Ionic basis of ventricular arrhythmias in remodeled rat heart during long-term myocardial infarction

Franck Aimond\textsuperscript{a}, Julio L. Alvarez\textsuperscript{b}, Jean-Michel Rauzier\textsuperscript{a}, Paco Lorente\textsuperscript{a}, Guy Vassort\textsuperscript{a,*}

\textsuperscript{a}Unité de Recherches INSERM U-390, Physiopathologie Cardiovasculaire, IFR N°3 Communications Cellulaires Normales et Pathologiques, CHU Arnaud de Villeneuve, F-34295 Montpellier, France

\textsuperscript{b}Laboratorio de Electrofisiología, Instituto de Cardiología y Cirugía Cardiovascular, La Habana, Cuba

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Abstract

Objective: Deleterious electrical abnormalities evolve during myocardial infarction. The goal of this study was to analyse current changes during the late decompensated phase of heart disease induced by coronary ligation and to compare them in various heart regions.

Methods: Young rats were submitted to left coronary ligature. After 4–6 months, cells were enzymatically dissociated and isolated from the upper part basal region of the left ventricle, as well as from the septum, apex and the right ventricle before being studied under whole-cell patch-clamp.

Results: Basal L-type Ca current, \( I_{\text{Ca,L}} \), elicited at +10 mV did not exhibit regional dependence neither in control nor after post-myocardial infarction (PMI). \( I_{\text{Ca,L}} \) showed both a significantly reduced peak amplitude (17.1±2.8 pA/pF versus 9.9±1.4 pA/pF in seven control and seven PMI hearts, \( n=32 \) and 40, respectively) and a slower inactivation, such that the amount of inward charges during a 200 ms-depolarizing pulse was nearly unchanged. \( \beta \)-Adrenergic stimulation was less effective in increasing \( I_{\text{Ca,L}} \) in PMI cells but it slowed inactivation further. Significant differences in the K currents were observed. A regional distribution was seen for \( I_{\text{K}} \) only, with the largest amplitude in the right ventricle (in pA/pF: 23.1±2.4, 18.2±3.9, 14.8±2.4, 8.3±1.7 in the right ventricle, apex, septum and left ventricle, respectively \( n=8, 7, 8 \) and 9). This was also true in failing heart cells despite \( I_{\text{K}} \) being halved in each of the four regions (in pA/pF: 12.2±2.5, 11.2±1.9, 5.1±1.0 and 4.8±1.0, respectively \( n=12, 12, 11 \) and 13). \( I_{\text{K}} \) was also significantly reduced by 20% in the PMI cells. Two-way analyses of variance demonstrated the absence of interaction between the topographical origin of the cells and the physiological state of the rats. The \( \alpha \)-adrenergic agonist, methoxamine significantly reduced \( I_{\text{Ca,L}} \) and \( I_{\text{K}} \) to the same extent in both sham and PMI cells, by about 35% and 20% respectively.

Conclusions: Long-term left coronary occlusion induces significant alterations in both Ca and K currents that occur with similar amplitude in both ventricles. They include a marked reduction in \( I_{\text{K}} \) amplitude as well as a slowing of \( I_{\text{Ca,L}} \) inactivation. Both factors could contribute to the disturbances in cellular electrical behaviour and the occurrence of arrhythmias in the post-myocardial infarcted heart.

Keywords: Ventricular arrhythmia; Ca-current; K-current; Heart failure; Remodeling

1. Introduction

Left ventricular post-infarct remodeling consists of ventricular dilatation and eccentric hypertrophy accompanied by an increased amount of fibrous tissue. Heart failure is often the late evolving phase of this adaptive process. Post-myocardial infarction and heart failure also time dependently involve complex biochemical, neuro-hormonal and electrophysiological changes [1]. Myocardial cells and tissues isolated from failing animals and human hearts reveal abnormalities in electrical activities. Particularly, the prolongation of action potential repolarisation [2] might predispose to dispersion of repolarisation and development of after-depolarisation, which in turn can induce various arrhythmias [3].
Various membrane currents defects have been described in this pathology. K⁺ currents play a major role in initiating and modulating the repolarization phase of action potential. In rat ventricular myocytes, together with the inward rectifying K⁺ current, \( I_{K1} \), two outward voltage-dependent K⁺ currents are involved in the repolarization phase. The rapidly activating and inactivating current, \( I_{Ks} \), is sensitive to 4-aminopyridine (4-AP) and the slow activating, non-inactivating current, \( I_{K} \), is more sensitive to tetraethylammonium chloride (TEA) [4]. Like in failing human hearts [5,6], a major down-regulation of \( I_{K} \) has been recently reported in dogs with pacing-induced heart failure [7] and in rats after post-infarct remodeling [8,9]. Such a feature had been observed in most pathological conditions [2] and by itself could account for electrical activity abnormalities. Regional differences in the electrophysiological properties of the ventricular tissues of the heart were previously outlined, particularly in [11]. In rat heart ventricle, marked differences in the density of \( I_{Ks} \) have been reported in the left ventricular wall, septum and apex cells [10]. Moreover, pressure overload-induced hypertrophy reduces \( I_{Ks} \) density with a tendency to homogenize the ionic profile between the studied regions [11].

A specific attention has also been devoted to the L-type Ca²⁺ current, \( I_{CaL} \). Since a reduction in transmembrane Ca influx could contribute to the pathophysiology of heart failure, literature reports are quite variable with species and models of hypertrophy [2]. A similar variability occurs in post-myocardial infarction and heart failure. \( I_{CaL} \) density is reported to be unaffected in pacing-induced failing dog heart [7]. It is also unaffected [8,12], or decreased in post-infarction remodeled rats [13] as well as in failing guinea pig after aortic constriction, an effect that might be related to the cell size [14].

The chronic infarcted rat heart as a model of left ventricular dysfunction is clinically relevant and has predicted results of pathophysiological and pharmacological studies in man [15,16]. During this late phase of evolving heart failure, far from acute infarction, necrosis, failure to adaptation and ageing combined their effects. In this model, both ventricles undergo hypertrophy. The left ventricle may also show dilatation within the context of pump failure while the right atrial pressure is increased [15,17]. In this context, we chose to investigate the cellular ionic basis of arrhythmias four to six months after occlusion of the left anterior coronary artery. Alterations in both the inwardly rectifying and the early outward currents were compared in the apex, septum, left and right ventricles. Measurements of various K⁺ channel subunit proteins were performed to analyse the effects of myocardial infarction on protein level expression. Changes in Ca²⁺ current that mediate changes in action potential configuration and decrease of contractile activity were also investigated. The reduction in K⁺ currents and the slowing of Ca²⁺ current inactivation results support the increase changes in action potential duration and the anomalous electrical activities time course observed in this model.

2. Methods

The 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. Experimental myocardial infarction

Male Wistar rats weighing 180–230 g underwent left anterior coronary artery ligation according to Pfeffer et al. [15]. Briefly, rats were anaesthetized with a mixture of 150 mg/kg IP ketamine and 15 mg/kg IP chlorpromazine before being intubated and ventilated. After median-left thoracotomy and opening of the pericardium, the left main coronary artery was occluded with a 7-0 silk suture at the most proximal point below the left atrial appendage. Successful occlusion was recognized by pallor of the anterior left ventricular free wall and by the occurrence of immediate regional dyskinesia. Sham-operated rats were submitted to the same treatment except the coronary artery ligation. Rats were then allowed to recover in individual cages. Rats surviving the ligation (70% at 4 months, not including the initial death during the surgery and the first two weeks) and shams received similar housing conditions, including ad libitum food, water, and a 12-h day/night cycle. Four to six months after operation, rats with post-myocardial infarction (PMI; \( n=7 \)) and sham (\( n=7 \)) were sacrificed for electrophysiological and Western-blot experiments.

2.2. Physiological variables

In parallel series of experiments, several physiological variables were estimated after four to six months in rats that underwent the same operation and showed similar range of infarcted scar size. After anaesthesia and tracheotomy, some rats underwent hemodynamic invasive study. The right carotid artery was cannulated with a polyethylene catheter connected to a pressure transducer (Baxter) to record maximal aortic and left ventricular end-diastolic pressures. On other rats, under mild anaesthesia, cardiac ultrasound studies were performed with a Challenge ultrasonograph (ESAOTE BIOMEDICA) using a dynamically focused 7.5 mHz annular array transducer. Measurements of left ventricular end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were generally obtained from the parasternal short axis, but occasionaly echoes were best defined from the long axis view.

In vivo Holter monitoring [18] was performed by telemetry in untethered rats at least a week (for periods up to four
months) after intraperitoneal implantation of the transmitter (Data Science International) during mild anaesthesia [19].

2.3. Ventricular myocytes isolation

Ventricular myocytes were isolated from the heart of urethane-anaesthetized (2 g/kg, IP) sham or ligated rats as previously described [20]. The heart was first perfused for 5 min at 35°C with a nominally Ca-free HEPES-buffered solution containing (mM): NaCl 117, KCl 5.7, NaHCO₃ 4.4, KH₂PO₄ 1.5, MgCl₂ 1.7, HEPES 21, glucose 11, taurine 20, and then for 50 min with the same solution containing also 20 μM Ca²⁺ and 1.2 mg/ml collagenase (Type A, Boehringer). The heart was then removed from the perfusion set-up and cut in four pieces: right ventricle, septum, apex and upper part of the left ventricular free wall above the scar. Each sample was gently dissociated through the bore of a large-tip pipette followed by two decantations to separate dead cells. The cells were then suspended in HEPES buffer with 1 mM Ca²⁺ and 0.5% bovine serum albumin (pH 7.4). The yield of well-striated, elongated cells was near 60% for sham and 40% for PMI animals. Cell size was estimated after isolation by measuring length and width with either a goniometer or, most often from pictures of the screen of a video monitor.

2.4. Voltage clamp recording

2.4.1. Pulse protocol and data analysis

Recordings of the K⁺ and Ca²⁺ currents were performed in parallel on two set-up using the whole-cell configuration of the patch clamp technique at room temperature (22±2°C). To ensure voltage clamp quality, electrode resistance was between 0.9 and 1.1 MΩ. Junctional potentials were zeroed with the electrode in the standard solution. After establishing the whole-cell configuration, the capacitive transients elicited by symmetrical 2-mV voltage steps (E_m) from −70 mV were used for calculation of cell capacitance (C_m), access resistance (R_s), and input impedance [20]. The uncompensated series resistance was 4.1±0.3 and 4.3±0.28 MΩ in sham and PMI cells, respectively. The residual R_s was 2.08±0.15 and 2.2±0.14 MΩ, respectively since R_s could be compensated up to 50% without oscillations. The averaged decay time constant of the capacitive transient was 0.6±0.1 and 0.7±0.4 ms in sham and PMI cells. Due to the presence of a residual series resistance, the membrane potential (V_m) deviates from the command potential (V_c) according to the equation:

\[ V_m = V_c \left(1 - \frac{R_s}{(R_s + R_m)}\right) \]

where \( \frac{R_s}{(R_s + R_m)} \) is the error factor. From the positive slope of the current/voltage relationships we obtained \( R_s + R_m \) which gives a good estimate of the lowest \( R_m \) value. Therefore, the maximal error factor was estimated to be 0.026±0.001 and 0.043±0.001 during the flow of the large \( I_{ca} \) and \( I_{cal} \) respectively.

Cells showing inadequacies in voltage control such as steep increase in inward current amplitude in the negative slope region of the current/voltage relationship, ‘abominable notches’, excessively long time to peak and/or kinetic changes at a given test pulse as current amplitudes varied with prepulse membrane potentials, were discarded.

For action potential recordings, 2-ms suprathreshold stimuli were applied at 1 Hz. Then, voltage traces were filtered at 3 kHz and digitized at a sampling interval of 40 μs.

L-type Ca²⁺ currents, \( I_{cal} \), were recorded using a patch clamp amplifier (model RK-400; Biologic, Claix, France) and filtered at 3 kHz. Current traces were digitized at a sampling interval of 20–50 μs with a 12-bit analog to digital converter (LabMaster DMA, Scientific Solutions, USA) and the ACQUISI software (version 2; CNRS Licence, France) and stored on a computer hard disk. Current amplitude was estimated as the difference between peak inward current and the current level at the end of the 200-ms pulse. Current/voltage (I/V) relationships and availability curves were constructed using a standard double-pulse voltage protocol [20]. Normalization of current amplitude at the test pulse by the maximal current recorded as a function of prepulse potential gave the availability curve. The experimental points were fitted to a Boltzmann function:

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

where \( V_p \) is the prepulse potential, \( V_{1/2} \) the potential for half availability and \( s \) a slope factor. The peak current values of the \( I/V \) relationships were converted to conductance, assuming no contamination with the inactivation process. Conductance at each membrane potential was normalized by the maximal conductance to obtain the activation curve. Experimental points were then fitted to a Boltzmann function.

Inactivation time course of currents was described by fitting the current traces between the inward peak and the end of the pulse using the fitting procedures of ACQUISI software. Current trace was best fitted by the equation:

\[ y = A_{fast} \cdot e^{-t/\tau_{fast}} + A_{slow} \cdot e^{-t/\tau_{slow}} + A \]

with \( A_{fast} \) and \( A_{slow} \) being the maximal amplitude, and \( \tau_{fast} \) and \( \tau_{slow} \) the time constant of the fast and slow components of inactivation, respectively. Recovery from inactivation was studied by a double-pulse protocol. Recovery from inactivation or reactivation, was obtained by the ratio of current at the second pulse/current at the first pulse vs time interval.

K⁺ currents were recorded every 4 s using a patch-clamp amplifier (model RK-300; Biologic, Claix, France) and filtered at 3 kHz. Current traces were digitized at 200
μs with a 12-bit analog-to-digital converter (LabMaster DMA, Scientific Solutions, USA) and pClamp 6 software (Axon Instruments, Foster City CA, USA). A detailed kinetical analysis of the fast transient outward current, \( I_{\text{to}} \), was performed. \( I_{\text{to}} \) activation characteristic was determined by applying 6 ms prepulses within the range −40 to +50 mV in 10-mV increment that were followed by a −40 mV, 120-ms pulse. Its inactivation was established by applying a 200-ms prepulse within the range −85 to +30 mV in 5-mV increment that was followed by a +50 mV–200 ms pulse. A two-pulse protocol (+50 mV, 250 ms) with random interval durations (10 to 3000 ms) allowed to determine the reactivation curve. Activation and inactivation curves were fitted to Boltzmann functions.

2.4.2. Solutions and drugs

For experiments, a cell aliquot was put in a Petri dish containing the control solution (mM): NaCl 117, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.7, glucose 10 and HEPES 10; pH was adjusted to 7.4 with NaOH. After achieving whole cell patch clamp configuration, the cell was exposed to different extracellular solutions by positioning it at the extremity of one of six capillaries (inner diameter of each capillary was 250 μm). Such a system allowed rapid changes of solution (<2 s). For K⁺ current recordings, to the above described control solution 50 μM tetrodotoxin (TTX) and 2 mM CoCl₂ were added to block respectively Na⁺ and Ca²⁺ currents. At 2 mM, CoCl₂ also blocked the steady state K⁺ current [21]. The internal solution contained (mM): KC1 120, MgCl₂ 6.8, Na₂ATP 5, Na₂creatine phosphate 5, Na₂GTP 0.4, EGTA 10, and HEPES 20; pH was adjusted with KOH to 7.2 such as total K⁺ was 145 mM. For Ca²⁺ current recordings, the control solution was (mM): TEACl 140, CaCl₂ 5, MgCl₂ 2, glucose 10, HEPES 10; pH was adjusted to 7.4 with NaOH. In some cases, NaCl was 30 and TEA 110 mM. In each experiment, 50 μM TTX was added to the extracellular solution. The intracellular filling pipette solution contained (mM): CsCl 100, TEACl 20, EGTA 10, HEPES 10, Na₂ATP 5, Na₂GTP 0.4; pH was adjusted to 7.3 with CsOH. Nifedipine (Bayer, Germany) was prepared as a 10-mM stock solution in absolute ethanol and dissolved to a final concentration of 3 μM. Nifedipine-containing solutions were continuously protected from light. Methoxamine and isoprorenaline, α₁- and β-adrenergic agonists and all salts were from Sigma (L’Isle d’Abeau, France).

2.5. Western-blot analysis

Proteins from membrane fractions prepared from isolated ventricular myocytes were loaded (100 μg per lane) on 7.5% acrylamide gels. After electrophoretic transfer, the nitrocellulose membranes were incubated overnight at 4°C with the different antibodies. Kv4.2 (generously provided by Dr G.-N. Tseng), Kv2.1 (generously provided by Dr J.M. Nerbonne), Kv1.5 (Alamone lab) and Kir6.2 (raised against the peptide sequence 19–39, EDPAEPRYRAR-QRRARFVSKK of Kir6.2) antisera were used at dilution 1:1000, 1:200, 1:200 and 1:1000 respectively. Bound primary antibody was revealed using a secondary peroxidase-conjugated anti-rabbit IgG antibody (1:10000) and ECL detection according to the manufacturer’s instructions. The blots were quantified by a digital imaging system (Scion Image). The blots were stripped for 20 min at 50°C in a Tris buffer containing 62.5 mM Tris, 2% SDS and 10 mM mercaptoethanol adjusted to pH 6.7.

2.5.1. Statistical analysis

All averaged values and error bars represent mean±s.e.m. To evaluate the respective effects of post-infarction heart failure and topographical origin of cells, as well as the interaction between both factors, we used two-way analyses of variance and, in the case of a significant F-ratio, multiple comparison through the Fisher’s protected least significant difference procedure. For Western blots, densitometric units obtained for PMI animals were normalized to those obtained for sham animals and were statistically compared by a Student’s t-test. For each method, significance was assumed at \( P < 0.05 \).

3. Results

3.1. Characterisation of the PMI rats

Four to six months after the coronary ligation, scar size was 24±3% (\( n = 7 \)) of the left ventricular free wall area; necrosis was transmural as checked at the late stage of cell dissociation. In parallel experimental series, several measures were performed in rats that underwent the same operation. Hemodynamic measurements demonstrated a significant increase in end-diastolic pressure from 2.5±0.5 to 17.1±13.5 mm Hg (\( n = 9 \)). In vivo Holter recordings demonstrated various spontaneous ventricular premature complexes. They occurred with a quite variable frequency from a few to several thousand extrasystoles per hour (Fig. 1A). Ventricular tachycardia was occasionally observed. M-Mode echocardiography showed that PMI rats had a dilated left ventricle. both LVEDD and LVESTD were significantly increased to 10.5±0.4 and 8.3±0.6 mm compared to basal values of 7.3±0.4 and 3.8±0.5 mm in sham rats of the same age respectively (\( n = 8; P < 0.0001 \)) (Fig. 1B).

3.2. Morphometric changes

Table 1 summarizes the heart and body weights as well as length and width values of cells isolated from the four defined regions (right ventricle, septum, apex and upper part of the left ventricle above the scar) from sham and 4 to 6 month-ligated rats. In PMI rats, left ventricle was
markedly dilated and heart weight/body weight ratio was significantly increased despite the free wall of the infarcted area was very thin and consisted of connective tissue. In both types of animals, cell dimensions were very similar in the four zones while length and width were both significantly increased in the PMI rats. The mean capacitance of the cells investigated under whole-cell patch-clamp in this study was 307.3±10.6 and 321.5±11.8 pF; n = 65 and n = 97 from five sham and seven PMI rats, respectively.

### 3.3. Action potential measurement

Typical action potential recordings in a control and a PMI cell under whole-cell current-clamp conditions are illustrated in Fig. 2. In average, PMI cells demonstrated no significant change in resting potential (−82.8±1.0 mV and −81.5±1.5 mV in sham (n = 23) and PMI cells (n = 16), respectively) nor in action potential amplitude (94.5±4.0 mV and 99.8±3.6 mV in sham and PMI cells, respectively.)

Table 1

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<th>Caracteristics of animals and isolated ventricular cells in control and after long-term left coronary artery ligation*</th>
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<tr>
<td><strong>Body Weight (BW)</strong></td>
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<td>Sham</td>
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* LV: left ventricle; RV: right ventricle; L: length and W: width in μm. Body and heart weights in g. m, n: number of hearts and cells, respectively.

b P <0.05.
c P <0.0001.
However, a marked difference existed between action potential duration (APD, inset Fig. 2). These results are consistent with intracellular action potential recordings performed in papillary muscles isolated from sham and PMI rat hearts [18].

### 3.4. Potassium currents

#### 3.4.1. Current characteristics

To monitor $K^+$ currents, ventricular cells were held at −80 mV in a TTX- and Co$^{2+}$-containing solution to inhibit the Na$^+$ and Ca$^{2+}$ inward currents. Three major $K^+$ currents i.e. the transient outward current, $I_o$, the slowly inactivating delayed rectifier current, $I_{K1}$, and the inward rectifier $I_{K1}$, were evaluated in the four selected ventricular regions. Current densities were estimated at −130 mV for $I_{K1}$, at the end of the 300 ms pulse to +50 mV for $I_{K1}$, and $I_o$ was estimated as the difference between peak and steady currents elicited at +50 mV. $I_{K1}$ and $I_o$ were suppressed by 20 mM TEA and by 4 mM 4-AP respectively. $I_o$, obtained by the difference in peak and steady current, was similar to the 4-AP sensitive current. In sham animals, analyses of variance of the $I_{K1}$ and $I_{K1}$ densities did not show significant difference between the four regions, despite $I_{K1}$ density in apex was slightly larger than in left ventricle and septum. However, there was a clear regional distribution of $I_o$ density with the right ventricular cells showing the largest values: 23.1±2.4 pA/pF compared to 18.2±3.9 pA/pF in apex, 14.8±2.4 pA/pF in left ventricle and 8.3±1.7 pA/pF in septum cells ($P<0.005$). In PMI cells, there was no significant regional distribution in $I_{K1}$ and $I_{K1}$ densities either, whereas regional differences in $I_o$ density were similarly observed. The highest $I_o$ densities were also observed in the right ventricle (12.2±2.5 pA/pF) and in the apex (11.2±1.9 pA/pF) while $I_o$ densities were lower in the left ventricle (5.1±1.0 pA/pF) and in the septum (4.8±1.0 pA/pF; $P<0.005$) (Fig. 3). The main observation was that $I_o$ and $I_{K1}$, but not $I_{K1}$, were significantly reduced in the PMI-rat cells. On the average, $I_o$ and $I_{K1}$ decreased by about 50 and 20%, respectively. In addition, two-way analyses of variance demonstrated the absence of interaction between the topographical origin of cells and the physiological state of the rats, so that similar myocardial infarction-induced long-term effects could be noticed in each region for $I_o$ and $I_{K1}$ (Fig. 3). Moreover, after chronic infarction, the time constants of inactivation of both $I_o$ and $I_{K1}$ were significantly increased from 46.2±2.9 to 56.3±3.3 ms ($P<0.05$) and from 59.9±2.6 to 85.9±4.2 ms ($P<0.01$), respectively, when measured at +50 mV and −130 mV in 33 and 44 cells, respectively, isolated from 5 sham and 7 PMI rats. The effects on $I_o$ were independent of the voltage since a similar relative reduction in $I_o$ elicited from −40 to +50 mV was observed in the four ventricular regions of PMI rats (Fig.
4). The activation, inactivation and reactivation curves of $I_{K_4}$ were also established. No significant changes were recorded in the two models (Fig. 5).

Modulation of the $K^+$ currents by $\alpha_1$-adrenergic agonists was evaluated in sham and PMI cells. The application of the $\alpha_1$-adrenergic agonist methoxamine at 100 $\mu$M significantly reduced $I_{K_4}$ and $I_{K_1}$ amplitude (35.0 ± 3.7 and 19.7 ± 4.3%, respectively) but left unaffected $I_{K_1}$ in sham animals. Methoxamine decreased $I_{K_4}$ and $I_{K_1}$ to the same extent (39.4 ± 3.3 and 17.0 ± 1.7%, respectively) and did not affect $I_{K_1}$ in PMI rats (Fig. 6).

3.4.2. Changes in $K^+$ channel subunit protein expression

To understand whether changes in $K^+$ current amplitude resulted from alterations in biophysical characteristics or in amount of channel protein, the expression levels of $K^+$ channel $\alpha$-subunits protein were checked by Western blots. Fig. 7 illustrates results obtained on the Kv4.2, Kv1.5, Kv2.1 and Kir6.2 proteins. The Kv4.2 immunoreactive protein appeared most often in two bands at 68 and 66 kDa. Densitometric measurement shows that this protein was decreased by about 26% in PMI ($n = 6$) compared to sham hearts ($n = 4$) ($P < 0.05$). The three other subunit proteins measured at 75, 130 and 47 kDa for Kv1.5, Kv2.1 and Kir6.2, respectively, were not significantly changed into $K_1$.

3.5. L-type Ca$^{2+}$ current

3.5.1. Current characteristics

L-type Ca$^{2+}$ currents, $I_{Ca_L}$ elicited at +10 mV from a holding membrane potential at −90 mV had similar amplitude in cells isolated from right ventricle, septum, apex and base of left ventricle of sham rat hearts (Fig. 8A). They were also of equal amplitude in the four same regions of PMI rats. Thus in the following, data obtained
Fig. 4. Comparison of the current/voltage relations established for the early outward current $I_o$, in sham and PMI cells isolated from the upper part of the left ventricle (LV), septum, apex and right ventricle (RV). (Cells were isolated from 5 sham and 7 PMI-rat hearts). * $P < 0.05$. $I_o$ was measured as the difference between the peak outward current and the current at the end of 300 ms depolarizing pulses.

from the different regions are pooled. The peak amplitude of $I_{Ca}$, was, however, significantly reduced in the PMI rats (Fig. 8B). $I_{Ca}$ recorded in the PMI cells exhibited a slower inactivation, particularly in the late phase such that some inward current was still flowing at the end of the 200 ms depolarization. Time-to-peak current was similar in both cell types (4.8 ± 0.1 and 4.7 ± 0.2 ms) as well as the fast time constant of inactivation, $\tau_{fast}$ (5.9 ± 0.3 and 6.8 ± 0.4 ms in sham and PMI, respectively). However, the slow time constant of inactivation, $\tau_{slow}$, was significantly increased from 47.8 ± 1.6 ms in sham ($n = 32$) to 65.5 ± 3.4 ms ($n = 40$) in the PMI cells. Slower inactivations were generally associated with $I_{Ca}$ of lesser amplitude. As a result, the quantity of charges carried by the Ca$^{2+}$ current over a 200-ms depolarizing period at +10 mV in a PMI cell was roughly similar to the one estimated in sham cells (Fig. 8C). Reduction in peak amplitude and slowing of inactivation were equally observed in the four regions. Besides, $I_{Ca}$ did not show differences in the voltage dependence of its kinetics. As shown on the current-voltage relations, maximal peak current occurred at 0 mV in both sham and PMI cells (Fig. 9A) and the steady-state activation and inactivation curves established on the two cell types were superimposable (Fig. 9B). Note however, that the relief from inactivation following high-voltage prepulses was significantly larger in the PMI cells (Fig. 9B). Recovery from inactivation curves studied in sham ($n = 5$) and PMI ($n = 7$) cells did not show significant difference (Fig. 9C).

There was no sign of a low threshold Ca$^{2+}$ current, $I_{Ct}$, in any of the cells investigated in sham or PMI rats.

3.5.2. Ca$^{2+}$ current pharmacology

Pharmacology of Ca$^{2+}$ currents could be altered during hypertrophy and failure. Namely, the maximal increase in $I_{Ca}$ induced by isoproterenol is much less in hypertrophied cells [20]. β-Adrenergic stimulation was investigated in PMI cells (Fig. 10). Relative increase in peak $I_{Ca}$ induced by applying 1 μM isoproterenol was not significantly different in sham and PMI (57 vs. 48%) myocytes. However, the tendency toward reduced responsiveness to β-adrenergic stimulation is consistent with previous data from patients with heart failure [22]. Furthermore, the application of isoproterenol slowed $I_{Ca}$ inactivation in PMI cells in such a way that the amount of charges carried by Ca$^{2+}$ ions increased significantly more after β-adrenergic stimulation in the PMI (45%) than in the sham (20%) (Fig. 10C). In the presence of isoproterenol,
Fig. 5. Kinetics of the transient outward current \( I_o \). (A) Voltage-dependent activation and inactivation of \( I_o \) in sham \((n=7)\) and PMI \((n=10)\) cells. The relations are fitted by a Boltzmann equation. Half-activation of \( I_o \) occurred at \(-4.8\pm0.7\) mV in sham cells and at \(-6.4\pm0.6\) mV in PMI cells. Half-inactivation was respectively at \(-39.5\pm0.2\) mV and \(-38.1\pm0.2\) mV in sham and PMI cells. (B) Recovery from inactivation of \( I_o \) elicited by 200 ms, +50 mV depolarizing pulses applied at various aleatory intervals after the conditioning pulse. Both curves were well fitted by a single exponential with \( \tau=40.5\pm2.6 \) and \( 34.8\pm3.2 \) ms respectively in sham \((n=5)\) and PMI \((n=4)\) cells.

Fig. 6. Inhibitory effects of \( \alpha\)-adrenergic stimulation in sham and PMI heart cells. The application of 100 \( \mu M \) methoxamine reduced both \( I_o \) and \( I_k \) to the same extent in sham \((C_m=294\) pF\) and PMI \((C_m=304\) pF\) cells, both from apex. \( I_o \) was unaffected \((n=8\) and 12, from 4 sham and 6 PMI hearts, respectively).

4. Discussion

Arrhythmias are common features of post-myocardial infarction and heart failure. In the present study, during the late decompensated phase investigated four to six months after left coronary artery ligation in rat, cells isolated from different ventricular regions were similarly hypertrophied including in the right ventricle. Besides these morphological changes, the most salient electrophysiological variations induced at this late stage are twofold. First, we demonstrate that the previously reported decrease in \( I_o \) density in various pathological conditions occurs to the same extent in the four regions independently of original current density. Second, \( I_{Ca,L} \) peak amplitude is similarly reduced; this is accompanied by a slowing of inactivation that allows for an expected little change in Ca influx during an action potential. Reduction in K currents and slowing of \( I_{Ca,L} \) inactivation are potential strong arrhythmogenic factors.

4.1. Chronic infarction-induced slowing of \( I_{Ca,L} \) inactivation

In the PMI cells, four to six months after the ligation,
Fig. 7. Representative comparison of K⁺ channel protein expression in sham and PMI rats. (A) Western blot analysis of Kv channel-subunit immunoreactive proteins (Kv4.2, Kv1.5, Kv2.1 and Kir6.2) from isolated myocytes of sham (n=4) and PMI (n=6) rats. (B) Bar graph of Kv immunoreactivities after densitometric measurements of the signal for the proteins (Kv4.2, Kv1.5, Kv2.1 and Kir6.2) using a digital imaging system. Densitometric data for sham (□) and PMI (■) animals were averaged and normalized to those obtained for sham animals.

$I_{\text{CaL}}$ was significantly reduced to the same extent in the four selected ventricular regions, with a concomitant slowing of its inactivation. The other voltage dependent characteristics that we checked (availability and reactivation) were not significantly affected. In various pathophysiological models of compensated and decompensated hypertrophy, $I_{\text{CaL}}$ density has been reported to be unchanged, increased or decreased with, in a few cases, a slowing of inactivation [2]. $I_{\text{CaL}}$ is unchanged after 3 to 4 weeks of pacing-induced heart failure in dogs [7] and in cardiomyopathic human heart [23] but it is reduced in hypertrophied failing guinea pig heart [14]. Following extensive healed myocardial infarction in rat, $I_{\text{CaL}}$ density is unchanged after 3 to 4 weeks despite a simultaneous apparent decrease in dihydropyridine binding sites [8,12]. Later, however, $I_{\text{CaL}}$ density decreases [13]. Similarly, a decrease in the rate of $I_{\text{CaL}}$ inactivation was observed only in a few cases that include congestive heart failure of cardiomyopathic hamster [24], or late infarct-induced hypertrophy [13] while inactivation is hardly affected within the first 3 to 4 weeks [8]. This observation might in part account for the prolonged Ca\textsuperscript{2+} handling reported in myocardium from patients with end-stage heart failure [25]. Consequently in the PMI cells, the integrated amount of charges flowing during an action potential would not be reduced or even enhanced (Fig. 8) as was also seen in senescent myocytes [26]. Such is even more true during \(\beta\)-adrenergic stimulation. Despite a fade increase in peak $I_{\text{CaL}}$, Ca\textsuperscript{2+} influx is markedly enhanced by isoproterenol such as the estimated increase in intracellular Ca\textsuperscript{2+} concentration is significantly larger in PMI than in control cells.

Calcium current is altered density is reduced in the PMI cells. This could result either from reduced Ca\textsuperscript{2+} channel
Fig. 10. Effects of β-adrenergic stimulation on $I_{Ca}$ elicited in sham and PMI cells. (A) Time course of the increase in $I_{Ca}$ elicited by $+10 \text{mV}$ depolarizing pulses applied every 8 s on a diseased cell after applying 1 μM isoproterenol. The upper inset shows superimposed original current traces (left) as well as the integration of entering charges (right) during the 200 ms depolarizing pulse at $+10 \text{mV}$ before and after the β-adrenergic stimulation ($C_m=334 \text{ pF}$, from the left ventricle). (B) Comparison of the quantity of entering charges in both conditions in 7 cells isolated from 5 sham and 7 PMI-rat hearts respectively.

Fig. 9. Comparison of the Ca$^{2+}$ current characteristics in sham and PMI cells. (A) Current/voltage relations of $I_{Ca}$ densities in sham and PMI cardiomyocytes. The amplitude of $I_{Ca}$ was reduced by about 45% in the PMI cells at every depolarizing potential. (B) There was no significant difference between the steady-state activation ($d_v$) and the steady-state inactivation ($I_v$) in the two cell types except that relief of inactivation following large prepulses was more marked in PMI cells. The data were fitted by Boltzmann functions with half-activation at $-13.6\pm0.3$ and $12.5\pm0.4 \text{mV}$ and slope factor of $5.9\pm0.3$ and $6.1\pm0.3 \text{mV}$, with half-inactivation at $-25.3\pm0.3$ and $-22.3\pm0.4 \text{mV}$ and slope factors of $10.1\pm0.4$ and $8.9\pm0.2 \text{mV}$ for cells isolated from sham (open) and PMI (filled) hearts, respectively ($P=ns$). In A and B, the curves were established on 14 and 15 cells isolated from 5 sham and 7 PMI rats and pooled from the four regions. (C) Recovery from inactivation at $-90 \text{mV}$ after a 200-ms depolarizing pulse to $+10 \text{mV}$ in sham (n=5) and PMI (n=6) cells were well fitted by the same double exponential function with $\tau_1=40.3\pm2.7 \text{ms}$ and $\tau_2=354.9\pm67.5 \text{ms}$.

protein expression relative to cell size increase, or from alterations in the Ca$^{2+}$ channel behaviour. Four main possibilities specific to chronic cardiac failure compared to the early stages of compensated hypertrophy can account for these changes in $I_{Ca}$ amplitude and kinetics. They include a functional expression of new Ca$^{2+}$ channel isoforms as suggested by the re-emergence of the fetal pattern of $\alpha_1$-subunit of the L-type Ca$^{2+}$ channel in a similar model of remodeling [27]. A second aspect relates alterations in the β-subunit of the Ca$^{2+}$ channel, whose expression is known to enhance DHP-sensitive Ca$^{2+}$ channel current and accelerates its kinetics [28]. This hypothesis is supported by the decrease in DHP binding sites in the myocardium of patients with end-stage heart failure [29]. Third, the level of Gi protein, whose $\alpha_1$-subunit competes with the Ca$^{2+}$ channel β-subunit [28] is known to be enhanced under several pathological conditions [30]. An increase in the Gi-protein inhibitory tone is suggested by the larger relief from inactivation during high depolarising prepulses, an effect already described for the N- and T-type Ca$^{2+}$ channel [28,31]. Alterations in the
cytoskeleton should also be considered. Inactivation of \( I_{\text{Ca,L}} \) is slowed by the cytoskeletal stabilizers taxol and phalloidin [32] and it has also been shown that free, polymerized, and total \( \beta \)-tubulins are increased in hypertrophied myocardium [33,34].

4.2. Regionally heterogeneous \( K^+ \) currents are similarly reduced after chronic infarction

The present results extend the observation of heterogeneity in \( I_{\text{to}} \) density previously described in rat left ventricular cells [10] to the right ventricle, where \( I_{\text{to}} \) exhibits significantly larger amplitude than in the basal left ventricular and septal cells. Furthermore, \( I_{\text{to}} \) and \( I_{\text{k1}} \) were significantly reduced in diseased cells independently of their regional origin. In most physiopathological conditions, the main abnormality in membrane current that underlies action potential prolongation is a down-regulation of \( I_{\text{to}} \) [2]. Such has been reported in myocytes isolated from failing heart dogs [7] and PMI rats [8,9] as well as in human ventricular myocytes isolated from patients with terminal heart failure [5,35]. In the dog pacing-induced heart failure [7], the reduction in \( I_{\text{to}} \) was attributed to a reduction in channel number since neither single-channel conductance nor open probability was affected. In the rat model, the changes in ionic current density have been related, at least in part, to the more than 50% reduction in the mRNA and protein levels of Kv4.2 (putative \( I_{\text{to}} \)) and Kv2.1 (\( I_{\text{k1}} \); putative \( I_{\text{to}} \)) [36]. In the same model 3 to 5 months later, we observed a decrease in density and amplitude of \( I_{\text{to}} \) and \( I_{\text{k1}} \) in the cells isolated from each region. The decrease in \( I_{\text{to}} \) amplitude also correlates with a decrease in Kv4.2 protein; moreover, this is accompanied by alterations in channel behaviour: \( I_{\text{to}} \) inactivation (and also \( I_{\text{k1}} \) inactivation) is slowed in the PMI cells as was already mentioned in hypertrophied rat cells [10]. Reexpression of other isofoms as well as alterations in the cytoskeleton or in the \( K^+ \) channel \( \beta \)-subunit might be anticipated to account for these changes in kinetics and, in part in amplitude, as with the Ca current.

In the PMI cells, a decrease in Kv1.5-protein level was observed although it was not significant. Kv1.5 underlies the delayed rectifier (\( I_{\text{k1}} \)) in human heart [35,37]. In rat heart, Kv 1.5 \( \alpha \)-subunit is assumed to contribute to the formation of functional channels carrying \( I_{\text{k1}} \) in association with other \( K^+ \) \( \alpha \)-subunits since \( K^+ \) channels are heterotetramers. Kv 1.5 protein level was not changed in the PMI-rat model 3 weeks after ligation [36]. It is interesting to note that this \( \alpha \)-subunit protein is reduced in human atrial cells with chronic atrial fibrillation [37]. The expression of Kv2.1 \( \alpha \)-subunit (another protein potentially involved in \( I_{\text{k1}} \) channels) was not different in PMI cells late after myocardial infarction (Fig. 7) although it was decreased after 3 weeks [36]. However in the latter case, Kv2.1 expression was estimated in cardiac tissue not isolated cardiomyocytes; this difference might also be accounted for either by a transient down-regulation of Kv2.1 protein expression during infarction or by the difference between the two animal species (male Wistar/ female Sprague-Dawley). Considering that Kv1.5 and/or Kv2.1 subunit proteins form \( I_{\text{k}} \) functional channels, our results are in agreement with the fact that no change in \( I_{\text{k}} \) current densities was observed. Furthermore, we also found no change in the amount of Kir6.2 protein which, in association to an ATP-binding cassette protein (SUR1) is assumed to carry the ATP sensitive \( K^+ \) current (\( I_{\text{K-ATP}} \)). No change in Kir6.2 mRNA level was also noted in a model of myocardial ischemia that, besides, induces an up-regulation of Kir6.1 mRNA and protein levels [38].

The effects of \( \alpha_1 \)-adrenergic agonists on \( K^+ \) currents in isolated rat ventricular myocytes have been previously characterized [39,40]. We confirm that both \( I_{\text{to}} \) and \( I_{\text{k1}} \) in sham cells are significantly reduced in the presence of methoxamine while \( I_{\text{k}} \), the remaining current at the end of the 300 ms-pulse in the presence of Co\(^{3+} \) ions was unaffected. These two currents, which were reduced in PMI cells, were further reduced to similar extent by the \( \alpha_1 \)-adrenergic agonist. This is in line with the fact that, unlike \( \beta \)-adrenergic receptor density which consistently decreased in failing hearts, \( \alpha_1 \)-adrenergic receptor density appears to be unaffected or increased in some forms of heart failure [41].

Ventricular arrhythmias are common features in human heart failure and many other pathological situations. In the present model, various ventricular electrical abnormalities were recorded by telemetric ECG monitoring in untreated rats (Fig. 1A) [19]. Besides fibrosis with possible impairment of cellular coupling, alterations in ionic currents as shown in this study might play a key role in the arrhythmogenicity. One cannot also exclude that during this single cell study even more than during others, there was a bias induced by cell dissociation or erratic selection for patch. So that partly damaged cells would be eliminated; despite, the latter due to their short action potentials would heavily contribute to post-myocardial arrhythmia. The importance of \( I_{\text{to}} \) in the pathogenesis of cardiac arrhythmias is suggested by its constant reduction in various arrhythmogenic substrates including heart failure [2]. \( I_{\text{k1}} \) also contributes to the cellular electrical behaviour, and disturbances in the gating of \( I_{\text{k1}} \) are likely to be arrhythmogenic [42]. This effect could mimic the \( K^+ \) conductance decreases at voltages around the resting potential reported in the present study and in failing dogs and rats [7–9]. Besides reductions in \( K^+ \) currents, the slowly inactivating \( I_{\text{Ca,L}} \) might be another potential arrhythmogenic source by providing inward charges at the plateau level. A last important feature of this study in rat heart, is that myocardial infarction after long-term left coronary artery ligation induces similar ionic current alterations in the various ventricular regions including the right ventricle so that such as this tissue might as well be the source of anomalous activity.
5. Abbreviations

- Cm: membrane capacitance
- PMI: post-myocardial infarction
- APD: action potential duration
- \( I_{Ca} \): T-type Ca\(^{2+}\) current
- \( I_{CL} \): L-type Ca\(^{2+}\) current
- \( I_{to} \): transient outward K\(^{+}\) current
- \( I_{K} \): delayed outward K\(^{+}\) current
- \( I_{K1} \): inward rectifying K\(^{+}\) current
- I/V: current/voltage

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