Pacing-induced heart failure causes a reduction of delayed rectifier potassium currents along with decreases in calcium and transient outward currents in rabbit ventricle

Yukiomi Tsuji\textsuperscript{a}, Tobias Opthof\textsuperscript{b}, Kaichiro Kamiya\textsuperscript{a}, Kenji Yasui\textsuperscript{a}, Weiran Liu\textsuperscript{a}, Zhibo Lu\textsuperscript{a}, Itsuo Kodama\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}Department of Circulation, Division of Regulation of Organ Function, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

\textsuperscript{b}Department of Medical Physiology, Utrecht University Medical Center, Utrecht, The Netherlands

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Abstract

**Objective:** Heart failure in patients and in animal models is associated with action potential prolongation of the ventricular myocytes. Changes in several membrane currents have been already demonstrated to underlie this prolongation. However, information on the two components ($I_{K_s}$ and $I_{K_t}$) of the delayed rectifier potassium current ($I_{K}$) in rapid pacing induced heart failure is lacking. **Methods and results:** Action potentials and whole-cell currents, $I_{K_s}$, $I_{K_t}$, $I_{K}$, and $I_{Ca}$ were recorded in apical myocytes of left ventricle from 10 rabbits subjected to left ventricular pacing at 350–380 beats/min for 3–4 weeks and 10 controls with sham operation. Action potential duration at 90\% repolarization (APD\textsubscript{90}) was prolonged in myocytes from failing hearts compared to controls at both cycle lengths of 333 and 1000 ms. Both E-4031-sensitive and -resistant components of $I_{K}$ ($I_{K_s}$, $I_{K_t}$) in myocytes from failing hearts were significantly less than those of control hearts; tail current densities of $I_{K_s}$ and $I_{K_t}$ following depolarization to +50 mV were 0.62±0.05 vs. 0.96±0.12 pA/pF ($P<0.05$), and 0.27±0.08 vs. 0.52±0.08 pA/pF ($P<0.05$), respectively. There was no significant difference between control and failing myocytes in the voltage- and time-dependence of activation of total $I_{K}$, $I_{K_s}$, and $I_{K_t}$. The peak of L-type Ca\textsuperscript{2+} current ($I_{Ca,L}$) was significantly reduced in myocytes from failing hearts (at +10 mV, −9.29±0.52 vs. −12.82±1.63 pA/pF, $P<0.05$), as was the Ca\textsuperscript{2+}-independent transient outward current ($I_{to}$; at +40 mV, 4.8±0.9 vs. 9.6±1.3 pA/pF, $P<0.05$). Steady state I–V curve for $I_{K}$ was similar in myocytes from failing and control hearts. **Conclusions:** Decrease of $I_{K}$ (both $I_{K_s}$ and $I_{K_t}$) in addition to reduced $I_{Ca,L}$, may underly action potential prolongation at physiological cycle length and thereby contribute to arrhythmogenesis in heart failure. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Arrhythmia (mechanisms); Heart failure; Membrane currents; K-channel

This article is referred to in the Editorial by S. Nattel (pages 188–190) in this issue.

1. Introduction

Congestive heart failure carries a grim prognosis. Five year survival is well below 40\% [1] with half of deaths classified as sudden [2,3]. These deaths are probably caused by ventricular arrhythmias or by electromechanical dissociation [4–6]. Electrophysiological remodeling is associated with the progression of hypertrophy and heart failure [7]. Heart failure in patients and in several animal models is associated with action potential prolongation of the ventricular myocytes [8–10], albeit that this prolongation is less prominent at physiological cycle length [9]. Action potential prolongation in itself may be expected to decrease the propensity to (reentrant) arrhythmias [11]. However, this no longer holds when the prolongation becomes excessive, thereby leading to early afterdepolarization.
zations [12], or when the prolongation is regional and thereby increases dispersion in action potential duration and refractoriness [13].

One of the available models of heart failure [9,14–16], the rapid pacing model, shows severe reduction in cardiac output, based on impaired contractile function, and increase of systemic vascular resistance, left ventricular wall stress, and of several neurohumoral factors [15,16]. Rapid pacing of the ventricles leads to heart failure within 2–3 weeks in rabbits [17,18], and within 3–4 weeks in pigs [19] and dogs [20,21]. Unfortunately, action potential prolongation associated with heart failure is often assessed at unphysiological long cycle length [18,20,21] and absent at physiological cycle length [18].

Changes in membrane currents underlying prolongation of the ventricular action potential are incompletely understood. The transient outward K+ current (I_{to}) is downregulated by some 65% in rabbit [18] and in dog [21] and also substantially in myocytes from patients [22] with end-stage heart failure (review [23]). The L-type inward Ca2+ current (I_{Ca,L}) was found to be unchanged in rabbit [18] and in dog [21], whereas a reduction by 40% was demonstrated in the pig [19]. In all three species the responsiveness of I_{Ca,L} to β-adrenoceptor stimulation was blunted by 40% or more [18,19,21]. By and large, there is a tendency to a decrease in I_{Ca,L} in the more severe stages of hypertrophy and heart failure (reviews [7,8]). Measurement of membrane currents at room temperature and of action potentials at physiological temperature [19,21] hampers understanding of electrophysiological changes relevant for arrhythmias in heart failure.

The delayed rectifier (I_K), relevant for repolarization [24] and first described by Noble and Tsien [25] in 1969 and later dissected into rapid (I_{Kr}) and slowly activating components (I_{ks}) by Sanguinetti and Jurkiewicz [26], has been demonstrated in guinea-pig [26], dog [27], rabbit [28] and human ventricular myocytes [29]. Information on I_K and I_{Kr} is lacking in pacing-induced models of heart failure [18–21] and in patients with end-stage heart failure [7]. In the present study we demonstrate for the first time downregulation of both I_K and I_{Kr} by about 50% in rabbits with pacing-induced heart failure in association with action potential prolongation at physiological stimulation frequency and at physiological temperature.

2. Methods

Experiments were performed on Japanese white rabbits weighing 2.0 to 3.0 kg. All rabbits were fed and housed according to institutional guidelines at Nagoya University. This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

2.1. Animal model

Rabbits were anesthetized with a mixture (1 ml/kg im.) of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg). Supplemental anesthesia was provided by thiAmyl sodium (25 mg/kg) iv. They were intubated and ventilated artificially with room air supplemented with oxygen (tidal volume 10 ml/kg; frequency 25 /min). After opening the left fourth intercostal space, part of the pericardium was cut to expose the anterior surface of the heart. An unipolar pacing lead (Medtronic, USA: 6491) fixed to the anterobasal region of the left ventricular free wall was connected to a pacemaker (Medtronic, USA: 5985), which was implanted subcutaneously in the back. The chest was closed in layers. Post-operatively, cefotiam hydrochloride (10 mg/kg) was given for 5–7 days. Two weeks after the operation, i.e. following full recovery from surgery, the left ventricle of each rabbit was paced at 350–380 beats/min, and pacing was continued for 3–4 weeks. The pacemaker implantation was carried out in a total of 26 rabbits; 16 were paced to induce heart failure, the remaining 10 were unpaced as controls. All 16 paced rabbits showed physical signs of heart failure 3–4 weeks after the initiation of pacing. Six rabbits died during the observation period probably due to serious pump failure. The remaining 10 rabbits were sacrificed for electrophysiological experiments. All 10 control rabbits survived the observation period.

To monitor the progress of heart failure during the observation period, two-dimensional echocardiography was carried out periodically together with recordings of scalar electrocardiograms (ECGs). The recordings were performed under anesthesia by a mixture (1 ml/kg im.) of ketamine hydrochloride (30 mg/kg) and xylazine (5 mg/kg). The pacing was turned off transiently for the recordings in sinus rhythm. Electrophysiological experiments in single ventricular myocytes from paced hearts were carried out as soon as fractional shortening (FS) was <22%.

2.2. Electrophysiological experiments

Myocytes were isolated enzymatically from the apex of the left ventricle by the same procedure as recently described [28]. In brief, the hearts were perfused on a Langendorff apparatus with normal Tyrode’s solution (gassed with 100% O2 at 34°C) for 3–5 min, then with Ca2+-free Tyrode’s solution for 10–15 min and finally with 0.12 mg/ml collagenase (Yakult, Japan) containing Ca2+-free solution for 15 min. The hearts were subsequently washed with high-K+ storage solution (Kraftbrühe solution: KB solution) for 5 min. The apical region of the left ventricle was separated and minced in the KB solution to obtain a suspension of myocytes. The standard whole-cell patch-clamp method was used for recording the membrane potential and currents.

An aliquot of the cell suspension was placed in a
recording chamber on the stage of an inverted microscope (Diaphoto, Nikon Co., Tokyo). The cells were superfused with normal Tyrode’s solution at 3 ml/min. The bath temperature was maintained at 34°C. Suction pipettes of borosilicate glass had a resistance of 3–5 MΩ after filling with the pipette solution. Junction potential of the pipette was 4–10 mV (5.6 mV in average). Resting membrane potential recorded by the current clamp mode was corrected for the value. The cell capacitance was measured by small voltage steps from a holding potential of −50 mV. The cell capacitance and series resistance ($R_s$) were compensated for by about 50–70%, giving rise to the values of $R_s$ at 2–5 MΩ (4.2 MΩ in average) and the time constant of capacitive surge at 450–500 μs (479 μs in average). Command potentials generation and data acquisition were performed with a patch-clamp amplifier (Axopatch 200B) controlled by pCLAMP software (Axon Instruments) and a PC. Action potentials were recorded in current-clamp mode and were elicited by injecting 5 ms long rectangular pulses of depolarizing current through the pipette. Voltage signals were displayed on an oscilloscope. Current signals were filtered at 1 kHz, and digitized at a sampling frequency of 2 kHz.

When the delayed rectifier $K^+$ current ($I_{K1}$) was measured, the myocytes were superfused with Na$^+$-free, K$^+$-free solution including $N$-methyl-$d$-glucamine (NMG) to eliminate relatively large inward rectifier $K^+$ current ($I_{K1}$). It has been proposed that the rapidly activating component of $I_K$ ($I_{Kr}$) is greatly suppressed in extracellular K$^+$-free condition in some animal species such as guinea pigs and dogs [27,30]. However, in our previous study [28] on $I_{Kr}$ of rabbit ventricular myocytes, we showed that although the current density during depolarization in NMG solution was significantly larger than in Tyrode’s solution, there was no significant difference in the tail current density between the two solutions. The two components of $I_K$, $I_{Kr}$, and $I_{K1}$, were pharmacologically separated by the application of a selective blocker, E-4031 (5 μM). The E-4031-sensitive component was obtained by digital subtraction of the E-4031-resistant component from the total $I_K$ current. The E-4031-sensitive and -resistant components were measured as indexes of $I_{Kr}$ and $I_{K1}$, respectively. The E-4031-resistant component was confirmed as $I_{K1}$ by elimination with 30 μM chromanol-293B, as has been shown by Cheng et al [28] in rabbit ventricular myocytes. $I_{Ca-L}$ and $I_{K1}$ were measured in Tyrode’s solution including 3 μM nisoldipine for blockade of $I_{Ca-L}$. In experiments to measure $I_{Ca-L}$, KCl in the external (Tyrode) solution and pipette solution was replaced by CsCl to avoid the contamination of $K^+$ currents. For comparison of currents derived from control and failing hearts, current amplitudes were normalized to the cell capacitance.

### 2.3. Solutions

Tyrode’s solution for cell isolation and the recording of action potentials was composed of (in mM): NaCl, 143; KCl, 5.4; CaCl$_2$, 1.8; MgCl$_2$, 0.5; Na$_2$HPO$_4$, 0.25; HEPES, 5.0; and glucose, 5.6, pH adjusted to 7.4 with NaOH. The Ca$^{2+}$-free Tyrode’s solution was as above without CaCl$_2$. The KB solution contained (in mM): L-glutamic acid, 50; KCl, 40; KH$_2$PO$_4$, 20; taurine, 20; HEPES, 10; MgCl$_2$, 3; glucose, 10; EGTA, 0.5, pH adjusted to 7.4 with KOH. The Na$^+$-free, K$^+$-free NMG solution contained (in mM): $N$-methyl-$d$-glucamine, 149; MgCl$_2$, 5; CaCl$_2$, 0.65; HEPES, 5 and nisoldipine, 0.003, pH adjusted to 7.4 with HCl. The internal pipette solution contained (in mM): KOH, 50; KCl, 80; aspartate, 40; HEPES, 5; EGTA, 10; MgATP, 5; sodium creatinine phosphate, 5; and CaCl$_2$, 0.65 (pH 7.2, pCa 8.0). The pipette solution for the measurement of $I_{Ca-L}$ contained (in mM): CsCl, 80; CsOH, 60; aspartate, 40; HEPES, 5; EGTA, 10; MgATP, 5; sodium creatinine phosphate, 5; CaCl$_2$, 0.65 (pH 7.2, pCa 8.0).

### 2.4. Statistics

The pCLAMP program (Axon Instruments) was used in data analysis. Data were expressed as mean±SEM. Statistical analysis was performed using unpaired Student’s $t$-test. Values of $P<0.05$ were considered significant.

### 3. Results

#### 3.1. Characteristics of heart failure

Long-term (3–4 weeks) rapid ventricular pacing decreased fractional shortening, increased end-systolic and end-diastolic diameter, and left atrial diameter. It reduced the wall thickness of the interventricular septum and the posterior wall of the left ventricle (Table 1). Table 2 summarizes the ECG data obtained from the failing and control rabbits. The QT interval was corrected (QTc) according to the method of Carlsson et al [31] for rabbits with the formula QTc = QT − 0.175(RR-300). In the failing rabbits 3–4 weeks after pacing, the QT interval and the QTc interval were significantly prolonged by 9.2% and

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 10)</th>
<th>Paced (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>FS (%)</td>
<td>59.1±0.1</td>
<td>20.4±0.1**</td>
</tr>
<tr>
<td>LVED diameter (mm)</td>
<td>14.5±0.2</td>
<td>17.0±0.6**</td>
</tr>
<tr>
<td>LVES diameter (mm)</td>
<td>8.3±0.3</td>
<td>14.7±0.8**</td>
</tr>
<tr>
<td>IVS (mm)</td>
<td>2.9±0.1</td>
<td>2.3±0.1**</td>
</tr>
<tr>
<td>PW (mm)</td>
<td>3.0±0.1</td>
<td>2.3±0.2**</td>
</tr>
<tr>
<td>LA diameter (mm)</td>
<td>9.0±0.3</td>
<td>13.8±0.3**</td>
</tr>
</tbody>
</table>

*Values are mean±S.E.M. FS = fractional shortening; LVED = left ventricular end-diastolic; LVES = left ventricular end systolic; IVS = interventricular septum; PW = posterior wall of left ventricle; LA = left atrium; $n =$ number of rabbits.  
** $P<0.01$ denotes significant difference from control.
Table 2
ECG data of paced and control rabbits

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After 3–4 weeks</th>
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<tbody>
<tr>
<td><strong>Paced rabbits</strong> (<strong>n</strong>=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>215±13</td>
<td>225±11</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>40.3±1.0</td>
<td>40.2±0.7</td>
</tr>
<tr>
<td>QTc interval (ms)</td>
<td>140.7±3.7</td>
<td>153.7±3.6</td>
</tr>
<tr>
<td>QT interval (ms)</td>
<td>145.8±3.6</td>
<td>155.7±3.3</td>
</tr>
<tr>
<td><strong>Control</strong> (<strong>n</strong>=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>205±17</td>
<td>202±10</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>40.1±0.5</td>
<td>40.0±0.5</td>
</tr>
<tr>
<td>QTc interval (ms)</td>
<td>141.3±3.6</td>
<td>141.4±2.9</td>
</tr>
<tr>
<td>QT interval (ms)</td>
<td>146.8±3.5</td>
<td>146.6±3.1</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M. QTc = corrected QT interval; n = number of rabbits.

6.8%, respectively from the baselines before pacing. QRS duration was unchanged after pacing. Heart rate (HR) after pacing tended to be increased, but the difference from the baseline did not reach statistical significance. For comparison, the control rabbits showed no changes in the measured ECG parameters after the corresponding observation period. All failing rabbits had considerable amounts of ascites and pleural effusion. They showed increased heart, lung and liver weight both absolute and relative to body weight (Table 3).

3.2. Action potential changes in heart failure

Action potentials in myocytes from failing hearts differed in shape and duration compared with control hearts. Fig. 1A shows traces at 333 ms and 1000 ms cycle length (CL). Control myocytes typically have a prominent notch in phase 1 at 333 ms and 1000 ms, which is markedly attenuated in myocytes from failing hearts. Action potential duration (APD90), measured at 90% of repolarization, was prolonged in myocytes from failing hearts compared with control hearts at both 333 and 1000 ms (Fig 1A, Table 4). Fig. 1B shows the relationship between CL and APD90. With increasing CLs, the difference in APD90 between normal and failing myocytes became more pronounced. Action potential duration, measured at 50% repolarization, resting membrane potential and action potential amplitude did not differ between the two groups.

The cell capacitance – an estimate for cell surface – was comparable in myocytes isolated from control (165±6.6 pF, **n**=71) and failing (170±8.9 pF, **n**=58) hearts.

3.3. Delayed rectifier in control and failing myocytes

We studied the two components of **I**<sub>k</sub> in control and failing hearts. At first, total **I**<sub>k</sub> was activated by applying voltage clamp steps for 1.5 s at 0.1 Hz from a holding potential of −50 mV to a range of depolarizing levels between −40 mV and +50 mV. E-4031 (5 μM) was then applied to see the E-4031-resistant component (**I**<sub>Kr</sub>). The measurements of **I**<sub>k</sub> were carried out twice at 10 and 15 min after application of E-4031 to monitor time-dependent
rundown of $I_{Ks}$. Data obtained from myocytes showing $I_{Ks}$ reduction $>10\%$ from 10 to 15 min were not included in the results. The E-4031-sensitive component was obtained as an index of $I_{Kr}$ by digital subtraction of total $I_{K}$ and $I_{Ks}$ estimated 10 min after application of E-4031.

Representative tracings are shown in a control and a failing myocyte in Fig. 2. In the failing myocyte, amplitudes of the time-dependent outward current during depolarization ($I_{Kstep}$) and the tail current on repolarization ($I_{Ktail}$) of total $I_{K}$ were smaller than those in the control myocyte. The corresponding amplitude of the E-4031-resistant component ($I_{Kr}$) and those of the E-4031-sensitive component ($I_{Kr}$) of the failing myocyte were also smaller than the control myocyte. The E-4031-sensitive current had relatively large $I_{Ktail}$ compared with $I_{Kstep}$.

Fig. 3A shows the current–voltage ($I$–$V$) relationship of $I_{Kstep}$ (upper panel) and $I_{Ktail}$ (lower panel) of total $I_{K}$ in control and failing myocytes. The current amplitude was normalized to cell capacitance and plotted as a function of test potentials. Densities of $I_{Kstep}$ and $I_{Ktail}$ in failing myocytes were both significantly lower than those in control myocytes from +10 mV to more positive potentials (at +50 mV, 0.67±0.14 vs. 1.34±0.24 pA/pF for $I_{Kstep}$; 0.87±0.12 vs. 1.47±0.20 pA/pF for $I_{Ktail}$). Fig. 3B illustrates the $I$–$V$ relationship of $I_{Kstep}$ and $I_{Ktail}$ of the E-4031-resistant component ($I_{Kr}$). Densities of $I_{Kstep}$ and $I_{Ktail}$ in failing myocytes were significantly lower than those in control myocytes at +30 mV to more positive potentials (at +50 mV, 0.47±0.11 vs. 0.81±0.13 pA/pF for $I_{Kstep}$; 0.27±0.08 vs. 0.52±0.08 pA/pF for $I_{Ktail}$). Fig. 3C illustrates the $I$–$V$ relationship of $I_{Kstep}$ and $I_{Ktail}$ of the E-4031-sensitive component ($I_{Kr}$). Densities of $I_{Kstep}$ and $I_{Ktail}$ in failing myocytes were significantly lower than those in control myocytes at +20 mV to more positive potentials (at +50 mV, 0.20±0.08 vs. 0.53±0.10 pA/pF for $I_{Kstep}$; 0.62±0.07 vs. 0.96±0.12 pA/pF for $I_{Ktail}$).

Voltage-dependence of activation was determined by normalizing $I_{Ktail}$ at each test potential to the current at the most positive test potential, and analyzed by Boltzmann fits. Half activation voltage of total $I_{K}$ was $-7.6±3.5$ mV in failing myocytes ($n=19$), and $-6.3±2.3$ mV in controls ($n=14$) (n.s.). Corresponding slope factors were 11.8±1.9 mV in failing myocytes, and 13.7±1.0 mV in controls (n.s.). Half activation voltage of the E-4031-resistant component ($I_{Kr}$) was $0.2±0.2$ mV in the failing myocytes, and $1.2±1.9$ mV in the controls (n.s.). Corresponding slope factors were 14.3±2.2 mV in the failing myocytes, and 12.4±1.5 mV in the controls (n.s.). Half activation voltage

| Table 4 |
| Action potential characteristics of myocytes from control and failing hearts* |
| CL (ms) | Control ($n=20$ cells from 6 rabbits) | |
| | APD$_{50}$ (ms) | APD$_{90}$ (ms) | RMP (mV) | APA (mV) | NA (mV) |
| 333 | 138.5±5.8 | 169.8±6.5 | −78.7±1.4 | 125.2±1.8 | 8.0±1.2 |
| 1000 | 193.2±5.4 | 225.3±6.3 | −79.2±0.9 | 127.2±0.7 | 9.2±0.7 |
| CL (ms) | Heart failure ($n=25$ cells from 8 rabbits) | |
| | APD$_{50}$ (ms) | APD$_{90}$ (ms) | RMP (mV) | APA (mV) | NA (mV) |
| 333 | 158.6±12.5 | 201.2±13.5* | −79.7±1.1 | 126.5±2.4 | − |
| 1000 | 212.3±16.3 | 259.6±18.8* | −78.8±1.4 | 128.0±1.3 | − |

*Values are mean±S.E.M. CL = cycle length; RMP = resting membrane potential; APA = action potential amplitude; NA = notch amplitude; $n$ = number of rabbits.

P<0.05 denotes significant difference from control.
of the E-4031-sensitive component ($I_{Ks}$) was $-9.4 \pm 3.9$ mV in the failing myocytes, and $-7.1 \pm 1.5$ mV in the controls (n.s.). Corresponding slope factors were $8.9 \pm 2.1$ mV in the failing myocytes, and $11.7 \pm 1.5$ mV in the controls (n.s.).

The activation of the E-4031-resistant component ($I_{Kr}$) at $+50$ mV was approximated by a single exponential function with a time constant of $495 \pm 64$ ms in control ($n=6$) and $513 \pm 50$ ms in failing myocytes ($n=6$, n.s.). Deactivation of $I_{Ks}$ tail following the depolarization to $+50$ mV was fitted by a dual exponential function. The fast and slow time constants for the deactivation at $-50$ mV in failing myocytes ($200 \pm 60$ and $1100 \pm 180$ ms, $n=6$) were similar to those in control myocytes ($185 \pm 45$ and $952 \pm 189$ ms, $n=6$; both n.s.). The activation of the E-4031-sensitive component ($I_{Kr}$) at $0$ mV was approximated by a single exponential function with a time constant of $401 \pm 62$ ms in control ($n=6$) and $385 \pm 42$ ms in failing myocytes ($n=6$, n.s.). Deactivation of $I_{Kr}$ tail following the depolarization to $+50$ mV was fitted by a dual exponential function. The fast and slow time constants for the deactivation at $-50$ mV in failing myocytes ($90 \pm 28$ and $555 \pm 110$ ms, $n=6$) were significantly longer than those in control myocytes ($45 \pm 11$ and $350 \pm 74$ ms, $n=6$; both $P<0.05$).

3.4. $L$-type $Ca^{2+}$ current ($I_{Ca-L}$)

$I_{Ca-L}$ was examined by application of voltage-clamp steps for $200$ ms from a holding potential of $-50$ mV to different depolarizing levels up to $+40$ mV at $0.1$ Hz. Fig. 4 shows representative traces of $I_{Ca-L}$ (upper panel) and the current-voltage relationship of the peak $I_{Ca-L}$ density in the control and the failing myocytes (lower panel). $I_{Ca-L}$ density from $-20$ mV to $+20$ mV was significantly decreased in the failing myocytes.

3.5. Transient outward current ($I_{to-t}$) and inward rectifier $K^+$ current ($I_{Kr}$)

Whole-cell currents were elicited by application of $300$ ms hyperpolarizing or depolarizing voltage steps between $-100$ mV and $+40$ mV from a holding potential of $-50$ mV to inactivate $Na^+$ channel at $0.1$ Hz. $I_{to-t}$ was measured as the difference between the peak outward current and the sustained current at the end of the depolarizing pulse. Fig. 5 shows representative traces (upper panel) of $I_{to-t}$ and the current-voltage relationship (lower panel) of $I_{to-t}$ density in control and failing myocytes. $I_{to-t}$ density was significantly decreased in failing myocytes from $-10$ mV to more positive voltage and $I_{to-t}$ was reduced by about $50\%$ at all
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Fig. 4. I_{Ca-L} is decreased in heart failure. I_{Ca-L} was measured in Tyrode’s solution, in which KCl was replaced by cesium chloride. It was elicited by applying depolarizing potentials from a holding potential of −50 mV to various levels ranging from −40 to +40 mV for 200 ms. Upper panel: representative current tracing of I_{Ca-L} in a control (left) and a failing myocyte (right). Lower panel: the average current-voltage relation of I_{Ca-L} plotted for failing myocytes (●, n = 10 cells from 8 rabbits) and control (○, n = 8 cells from 6 rabbits). *P < 0.05 vs. control.

Fig. 5. I_{to1} is decreased in heart failure. I_{to1} was measured in Tyrode’s solution, with 3 μM nisoldipine for blockade of I_{Ca-L}. It was elicited by applying depolarizing potentials from a holding potential of −50 mV to various levels ranging from −40 to +40 mV for 300 ms. Upper panel: representative current tracing of I_{to1} in a control (left) and a failing myocyte (right). Lower panel: the average current-voltage relation of I_{to1} plotted for failing myocytes (●, n = 10 cells from 8 rabbits) and control (○, n = 11 cells from 6 rabbits). *P < 0.05 vs. control.

voltages. Time course of I_{to1} inactivation was similar for control and failing myocytes.

To elicit I_{K1}, 300 ms hyperpolarizing or depolarizing voltage steps between −100 and −20 mV were applied from a holding potential of −40 mV at 0.1 Hz (Fig. 6). The steady-state currents at the end of the clamp pulses were measured as an index of I_{K1}. There was no significant difference in the density of I_{K1} between control and failing myocytes.

4. Discussion

In the present study of pacing-induced heart failure in rabbits, we observed a substantial reduction in I_{K} density. We also observed a decrease in I_{to1} and I_{Ca-L} densities, but noted unchanged I_{K1} density. Both E-4031-resistant and -sensitive components of I_{K} (I_{Ks} and I_{Kr}) were reduced in failing myocytes compared with control myocytes. The changes in membrane currents were assessed at physiological temperature and were associated with prolonged action potentials at physiological cycle length as well as prolongation of QT and QTc intervals in ECG.

4.1. Downregulation of membrane currents in heart failure

Although a decrease in -unseparated- delayed rectifier current has been demonstrated in ventricular myocytes obtained from cats with hypertrophy resulting from pulmonary [32] and abdominal banding [33], our study is the first to show a substantial (about 50%) reduction in both components of the delayed rectifier in an animal model of heart failure based on rapid pacing. Especially the downregulation of I_{K1}, which constitutes a current with marked regional dispersion even in normal hearts (see below), may contribute to increased dispersion in action potential...
were applied from a holding potential of 2 mV our study and another study in the rabbit of Rozanski et al. myocytes from failing canine ventricle. This suggests an rapid pacing [38]. There were three differences between `failing action potential' into a `normal action potential' in that this downregulation starts as early as after 1 week of -mimicking the effect of activation of $I_{Ks}$ with those in the pig [19]. Recently, it was demonstrated directly following the upstroke of an action potential decreased $I_{Ks}$ shown a significant reduction of $I_{Ks}$ the right ventricle [36]. In atrial myocytes isolated from made until more data are available.

density along with converts a `normal action potential' into a `failing action potential' (reviews [7,8]).

4.2. Physiological implications

Both delayed rectifier currents have been described in dog ventricle [27,39], in rabbit ventricle [28], in guinea pig ventricle [40] and in human ventricle [29]. $I_{Kr}$ has also been demonstrated at the single channel level in rabbit ventricle [41]. Interestingly, $I_{Kr}$ distribution in the ventricle is regionally different and may contribute to the dispersion of repolarization [42]. First, transmural left ventricular differences in density of $I_{Kr}$ have been demonstrated in dog with a weaker $I_{Kr}$ in M-cells than in subendocardial and subepicardial cells [27] and in guinea pig with lower density in subendocardial compared to subepicardial cells [40]. Secondly, $I_{Kr}$ is larger at the base than at the apex of the rabbit ventricle whereas $I_{Kr}$ is larger at the apex than at the base [28]. Thirdly, $I_{Kr}$ is about two times larger in right than left canine ventricular midmyocardial cells [39].

Animal studies are still very useful, because the picture in human studies is far from clear. In ventricular myocytes from normal donor hearts not used for transplantation, Beuckelmann et al. [22] were unable to show delayed rectifier current. Iost et al. [43] described $I_{Kr}$, but no $I_{Ks}$ in normal human ventricular myocytes. The occurrence of $I_{Ks}$ and $I_{Kr}$ was described in myocytes from apparently normal right ventricles which were obtained from failing explanted hearts [30]. Whether or not these hearts may be considered normal is crucial for conclusions, because, Beuckelmann et al. [22] did show some delayed rectifier current in about half of myocytes from failing hearts. In cells from hearts with end-stage dilated and ischemic cardiomyopathy, $I_{Kr}$ but no $I_{Ks}$ could be demonstrated at the single channel level [44]. This may be due to much smaller single channel conductance of $I_{Ks}$ [45]. Because delayed rectifier current is very sensitive to cell isolation artifacts [46], definite conclusions with respect to heart failure in man cannot be made until more data are available.

Kääb et al. [21] showed previously that inhibition of $I_{Ca-L}$ converts a ‘normal action potential’ into a ‘failing action potential’ in myocytes from normal canine ventricle, whereas current injection during a brief 8 ms period directly following the upstroke of an action potential -mimicking the effect of activation of $I_{Ca-L}$ -reverts a ‘failing action potential’ into a ‘normal action potential’ in myocytes from failing canine ventricle. This suggests an
important role for $I_{o1}$ in action potential changes during the progression of heart failure. Indeed, downregulation of $I_{o1}$ appears to present a common factor in all animal models of heart failure and also in end-stage heart failure in patients [7,8–23,27,29,35]. However, these observations are often made at very long cycle length and $I_{o1}$ is strongly rate dependent. At high heart rates it may fail to recover from inactivation almost completely [47] and its behavior is very much different at steady state cycle length and after sudden changes in cycle length [48]. Recovery from inactivation is actually so slow that in rabbit atrium a transition of 0.1 to 4 Hz will leave peak $I_{o1}$ current intact for only 3% [49]. We wish also to emphasize that Kääb et al. [21] noticed that despite the substantial downregulation of $I_{o1}$ in myocytes from canine ventricle, these cells were more sensitive to 4-aminopyridine, a blocker of $I_{o1}$, than myocytes from normal hearts. This may be explained if the alternative currents for final repolarization in heart failure, $I_{k1}$ and $I_{k2}$, are very small in heart failure.

4.3. Limitations

In rabbits, unlike guinea pigs and dogs, activation time constants of $I_{k1}$ and $I_{k2}$ are in a similar range (300–600 ms) [28]. Accordingly, it is difficult to discriminate the two components using a protocol of different pulse duration. Therefore, we used E-4031 to separate the two components; E-4031-resistant and -sensitive components were measured as indexes of $I_{k1}$ and $I_{k2}$, respectively. Validity of this method depends on the stability of $I_{k1}$ and $I_{k2}$ during the 10 min exposure to E-4031. If $I_{k2}$ is running down substantially over the time, the E-4031-sensitive component would include not only real $I_{k1}$ but also the rundown of $I_{k2}$. We cannot completely rule out this possibility, but it is unlikely for the following reason: $I_{k2}$ (E-4031-resistant component) was measured twice, 10 and 15 min after application of E-4031, and the data from the myocytes showing rundown >10% were not included in the results. It was observed in our different series of experiments in rabbit ventricular myocytes that $I_{k1}$ and $I_{k2}$ estimated by E-4031-resistant and -sensitive components of $I_{k}$ using the same protocol as in the present study were virtually identical to $I_{k1}$ and $I_{k2}$ estimated by chromanol 293B (30 μM, 10 min bath application) -sensitive and -resistant components of $I_{k}$ (unpublished data).

Although one simulation study [24] emphasized the significance of $I_{k2}$ and $I_{k1}$ for final repolarization, another simulation study shows that in heart failure a mere reduction in membrane currents will not mimic action potential prolongation unless deviations for calcium handling proteins such as the SR Ca$^{2+}$-ATPase and the sarcolemmal Na$^{+}$/Ca$^{2+}$ exchanger are included in the program [50]. This may underscore the significance of altered calcium handling for action potential changes [10,11,52].

5. Conclusion

The downregulation of delayed rectifier potassium currents ($I_{k1}$ and $I_{k2}$) in heart failure, may render these currents important for action potential prolongation at physiological cycle length and for arrhythmogenesis in heart failure.

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References


[35] Lodge NJ, Normandin DE. Alterations in I_{K1}, I_{K2} and I_{Ks} density in the BIO TO-2 strain of syrian myopathic hamsters. J Mol Cell Cardiol 1997;29:3211–3221.


