INFLUENCE OF $[Ca^{2+}]_O$ ON CARDIOMYOCYTE INJURY BY DEFIBRILLATOR-LIKE SHOCKS

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Abstract: *Electrical defibrillation, the most effective treatment of life-threatening arrhythmias, such a ventricular fibrillation, is based on the application of shocks to the heart for the reversal of the abnormal rhythm. However, depending on their intensity, defibrillating shocks may cause cell injury and even death. Massive* Ca^{2+} *influx due to electrical sarcolemmal damage contributes to both cell injury and membrane repair. This study aimed at investigating how variation in the extracellular* Ca^{2+} *concentration* ($[Ca^{2+}]o$) *affects the susceptibility of isolated cardiomyocytes to lethal injury by high-intensity electrical fields. The results indicate that doubling the physiological* $[Ca^{2+}]o$ *did not make the cells more vulnerable to high-intensity electric stimulation, but a 50% decrease in* $[Ca^{2+}]o$ *made the cells less susceptible to the shock-induced lethal injury. In order to make defibrillation procedures safer, studies have shown ways to improve defibrillators or to protect the myocardium with drugs. This study reveals a new point of view in which a safer defibrillation approach might be achieved by changing the medium around the cell.*

Keywords: *Cardiac myocyte, defibrillation, extracellular calcium concentration, electrical injury.*

Introduction

Electrical stimulation of the heart is clinically used to regulate the heart rhythm, both for pacemaking and for correction of rhythm abnormalities. Automatic external defibrillators (AEDs), designed for emergency use by untrained persons and available in some shopping malls and airports, are able to analyze the heart rhythm as to detect the occurrence of arrhythmias before delivering an electric shock to the heart [1]. On the other hand, some chronic, severe arrhythmias, such as atrial fibrillation, demand the use of implantable cardioverter defibrillators, which are inserted into the chest of patients and electrically stimulate the heart upon the detection of arrhythmia [2]. The increasing utilization of these devices makes it important to study the effects of electrical fields on the heart as to improve the efficacy and safety of cardiac electrical stimulation.

High-intensity electrical fields may produce microlesions in the cell membrane, a process known as electroporation. This phenomenon occurs because of the great field-induced polarization of the membrane, which leads to phospholipid reorganization and formation of hydrophilic pores [3]. The electroporated membrane loses its selective permeability, allowing the flux of water, ions and other molecules, which causes osmotic and electrochemical imbalance, as well as loss of electrical excitability. A rapid, massive influx of Ca^{2+} ions into the myocardial cells may result in Ca^{2+} overload, arrhythmias, hypercontracture, cytoskeletal disruption and death [4, 5, 6, 7, 8, 9, 10]. On the other hand, the transient increase in intracellular Ca^{2+} concentration is recognized as an important requisite for membrane repair [11, 12].

One way to increase the safety of the defibrillation procedure is to attempt to minimize cell damage, by identifying factors may be involved in the production of the deleterious effects of electrical stimulation. Considering the dual role of Ca^{2+} on cell vulnerability to electrical damage, the aim of this study was to determine if the extracellular $[Ca^{2+}]$ concentration ($[Ca^{2+}]o$) affect the vulnerability of isolated ventricular myocytes to lethal damage by to defibrillator-like, high-intensity electrical shocks.

Material and Methods

Cell preparation – Cardiomyocytes were isolated from the left ventricle of adult (4-6 month-old) male Wistar rats by coronary perfusion with collagenase I [13]. The protocols for animal care and use were approved by the institutional Committee of Ethics in Animal Use (CEUA/IB/UNICAMP – protocol 2942-1(B)).

The cell suspension was plated on a perfusion chamber which allows electric field estimation [9, 14] and perfused (~3 mL/min) with Tyrode´s solution (in mM: 140 NaCl; 6 KCl; 1.5 $MgCl_2$; 10 HEPES; 11.1 glucose; pH 7.4) containing 0.5, 1.0 or 2.5 mM $CaCl_2$. All experiments were carried out at room temperature (23±1 °C).

Electric Field Stimulation – Cells were electrically stimulated at 0.5 Hz with biphasic voltage pulses (10 ms total duration) using a low-power stimulator (CEB/UNICAMP, Campinas, Brazil). The stimulus amplitude was decreased until contractions ceased in order to determine the threshold stimulation field amplitude (E_T). Once E_T was determined, the cell was paced (0.5 Hz, 1.2 x E_T) for 30 s. Two seconds after the

last stimulus, a high-power stimulator (S48K, Grass, West Warwick, USA) delivered a single defibrillator-like, monophasic pulse (10 ms duration). The pulse amplitude was adjusted to produce an electric field of which the intensity was a factor (from 8 to 30) of E_T. Initially, the applied high-intensity field was 8 x E_T. The cell then was rested until it recovered quiescence and full responsiveness to near-E_T stimulation. Next, the protocol was repeated, increasing the amplitude of the defibrillator-like pulse until cell death occurred [9, 14].

Only one cell was selected per chamber based on the following set of criteria: 1) the major cell axis was parallel to the electric field lines; 2) cell was located 2 mm away from the electrodes; 3) presence of clear striations, as well as preserved structure and contractile function [9, 14].

Experimental Groups and Statistical Analysis – Three experimental groups were defined according the $[Ca^{2+}]o$. The E_T values and cell length were compared among the groups with one-way analysis of variance.

The lethality curve (i.e., the relationship between the probability of lethal injury and the electrical field intensity) was determined for each experimental group based on the maximum non-lethal and the minimum lethal amplitude of the defibrillator-like shocks [9, 14]. The curves were compared with Mantel-Cox test. To provide a mean parameter of the cell sensitivity to the electric field, a sigmoidal function was fitted to the lethality curves:

$$P = \frac{1}{1+\left(\frac{L50}{X}\right)^n} \quad (1)$$

where P is the probability of lethality, X is the electric field amplitude; L50 is the X value for P = 0.5; and n is the Hill coefficient. In all regressions, R^2 was greater than 0.90.

Values are presented as means ± standard error. For comparisons, $p < 0.05$ was considered as indicative of statistically significant difference. Statistical analysis was performed with Prism 5.03 (GraphPad Software, Inc, San Diego, USA).

Results

No significant difference was detected in the values of E_T and cell length among the cell groups (Table 1, P> 0.13).

Table 1: Cell length and threshold stimulation field (E_T) (mean ± standard error) determined in isolated rat ventricular myocytes perfused with different $[Ca^{2+}]_O$. N is the number of cells in each group.

$[Ca^{2+}]_O$ (mM)	E_T (V/cm)	Cell length (µm)	N
0.5	3.19±0.08	132.3±2.6	13
1.0	2.96±0.17	138.1±6.6	8
2.5	3.33±0.10	143.3±5.2	8

Sublethal shocks usually caused spontaneous activity, consisting of full contractions and cell length oscillations in absence of electrical stimulation. More intense spontaneous activity was observed in the cell end closer to the anode, where electroporation is considered to be more severe [8, 10, 15, 16]. After lethal shocks, irreversible hypercontracture and loss of cell structural integrity occurred within 1-10 min.

The lethality curves determined at different $[Ca^{2+}]o$ values are shown in Figure 1. It should observed that 1.0 mM is a near-physiological $[Ca^{2+}]o$, as the concentration of ionized Ca^{2+} in the blood of Wistar rats is ~1.3 mM [17]. It is clear the presence of a rightward shift in the lethality curve when $[Ca^{2+}]o$ was reduced by 50%, to 0.5 mM (p= 0.03, Mantel-Cox test vs. 1.0 and 2.5 mM $[Ca^{2+}]o$), which indicated that higher electric field intensity was required to produce lethal injury. At 0.5 mM $[Ca^{2+}]o$, the estimate of the mean field intensity required to cause death in 50% of the cells was ~15-20% greater than those at 1 and 2.5 mM $[Ca^{2+}]o$. However, the lethality curves determined at 1 and 2.5 mM $[Ca^{2+}]_O$ were not statistically different (p= 0.86, Mantel-Cox test), although for the lower field intensity range (up to 65 V/cm), the probability of cells death was apparently greater at 2.5 mM $[Ca^{2+}]_O$.

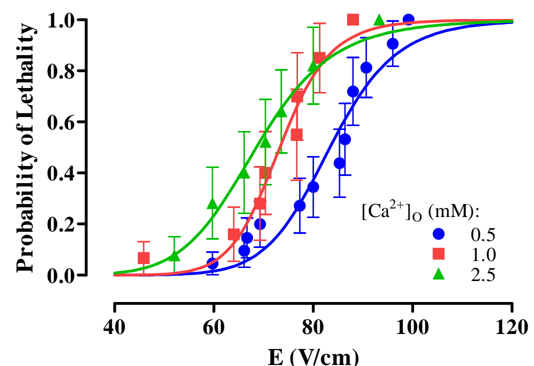

Figure 1: Lethality curves in which the probability of lethality was determined as a function of electric field intensity applied to isolated rat ventricular myocytes exposed to different extracellular Ca^{2+} concentrations ($[Ca^{2+}]o$): 0.5 (blue), 1.0 (red) or 2.5 (green) mM. Data are presented as mean ± standard error. The parameters of the fitted curves are presented in Table 2.

Table 2: Electric field intensity estimated to cause lethal injury to 50% of the myocyte population (L50), determined during exposure to different extracellular Ca^{2+} concentrations ($[Ca^{2+}]_O$). Means ± standard error values of L50 and Hill coefficient (n), as well as the number of cells in each group (N), are presented.

$[Ca^{2+}]_O$ (mM)	L50 (V/cm)	n	N
0.5	83.4±1.0	12.5±1.9	13
1.0	73.3±0.7	14.4±2.1	8
2.5	68.6±0.7	8.9±0.9	8

Discussion

The direct effect of $[Ca^{2+}]$o on electroporation was modeled by DeBruin and Krassowska [15]. The authors showed that changes in $[Ca^{2+}]$o, in contrast with the variation in the concentration of others ions, do not affect the density of the pores created by strong electrical fields. While this prediction excludes $[Ca^{2+}]$o as an important determinant of the conductance of the electroporated membrane, $[Ca^{2+}]$o is expected to influence Ca^{2+} influx by determining its driving force. The Ca^{2+} equilibrium potential calculated with the Nernst equation, assuming 0.2 μM as the diastolic $[Ca^{2+}]$ in the cytosol [18], was ~100, 109 and 120 mV at 0.5, 1.0 and 2.5 mM $[Ca^{2+}]$o, respectively. Both lowering $[Ca^{2+}]$o from 1.0 to 0.5 mM and increasing it from 1.0 to 2.5 mM are expected to affect the driving force for Ca^{2+} influx by ~10 mV. However, only the former maneuver affected the myocyte response to high-intensity electrical fields. Thus, it seems unlikely that the death probability could be linearly related to peak Ca^{2+} influx alone

The elevation of the cytosolic intracellular Ca^{2+} concentration ($[Ca^{2+}]$i) is known to activate the repair mechanisms that lead to membrane pore resealing after the electroporation [11, 12]. On the other hand, once the cell membrane is disrupted by a defibrillator-like shock, a massive Ca^{2+} influx also promotes alterations that may lead to cell death [4, 5, 6, 7, 8, 9, 10]. One possibility to explain the present results is the difference in the temporal pattern of increase in $[Ca^{2+}]$i upon membrane electroporation. It is plausible to suppose that the greater the driving force for Ca^{2+} influx (i.e., the greater the $[Ca^{2+}]$o), the faster $[Ca^{2+}]$i will rise. Assuming that the Ca^{2+} influx at 0.5 mM $[Ca^{2+}]$o causes a $[Ca^{2+}]$i increase sufficient to trigger efficient membrane repair, if the mechanisms associated to the promotion of irreversible cell injury by excess Ca^{2+} (i.e., protein aggregation, phosphate precipitation, proteolysis, mitochondrial dysfunction [19] are activated before substantial repair can take place, the cell will not survive. Thus, the faster the $[Ca^{2+}]$i rise, the greater should be the probability of cell death. Lowering $[Ca^{2+}]$o might thus result in slower increase in $[Ca^{2+}]$i (vs. that at 1 mM $[Ca^{2+}]$o), thus allowing membrane repair to ensue before irreversible damage occurs.

It should also be considered that the sarcoplasmic reticulum and mitochondria act as powerful Ca^{2+} buffers, as they take up Ca^{2+} from the cytosol. This buffering may contribute to delay the rise in $[Ca^{2+}]$i upon electroporation. Inhibition of the sarcoplasmic reticulum Ca^{2+} uptake increases the level and duration of electroporation-induced $[Ca^{2+}]$i elevation, whereas inhibition of mitochondrial Ca^{2+} uptake leads to induction of cell death by typically sublethal shock intensities (Oliveira PX, Goulart JT, Bassani JWM, Bassani RA, unpublished observations). The capacity of these stores, however, is finite, and some of it might be not far from saturation in cells exposed to physiological $[Ca^{2+}]$o. For instance, imposing a large $[Ca^{2+}]$o entry into the cytosol by membrane electroporation or other means not only does fail to increase the sarcoplasmic reticulum Ca^{2+} content, but also causes spontaneous Ca^{2+} release from this organelle [7, 20]. Thus, at both 1.0 e 2.5 mM $[Ca^{2+}]$o, the influx would possibly increase $[Ca^{2+}]$i to critical levels earlier than the repair mechanisms could limit further Ca^{2+} overload, so that the rate of Ca^{2+} entry would not make much difference.

Conclusion

This study shows that decreasing $[Ca^{2+}]$o to 50% of the physiological level improves the chances of cell survival after exposure to strong electrical fields. This information might be useful for devising approaches to enhance defibrillation safety.

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