulled with a microelectrode puller (model P-97; Sutter Instrument, Novato, CA, USA) and had a resistance of 2–5 MΩ when filled with electrode internal solution. Membrane currents were recorded in the voltage-clamp mode using a patch-clamp amplifier (Axopatch B; Axon Instruments, Burlingame, CA, USA). Experimental protocols and data acquisition

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Statistical Analysis

All statistical data are presented as means ± standard deviation. Non-paired t-test was used to compare the physiological variations between 2 groups. The statistical analysis was carried out using SPSS software for Windows (version 13.0; Chicago, IL, USA). A difference at P<0.05 was considered statistically significant.

Results

RA and RV Hypertrophy in MCT-Treated Rats

Table 2 summarizes the animals’ characteristics 4 weeks after MCT injection. The heart rate, as measured from the ECG recording, was 361.6±11.6 beats/min (n=22) in MCT-treated rats, which was significantly lower than that in the controls (426.5±11.5 beats/min, n=11). Hemodynamic measurements revealed that the RV pressure was significantly higher in MCT-treated rats than in the controls. The RA/BW, RV/BW, and RV/(LV+IVS) ratios were significantly higher in MCT-treated rats than in the controls. Cardiomyocyte hypertrophy was also confirmed by the measurement of cell capacitance, which was significantly larger in MCT-treated rats than in the controls. On average, Cm was 62.7±2.2 pF (9 hearts with 87 cells) and 85.3±4.1 pF (12 hearts with 95 cells) for the controls and MCT-treated rats, respectively. ECG recording demonstrated that all the animals were in sinus rhythm (data not shown). The P-wave voltage was significantly higher and the QT interval longer in MCT-treated rats than in control rats. Right-sided deviation of the electrical axis was prevalent in MCT-treated rats, compared with control rats (P<0.05, Table 2).

I_{Ca,L} Recording in Cs-Tyrode Solution

Fig 1A shows representative recordings in RA myocytes from control and MCT-treated rats on Day 28 after injec-
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...tion. I\textsubscript{CaL} was continuously monitored after rupture of the membrane patch by applying 300-ms square pulses from −50 mV to 0 mV at an interval of 0.1 Hz. The current–voltage (I–V) relationships measured at the initial peak and the end of the pulses are shown in Fig 1B. It is evident that the initial current peaks at test potentials more positive than −30 mV were significantly smaller in RA cells from MCT-treated rats than in those from saline-treated rats. The depolarization-activated inward current was confirmed to be I\textsubscript{CaL}, as the current was completely inhibited with 0.3 μmol/L nisoldipine (Fig 2). The average I\textsubscript{CaL} density at 0 mV, thus measured, was 2.0±1.8 pA/pF (3 hearts with 10 cells) in MCT-treated rats and 5.8±2.1 pA/pF (4 hearts with 11 cells) in control rats (P<0.05).

The same protocol was applied to LA cells from saline- and MCT-treated rats (Fig 1C). The average I\textsubscript{CaL} density at 0 mV was 6.7±0.6 pA/pF (6 hearts with 23 cells) in MCT-treated rats and 6.5±1.1 pA/pF (4 hearts with 11 cells) in control rats (P<0.05).

The steady-state activation of I\textsubscript{CaL} was estimated from conductance measurements at the time of peak I\textsubscript{CaL}, by dividing the peak current amplitude at different potentials, V, by the corresponding driving force V–V\textsubscript{rev}, where V\textsubscript{rev} is the reversal potential of the current. The value of V\textsubscript{rev} was determined from a linear fit through the current values at potentials more positive than the peaks of the I–V curves. The steady-state activation thus obtained is shown in Fig 1D. The data were fitted with the Boltzmann equation:

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp \left( \frac{V_{1/2} - V}{s} \right)}
\]

where V\textsubscript{1/2} is the half activation potential and “s” the slope factor. The V\textsubscript{1/2} value was more positive in the RA cells of MCT-treated rats (−4.03±1.78 mV, 2 hearts with 8 cells) than in those of control rats (−15.54±1.75 mV, 2 hearts with 6 cells; P<0.05). The “s” value was 6.26±0.29 (2 hearts with 6 cells) in MCT-treated rats and 6.31±0.22 (2 hearts with 8 cells) in the controls, and there was no statistical significance.

The inactivation property was examined using a double-pulse protocol (see Fig 1D). We fitted the steady-state inactivation relationship with the Boltzmann function:

\[
\frac{I_{\text{max}}}{I} = \frac{1}{1 + \exp \left( (V_{1/2} - V)/s \right)}
\]

where V\textsubscript{1/2} is the half activation potential and “s” the slope factor. The V\textsubscript{1/2} value was more positive in the RA cells of MCT-treated rats (−4.03±1.78 mV, 2 hearts with 8 cells) than in those of control rats (−15.54±1.75 mV, 2 hearts with 6 cells; P<0.05). The “s” value was 6.26±0.29 (2 hearts with 6 cells) in MCT-treated rats and 6.31±0.22 (2 hearts with 8 cells) in the controls, and there was no statistical significance.
0.28 mV for the controls and 6.01±0.45 mV for the MCT-treated rats. No statistically significant difference was detected for the inactivation property.

The time course of $I_{CaL}$ inactivation was also analyzed. The time-dependent inward current upon depolarization was almost completely inhibited by nisoldipine (Fig 2A). We analyzed quantitatively the inactivation time course by the least squares fit with the sum of 2 exponential functions:

$$I = A_1 \times \exp \left( \frac{t}{\tau_{fast}} \right) + A_2 \times \exp \left( \frac{t}{\tau_{slow}} \right) + I_{sus},$$

where $\tau_{fast}$ and $\tau_{slow}$ indicate the time constant of the fast and slow exponential components, respectively; $A_1$ and $A_2$ are the amplitude of the fast and slow exponential components, respectively; $I_{sus}$ denotes the amplitude of the sustained component.

Figs 2B and C summarize the data obtained from 5 cells from control rats and 7 cells from MCT-treated rats. The time constant of the fast component was larger at all test potentials in the RA cells from MCT-treated rats compared with control rats (Fig 2Ba), whereas the slow component did not differ significantly between the 2 groups (Fig 2Bb). No significant differences were observed for the relative amplitudes of the fast, slow, and sustained components.

$I_{CaT}$ Recorded in Na+/K+-Free Solution

Fig 3A shows representative traces obtained with a 300-ms depolarizing pulse to −10 mV from holding potentials of −90 and −50 mV. Although the 2 traces were almost superimposable in the control cells (Fig 3Aa), a marked difference was observed for the cells from MCT-treated rats (Fig 3Ab). The difference current, shown in the lower panel, clearly indicates the existence of a low voltage-activated Ca$^{2+}$ current, and its magnitude is much larger in RA cells from MCT-treated rats than in RA cells from control rats. The I–V relationships for $I_{CaT}$, defined as the difference current, are shown in Fig 3B, in which the initial peak and the end (filled circles) of the pulses are plotted against the test potentials. Data are mean±SEM for 10 cells from saline-treated rats (a) and for 9 cells from MCT-treated rats (b).
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–30 mV, was 3.0±0.1 pA/pF (4 hearts with 9 cells) in MCT-treated rats. This value was significantly larger than that in control rats (1.0±0.04 pA/pF, 4 hearts with 10 cells). LA cells were also examined, but no significant difference was detected in the current density of I_{CaT} (ie, the I_{CaT} amplitude at –30 mV was –0.45±0.24 pA/pF (4 hearts with 10 cells) in the control rats, and –0.31±0.08 pA/pF (7 hearts with 15 cells) in the MCT-treated rats).

The time course of inactivation of I_{CaT} was analyzed only in RA cells from MCT-treated rats. The I_{CaT} was fitted with a single exponential function, and the time constant was plotted against the test potential (Fig 4A).

Although the voltage-dependent properties of I_{CaT} recorded in MCT-treated rat RA cells were quite similar to those of I_{CaT} reported previously in cardiomyocytes, we found several differences in the pharmacologic properties. In the experiments shown in Fig 3B, I_{CaT} was elicited by a depolarizing pulse to –50 mV, from the holding potential of –90 mV. As illustrated in typical traces, I_{CaT} in MCT-treated rat RA cells was not blocked by 50–100 μmol/L Ni^{2+} (Fig 4Ba), which is in contrast to previous reports that cardiac I_{CaT} is completely blocked by low Ni^{2+} concentration (40 μmol/L).13 The current was not decreased by increasing the TTX concentration up to 50 μmol/L, excluding the possibility that the current was carried via I_{Na}. On the other hand, the low voltage-activated inward current was markedly suppressed by replacing external CaCl_{2} with equimolar MgCl_{2} (Fig 4Bb), confirming that the current was carried by Ca^{2+}. Mibefradil at 10 μmol/L, which is known to block I_{CaT}, almost completely blocked I_{CaT} (Fig 4Bc, 2 hearts with 5 cells). Efonidipine is also known to block I_{CaT} and 1 μmol/L efonidipine inhibited I_{CaT} slightly in the present study (Fig 4Bd). In 4 cells from 2 hearts, 1 μmol/L efonidipine inhibited I_{CaT} by only approximately 30%.

RT-PCR Analysis of Ca^{2+} Channels

Fig 5 shows that the expression levels of CaV1.2 and β2 normalized to the β-actin expression level were significantly lower in the MCT-treated rats than in the control rats (CaV1.2 control vs MCT: 0.96±0.06 vs 0.76±0.06 (n=6), P<0.05; β2: 1.00±0.04 vs 0.88±0.03 (n=6), P<0.01) (Fig 6A). The expression level of β3 mRNA was significantly higher in the MCT-treated rats than in the control rats [β3 (control vs MCT): 0.82±0.10 vs 1.22±0.14 (n=6), P<0.01]. No significant difference was detected in the expression levels of CaV1.3, CaV3.1, and CaV3.2 subunits (Fig 5A and B).
CaV1.3, CaV3.1, and CaV3.2 (CaV1.3 (control vs MCT): for CaV1.3, 0.79±0.02 vs 0.83±0.05 (n=6); for CaV3.1, 0.91±0.04 vs 0.80±0.07 (n=6); and for CaV3.2, 0.96±0.05 vs 0.91±0.08 (n=6)) (Fig 5B).

Immunoblot Analysis

The anti-CaV1.2 antibody recognized proteins of 180 kDa and approximately 75 kDa, indicating the presence of its truncated form (Fig 6A, Left panel). The anti-CaV3.1 antibody specifically detected a single protein 140 kDa (Fig 6A, Right panel). The amount of CaV1.2 was significantly decreased in the RA of MCT-treated rats. After stripping, the blots were reprobed with antibodies for GAPDH, to verify equal loading in each lane (Fig 6A, Lower panels).

Densitometric quantification was performed by normalizing the immunoblot signals of the CaV1.2 and CaV3.1 subunits to that of GAPDH (Fig 6B). This revealed a significant decrease in the amount of CaV1.2 in the RA of MCT-treated rats, and no significant differences in the CaV3.1 levels between control and MCT-treated rats. Neither CaV1.2 nor CaV3.1 in the LA was affected by MCT treatment.

Discussion

Major Findings

The present study was carried out to investigate electrical and molecular remodeling of L- and T-type Ca\(^{2+}\) channels in atrial cells under MCT-induced PH, and the following results were obtained.

1. MCT-treatment resulted in marked RA hypertrophy that was evident on anatomical, hemodynamic and ECG measurements.

2. The I\(_{Ca,T}\) density was significantly decreased in RA cells of MCT-treated rats, accompanied by a positive shift of steady-state activation and delayed inactivation.

3. RT-PCR analysis revealed a significant reduction in mRNA expression of the L-type Ca\(^{2+}\) channel \(\alpha\)t (CaV1.2) subunit and accessory \(\beta\)t subunit, and an increase in the \(\beta\)s subunit in MCT-treated cells.

4. On the other hand, the low voltage-activated Ca\(^{2+}\) current was more marked in RA cells of MCT-treated rats than those of control rats. The current was clearly decreased by removing external Ca\(^{2+}\), confirming that it was carried by Ca\(^{2+}\). The I-V relationship and the time course of inactivation resembled closely those of I\(_{Ca,T}\). However, the low voltage-activated Ca\(^{2+}\) current was only slightly inhibited by 10–100 \(\mu\)mol/L Ni\(^{2+}\), 1 \(\mu\)mol/L efonidipine, or additional application of 50 \(\mu\)mol/L TTX.

5. No significant difference was observed in the mRNA expression of \(\alpha\)G and \(\alpha\)II, or the protein level of \(\alpha\)G subunit.

All these findings indicate that PH causes RA hypertrophy, associated with alteration of the electrophysiological molecular properties of the Ca\(^{2+}\) channels in RA cells.

I\(_{Ca,T}\) Remodeling in MCT-Treated Rat Atria

The reduced I\(_{Ca,T}\) density was paralleled by decreased channel expression of the pore-forming CaV1.2 subunit, which suggests that transcriptionally mediated I\(_{Ca,T}\) down-regulation occurs in RA cells in MCT-induced PH. In addition, the reduction in \(\beta\)t subunit expression would facilitate I\(_{Ca,T}\) downregulation by reducing the number of functional channels in the cell membrane. However, the positive shift of activation and delayed inactivation cannot be explained entirely by this hypothesis. One possible explanation for the mechanism is impaired channel phosphorylation in RA cells in MCT-induced PH. It is well known that diseased hearts are associated with activation or inhibition of various types of protein kinases and/or phosphatases, and it was demonstrated that cAMP-mediated phosphorylation of the L-type Ca\(^{2+}\) channel induces a negative shift in the voltage dependence of I\(_{Ca,L}\) activation and delayed inactivation. Moreover, it has been reported that basal cAMP-dependent phosphorylation of the L-type Ca\(^{2+}\) channels is impaired in CHF. Interestingly, our data showed that \(\beta\)t mRNA was decreased, but \(\beta\)s mRNA was increased, in hypertrophied RA cells, in contrast to a previous study in which the levels of mRNA expression for both the \(\beta\)t and \(\beta\)s subunits were equally decreased in a rapid atrial pacing animal model. The function of auxiliary \(\beta\) subunits of the L-type Ca\(^{2+}\) channel is not only to promote the membrane expression of CaV1.2 subunits, but also to regulate inactivation kinetics. Therefore, it is likely that alternation of the \(\beta\)t and \(\beta\)s subunits influences the inactivation kinetics in hypertrophied RA cells.

T-Type Ca Channel Current Reappears in MCT-Treated Cells

I\(_{Ca,T}\) has been shown to reappear in ventricular cardiomyocytes in some pathologic processes in animal models, such as ventricular hypertrophy and myocardial infarction. The increase in I\(_{Ca,T}\) density observed in the hypertrophied RA cells in the present study might be in line with those findings. We also found that I\(_{Ca,T}\) was not inhibited by approximately 100 \(\mu\)mol/L Ni\(^{2+}\), which contradicts previous reports that cardiac I\(_{Ca,T}\) is completely blocked by a low Ni\(^{2+}\) concentration (40 \(\mu\)mol/L). Although a previous report described postnatal evolution of I\(_{Ca,T}\) density in rat atrial cardiomyocytes and demonstrated that rat neonatal I\(_{Ca,T}\) is poorly sensitive to Ni\(^{2+}\) (IC\(_{50}\), 160 \(\mu\)mol/L). It was also noted that the I\(_{Ca,T}\) density in adult rat RA cells in their study was comparable to that of the control cells in the present study. It is interesting to note that the CaV3.2- and CaV3.1-related currents, both of which are responsible for cardiac I\(_{Ca,T}\), differ strongly in their Ni\(^{2+}\) sensitivity (IC\(_{50}\), 13 \(\mu\)mol/L and >150 \(\mu\)mol/L, respectively). In this respect, the Ni\(^{2+}\) sensitivities of neonatal and hypertrophied RA cells are closer to that of CaV3.1-related currents.

Takebayashi et al have reported that T-type channels are abundantly expressed in hypertrophied RV cardiomyocytes, and they have also demonstrated that the CaV3.2-subunit is a central mechanism for the increased I\(_{Ca,T}\) density in hypertrophied RV cells. In contrast, post-transcriptional activation of the CaV3.1 isoform may play a major role during RA hypertrophy, because in the present study the mRNA levels for CaV3.1 and CaV3.2 and the protein level of the CaV3.1 subunit were not increased in hypertrophied RA cells. It has been shown that not only native I\(_{Ca,T}\), but also recombinant I\(_{Ca,T}\), is modulated by various intracellular and extracellular substances. It is possible that unidentified factors or subunits may control T-type gene expression or function during hypertrophy.

It is often proposed that T-type Ca\(^{2+}\) channels are involved in pacemaking activity. In the diseased heart, an increase in the inward current at negative potentials via re-expressed I\(_{Ca,T}\) may act as an abnormal pacemaker current, and the resultant intracellular Ca\(^{2+}\) overload may facilitate arrhythmogenesis. In fact, it has been shown that mibefradil protects against atrial remodeling in a dog model of AF. It is likely that reappearance of I\(_{Ca,T}\) in hypertrophied RA cells...
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contrtributes to the arrhythmogenesis under PH. In addition, the increase in Ca²⁺ influx through T-type Ca²⁺ channels may contribute to the development of hypertrophy of atrial cells, in a way similar to ventricular cells. Further studies are clearly necessary to elucidate the functional role of T-type Ca²⁺ channels in electrical and anatomical remodeling of atrial myocytes.

Possible Mechanisms of MCT Action

Because both downregulation of Ical and reappearance of ÏCaT were observed in hypertrophied RA cells, but not in LA cells, the primary effects of MCT appear to be hemodynamic. On the other hand, there is increasing evidence that oxidative stress contributes to the pathogenesis of anatomic and electrical remodeling. Farahmand et al have shown that in the early stages of MCT-induced PH, RV hypertrophy is accompanied by an increase in the antioxidant reserve. In addition, atrial electrical remodeling in AF has been shown to result from increased oxidant production and electrical remodeling in dogs subjected to rapid atrial pacing can be prevented by agents that decrease oxidant production. Taken together, it is possible that increased hemodynamic wall stress resulting from MCT treatment increases oxidant production in RA cells, which in turn leads to the down-regulation of Ical and reappearance of ÏCaT, along with RA hypertrophy.

We conclude that experimental PH with MCT results in RA overload and causes discrete changes in the electrophysiological properties of Ical and ÏCaT in RA cardiomyocytes.

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