

CALORIMETRY OF ISOLATED HEARTS (II) ENERGETIC OF Ca²⁺ HOMEOSTASIS IN DIFFERENT ANIMAL MODELS AND DURING ISCHEMIA – REPERFUSION

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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 Ca²⁺ movements. Finally, it is briefly commented the application of calorimetry to study Ca²⁺ homeostasis during a model of stunning induced by no-flow ischemia and reperfusion in entire hearts by using selective inhibitors of cellular Ca²⁺ 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

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 $1 \text{ mW}\cdot\text{g}^{-1}$ for cat, $1.8 \text{ mW}\cdot\text{g}^{-1}$ for rabbit, $2.5 \text{ mW}\cdot\text{g}^{-1}$ for guinea-pig, and 3.5 to $4 \text{ mW}\cdot\text{g}^{-1}$ 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 $12.7 \text{ mW}\cdot\text{g}^{-1}$ 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 calorimetric 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 $1.68 \text{ mW}\cdot\text{g}^{-1}$ with thermopiles [9]. So, according to the Gibbs and Loiselle predictions it was expected a basal or resting heat rate between 8 and $14 \text{ mW}\cdot\text{g}^{-1}$ 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 $5 \text{ mW}\cdot\text{g}^{-1}$ for human hearts and $40 \text{ mW}\cdot\text{g}^{-1}$ 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 $1.5 \text{ ml}\cdot\text{min}^{-1}$ 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 $24.7 \pm 3.4 \text{ mW}\cdot\text{g}^{-1}$ ($n = 5$) and Hr was $18.6 \pm 4.7 \text{ mW}\cdot\text{g}^{-1}$ ($n = 4$) in wild-type mice (WT). This value is about 3.7 fold the Hr of rat hearts under the same cardioplegic solution ($5 \text{ mW}\cdot\text{g}^{-1}$), 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} ($6.1 \text{ mW}/4 \text{ Hz}$). 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 $477 \text{ kJ.mol}^{-1} \text{ O}_2$ [13] or other expressions for interconversion of units, such as $1 \text{ mL O}_2 \cdot \text{g}^{-1} \text{ dw.min}^{-1}$ equivalent to $74 \text{ mW.g}^{-1} \text{ ww}$, and $4.5 \text{ g ww.g}^{-1} \text{ dw}$ (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 $10.7 \mu\text{mol O}_2 \cdot \text{g}^{-1} \cdot \text{s}^{-1}$ [14] and $130\text{-}150 \mu\text{L.min}^{-1} \cdot \text{g}^{-1}$ [15] are equivalent to resting heat rates of 85 mW.g^{-1} and $30\text{-}49 \text{ mW.g}^{-1}$, 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.

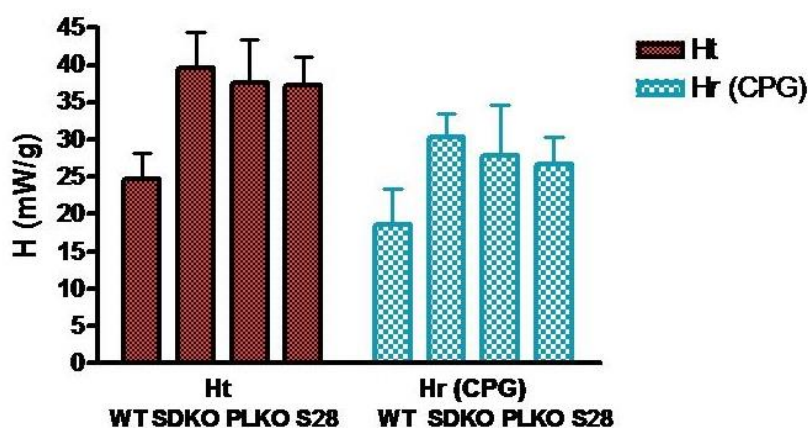


Figure 1: Total heat rate (Ht) and resting heat rate (Hr) obtained from isolated mouse hearts perfused inside the flow calorimeter. Ht was obtained at a heart rate of 4 Hz, while Hr was obtained by perfusing Krebs with 25 mM K-0.5 mM Ca (CPG). Mice belonged to 4 strains of C57BL/6: wild-type (WT), SDKO, PLNKO (PLKO), and RyR2-S2814D (S28). Results are shown as mean \pm 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 sinus 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 (Hr) 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 [Ca²⁺]_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 Ca²⁺ [6]. That [K⁺]_o depolarized the membrane potential to about -45 mV [17] in which L-type Ca²⁺ channels could be activated, with the consequent active removal of cytosolic Ca²⁺ 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 Ca²⁺ influx through L-channels releases Ca²⁺ 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 ⁸⁶Rb 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 Ca²⁺ entered through L-channels, since it was sensitive to nifedipine, a more selective Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ in cardiomyocytes under resting condition with the fluorophores Fluo-4 or Fura-2 and Rhod-2 respectively. In rat and guinea-pig cardiomyocytes there were found some differences in Ca²⁺ homeostasis [19, 22]. In resting rat cardiomyocytes the high-K⁺ solution transiently increased the relative fluorescence of both, Fura-2 ([Ca²⁺]_i) and Rhod-2 ([Ca²⁺]_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 [Ca²⁺] in both cytosol and mitochondria, being SERCA the main energetic consumer, with a slight but Ca²⁺-dependent increase in diastolic tone. The mitochondrial Ca²⁺ uptake contributed to cytosolic Ca²⁺ 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 Ca²⁺. Addition of 10 mM pyruvate (Pyr) also increased Hr and resting pressure, since it stimulates the aerobic metabolism and improves ΔΨ_m for Ca²⁺ uptake [19]. Figure 2 shows the Hr values under CPG demonstrating the dependence on [Ca²⁺]_o and the role of UCam in Ca²⁺-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 Ca²⁺ uptake [23] such rise in Hr may be related to the Ca²⁺ 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 $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]$ 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 $[\text{Ca}^{2+}]_m$ 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 $5.8 \text{ mW}\cdot\text{g}^{-1}$) 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].

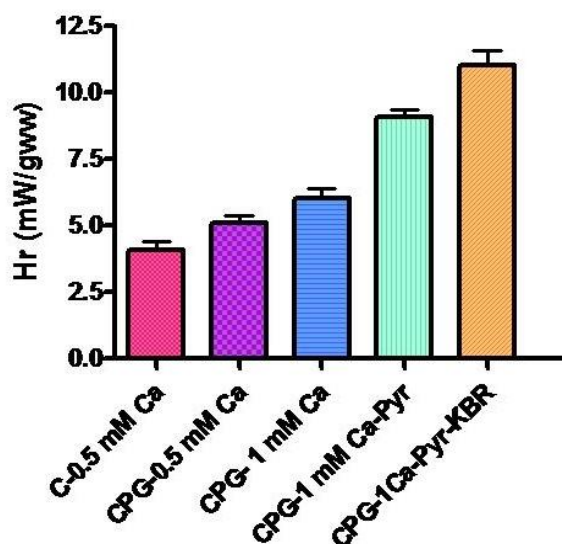


Figure 2: Resting heat rate (Hr) obtained from isolated rat hearts perfused inside the flow calorimeter and exposing to the following treatments: control Krebs.0.5 mM Ca (C-0.5 Ca), Krebs with 25 mM K and 0.5 mM Ca (CPG-0.5 Ca), CPG-1 mM Ca, CPG-1 mM Ca and 10 mM pyruvate (CPG-1 mM Ca-Pyr), and CPG-1 mM Ca and 10 mM pyruvate and 5 μM KB-R7943 (CPG-1 mM Ca-Pyr-KBR), Results are shown as mean \pm 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 calorimetric 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 consecutive 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 $[\text{Ca}^{2+}]$ - 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].

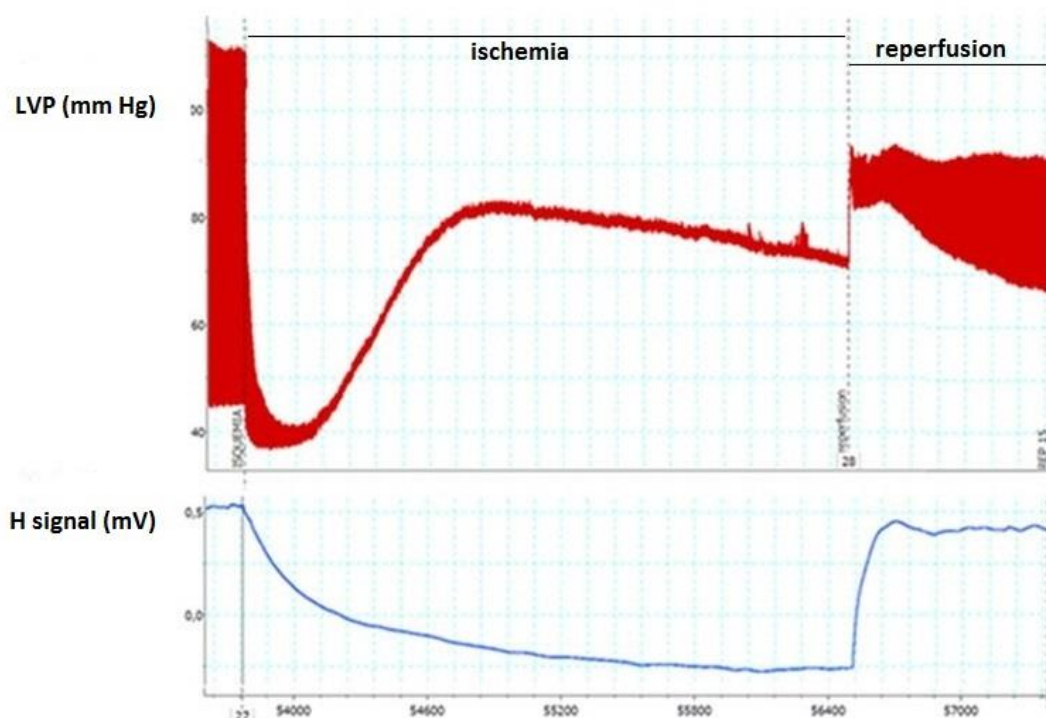


Figure 3: Typical analogic/digital recording (A/D) obtained from an isolated rat heart stimulated at 3 Hz, before and during exposure to a period of no-flow ischemia and the first 15 minutes of reperfusion. It shows the left intraventricular pressure (LVP, upper) and the simultaneous signal of total heat rate (H, lower, measured in mV). This H signal has to be converted to the real Ht (in mW) by subtracting the baseline H signal obtained without muscle and considering the calibration factor (in $\text{mW}\cdot\text{mV}^{-1}$). 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 $[\text{Ca}^{2+}]_m$, 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 $[\text{Ca}^{2+}]_m$ but reduced the Ca^{2+} transients in rat cardiomyocytes [37]. Moreover, other drugs which reduce the $[\text{Ca}^{2+}]_m$ (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, calorimetric 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 $[\text{Ca}^{2+}]_m$ 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 $[\text{Na}^+]_i$ and $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$. 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 $[\text{Na}^+]_i$ 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 $[\text{Na}^+]_i$ 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 sarcoereticular 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 30°C [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 $[\text{Na}^+]_i$ 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 30°C 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 37°C 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 30°C (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 ($H_a = (H_t - H_r)/HR$), 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 $\text{mJ}\cdot\text{g}^{-1}$) 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 ($6.05 \pm 0.04 \text{ mmHg}\cdot\text{g}\cdot\text{mW}^{-1}$) at 37°C. However, after 20 minutes of ischemia and 45 minutes of reperfusion P/Ht was reduced in the euthyroid hearts (to $3.6 \pm 0.6 \text{ mmHg}\cdot\text{mW}^{-1}\cdot\text{g}$) while the HpT hearts developed a more economical contractile state (P/Ht of $9.7 \pm 1.7 \text{ mmHg}\cdot\text{mW}^{-1}\cdot\text{g}$) [40]. So, hyperthyroidism was cardioprotective in the moderate stunning, also increasing PICR from $77.5 \pm 3.2\%$ in euthyroid to $108.8 \pm 11.6\%$ 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 ($-\text{P}/\text{P} = -\text{dP}/\text{dt}/\text{P}$) 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 $-\text{P}/\text{P}$, 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 $-\text{P}/\text{P}$. 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 $5 \text{ mg}\cdot\text{kg}^{-1}$ Gst 1 day before the I/R experiment was more cardioprotective than Gst perfusion on isolated hearts [39].

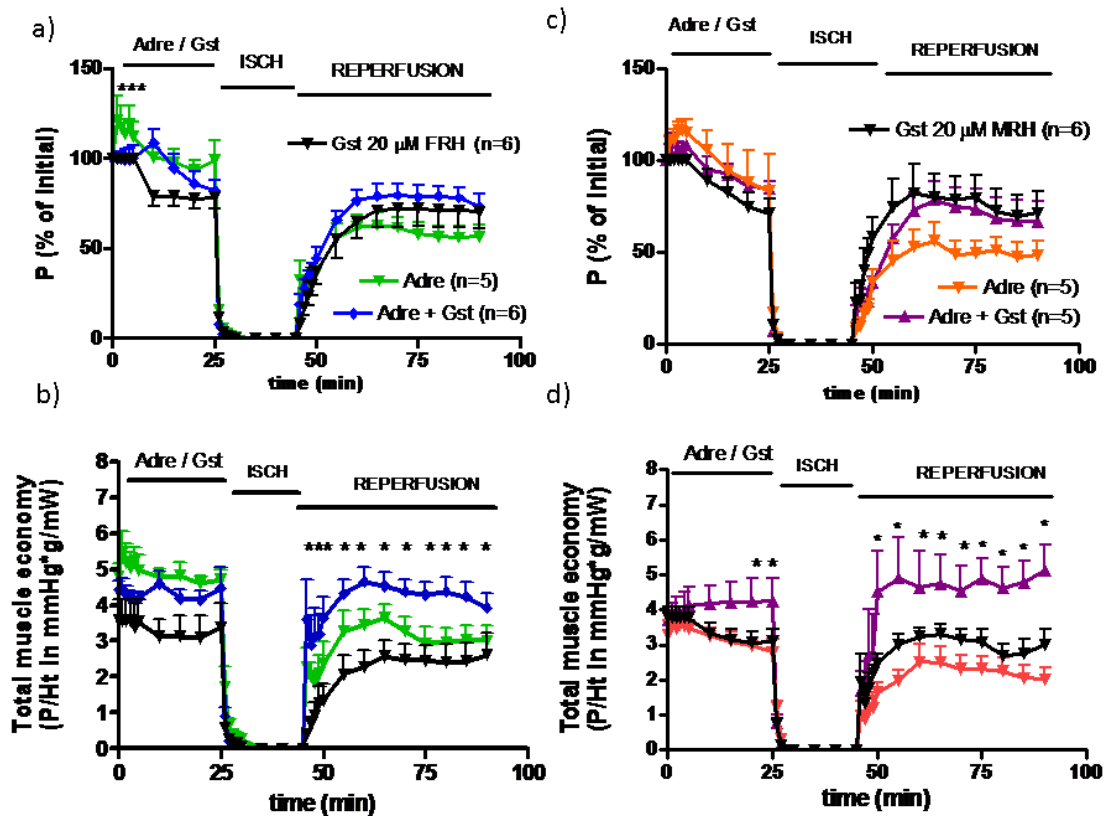


Figure 4: Effects of perfusing the phytoestrogen 20 μM genistein (Gst) in the absence and the presence of 0.05 μM adrenaline (Adre) before exposing to 20 minutes ischemia and 45 minutes reperfusion (with Krebs) in isolated female (FRH, in a and b) and male (MRH, c and d) rat hearts. See the maximal pressure 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 calorimetric 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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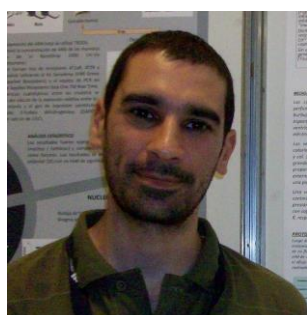
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