Ionic basis of the action potential prolongation in ventricular myocytes from Syrian hamsters with dilated cardiomyopathy

Dominique Thuringer, Edith Deroubaix, Alain Coulembe, Edouard Coraboeuf, Jean-Jacques Mercadier *

Laboratoire de Cardiologie Moléculaire et Cellulaire, Université de Paris XI, CNRS URA 1159, Paris, France

Received 21 June 1995; accepted 29 November 1995

Abstract

Objective: The aim of our study was to determine the main electrophysiological alterations associated with cardiac dilation in MS200 strain Syrian hamsters, a model of genetically determined cardiomyopathy. Methods: Ventricular action potentials (APs) were recorded with standard microelectrodes in isolated hearts from 120-day-old cardiomyopathic (strain MS200) and age-matched control (strain CHF148) Syrian hamsters. Ionic currents were recorded from single ventricular myocytes using the whole-cell patch-clamp technique. Results: In MS200, AP was prolonged and the plateau phase was markedly increased as compared to CHF148. Differences in both AP duration and 4-aminopyridine-induced AP lengthening between epicardial and endocardial tissues were less marked in MS200 than in CHF148. Cell size and membrane capacitance were not higher in MS200 than in CHF148 myocytes, indicating the absence of cell hypertrophy in myopathic ventricles. The I-type calcium current (I_{Ca,L}) density was significantly reduced in MS200 and the voltage-dependence of both steady-state activation and inactivation was altered. The voltage-dependent outward current was composed of both transient (I_{K1}) and sustained (I_{Ks}) components, respectively sensitive and insensitive to 4-aminopyridine. I_{K1} density was strongly depressed in MS200 compared to CHF148, whereas I_{Ks} density was only slightly reduced. The conductance-voltage and steady-state inactivation relationships for I_{Ca,L} were shifted to more positive potentials in MS200. The I_{Ca,L} recovery process was markedly slower in MS200 than in CHF148. Steady-state current-voltage relationships, in the physiological voltage range, were superimposable in MS200 and CHF148. Conclusions: In ventricular myocytes from dilated heart of MS200 Syrian hamsters, I_{Ca,L} is more drastically depressed than I_{K1}. Such an observation might partially explain dilation-induced AP lengthening.

Keywords: Patch-clamp; Cardiomyopathic hamster, ventricular myocytes; Calcium channel, L-type; Membrane potential; Potassium channel, transient outward; Heart failure

1. Introduction

Syrian hamster hereditary cardiomyopathy is a reproducible experimental model which illustrates the development of the major features of heart muscle disease including hypertrophy, dilation and failure [1–8]. In several strains of cardiomyopathic Syrian hamsters such as BIO 14.6, the disease appears in three histological and clinical phases [1,6]. At 4–5 weeks of age, animals develop spotty cellular myolysis with little surrounding inflammation, but no clinical sign of congestive heart failure. After approximately 20 weeks, progressive ventricular hypertrophy ensues. At 30–40 weeks, ventricular dilation increases with time, leading to death from congestive heart failure or arrhythmia, generally within a year. In contrast, the more recent strain BIO 53–58 (or BIO TO-2 and its derivative MS200 used here) gradually develops a dilated form of cardiomyopathy at approximately 4–20 weeks of age, characterized by enlarged cardiac chambers and thin ventricular walls, without detectable hypertrophy [2]. BIO 53–58 hamsters also have a significantly shorter life-span and show reduced cardiac function at an earlier age than BIO 14.6 hamsters. Although the cause of myopathy in
BIO 53–58 (and MS200) is not fully understood, alterations to membrane proteins involved in cellular handling of calcium may be determining factors in the arrhythmogenicity of hamster hearts [9–11].

The few reported studies of the electrical properties of Syrian hamster cardiac myocyte membranes have been limited to strains developing the hypertrophic form of cardiomyopathy, such as BIO 14.6, especially in the late stages of the disease. One of the changes in the cardiac electrical properties of BIO 14.6, consistently reported in all animal models of hypertrophy [12,13], is a prolongation of the action potential [4,14]. This has been attributed to altered Ca2+-signaling mechanisms [9–11,15–17]. In ventricular myocytes from 8- to 10-month-old BIO 14.6, the L-type Ca2+ current density was found to be either unchanged [17] or reduced [11,16] compared to age-matched control hamsters, whereas an increased T-type Ca2+ current [17] and a slowly inactivating Na+-Ca2+ exchange current [16] were observed in cells from diseased animals. However, the regulation of outward K+ currents, which could also contribute to lengthening of the action potential in cardiomyopathic Syrian hamster, is unknown.

We studied alterations in the action potential and underlying changes in ionic currents (steady-state current, inward Ca2+ current, Ca2+-independent transient outward current) in two groups of hearts and myocytes isolated from 120-day-old cardiomyopathic (MS200) and normal (CHF148) Syrian hamsters, a time when MS200 are expected to develop ventricular dilation without hypertrophy. We show that, in dilated hearts from MS200, the action potential plateau was markedly increased in amplitude and duration, while in single MS200 ventricular myocytes the L-type Ca2+ current density was reduced by 37% whereas the Ca2+-independent transient outward current density was even more markedly depressed (by 71%).

2. Methods

Animals were purchased from Canadian Hybrid Farm (Halifax, Canada) and maintained on a 12 h light/dark cycle in our air-conditioned vivarium. Ten cardiomyopathic male hamsters (MS200; created by Michael Sole and derived from Bio 53–58) and ten age-matched controls (CHF148; a normal albino hamster from Canadian Hybrid Farm) were used at 120 days of age, a time when MS200 are expected to develop ventricular dilation without hypertrophy. No commonly accepted sign of congestive heart failure was observed in MS200 hamsters. The animals did not gain excessive weight through generalized fluid accumulation or subcutaneous edema, and did not exhibit fatigue nor hyperpnea. The investigation was performed in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, published by HMSO, London.

2.1. Solutions

Ca2+-free Tyrode solution was composed as follows (in mM): 136 NaCl, 5.4 KCl, 1.1 MgCl2, 5 N-(2-hydroxyethyl) piperazine-N’(2-ethanesulfonic) acid (HEPES), 20 NaHCO3, 0.4 NaH2PO4, and 20 glucose; pH was adjusted to 7.3 with NaOH. Normal Tyrode solution also contained 1.8 mM CaCl2. KB solution was composed as follows (in mM): 10 taurine, 70 K-glutamate, 25 KCl, 10 KH2PO4, 0.5 ethylene glycol-bis [β-aminoethyl-ether) N,N,N’,N’-tetraacetic acid (EGTA) and 20 glucose; pH was adjusted to 7.3 with KOH. For Ca2+ current measurements, the intracellular pipette solution contained (in mM): 110 CsCl, 20 TEA-Cl, 2 MgCl2, 15 EGTA, 10 HEPES, 5 Mg-ATP and 10 glucose; pH was adjusted to 7.3 with CsOH. When the whole-cell configuration was obtained, normal Tyrode solution was replaced by external Na+-free and K+-free solution containing (in mM): 136 TEA-Cl, 20 CsCl, 5 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose and 4 4-aminopyridine (4-AP); pH was adjusted to 7.3 with CsOH. To record steady-state and transient outward currents, the intracellular pipette solution was a nominally Ca2+-free solution containing (in mM): 115 K-aspartate, 10 KCl, 4 KH2PO4, 3 MgCl2, 10 HEPES, 5 EGTA, 5 Mg-ATP, 5 di-Tris-phosphocreatine and 10 glucose; pH was adjusted to 7.3 with KOH. The steady-state currents elicited in the voltage ramp protocol were recorded in normal Tyrode solution (also containing 0.5 CdCl2), whereas the Ca2+-independent transient outward current, Ito, was recorded in external Na+-free solution containing (in mM): 135 choline chloride, 1 MgCl2, 1.8 CaCl2, 0.5 CdCl2, 10 HEPES, 0.01 atropine sulfate, 0.001 tyanidine and 10 glucose; pH was adjusted to 7.4 with KOH and the final concentration of K+ ions was 5.4 mM. In these conditions, sarcolemal calcium current and both Na+ and Ca2+ currents were suppressed, and the contributions of putative muscarinic and Ca2+ activated K+ currents were minimized. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Cell isolation

Single myocytes were isolated by using an enzymatic dissociation procedure [11]. On the day of the study, animals were weighed, anesthetized with pentobarbital sodium (30 mg/kg i.p.; Sanofi, France), and anticoagulated with heparin sodium (400 IU/kg i.p.; Roche, France). The excised heart was quickly mounted on a Langendorff-type apparatus and perfused in retrograde fashion via the aorta for 5 min with nominally Ca2+-free Tyrode solution at a flow rate of 5–10 ml/min. Enzymatic digestion was achieved by recirculating Ca2+-free Tyrode solution containing 2 mg/ml collagenase (type A; Boehringer Mannheim Corp., Germany) and 0.2 mg/ml protease (type XIV; Pronase E; Sigma Chemical Co., St. Louis, MO) for
12 to 18 min. The heart was then washed with KB solution for 5 min. All perfusates were gassed with 95% O$_2$/5% CO$_2$ and maintained at 37°C. Ventricles were separated from atria and cut into small pieces with fine scissors. Because of the thinness of ventricular walls of MS200 hearts, we could not separate subepicardial and subendocar-dial tissue layers, and thus, epicardial and endocardial cells were not examined separately. Cells were dispersed by gentle agitation of tissue pieces and stored in KB solution at room temperature for at least 1 h before use. The cells used for capacitance and current measurements were chosen at random.

2.3. Experimental protocols

Action potential measurements were performed on isolated hearts according to the Langendorff technique with constant pressure (70 cm water, equivalent to about 7 kPa). The isolated heart was continuously perfused with normal Tyrode solution gassed with 95% O$_2$/5% CO$_2$. Transmembrane action potentials were recorded from the epicardial surface of left ventricles by means of standard floating microelectrodes (15–30 MΩ). The apex of the heart was attached to a strain gauge (Statham UC2) for tension recording. Action potentials were recorded using a M707 amplifier (WP Instruments), stored digitally on audio tape (Biologic DTR 1202 DAT Recorder, Bio-Logic, Claix, France) and further digitized with a microcomputer (Tandon, MCS 486) using an S200 interface (Cambridge Research System, Cambridge, UK). The heart was electrically driven (2- to 3-ms pulses) at a constant frequency (about 2 Hz; 10–20% higher than the spontaneous frequency) by means of dipolar silver electrodes located on the right atrium. In some cases, an L incision was made in the left ventricular wall (care being taken of avoiding large coronary vessels) and the muscle gently folded back in order to record action potentials on both the endocardial and epicardial surfaces. Action potential durations (APDs) were measured at 0 mV and -60 mV, and are denoted APD$^0$ and APD$^-60$ respectively. The experimental temperature was 24 ± 0.5°C.

Single ventricular myocytes were placed in Petri dishes and superfused with normal Tyrode solution followed by modified external solution at room temperature (21–24°C). A flow of solution (50–100 μl/min) from a series of five pipette outlets continuously superfused the cell from which the recording was made. During superfusion with normal Tyrode solution (1.8 mM CaCl$_2$), 70–90% of cells were Ca$^{2+}$-tolerant and rod-shaped, with clearly marked striations. Ionic currents were recorded in the whole-cell patch-clamp configuration with a RK300 amplifier (BioLogic). Pipettes with a resistance of 2–3 MΩ were pulled from Pyrex capillaries (Corning code 7740, Corning, NY) and were not fire-polished. After cancelling pipette capacitance electronically, the membrane was perforated and the cell membrane capacity current was measured before compensating resistance in series with the membrane. A sequence of ten hyperpolarizing pulses of 10 mV amplitude and 10 ms duration was imposed on the cell membrane at a frequency of 10 Hz. The capacitative currents were then averaged. Cell capacity (C$_m$) was calculated as the ratio of the numerical integration of the average current transient (total charge) to the magnitude of the hyperpolarizing pulse. Resistance in series with the cell membrane was compensated for, but the cell capacitative current and leakage current were not. Cell currents were digitized at 6 kHz by using Acquisl software (G. Sadoc, CNRS-URA 1121, Orsay, France) on a Tandon MCS 486 microcomputer, then analysed and printed on an HP Laserjet III (Hewlett-Packard, San Diego, USA). Current densities are expressed as means (±s.d.) in pA/pF.

Inward Ca$^{2+}$ currents were measured as the difference between inward peak and steady-state currents (steady state at the end of a 350-ms test pulse from a holding potential of -80 mV; stimulation frequency of 0.5 Hz). The transient outward current, $I_{to}$, was measured as the difference between total peak and steady-state outward currents (steady state at the end of the 500-ms test pulse from a holding potential of -80 mV; stimulation frequency of 0.2 Hz). Steady-state currents were elicited from a holding potential of -80 mV by a voltage ramp of 30 s, from -120 to +60 mV (stimulation frequency of 0.02 Hz). For each strain of hamsters, the steady-state current averaged from different experiments was normalized to the mean cell capacity (C$_m$). For Ca$^{2+}$ current inactivation measurements, the membrane was held for 2 s at potentials ranging from -50 to +10 mV (5-mV increments) before applying the test potential of +10 mV for 300 ms (stimulation frequency of 0.1 Hz). To determine the voltage dependence of $I_{to}$, inactivation, 2-s prepulses from -110 to +10 mV (5-mV increments) and a test potential of +60 mV for 700 ms were used (stimulation frequency of 0.1 Hz). Data for steady state inactivation were fitted using the non-linear least-squares gradient-expansion algorithm of Marquardt, according to the following Boltzmann relation:

$$I/I_{\text{max}} = 1/[1 + \exp((V_m - V_d)/k)]$$

where $V_m$ is the membrane potential, $V_d$ the potential at which the current is half-maximally inhibited, and k the slope factor. The steady-state activation curve was also fitted using the non-linear least-squares gradient-expansion algorithm of Marquardt, according to the Boltzmann relation:

$$I/I_{\text{max}} = 1/[1 + \exp((V_{0.5} - V_m)/k)]$$

where $V_{0.5}$ is the potential at which current is half-maximally activated. For the transient outward current, we assumed that $I_{to}$ is carried only by K$^+$ ions and that it reverses at the potassium equilibrium potential $V_K$ (−85
mV in our experimental conditions). The steady-state activation curve for \( I_{\text{to}} \) was constructed by calculating the chord conductance \( (G) \) from peak \( I_{\text{to}} \) amplitudes corrected for driving force \( (V_m - V_K) \) at each test potential \( (V_m) \), and normalized to the maximum chord conductance \( (G_{\text{max}}) \). As maximum experimental values did not reflect \( G_{\text{max}} \), we determined the latter using a computer-calculated fit to the Boltzmann relation, according to the equation:

\[
G = G_{\text{max}} /[1 + \exp((V_{0.5} - V_m)/k)]
\]

To investigate \( I_{\text{to}} \) recovery from inactivation, a two-pulse protocol was used: two identical depolarizing pulses from -80 to +40 mV were applied every 15 s at selected intervals ranging from 4 to 5000 ms. The peak amplitude of \( I_{\text{to}} \) elicited by the second pulse (test) was normalized to its value measured during the first pulse (control) and plotted against the interpulse duration. Data could be described by the following two-exponential function:

\[
\text{fraction of } I_{\text{to}} = 1 - a \exp(-t/\tau_1) - b \exp(-t/\tau_2)
\]

where \( t \) is the interpulse interval, \( \tau_1 \) and \( \tau_2 \) are the fast and slow time constants of the recovery process, respectively.

2.4. Statistical analysis

All data are presented as means ± s.d. (standard deviation). One-way analysis of variance (ANOVA; SigmaStat) was used to compare mean values in the different treatment groups (Dunnett’s test). \( P \) values of less than 0.05 were considered significant.

3. Results

3.1. Ventricular action potentials

Fig. 1 shows superimposed action potentials (APs) recorded from ventricular myocytes of diseased (MS200) and control (CHF148) hamsters aged 120 days. Each trace is the average of APs recorded from several cells (57 cells from three CHF148 hamsters and 69 cells from five MS200 hamsters). The amplitude of the repolarizing two-plateaus phase of AP was higher in MS200 than in CHF148. The AP duration was also markedly prolonged in MS200 compared to CHF148 at both 0 mV (APD\(_{\text{c}}\)) and -60 mV (APD\(_{\text{-60}}\)), as shown in Table 1. The maximum diastolic potential (MDP) was slightly (by about 3 mV) but signifi-

![Fig. 1](image1.png)

Fig. 1. Averaged action potentials recorded in epicardial ventricular muscles from 120-day-old MS200 and CHF148 hamsters (41 impalements from 3 normal left ventricles and 71 impalements from 5 dilated left ventricles). Isolated perfused hearts were stimulated electrically at 2 Hz.

![Fig. 2](image2.png)

Fig. 2. APs recorded from epicardial (EPI) and endocardial (ENDO) muscles in one CHF148 (left panel) and one MS200 (right panel) isolated perfused heart. Superimposed traces were obtained before (C) and 2 min after the addition of 4 mM 4-aminoypyridine (4-AP) to normal Tyrode solution. Horizontal arrows indicate zero mV (stimulation frequency of 2 Hz).
were significantly lower in MS200 than those observed from the whole ventricles. The fact that the effects of 4-AP,
were not systematically studied the rate of rise of APs in the present work, we observed that this rise was often slowed down in MS200 ventricles (for example, see Fig. 2, upper right traces) especially in stressed tissues bordering the necrotic regions. It has been previously reported that differences exist in both the amplitude and duration of the repolarizing plateau phase of APs due to differences in $I_{\text{to}}$ density between myocytes spanning the cardiac ventricular wall [18]. For this reason, we compared APs from epicardial and endocardial surfaces of perfused hamster hearts, and tested their sensitivity to 4-AP in the two strains. Fig. 2 shows representative APs recorded from a CHF148 heart (left panel) and a MS200 heart (right panel), before and after 2-min exposure to 4 mM 4-AP. It can be seen that, in the CHF148 heart perfused with normal Tyrode solution, the repolarizing two-plateau phase of APs, especially the initial plateau, was shorter in epicardial than in endocardial muscle, i.e. the initial repolarization was faster in the former than in the latter. This suggests that, in the Syrian hamster as in other species, the transient outward repolarizing current, $I_{\text{to}}$, is larger in epicardial than in endocardial myocytes. However, although being not identical, a comparable lengthening effect of 4-AP on APs was observed in the two tissues, indicating that a substantial $I_{\text{to}}$ exists in myocytes throughout the ventricular wall. The differences in AP duration and 4-AP-induced lengthening of APs between epicardial and endocardial muscle were still less marked in MS200 than in CHF148 (Fig. 2, right panel). Three other experiments gave quite similar results. For these reasons, we measured ionic currents in cells isolated from the whole ventricles. The fact that the effects of 4-AP were significantly lower in MS200 than those observed in CHF148 suggests that $I_{\text{to}}$ density is markedly reduced in ventricular myocytes from MS200 hearts.

### 3.2. Cell membrane capacitance and cell size

To detect any hypertrophy of ventricular myocytes isolated from MS200 hearts, membrane capacitance ($C_m$) was determined in all the cells studied, as indicated in Methods. $C_m$ was not significantly higher in MS200 than in CHF148. Its mean value ($\pm$ s.d.) was $165 \pm 40$ pF for MS200 ($n = 35$ cells) vs $148 \pm 30$ pF for CHF148 ($n = 16$; $P = 0.13$). The dimensions of single myocytes isolated from left and right ventricles were also assessed by phase contrast microscopy. Because of the thinness of ventricular walls of MS200 hearts, we could not separate subepicardial and subendocardial tissue layers, and thus, epicardial and endocardial cells were not examined separately. The maximum length and the width (measured at the middle of the long axis) of MS200 myocytes did not differ significantly from those of CHF148 myocytes (Table 2). That confirmed previous results showing that, even at 120 days, the MS200 hamster does not develop cell hypertrophy, thus providing a model of ventricular dilation.

### 3.3. Inward calcium current

It has long been postulated that increased influx of Ca$^{2+}$ via voltage-sensitive Ca$^{2+}$ channels may cause cytosolic Ca$^{2+}$ overload, thus prolonging the action potential in cardiomyopathic Syrian hamsters [4,19]. However, recent data obtained with BIO 14.6, a model of genetically determined cardiac hypertrophy, have shown that there is a decrease in the high-threshold L-type Ca$^{2+}$ current ($I_{\text{Ca,L}}$) [16]. We thus compared $I_{\text{Ca,L}}$ characteristics in dilated MS200 and control CHF148. $I_{\text{Ca,L}}$ was elicited by depolarizing pulses applied from a holding potential of $-80$ mV, after blockade of outward K$^+$ currents through substitution of K$^+$ by Cs$^+$ in both the bath and pipette solutions. Representative recordings of $I_{\text{Ca,L}}$ in MS200 and CHF148 myocytes are shown in Fig. 3. The current amplitude was greater in CHF148 than in MS200. The current–voltage relationships shown in Fig. 4 indicate that the peak Ca$^{2+}$ current density was smaller, at potentials more negative than $+20$ mV, in MS200 than in CHF148. For instance, maximum $I_{\text{Ca,L}}$ density was 37% lower in MS200 than in CHF148 despite the regional variability of AP shapes, suggests that $I_{\text{to}}$ density is markedly reduced in ventricular myocytes from MS200 hearts.

### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>MDP (mV)</th>
<th>APA (mV)</th>
<th>APD$_{90}$ (ms)</th>
<th>APD$_{60}$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHF148</td>
<td>56</td>
<td>73.4±0.6</td>
<td>89.4±0.8</td>
<td>12.6±0.5</td>
<td>127.4±1.9</td>
</tr>
<tr>
<td>MS200</td>
<td>56</td>
<td>70.8±0.8</td>
<td>91.4±1.2</td>
<td>24.4±1.3</td>
<td>178.3±6.4</td>
</tr>
</tbody>
</table>

Values are means ($\pm$ s.e.m.); $n =$ number of impalements; MDP = maximum diastolic potential; APA = maximum action potential amplitude; APD$_{90}$ and APD$_{60}$ = action potential duration at 0 mV and -60 mV. Significantly different from CHF148 values; * $P < 0.01$ and ** $P < 0.001$.  

### Table 2

<table>
<thead>
<tr>
<th>Single ventricular myocytes</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>Length/width</th>
<th>n cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHF148 left ventricle</td>
<td>135±26</td>
<td>23±5</td>
<td>6.1±1.73</td>
<td>118</td>
</tr>
<tr>
<td>CHF148 right ventricle</td>
<td>134±22</td>
<td>22±5</td>
<td>6.47±1.80</td>
<td>93</td>
</tr>
<tr>
<td>MS200 left ventricle</td>
<td>124±22</td>
<td>23±6</td>
<td>5.64±1.44</td>
<td>70</td>
</tr>
<tr>
<td>MS200 right ventricle</td>
<td>123±22</td>
<td>20±5</td>
<td>6.39±1.6</td>
<td>242</td>
</tr>
</tbody>
</table>

Values are means $\pm$ s.d. of $n$ cells as indicated in the table. There is no significant difference in mean values between the different groups ($P > 0.05$).
Fig. 3. Inward calcium currents recorded in a CHF148 myocyte and an MS200 myocyte. Currents were elicited by 350-ms depolarizing voltage steps applied from a holding potential of -80 mV in 10-mV increments. Traces elicited by test pulses from -40 to +60 mV are shown. The dash at the beginning of each set of superimposed tracings denotes the zero current level. 

$C_{\text{m}}$ values were 137 pF and 145 pF for CHF148 and MS200, respectively.

CHF148 (-7.03 ± 2.76 pA/pF (n = 7) vs -11.22 ± 1.93 pA/pF (n = 6) respectively; $P < 0.01$). The potential corresponding to maximum current density was more positive in MS200 (+10 mV) than in CHF148 (0 mV), whereas the reversal potential was not different. It is worth noting that the T-type Ca$^{2+}$ current ($I_{\text{Ca,T}}$), the mean density of which is increased in hypertrophic ventricular myocytes from adult BIO14.6 [17], was rarely observed in CHF148 or MS200 myocytes and then was not further investigated.

The kinetic properties of the low-threshold $I_{\text{Ca,L}}$ were further compared in MS200 and CHF148 myocytes. As shown in Fig. 5, the voltage-dependence of Ca$^{2+}$ current activation and inactivation was also altered in MS200. $I_{\text{Ca,L}}$ inactivated at more positive potentials in MS200 than in CHF148 (0 mV), whereas the reversal potential was not different. It is worth noting that the T-type Ca$^{2+}$ current ($I_{\text{Ca,T}}$), the mean density of which is increased in hypertrophic ventricular myocytes from adult BIO14.6 [17], was rarely observed in CHF148 or MS200 myocytes and then was not further investigated.

The kinetic properties of the low-threshold $I_{\text{Ca,L}}$ were further compared in MS200 and CHF148 myocytes. As shown in Fig. 5, the voltage-dependence of Ca$^{2+}$ current activation and inactivation was also altered in MS200. $I_{\text{Ca,L}}$ inactivated at more positive potentials in MS200 than in CHF148 ($V_{\text{h}}$ was $-17.8 ± 2.3$ mV ($n = 7$) vs $-31.3 ± 3.8$ mV ($n = 6$; $P < 0.01$) with a slope factor of $4.0 ± 0.6$ mV vs $4.2 ± 0.6$ mV in MS200 and CHF148, respectively).

The steady-state activation curve was also shifted by 12 mV towards more positive potentials in MS200 cells ($V_{0.5}$ was $-8.2 ± 2.8$ mV ($n = 7$) vs $-20.6 ± 2.6$ mV ($n = 6$; $P < 0.01$) with a slope factor of $4.7 ± 0.3$ mV and $4.6 ± 0.6$ mV in MS200 and CHF148, respectively).

3.4. Calcium-independent transient outward current

The fact that the plateau phase is prolonged in MS200 cells, despite the decrease in $I_{\text{Ca,L}}$ density, suggests that components of outward repolarizing current are more reduced than $I_{\text{Ca,L}}$ is. Fig. 6 shows representative families of whole-cell membrane current recorded from a normal CHF148 (upper left) and a dilated MS200 (upper right) myocyte during 500-ms depolarizing pulses from -80 mV to +60 mV (10-mV increments). In both cells, depolarizations more positive than -40 mV evoked a transient outward current which appeared to reach steady-state at the end of the test pulse. The amplitude of the total outward current increased with more positive potentials in each cell, but was smaller at every voltage level in the MS200 cell. The external application of 4 mM 4-AP (lower traces) completely suppressed the rapidly inactivating component of this current, thus identifying it as the
transient outward current ($I_{to}$ or $I_{to}$) previously described in various species, but did not reduce the sustained component ($I_{ss}$). This is better illustrated in Fig. 7 (upper panel), which shows the current traces elicited by depolarization to +60 mV before and during exposure to 4 mM 4-AP. The two components of the outward current can be clearly distinguished by their different sensitivity to 4-AP in the two types of cell, but their absolute values were smaller in MS200 than CHF148. The mean current densities of $I_{to}$ and $I_{ss}$, determined at +60 mV in several cells from the two strains, are summarized in Fig. 7 (lower panel). The mean density of $I_{to}$, which was totally suppressed by 4-AP, was drastically reduced in MS200 as compared to CHF148 (32.3 ± 10.1 pA/pF (n = 8 CHF148 cells) vs 9.5 ± 6.7 pA/pF (n = 8 MS200 cells; P < 0.01)). A reduction in the sustained component $I_{ss}$ was also observed in MS200 cells, but the difference did not reach statistical significance at potentials less positive than +60 mV. The mean density of $I_{ss}$ was, at +60 mV: 18.1 ± 2.4 pA/pF (n = 8 CHF148 cells) vs 14.7 ± 3.3 pA/pF (n = 8 MS200 cells; P = 0.034) and was, at +70 mV (overshoot of ventricular APs): 9.1 ± 2.2 pA/pF (n = 8 CHF148 cells) vs 8.6 ± 2.9 pA/pF (n = 8 MS200 cells; P = 0.715).

Differences between normal and cardiomyopathic hamsters clearly appeared in the mean current density–voltage relationships for $I_{to}$ (Fig. 8). In the two groups of cells, $I_{to}$ began to activate between −40 and −20 mV and increased as depolarizing pulses were made more positive. $I_{to}$ density was significantly lower in MS200 than in CHF148 in the voltage range from −10 to +60 mV. Fig. 9 shows the voltage dependence of the activation (conductance) and inactivation of $I_{to}$, obtained by using the procedures described in the Methods section. The conductance–voltage relationship in MS200 cells was shifted by

---

Fig. 6. Voltage-dependent outward currents. Superimposed current tracings were elicited by 500-ms depolarizing steps from −80 mV to +60 mV in 10-mV increments. Currents were recorded in myocytes from a normal CHF148 hamster (left panel; $C_m$: 166 pF) and from a cardiomyopathic MS200 hamster (right panel; $C_m$: 160 pF), after abolishing inward currents ($I_{Na}$ and $I_{Ca}$; see Methods). Upper traces were obtained in control conditions and lower traces during 4 mM 4-AP superfusion. The dash at the beginning of each set of superimposed tracings denotes the zero current level.

---

Fig. 7. Depressing effect of dilated cardiomyopathy on the CAP-sensitive transient outward current. Superimposed tracings showing the effects of 4 mM 4-AP on the voltage-dependent outward current in ventricular myocytes from CHF148 and MS200 hamsters ($C_m$ values: 166 and 160 pF). Currents were elicited by a test pulse from −80 to +60 mV. The dash at the beginning of each set of superimposed tracings denotes the zero current level. The bar graph summarizes the effects of 4-AP on the transient outward current ($I_{to}$) and on the time-independent current ($I_{ss}$) measured at +60 mV, as indicated in the upper traces. Normalized values are means ± s.d. for 8 CHF148 and 8 MS200 cells.
3.5. Steady-state potassium current

Steady-state background currents are known to be efficient modulators of the action potential plateau shape in ventricular myocytes from various species. To investigate the possible participation of these currents in the prolongation of the repolarization phase of MS200 action potentials, the whole-cell current elicited by voltage ramps was recorded in MS200 and CHF148 ventricular cells. In our experimental conditions (external Tyrode solution with CdCl₂ and high-K⁺ pipette solution with EGTA), this current certainly contains several components, such as the inwardly-rectifying K⁺ current, some delayed K⁺ current, background Na⁺-K⁺ current, but no Ca²⁺-dependent current because of the intracellular presence of EGTA. Fig. 11

inactivation-voltage relationship of I_{out} was also shifted to the right for MS200 relative to CHF148. Although small (4 mV), this shift was significant (P < 0.05; Dunnett’s test). No significant change in slope factors was observed in these relationships. The time course of recovery from inactivation of I_{out} was studied using a double-pulse protocol. The amplitude of I_{out} elicited by the second pulse, normalized to its value during the first pulse, was plotted against interpulse duration for CHF148 and MS200 cells. In the two strains, the time course of I_{out} recovery could be described by the sum of two exponentials. Histograms shown in Fig. 10 demonstrate that the reactivation of I_{out} was slower in diseased than in normal myocytes, as a consequence of the drastic increase in the slow time constant τ₂ in MS200.
The results presented here indicate that: (1) The dilated cardiomyopathy of 120-day-old MS200 Syrian hamsters is characterized by a marked increase in both the amplitude and duration of the repolarizing two-plateaus phase of APs as compared to age-matched control CHF148 Syrian hamsters. This prolongation of AP duration is accompanied by a reduced effect of 4-AP. (2) Although not identical, AP shapes and their 4-AP sensitivity are not markedly different in epicardial and endocardial muscle, indicating that ionic current measurements can be performed in cells isolated from whole ventricles of Syrian hamster hearts. (3) Cell size and capacitance of single ventricular myocytes are not significantly different in the two strains, confirming the absence of cell hypertrophy in dilated MS200 ventricles. (4) Densities of the L-type Ca\(^{2+}\) current (\(I_{\text{Ca,L}}\)) and the Ca\(^{2+}\)-independent transient outward current (\(I_{\text{to1}}\)) are both markedly reduced in MS200 cells, but \(I_{\text{to1}}\) density is more drastically reduced than \(I_{\text{Ca,L}}\) density. (5) Activation- and inactivation-voltage relationships for \(I_{\text{Ca,L}}\) and \(I_{\text{to1}}\) are shifted towards more positive potentials and the overall recovery process of \(I_{\text{to1}}\) from inactivation is slower in MS200 than in CHF148 cells. (6) The steady-state outward current density–voltage relationship does not differ in MS200 and CHF148 cells.

The MS200 strain of Syrian hamsters was originally derived from BIO 53–58 hamsters which have been reported to develop a dilated form of cardiomyopathy [2]. However, no morphometric study of single ventricular myocytes has been reported until now in this model. Here, we demonstrate the absence of cell hypertrophy at the cellular level in MS200 hearts. We also consistently observed that cardiac ventricles were largely distended and characterized by very thin walls despite the presence of white necrotic regions. It seems, therefore, that the hypertrophic process which normally develops in residual healthy cells of injured hearts [20,21], does not develop or develops very slowly in MS200 hearts. Therefore, this strain of Syrian hamster appears as an interesting model of pure ventricular dilation without cell hypertrophy.

Several points are of interest and must be discussed concerning the reduction in the density of the inward calcium current and transient outward current observed here. First, a decrease in \(I_{\text{Ca,L}}\) density (no data on \(I_{\text{to1}}\)) has also been observed in hypertrophic ventricular myocytes from BIO 14.6 hamsters with end-stage heart failure [11,16] but not those with early-stage hypertrophy, i.e. in the absence of heart failure [17]. However, no commonly accepted sign of congestive heart failure (pulmonary edema, pleural effusion, ascites and hepatomegaly) was observed in 120-day-old MS200 hamsters. These observations suggest that the decrease in \(I_{\text{Ca,L}}\) density is related to cardiac dilation rather than heart failure. Such a conclusion is in good agreement with the drastic reduction in \(I_{\text{Ca,L}}\) density also reported in dilated human atria [22,23]. The second point is that a reduction in \(I_{\text{to1}}\) (but not \(I_{\text{Ca,L}}\)) density has been observed in most models of cardiac hypertrophy in rat [24–28], except in one-clip two-kidney Goldblat hypertensive rat [29]. It is, therefore, surprising that, in the Syrian hamster, a species in which the ventricular AP plateau is of the same double-step type as in the rat, a reduction in \(I_{\text{to1}}\) density was observed in the cardiomyopathic animal developing dilation without hypertrophy. Our experiments support the concept that the reduced expression of the \(I_{\text{to1}}\) channel is attributed to cardiac dilation rather than hypertrophy. Therefore, the question arises as to whether, in hypertrophic models too, the phase of distension which usually precedes the development of the hypertrophic process [30], might be the cause of \(I_{\text{to1}}\) reduction in exerting some early and long-lasting blocking effect on \(I_{\text{to1}}\) channel expression. Such a possible effect of dilation in the control of cardiac ionic currents during hypertrophy has been suggested previously for \(I_{\text{Ca,L}}\) [22,23].

Another possibility must be put forward: a reduction in \(I_{\text{to1}}\) density has been also associated with canine cardiomyopathy similar to that seen in Duchenne muscular dystrophy in human (no data on \(I_{\text{Ca,L}}\)) [31]. Therefore, the question arises as to whether alterations of \(I_{\text{to1}}\) (and perhaps \(I_{\text{Ca,L}}\)) observed in the MS200 Syrian hamster and possibly in...
other strains, are not the consequence of the hereditary cardiomyopathy involving a defect in the sarcolemma and sub-sarcolemmal apparatus such as the disruption of the dystrophin-glycoprotein complex reported in BIO 14.6 hamsters [32]. Further investigations are needed to determine the contribution of the ventricular dilation and chronic myocyte stretch to alterations of this current. The third point concerns the specific alterations in current kinetic parameters (activation– and inactivation–voltage relationships of \( I_{\text{Ca,L}} \) and \( I_{\text{to,1}} \); recovery from inactivation of \( I_{\text{to,1}} \)) observed in diseased MS200 myocytes. Such a result differs from those obtained from hypertrophied BIO 14.6 hamster heart [17] and hypertrophied rat hearts [33] for which activation– and inactivation–voltage relationships of \( I_{\text{Ca,L}} \) were found unchanged compared to controls. A comparable absence of alteration in \( I_{\text{to,1}} \) parameters, including recovery from inactivation, was also observed in the hypertrophied rat heart [27–29]. We cannot presently determine the cause of the alteration of \( I_{\text{Ca,L}} \) and \( I_{\text{to,1}} \) parameters. However, it is worth noting that, in ventricular myocytes isolated from dog hearts with a cardiomyopathy similar to Duchenne muscular dystrophy, \( I_{\text{to,1}} \) density was reduced but neither the steady-state inactivation–voltage relationship nor the kinetics of recovery from inactivation were altered [31]. In contrast, in myocytes dispersed from epicardium of dog hearts 5 days after coronary artery occlusion, the time course of \( I_{\text{to,1}} \) recovery was found to be slower than in myocytes dispersed from epicardium of noninfarcted hearts [34]. It could be that some alterations of \( I_{\text{to,1}} \) parameters (shifts of activation– and inactivation–voltage relationships) observed in diseased MS200 myocytes results from a decrease in the density of external fixed negative charges or an increase in the density of internal fixed negative charges or both, i.e. some disturbances in the cell membrane composition or metabolic environment. The reason why a small but significant depolarization (of 3 mV) exists at resting membrane potential in perfused MS200 hearts, is also unknown. However, if the sarcoplasmic reticulum function is altered in MS200 hamsters causing a cytosolic Ca\(^{2+}\) accumulation, as previously reported in other strains of cardiomyopathic Syrian hamsters [9–11,16], the Na\(^+-/Ca\(^{2+}\) exchanger might be functional at resting membrane potential, i.e. delivering a small inward depolarizing current. Our results point to the complexity of the ionic mechanisms underlying ventricular AP of diseased MS200 hearts.

It is difficult to extend results obtained in single myocytes stimulated at long-lasting intervals to the whole heart driven at physiological frequencies. Despite this difficulty, our experiments shown in Fig. 2 clearly indicate that \( I_{\text{to,1}} \) participates sizeably in the development of AP repolarization in the normal hamster heart. This is no longer the case in the diseased heart. The weakness of the repolarizing effects of \( I_{\text{to,1}} \) is clearly due to its reduced density associated with the marked slowing of its recovery from inactivation. The shift of the inactivation–voltage relationship of \( I_{\text{to,1}} \) might somewhat increase its participation to repolarizing AP whereas the shift of the activation–voltage relationship must, on the contrary, add its depressive effect to those already indicated.

In summary, the main result of our study is that the dilated cardiomyopathy without hypertrophy results in a lengthening of the ventricular AP and a decrease in density of the transient outward current; two alterations that have been previously observed in the hypertrophied heart. In addition, dilated cardiomyopathy is associated with a reduction of the L-type calcium current density, an alteration mainly observed during the development of heart failure.

Acknowledgements

This work was supported by a grant from Procter and Gamble Pharmaceuticals, Fondation Simone et Cino del Duca, Fondation de France, Fondation pour la Recherche Médicale, and Caisse Régionale d'Assurance Maladie de l'Île de France. The authors are grateful to T.P. O'Neill (Procter and Gamble; USA) and L. Van Wersch (Procter and Gamble; France) for helpful comments and to D.D. Young for his help in restyling the English.

References


