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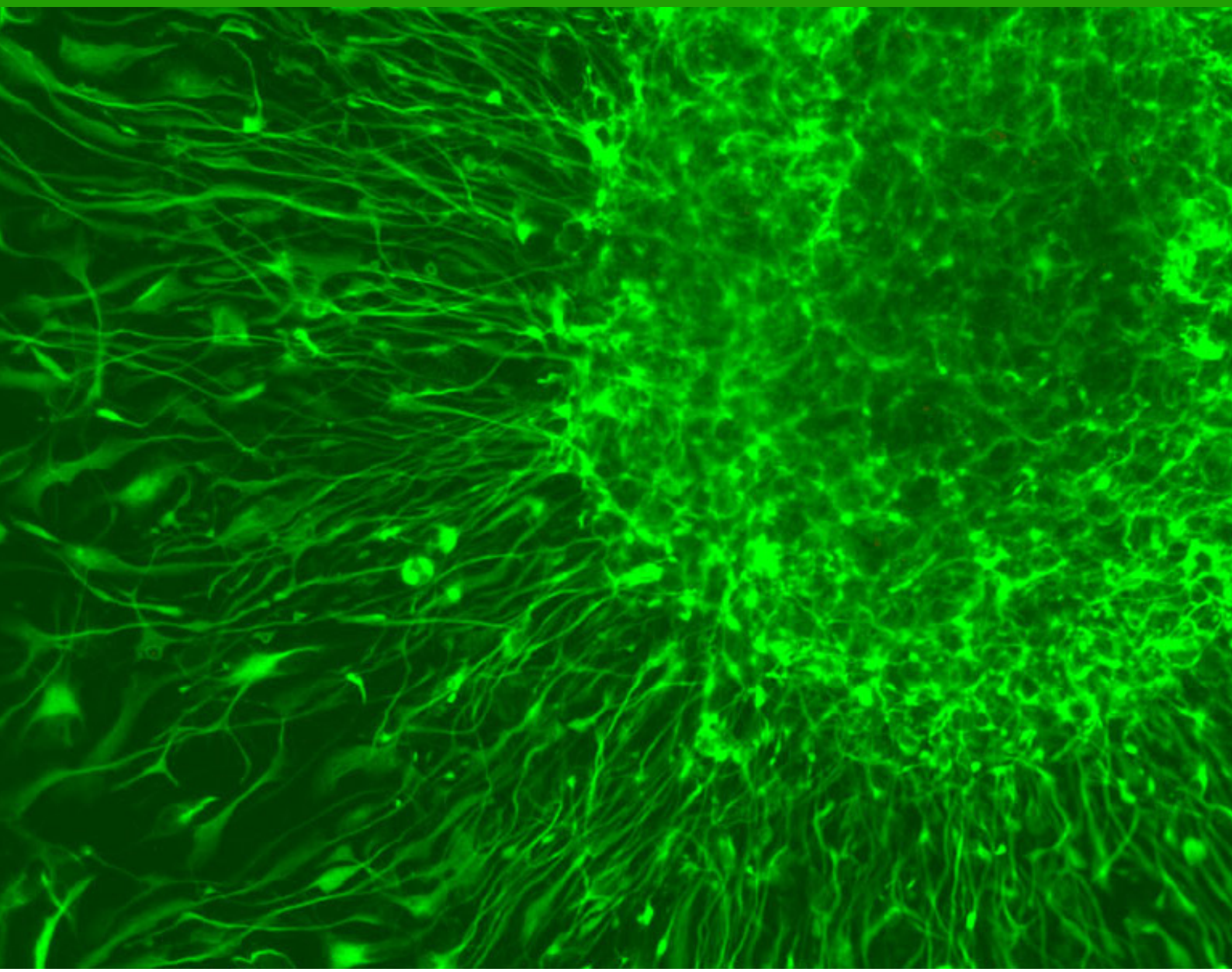

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Facultad de Ciencias Médicas; Universidad Nacional de La Plata;
La Plata, Buenos Aires, Argentina. Tel.-Fax: +54-211-4834833
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CALORIMETRY OF ISOLATED HEARTS (II) ENERGETIC OF Ca^{2+} HOMEOSTASIS IN DIFFERENT ANIMAL MODELS AND DURING ISCHEMIA – REPERFUSION

Alicia E. Consolini [1,*], María I. Ragone[1,3], Germán A. Colareda[1], Patricia Bonazzola[2,3]

[1]Grupo de Farmacología Experimental y Energética Cardíaca (GFEYEC), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), Argentina.
[2]Instituto de Investigaciones Cardiológicas, Universidad de Buenos Aires (UBA-CONICET) Argentina
[3]Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET)

* **Corresponding author: dinamia@biol.unlp.edu.ar**

Abstract

This review describes the application of calorimetry to study cardiac energetics in physiological, pharmacological and pathophysiological conditions of a perfused heart preparation. In particular, the factors influencing the resting heat rate (Hr), such as species differences among rat, mouse and guinea-pig hearts, as well as the mechanisms underlying the increased resting heat rate under a high $[K^+]$-cardioplegia were discussed. Our results give support to a functional interaction between the sarcoplasmic reticulum and mitochondria for myocardial Ca^{2+} movements. Finally, it is briefly commented the application of calorimetry to study Ca^{2+} homeostasis during a model of stunning induced by no-flow ischemia and reperfusion in entire hearts by using selective inhibitors of cellular Ca^{2+} transporters. Either in the presence or absence of perfusion, calorimetry allows to evaluate the total muscle economy. As application, calorimetry allows to detect a cardiac dysfunction still under unaltered contractility, demonstrating that it is a very sensitive methodology for studying pathological situations and pharmacological consequences.

Keywords: calorimetry, cardiac stunning, sarcoplasmic reticulum, mitochondria, calcium

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As described in the first part, calorimetry of entire small hearts allows studying the energetic of contraction and Ca^{2+} homeostasis under resting and active states, in different species, and in the presence or absence of perfusion. In the following paragraphs we describe the more recent findings about the role of sarcoplasmic reticulum (SR) and mitochondria in hearts, estimated from the calorimetric measurements under control conditions and after using ionic changes or selective pharmacological tools.

1. Energetic of Ca^{2+} homeostasis during resting state

1.1. Species differences in resting heat rate: from humans to mice

Considering the animal species, there is an inverse relation between the resting heat rate (Hr) and the body weight [1]. Thus, the estimations of Hr from papillary muscles with the thermopiles method were about 1 $mW.g^{-1}$ for cat, 1.8 $mW.g^{-1}$ for rabbit, 2.5 $mW.g^{-1}$ for guinea-pig, and 3.5 to 4 $mW.g^{-1}$ for rat [2]. Similar respective values were obtained with the flow calorimeter for small cardiac preparations such as the perfused rabbit interventricular septum [3,4] and the isolated entire rat ventricles [5,6]. Nevertheless, Gibbs and Loiselle [7] have critically analyzed the certain variability factors that affect the Hr values, such as the type and thickness of preparations, obtaining higher values when they are thin. Such influence was seen for guinea-pig heart preparations, which developed basal heat rates (Hr) between 2.6 in papillary and 12.7 $mW.g^{-1}$ in trabeculae under physiological conditions in different laboratories and methods [8], due in part to the generation of Ca^{2+} waves at low temperatures.

More recently, it was of interest to do the calorimetrical study of the mouse heart, because this species becomes the most popular mammal for molecular biology and provides researchers with a unique tool to explore the roles of particular genes and physiological functions. Although still limited the energetic study of mouse heart, on the basis of dimensional arguments Gibbs and Loiselle [7] predicted that mouse basal metabolism may be twice as high as that of rat and 8-fold higher than that of man's. Although there are few energetic determinations in human cardiac preparations, it was reported a basal or resting heat rate of 1.68 $mW.g^{-1}$ with thermopiles [9]. So, according to the Gibbs and Loiselle predictions it was expected a basal or resting heat rate between 8 and 14 $mW.g^{-1}$ for mouse hearts. Moreover, they analyzed a relation between basal and total myocardial metabolism, concluding that regardless species the net mechanical efficiency of hearts was about 20% when contracting at optimal length. Based on this concept, the authors demonstrate that in the whole heart preparations the basal metabolism has a large contribution to the total myocardial energy flux, and predict estimations of Hr of about 5 $mW.g^{-1}$ for human hearts and 40 $mW.g^{-1}$ for mice ones. The mouse heart will use about 1/3 of the energy per beat than that used by humans, and the difference between total and basal heat rate is very small in mouse heart, because they are operating much closer to the maximal oxidative capacity than human hearts [7]. Recently, we obtained the total and basal heat rate of mouse hearts in the flow calorimeter [3,6]. We have measured the total heat rate released by C57BL/6 mouse hearts perfused at a flow of 1.5 $ml.min^{-1}$ with Krebs-2.5 mM Ca^{2+} at 37°C and frequency of 4 Hz inside the flow calorimeter. The resting heat rate was measured after perfusing the cardioplegic solution of 25 mM K-0.5 mM Ca^{2+} Krebs (CPG). Figure 1 shows that Ht was 24.7 ± 3.4 $mW.g^{-1}$ (n = 5) and Hr was 18.6 ± 4.7 $mW.g^{-1}$ (n = 4) in wild-type mice (WT). This value is about 3.7 fold the Hr of rat hearts under the same cardioplegic solution (5 $mW.g^{-1}$), closed to that predicted by Gibbs and Loiselle [7]. In addition, the same measurements of heat production were done in mutant mice with phospholamban ablation (PLNKO mice)

[10], RyR2-S2814D knock-in mice [11], and mice resultant from crossbreeding PLNKO and RyR2-S2814D mice (SDKO mice) created by Dra Mattiazzi [12]. Results from Figure 1 show that hearts from SDKO developed an Ht similar to that of PLBKO and S2814D but higher than that of WT. The Ht show that those genetic alterations induce an extra energy consumption due to the high activity of SERCA and/or increased SR Ca^{2+} release which become in high cytosolic Ca^{2+} removal. Similar relationship was found in the respective values of Hr (Figure 1), which represented about 74% of total heat rate, more than the ratio found in rat hearts, in agreement with that suggested by Gibbs and Loiselle [7]. From these values of Ht and Hr of WT, and the heart rate (4 Hz) it can be calculated the active heat (Ha) as about 1.52 $mJ.g^{-1}$ (6.1 mW/4 Hz). This value agrees with the work output per beat (1.2 $mJ.g^{-1}$) calculated for mice from the cardiac output and stroke volume [7]. It is also possible to compare these Ht values with the oxygen consumption, by using the energetic equivalent of oxygen estimated in 477 $kJ.mol^{-1}$ O_2 [13] or other expressions for interconversion of units, such as 1 mL O_2 $.g^{-1}$ $dw.min^{-1}$ equivalent to 74 $mW.g^{-1}$ ww, and 4.5 g $ww.g^{-1}$ dw (where dw means dry weight and ww means wet weight) [7]. Then, some reports of oxygen consumption in wild-type C57BL/6 mouse hearts such as 10.7 µmol $O_2.g^{-1}.s^{-1}$ [14] and 130-150 $\mu L.min^{-1}.g^{-1}$ [15] are equivalent to resting heat rates of 85 $mW.g^{-1}$ and 30-49 $mW.g^{-1}$, respectively. The first report of oxygen consumption was for hearts beating at 500 beats *per* min while the second was for hearts at about 60 to 100 beats *per* min (rate pressure product of 5000-6000 mmHg.bpm). Then, our results of Ht about 25 $mW.g^{-1}$ in WT hearts beating at 180-240 bpm are in agreement with those obtained in the measurements of oxygen consumption.

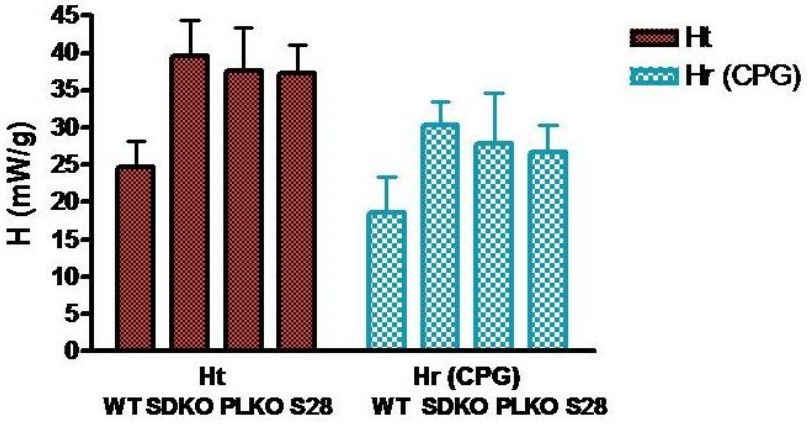

Figure 1: Total heat rate (Ht) and resting heat rate (Hr) obtained from isolated mouse hearts perfused inside the flow calorimeter. Ht was obtained at a heart rate of 4 Hz, while Hr was obtained by perfusing Krebs with 25 mM K-0.5 mM Ca (CPG). Mice belonged to 4 strains of C57BL/6: wild-type (WT), SDKO, PLNKO (PLKO), and RyR2-S2814D (S28). Results are shown as mean ± SEM (t-test between Ht and Hr: t= 9.321, p = 0.0026)

1.2. Effects of cardioplegia on basal metabolism

It is difficult to stop cardiac beating in order to measure Hr, especially at physiological temperature. The technique consists in separating the atria with the sinusal node and put a mechanical pressure or a little cut on the septum focus close to the aorta. Another strategy is to estimate the Hr from extrapolation at zero frequency a linear regression of the total

heat rate (Ht) versus the frequency of contraction [3]. The application of this method to the perfused rabbit interventricular septum gave values of Hr similar to those obtained in rat papillary muscles by the thermopiles method [8]. Alternatively, the cardioplegic solutions were a practical tool to determine Hr. Cold high-[K^+] crystalloid cardioplegia (CPG) [16] are used in some surgical procedures to arrest heart. Nevertheless, when increasing $[Ca^{2+}]_o$ under perfusion of 25 mM K^+ -Krebs (CPG) in isolated rat hearts, Hr was proportionally increased to about 5.1, 6, 7 and 8.3 $mW.g^{-1}$, respectively for 0.5, 1, 2 and 4 mM Ca^{2+} [6]. That $[K^+]_o$ depolarized the membrane potential to about -45 mV [17] in which L-type Ca^{2+} channels could be activated, with the consequent active removal of cytosolic Ca^{2+} and exothermic ATP consumption. In fact, Hr under 25 mM K-2 mM Ca-Krebs was significantly reduced by the L-channels blocker verapamil, while Hr under control Krebs (5-7 mM K^+) was unaffected by this drug [6,18]. Moreover, when $[K^+]_o$ increases from 6 to 25 mM during resting conditions, Hr has an initial transitory rise sensitive to caffeine and higher than the following stationary increase [5]. These results suggested that Ca^{2+} influx through L-channels releases Ca^{2+} from SR, which was reduced after activation of RyR2 channels by caffeine. The steady increase in Hr was associated to the activation of the Na^+, K^+-ATPase (measured by ^{86}Rb uptake-efflux experiments), which represents respectively the 95% of the steady-state and the 36% of the initial peak [5]. The Ca-dependence of Hr under high-$[K^+]_o$ was related to the active removal of Ca^{2+} entered through L-channels, since it was sensitive to nifedipine, a more selective Ca^{2+} channel blocker [19]. It has the advantage that it is not an inhibitor of the mNCX as verapamil is [20], therefore excluding the possibility that Hr reduction was due to inhibition of a mitochondrial Ca^{2+} cycling. In fact, the selective inhibitor of mNCX clonazepam did not reduce Hr under CPG [21]. However, tetrodotoxin (TTX) was able to reduce the high $[K^+]_o$-induced rise in Hr, suggesting that the slow depolarization may also activate a fraction of Na^+-channels, in a way that the rise in $[Na^+]_i$ further favors Ca^{2+} influx by the SL-NCX, forcing the cell to spend energy for maintain the gradients [19]. The underlying mechanisms of CPG were confirmed when measuring the changes in cytosolic and mitochondrial free Ca^{2+} in cardiomyocytes under resting condition with the fluorofores Fluo-4 or Fura-2 and Rhod-2 respectively. In rat and guinea-pig cardiomyocytes there were found some differences in Ca^{2+} homeostasis [19, 22]. In resting rat cardiomyocytes the high-K^+ solution transiently increased the relative fluorescence of both, Fura-2 ($[Ca^{2+}]_i$) and Rhod-2 ($[Ca^{2+}]_m$), with an exponential decay that was much slower for the Rhod-2 signal. The SERCA blockade with thapsigargin (Tpg) increased Rhod-2 fluorescence, while in rat hearts decreased Hr in about -0.44 ± 0.08 $mW.g^{-1}$. All these results suggested that CPG increased $[Ca^{2+}]$ in both cytosol and mitochondria, being SERCA the main energetic consumer, with a slight but Ca^{2+}-dependent increase in diastolic tone. The mitochondrial Ca^{2+} uptake contributed to cytosolic Ca^{2+} removal and was potentiated during SERCA inhibition, suggesting the functional interaction between SR and mitochondria. Consistently, the UCam blockade with KB-R7943 reduced the Rhod-2 signal and increased the resting pressure and Hr, in agreement with the increase in cytosolic Ca^{2+}. Addition of 10 mM pyruvate (Pyr) also increased Hr and resting pressure, since it stimulates the aerobic metabolism and improves $\Delta\Psi m$ for Ca^{2+} uptake [19]. Figure 2 shows the Hr values under CPG demonstrating the dependence on $[Ca^{2+}]_o$ and the role of UCam in Ca^{2+}-handling. Moreover, it was demonstrated the role of mKATP channels under high-K^+ medium, since the selective blocker 5-hydroxidecanoate (5-HD) increased Hr in about 10 $mW.g^{-1}$ [21]. Since it is known that mKATP current contributes to reduce the driving force for the mitochondrial Ca^{2+} uptake [23] such rise in Hr may be related to the Ca^{2+} dependent increase in metabolism. However, further addition of clonazepam reduced Hr

suggesting that energy consumption was related to a mitochondrial Ca^{2+} cycling between the uniporter and mNCX. In conclusion, under CPG the cardiac muscle consumes oxygen exothermically for maintaining the low $[Ca^{2+}]_i$ despite the Ca^{2+} influx through L-channels, Na^+ influx as a window current, SR Ca^{2+} release. The exothermic mechanisms for the active removal of Ca^{2+} are SERCA, SL-NCX and mitochondria, with a functional competition for Ca^{2+} between SR and mitochondria.

In guinea-pig cardiomyocytes, Hr was also increased by 25 mM K^+-medium as well as the Fluo-4 signal, indicating the increase in cytosolic $[Ca^{2+}]$ which is actively removed. Nevertheless, results with selective drugs showed differences with rat cardiomyocytes, suggesting that in guinea-pig hearts mitochondria would uptake Ca^{2+} via mNCX, rising the steady $[Ca^{2+}]_m$ and the Hr. It was reported that cytosolic Ca^{2+} was taken up by mitochondria through the reverse mode of mNCX [24] under other situations such as hypoxia [25]. Under 25 mM K^+-0.5 mM Ca^{2+}-medium, the Hr of guinea-pig hearts (about 5.8 $mW.g^{-1}$) was slightly increased with rising Ca^{2+} to 1 mM and reduced by nifedipine. However, it was not affected by the mNCX blocker CGP37157 as neither was the resting tone, suggesting that Ca^{2+} movements between cytosol and mitochondria were energetically compensated [22].

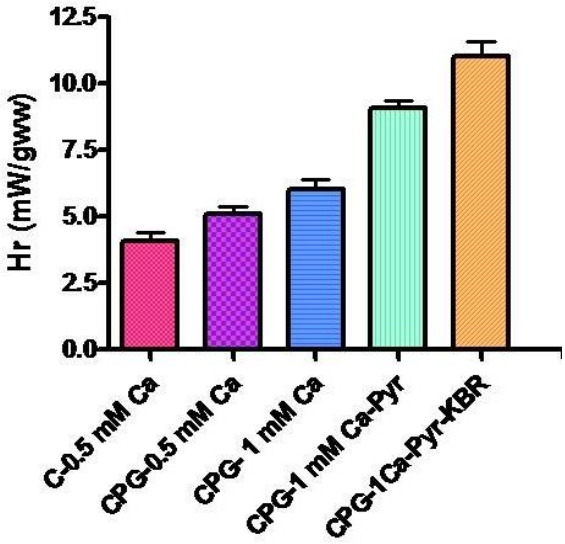

Figure 2: Resting heat rate (Hr) obtained from isolated rat hearts perfused inside the flow calorimeter and exposing to the following treatments: control Krebs.0.5 mM Ca (C-0.5 Ca), Krebs with 25 mM K and 0.5 mM Ca (CPG-0.5 Ca), CPG-1 mM Ca, CPG-1 mM Ca and 10 mM pyruvate (CPG-1 mM Ca-Pyr), and CPG-1 mM Ca and 10 mM pyruvate and 5 μM KB-R7943 (CPG-1 mM Ca-Pyr-KBR), Results are shown as mean ± SEM.

2. Energetic of ischemia-reperfusion: mitochondrial role in several models and animal species

As previously seen, the energetic measurements are sensitive enough to detect changes in the mechanisms of Ca^{2+} handling during the resting state or the steady-state beating. Then, it was expected that it could show the metabolic changes and the alterations in Ca^{2+} handling during a pathological condition in which both of them are affected, such as a model of ischemia and reperfusion (I/R). Such experimental models are used all over the world to study the underlying mechanisms associated to myocardial infarct

with many biochemical and biophysical methodologies which follow the changes in the mitochondrial state, such as apoptosis index, proteins from signal transduction chains, isolated mitochondrial properties, and oxidative injury, among others [26,27,28]. Nevertheless, there are few reports about the less drastic condition of "stunning" defined as a reversible state induced by a short period of ischemia from which contractility is partially recovered. The model of stunning allows to predict the first traces of dysfunction induced by a transitory coronary disease. So, it is useful to understand the genesis and find preventive pharmacological strategies. Moreover, it is known that a model of entire heart avoids the cellular consequences of isolation or biochemical reactions which change the mitochondrial metabolism and transporters. Because of that the continuous calorimetric determinations during the entire cycle of I/R contributed to the real knowledge about *in situ* cellular energetic processes. Despite it is not possible to detail the calorimetrical findings about the stunning models in rat and guinea-pig ventricles in this mini-review, we will briefly refer some points as examples of the methodological utilities.

2.1. Effects of ischemia on the heat components of a single beat

First of all, we studied the effects of ischemia on consecutives single beats (5 minutes apart) at 25°C in isolated rat hearts, in order to evaluate the consequences on each of the four heat components [29]. The no-flow ischemia induced a progressive and proportional fall in H1, H2, H3 and P, and a stronger reduction in H4 up to extinguish it even when contractility was still present. This behavior of H4 under ischemia was in agreement with that observed under hypoxia and with its mitochondrial origin. Nevertheless, there was a different myothermal effect under a less severe condition of ischemia, such as that induced by hypoperfusion. When the perfusion flow was reduced to about 25% of the initial, P and H3 (TDH) were gradually reduced with the number of single contractions, but the activation heat fractions (H1 and H2) remained unaffected [29]. These results suggested that the low-flow ischemia did not affect the Ca^{2+} transients but reduced contractility and TDH due to the loss of muscle tension and diastolic length. The $[Ca^{2+}]$- and O_2-dependent heat fraction H4 was also reduced under low-flow ischemia, in agreement with the reduced oxygen contribution to mitochondria [29].

2.2. Mitochondrial role on the stunning due to I/R in different species

Since the model of stunning requires a condition nearer to the pathophysiological, it was necessary to stimulate hearts at higher frequency and physiological temperature, maintaining a steady contractile state before and during I/R (Figure 3). In those conditions it was not possible to analyze a single beat or the heat components. Then, our following studies measured the total heat rate (Ht) and the total muscle economy (P/Ht) as energetical estimations for hearts before and during the exposure to I/R. We evaluated the cardioprotective effects of the cardioplegic solution of 25 mM K-0.5 mM Ca (CPG) at 30˚C. Cold cardioplegic solutions are protective against I/R dysfunction, mainly by the reduction of the total energy consumption when beating stopped [30], but it was not known the consequences on Ca^{2+} homeostasis and reversibility or stunning. In rat beating hearts the perfusion of CPG before I/R improved the post-ischemic contractile recovery (PICR) from about 55% to 77% of initial P, and reduced the diastolic contracture (LVEDP) typical of I/R. It was partially due to the maintaining of ATP and PCr levels and stimulation of sarcolemmal Ca^{2+} removal by SL-NCX [31].

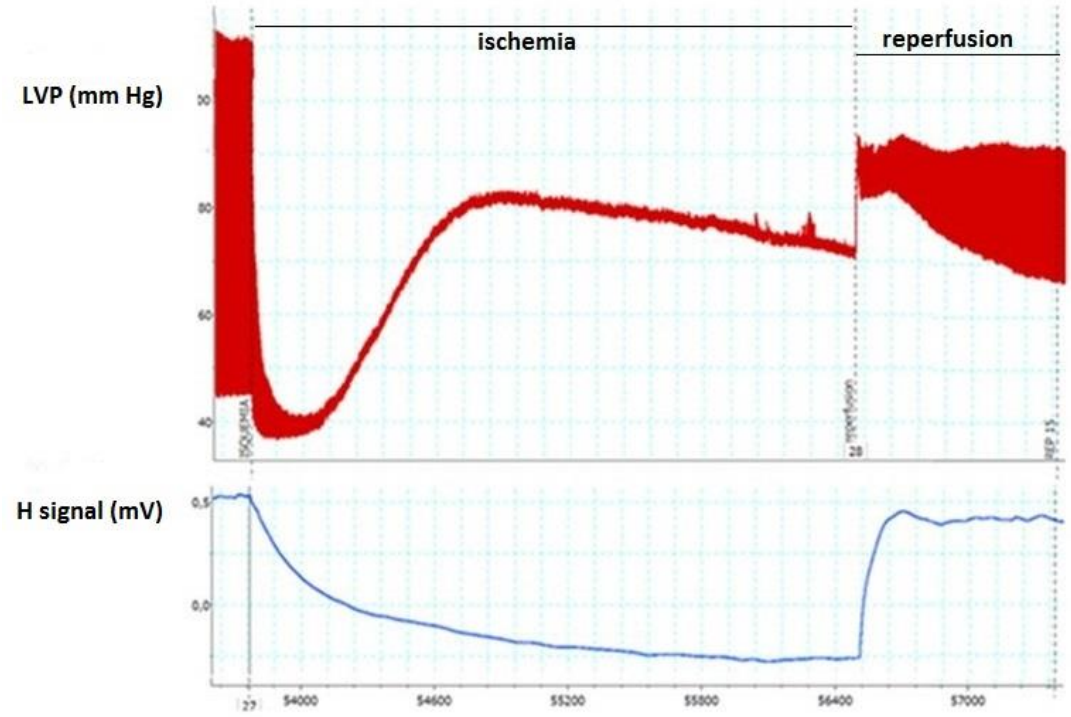

Figure 3: Typical analogic/digital recording (A/D) obtained from an isolated rat heart stimulated at 3 Hz, before and during exposure to a period of no-flow ischemia and the first 15 minutes of reperfusion. It shows the left intraventricular pressure (LVP, upper) and the simultaneous signal of total heat rate (H, lower, measured in mV). This H signal has to be converted to the real Ht (in mW) by substracting the baseline H signal obtained without muscle and considering the calibration factor (in $mW.mV^{-1}$). See details in [36].

In the last years there is a growing interest in understanding the interaction between SR and mitochondria, and some reports explored it under infarct due to drastic I/R [32, 33]. Now it is known that mitochondria participate in Ca^{2+} homeostasis, since they can accumulate Ca^{2+} in response to cytosolic changes during beating, with the consequent periodic oscillations in $[Ca^{2+}]_m$, being the definition of beat-to-beat changes dependent on the species [34, 35]. Then, we used calorimetry to evaluate the functional interaction between SR and mitochondria during the stunning consequent to I/R in two species, rat and guinea-pig, which have different cardiac Ca^{2+} handling. Our studies in ***rat heart*** concluded that CPG (25 mM K^+-0.5 mM Ca^{2+}-Krebs) improved PICR and total muscle economy (P/Ht) with reduced diastolic contracture in R, which was attributed to depletion of the SR Ca^{2+} [18,31,36]. Although the SR load explain the diastolic condition, it was not evident the mechanism of improvement in contractile recovery during R, by which it was supposed that CPG could stimulate mitochondrial metabolism and ATP synthesis during R. To evaluate the mitochondrial role under CPG, the mNCX was blocked with 10 µM clonazepam (Clzp) before I/R, and therefore the cardioprotection was reduced [36]. These results were in agreement with those reported by other authors who found that mNCX inhibition increased the $[Ca^{2+}]_m$ but reduced the Ca^{2+} transients in rat cardiomyocytes [37]. Moreover, other drugs which reduce the $[Ca^{2+}]_m$ (the UCam blocker Ru360 and the mKATP opener diazoxide) also reduced the CPG cardioprotection, but improved PICR in non-CPG hearts [21]. These results suggested that after depleting SR avoiding the ischemic contracture, the washing of CPG during the first 1 or 2 minutes of R would stimulate a Ca^{2+} cycling between mitochondria and SR, regulating the SR load and finally the PICR [36]. The role of SR

during I/R was also reported in mouse hearts, since during the first minutes of R there was an abrupt Ca^{2+} loss from SR which explains the diastolic contracture and stunning, while restoration of perfusion and metabolism favors the SR Ca^{2+} reuptake and contractile recovery [38]. In rat hearts, calorimetrical results in several other conditions suggested that the mitochondrial Ca^{2+} cycling through the mNCX and uniporter contributes to regulate the SR load during R and consequently the Ca^{2+} transients and PICR [39]. However, there is a threshold $[Ca^{2+}]_m$ which determines the limit between cardioprotection and dysfunction by Ca^{2+} overload. Contrarily to that described in rat, in guinea-pig hearts Clzp further improved PICR and muscle economy over the cardioprotection of CPG at 30°C, but without changes in the SR Ca^{2+} load and release [22]. These results and others suggested that the mNCX has a different role in guinea-pig and rat hearts. It is known that mNCX function depends on gradients of $[Na^+]_i$ and $[Ca^{2+}]_i$ which are dependent on the species [34]. In guinea-pig hearts the SR has a low Ca^{2+} content, and our results suggested that mNCX would compete with the SR in the cytosolic Ca^{2+} removal during the transient of $[Ca^{2+}]_i$. The function of mNCX was demonstrated also by fluorometrically measuring the cytosolic and mitochondrial Ca^{2+} levels in cardiomyocytes. Moreover, when adding ouabain to perfusion in order to increase $[Na^+]_i$ the role of mNCX in guinea-pig heart was changed to that previously seen in rat hearts [22]. Consequently, the difference on the mitochondrial role in reperfused guinea-pig and rat hearts treated with CPG is that the functional interaction between mitochondria and SR mainly depends on $[Na^+]_i$ and on the SR load. Mitochondria could act as a sink of Ca^{2+} competing with a leaky SR in guinea-pig hearts, or contribute as a source of Ca^{2+} to load the SR in rats. Moreover, Clzp and diazoxide were cardioprotectives during the CPG exposure of guinea-pig hearts [22], offering good perspectives of cardioprotection to other hearts with a low SR load, such as rabbit, dog and human hearts.

2.3. Temperature and hormonal influences on the energetic of stunning

Later, we assessed a more physiological model of stunning at 37°C [40, 41]. There is an influence of temperature on Ca^{2+} homeostasis during I/R, in such a way that the ischemic period at 37°C has to be shorten to 20 minutes in order to obtain the same degree of PICR than at 30°C. The sarcorreticular Ca^{2+} content after I, estimated from the reperfusion with 10 mM caffeine-36 M Na-Krebs, was reduced at the physiological temperature with respect to that obtained at 30°C, while the calorimetric output was increased, suggesting that maintaining the contracture was energetically more expensive at 37 than at 30ºC [39]. Results agree with the known temperature-dependent increment of the cytosolic Ca^{2+} removal rate through the active transporters, with the consequent increase in the energetic consumption. On this way, in rabbit hearts all Ca^{2+} transporters were reported to have a Q_{10} of 2-3 as well as the $[Na^+]_i$ and the activity of SL-NCX are sensitive to the influence of temperature on the Na, K-ATPase [42]. This agrees with reports in rat cardiomyocytes exposed to 10 minutes of simulated ischemia and reperfusion, in which hypothermia from 37 to 34 and 30ºC gradually increased the Ca^{2+} transients and cell shortening either before or after ischemia, and also increased the Ca^{2+}-sensitivity of myofilaments [43]. In spite of our results showed high energetical output under caffeine-low Na reperfusion, hearts steadily beating at 3 Hz and 37ºC have about the same total muscle economy (P/Ht of 5.5 ± 0.3 $mmHg.g.mW^{-1}$) than those beating at 1 Hz and 30ºC (P/Ht of 6.6 ± 0.8 $mmHg.g.mW^{-1}$). Considering that Ht includes the resting heat rate (Hr) and the energy of contractions (Ha = (Ht-Hr)/HR), the similarity in P/Ht suggests that cardiac metabolism and contractility are equilibrated. Our results are in agreement with the observations of Gibbs and Loiselle [7] who

compared different energetic studies in papillary muscles from different species and calculated the net mechanical efficiency as work/ (total – resting energy). The net efficiency remains in about 20% for the different species and conditions of beating at optimal length and afterload, still under inotropic agents as pyruvate, ouabain or isoprenaline, as other reported for the mechanical efficiency when energy was estimated as oxygen consumption [44]. The authors adjudicate this constancy to the large contribution of basal metabolism (Hr) to the total energy rate (Ht), both of which suffer the effects of species and temperature. In our experiments entire ventricles rarely remain in resting state at 37°C as necessary to estimate Hr, and pressure development could not be expressed in the same units of energy (as work is in $mJ.g^{-1}$) in order to have an adimensional estimation of muscle economy, as Gibbs and Loiselle did. Nevertheless, the P/Ht ratio resulted constant at different temperatures. So, it gave us the possibility of comparing the total muscle economy before and after ischemia.

In agreement with that, P/Ht was not significantly modified by hyperthyroidism (HpT) in rat hearts (6.05 ± 0.04 $mmHg.g.mW^{-1}$) at 37°C. However, after 20 minutes of ischemia and 45 minutes of reperfusion P/Ht was reduced in the euthyroid hearts (to 3.6 ± 0.6 $mmHg.mW^{-1}.g$) while the HpT hearts developed a more economical contractile state (P/Ht of 9.7 ± 1.7 $mmHg.mW^{-1}.g$) [40]. So, hyperthyroidism was cardioprotective in the moderate stunning, also increasing PICR from 77.5 ± 3.2% in euthyroid to 108.8 ± 11.6% of initial P in HpT, and reducing the diastolic contracture. The hyperthyroidism also changed the roles of mNCX and mK_{ATP} during the stunning associated to moderate I/R with respect to the euthyroid hearts [40].

In some situations calorimetry was more sensitive than the mechanical activity, such as in the perfusion of hearts with the phytoestrogen genistein (Gst) before the exposure to I/R. At 37°C there were not differences in contractile performance between the responses of female and male rat hearts to Gst, while in both sex it slightly reduced P/Ht during R. This result showed a high energetic consumption related to an increase in the relative relaxation rate (-P/P = -dP/dt/P) seen also during R [41]. Gst had a more different effect between female and male rat hearts when it was perfused at relatively low temperature (30°C), with a negative inotropism and low PICR in males but not in females, as well as in P/Ht [41]. Then, the energetics evidenced the balance between known effects of Gst, as Ca^{2+} current inhibition and SR Ca^{2+} load, in different degree at different temperatures. In other protocols, calorimetry showed that adrenaline reduced the total muscle economy (P/Ht) of control hearts while it increased –P/P, but simultaneous perfusion of Gst and adrenaline improved P/Ht during R by reducing the high energetical consumption due to adrenaline in both, female and male rat hearts (Figure 4). So, Gst limited the adrenaline-stimulated and unfavorable Ca^{2+} influx during I/R, and adrenaline masked the effect of Gst on –P/P. This situation of perfusing Gst together with adrenaline is more closed to the physiological condition, and predict certain benefits of Gst, which were afterward confirmed by the fact that *in vivo* treatment with 5 $mg.kg^{-1}$ Gst 1 day before the I/R experiment was more cardioprotective than Gst perfusion on isolated hearts [39].

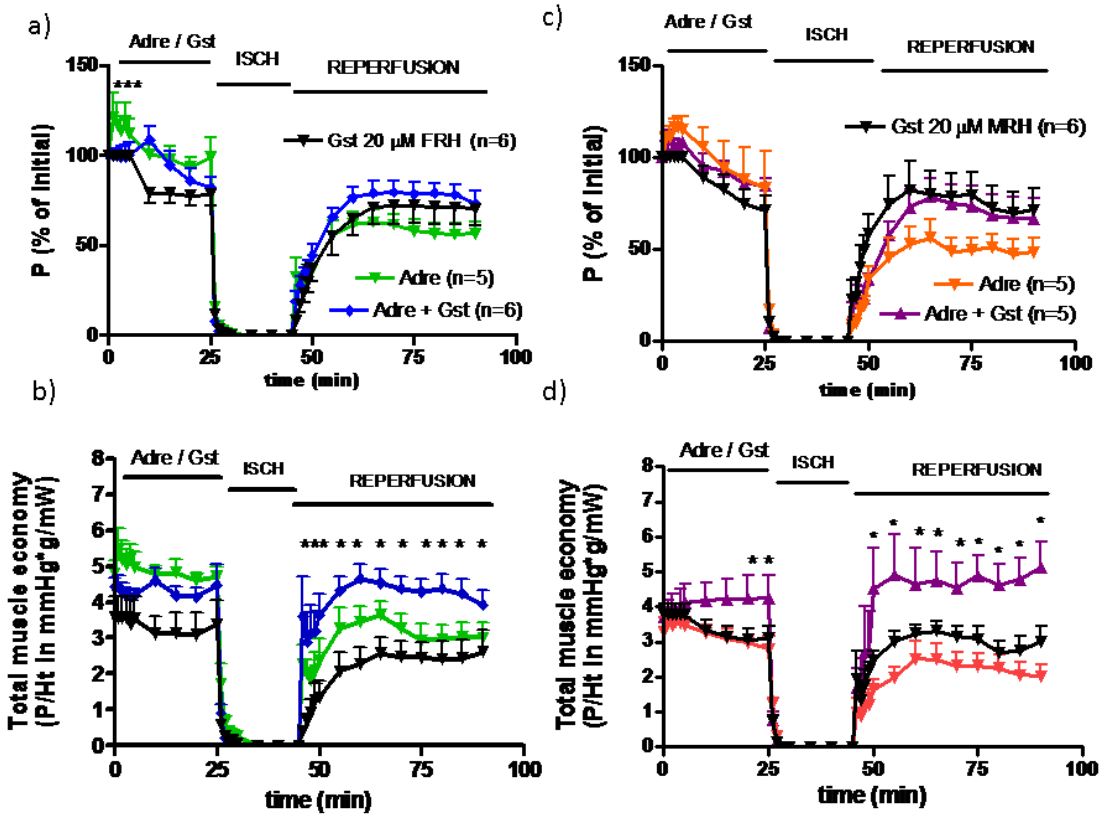

Figure 4: Effects of perfusing the phytoestrogen 20 µM genistein (Gst) in the abscence and the presence of 0.05 µM adrenaline (Adre) before exposing to 20 minutes ischemia and 45 minutes reperfusion (with Krebs) in isolated female (FRH, in a and b) and male (MRH, c and d) rat hearts. See the maximal pressure development (P, as % of initial) and the total muscle economy (as P/Ht), both shown as mean ± SEM (all had 2-way ANOVA $p<0.0001$, and *post-hoc* tests with * $p< 0.05$). Legends were described in a) and c).

In conclusion, this review shows the main findings related to the application of calorimetry to cardiac pathophysiology and pharmacology, showing that it allows to evaluate the role of SR and mitochondrial transporters in Ca^{2+} homeostasis during resting and active state. Moreover, it is the only calorimetrical method that allows to evaluate the heat released even in the absence of perfusion, and therefore to study a real model of ischemia/reperfusion without mitochondrial poisoning.

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References

[1] **Loiselle DS.** Cardiac basal and activation metabolism. *Basic Res Cardiol*. 1987; 82 Suppl 2: 37-50.

[2] **Gibbs C, Loiselle D.** The energy output of tetanized cardiac muscle: species differences. *Pflugers Arch*. 1978; 373(1): 31-38.

[3] **Ponce-Hornos JE, Ricchiuti NV, Langer LA.** On-line calorimetry in the arterially perfused rabbit interventricular septum. *Am J Physiol*. 1982; 243: H289-H295.

[4] **Bonazzola P, Ponce-Hornos JE.** Effects of caffeine on energy output of rabbit heart muscle. *Basic Res Cardiol*. 1987; 82(5): 428-436.

[5] **Ponce-Hornos JE, Marquez MT, Bonazzola P.** Influence of extracellular potassium on energetics of resting heart muscle. *Am J Physiol* 262: H1081-H1087, 1992.

[6] **Márquez MT, Consolini AE, Bonazzola P, Ponce-Hornos JE.** The energetics of the quiescent heart muscle: high potassium cardioplegic solution and the influence of calcium and hypoxia on the rat heart. *Acta Physiol Scand*. 1997; 160: 229-233.

[7] **Gibbs CL, Loiselle DS.** Cardiac basal metabolism. *Jpn J Physiol*. 2001; 51(4): 399-426.

[8] **Loiselle DS.** Cardiac basal and activation metabolism. *Basic Res Cardiol*. 1987; 82 Suppl 2: 37-50.

[9] **Hasenfuss G, Mulieri LA, Blanchard EM, Holubarsch C, Leavitt BJ, Ittleman F, Alpert NR.** Energetics of isometric force development in control and volume-overload human myocardium. Comparison with animal species. *Circ Res*. 1991; 68(3): 836-846.

[10] **Luo W, Grupp IL, Harrer J, Ponniah S, Grupp G, Duffy JJ, Doetschman T, Kranias EG.** Targeted ablation of the phospholamban gene is associated with markedly enhanced myocardial contractility and loss of beta-agonist stimulation. *Circulation Res.* 1994; 75: 401-409.

[11] **van Oort RJ, McCauley MD, Dixit SS, Pereira L, Yang Y, Respress JL, Wang Q, De Almeida AC, Skapura DG, Anderson ME, Bers DM, Wehrens XH.** Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. *Circulation.* 2010; 122: 2669-2679.

[12] **Mazzocchi G, Sommese L, Palomeque J, Felice JI, Di Carlo MN, Fainstein D, Gonzalez P, Contreras P, Skapura D, McCauley MD, Lascano EC, Negroni JA, Kranias EG, Wehrens XH, Valverde CA, Mattiazzi A.** Phospholamban ablation rescues the enhanced propensity to arrhythmias of mice with CaMKII-constitutive phosphorylation of RyR2 at site S2814. *J Physiol*. 2016; 594(11): 3005-3030.

[13] **Curtin NA, Woledge RA.** Energy changes and muscular contraction. *Physiol Rev.* 1978*;* 58: 690-761.

[14] **Kojic ZZ, Flogel U, Schrader J, Decking UK.** Endothelial NO formation does not control myocardial O_2 consumption in mouse heart. *Am J Physiol Heart Circ Physiol.* 2003; 85(1): H392-H397.

[15] **Koentges C, König A, Pfeil K, Hölscher ME, Schnick T, Wende AR, Schrepper A, Cimolai MC, Kersting S, Hoffmann MM, Asal J, Osterholt M, Odening KE, Doenst T, Hein L, Abel ED, Bode C, Bugger H**. Myocardial mitochondrial dysfunction in mice lacking adiponectin receptor 1. *Basic Res Cardiol.* 2015; 110(4): 37.

[16] **Dobson GP.** Organ arrest, protection and preservation: natural hibernation to cardiac surgery.*Comp Biochem Physiol B Biochem Mol Biol.* 2004; 139(3): 469-485.

[17] **Molyvdas PA, Sperelakis N.** Comparison of the effects of several calcium antagonistic drugs on the electrical activity of guinea pig Purkinje fibers.*Eur J Pharmacol.* 1983; 88: 205-214.

[18] **Consolini AE, Márquez MT, Ponce-Hornos JE.** Energetics of heart muscle contraction under high K perfusion: verapamil and Ca effects. *Am J Physiol*. 1997; 273: H2343-H2350.

[19] **Consolini AE, Ragone MI, Bonazzola P.** Mitochondrial and cytosolic calcium in rat hearts under high-K^+ cardioplegia and pyruvate: mechano-energetic performance. *Can J Physiol Pharmacol.* 2011; 89(7): 485-496.

[20] **Cox DA, Matlib MA.** Modulation of intramitochondrial free Ca^{+2} concentration by antagonists of Na^+-Ca^{+2} exchange. *TIPS* 1993; 14: 408-413.

[21] **Ragone MI, Consolini AE.** Role of the mitochondrial Ca^{2+} transporters in the high-[K^+]o cardioprotection of rat hearts under ischemia and reperfusion: a mechano-energetic study. *J Cardiovasc Pharmacol.* 2009; 54: 213-222.

[22] **Ragone MI, Torres NS, Consolini AE.** Energetic study of cardioplegic hearts under ischaemia/reperfusion and [Ca^{2+}] changes in cardiomyocytes of guinea-pig: mitochondrial role. *Acta Physiol (Oxf).* 2013; 207(2): 369-384.

[23] **Garlid KD, Dos Santos P, Xie ZJ, Costa AD, Paucek P.** Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K^+ channel in cardiac function and cardioprotection. *Biochim Biophys Acta*. 2003; 1606(1-3): 1-21.

[24] **Gunter TE, Yule DI, Gunter KK, Eliseev RA, Salter JD.** Calcium and mitochondria. *FEBS Letters*. 2004; 567: 96-102.

[25] **Griffiths EJ, Ocampo CJ, Savage JS, Rutter GA, Hansford RG, Stern MD, Silverman HS.** Mitochondrial calcium transporting pathways during hypoxia and reoxigenation in single rat cardiomyocytes. *Cardiovasc Res.* 1998; 39: 423-433.

[26] **Di Lisa F, Canton M, Menabò R, Kaludercic N, Bernardi P.** Mitochondria and cardioprotection. *Heart Fail Rev*. 2007; 12: 249-260.

[27] **González Arbeláez LF, Pérez Núñez IA, Mosca SM.** GSK-3β inhibitors mimic the cardioprotection mediated by ischemic pre- and postconditioning in hypertensive rats. *Biomed Res Int.* 2013; 317456.

[28] **Buja ML.** Mitochondria in ischemic heart disease. *Adv Exp Med Biol.* 2017; 982: 127-140.

[29] **Consolini AE, Marquez MT, Ponce-Hornos JE.** A comparison of no-flow and low flow ischemia in the rat heart: an energetic study. *Can J Physiol Pharmacol*. 2001; 79: 551-558.

[30] **Stowe DF, Varadarajan SG, An J, Smart SC.** Reduced cytosolic $Ca^{(2+)}$ loading and improved cardiac function after cardioplegic cold storage of guinea pig isolated hearts. *Circulation.* 2000; 102(10): 1172-1177.

[31] **Consolini AE, Quiroga P, Yuln G, Volonté MG.** Participation of Na/Ca-exchanger and sarcoplasmic reticulum in the high [K^+]-protection against ischaemia-reperfusion dysfunction in rat hearts. *Acta Physiol Scand.* 2004; 182(2): 121-132.

[32] **Ruiz-Meana M, Fernandez-Sanz C, Garcia-Dorado D.** The SR-mitochondria interaction: a new player in cardiac pathophysiology. *Cardiovasc Res.* 2010; 88: 30–39.

[33] **Shintani-Ishida K, Inui M, Yoshida K.** Ischemia-reperfusion induces myocardial infarction through mitochondrial Ca^{2+}overload. *J Mol Cell Cardiol*. 2012; 53(2): 233-239.

[34] **Griffiths E.** Species dependence of mitochondrial calcium transients during excitation-contraction coupling in isolated cardiomyocytes. *Biochem Biophys Res Commun*. 1999*;* 263: 554-559.

[35] **O'Rourke B, Blatter LA.** Mitochondrial Ca^{2+} uptake: tortoise or hare? *J Mol Cell Cardiol.* 2009; 46(6): 767-774.

[36] **Consolini AE, Ragone MI, Conforti P, Volonté MG.** Mitochondrial role in ischaemia-reperfusion of rat hearts exposed to high-K^+ cardioplegia and clonazepam: energetic and contractile consequences. *Can. J Physiol Pharmacol*. 2007; 85: 483-496.

[37] **Bell CJ, Bright NA, Rutter GA, Griffiths EJ.** ATP regulation in adult rat cardiomyocytes: time-resolved decoding of rapid mitochondrial calcium spiking imaged with targeted photoproteins*. J Biol Chem*. 2006; 281(38): 28058-28067.

[38] **Valverde CA, Kornyeyev D, Ferreiro M, Petrosky AD, Mattiazzi A, Escobar AL.** Transient Ca^{2+} depletion of the sarcoplasmic reticulum at the onset of reperfusion. *Cardiovasc Res.* 2010; 85: 671–680.

[39] **Consolini AE, Ragone I, Bonazzola P, Colareda GA.** Mitochondrial bioenergetics during ischemia and reperfusion. In: Mitochondrial Dynamics in Cardiovascular Medicine, Gaetano Santulli, editor. Springer/Nature Book, *Adv Exp Med Biol*. 2017; 982: 141-167.

[40] **Ragone MI, Bonazzola P, Colareda GA, Consolini AE.** Cardioprotective effect of hyperthyroidism on the stunned rat heart during ischaemia-reperfusion: energetics and role of mitochondria. *Exp Physiol*. 2015; 100(6): 680-697.

[41] **Colareda GA, Ragone MI, Consolini AE.** Sex differences in the mechano-energetic effects of genistein on stunned rat and guinea pig hearts. *Clin Exp Pharmacol Physiol*. 2016; 43(1): 102-115.

[42] **Bers DM.** Control of cardiac contraction by SR and sarcolemmal Ca fluxes. In: Bers DM ed. Excitation-Contraction Coupling and Cardiac Contractile Force. 2001; Chapter 6, pp. 245-272. Dordrecht, Netherlands, *Kluwer Acad. Publishers,* 2nd ed.

[43] **Ristagno G, Tantillo S, Sun S, Harry Weil M, Tang W.** Hypothermia improves ventricular myocyte contractility under conditions of normal perfusion and after an interval of ischemia. *Resuscitation*. 2010; 81: 898–903.

[44] **Suga H, Hisano R, Goto Y, Yamada O, Igarashi Y.** Effects of positive inotropic agents on the relation between oxygen consuption and systolic pressure volume area in canine left ventricle. *Circ Res* 1983; 53: 306-318.

About Authors

Prof. Dr. Alicia E. Consolini, PhD, is Titular Full Professor of Pharmacology in the Department of Biological Sciences, College of Exact Sciences, National University of La Plata (UNLP). She has been working in the subject for 27 years. She has published 33 articles on scientific peer review journals of physiology and pharmacology, and awarded three prices.

Prof. Dr. Maria Ines Ragone, PhD, is Adjunct Professor of Pharmacology in the Department of Biological Sciences, College of Exact Sciences, National University of La Plata (UNLP) and member of CONICET (Assistant researcher). She has been working in the subject for 13 years. She is coauthor of 17 articles on the scientific journals, and awarded one price.

Dr. Germán A. Colareda, PhD, is Post-doctoral fellow and teacher of Pharmacology in the Department of Biological Sciences, College of Exact Sciences, National University of La Plata (UNLP). He has been working in the subject for 8 years. He has 6 articles on the scientific journals.

Prof. Dr. Patricia Bonazzola, PhD, is Adjunct Professor of Biophysics, (School of Dentistry, University of Buenos Aires, UBA) and member of CONICET (Adjunct researcher) at the Cardiologic Research Institute (ININCA). She has been working in the subject for about 30 years. She has published more than 20 articles on the scientific-peer review journals of physiology and pathophysiology, and awarded four prices.