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Mitochondrial calcium handling in normotensive and spontaneously hypertensive rats: Correlation with systolic blood pressure levels

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ABSTRACT

The aim was to study the mitochondrial Ca^{2+} handling of mitochondria isolated from normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) hearts and to establish a possible correlation with systolic blood pressure (SBP). Mitochondrial swelling after Ca^{2+} addition, Ca^{2+}-retention capacity (CRC) by calcium green method, and membrane potential ($\Delta\Psi m$) were assessed. SBP was 124 ± 1 (WKY) and 235 ± 6 mm Hg (SHR). CRC, Ca^{2+} response and $\Delta\Psi m$ were lower in SHR than WKY mitochondria. The conclusion is: the more depolarized state of SHR than WKY mitochondria results in an abnormal Ca^{2+} handling and this event is closely associated with the SBP.

1. Introduction

Previous studies show that opening of the permeability transition pore of mitochondria (mPTP) is critically involved in regulating cell death by inducing a sustained and irreversible loss of inner mitochondrial membrane potential, coinciding with mitochondrial swelling and rupture (Javadov and Karmazyn, 2007; Kroemer et al., 2007). The proteins involved in mPTP formation are localized in cytoplasm (hexokinase), outer membrane (VDAC: voltage-dependent anion channel), inner membrane (ANT: adenine nucleotide translocator), and mitochondrial matrix (cyclophilin D) (Baines, 2009). In the presence of oxidative stress and mitochondrial Ca^{2+} overload protein components are assembled such as a pore forming of 1.0 to 1.3 nm radius, which allows the non-selective passage of molecules smaller than 1.5 kDa (Halestrap, 2009).

Homeostasis of mitochondrial Ca^{2+} is well maintained by a balance of Ca^{2+} uptake, sequestration and release mechanisms (Griffiths, 2009; Gunter and Sheu, 2009; Santo-Domingo and Demaurex, 2010). The main route for Ca^{2+} uptake is the ruthenium red-sensitive Ca^{2+} uniporter (Graier et al., 2007; Gunter and Pfeifer, 1990), which is in large part driven by the negative mitochondrial membrane potential ($\Delta\Psi m$). The main route for release of mitochondrial Ca^{2+} is the mitochondrial Na^{+}/Ca^{2+} exchanger (NCX) (Dedkova and Blatter, 2008; Gunter and Pfeifer, 1990; Hoppe, 2010).

The mitochondrial dysfunction has been increasingly associated with the development of hypertension (Lopez-Campistrous et al., 2008). It has been proposed that oxidative stress (Dikalov and Ungvari, 2013) and abnormalities in Ca^{2+} handling (Failli et al., 1997; Sugiyama et al., 1990; Williams et al., 2014) play important role in the development of hypertension. However, there is no agreement regarding the disorders of Ca^{2+} management in a model of compensatory hypertrophy. Thus, in earlier studies, Postnov's group reported an increase in the Ca^{2+}-accumulating capacity of cardiac mitochondria from spontaneously hypertensive rats (SHR) (Orlov et al., 1980). A subsequent study (Aguilera-Aguirre et al., 2002) showed that mitochondrial Ca^{2+} uptake and accumulation capacity of SHR are lower compared to normotensive rats.

Therefore, the aim of the present study was to characterize the Ca^{2+} handling and changes of potential ($\Delta\Psi$) of mitochondria isolated from SHR and to its control normotensive Wistar Kyoto (WKY) and to establish a possible correlation with the systolic blood pressure.

2. Material and methods

2.1. Animals

We used normotensive (Wistar Kyoto, WKY) and spontaneously hypertensive rats (SHR) of 5 months of age. All procedures followed during this investigation conform to 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) and to the guidelines laid down by the Animal Welfare Committee of La Plata School of Medicine.

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2.2. Systolic blood pressure measurement

For indirect systolic blood pressure (SBP) readings, the rats were placed in a chamber at 37 °C for 10 min, and then transferred to a standard setup with heating pad and acrylic restrainer, tail cuff and pulse sensor (Narco Biosystems, Houston, TX). The tail cuff was connected to a cylinder of compressed air through an arrangement of inlet and outlet valves that permitted inflation and deflation of the cuff at a constant rate. The tail cuff pressure was continuously recorded with a solid state pressure sensor (Sensym, Honeywell Sensing & Control, Inc.). The signals from the pulse and pressure sensors were conveniently amplified and then digitized with an analogy-digital board (DT16EZ, Data Translation, Inc., Marlboro, MA) mounted in a desktop computer. On-line display for controlling the procedure, and files for later processing, were obtained with appropriate software (Labtech Notebook Pro, Laboratory Technology Corp., Wilmington, MA). For each SBP determination the inflation and deflation readings were always recorded, as well as the compression interval (Fritz and Rinaldi, 2008).

2.3. Isolation of rat heart mitochondria

Rat heart mitochondria were obtained by differential centrifugation (Mela and Seitz, 1979). Left ventricle (LV) was washed and homogenized in ice-cold isolation solution (IS) consisting of 75 mM sucrose, 225 mM mannitol, and 0.01 mM EGTA neutralized with Trizma buffer at pH 7.4. After the tissue pieces were settled, the entire supernatant was discarded and fresh IS (5 ml) was added, and the mixture was transferred to a hand homogenizer. Proteinase (0.8 mg, bacterial, type XXIV, Sigma, formerly called Nagarse) was added just before starting the homogenization procedure. The whole homogenization procedure took no longer than 14 min in two steps of 7 min each (with 5 ml addition of fresh IS each). The homogenate was carefully transferred after each step to a polycarbonate centrifuge tube. After 5 min of 750 ×*g* of centrifugation to discard unbroken tissue and debris, the supernatant was centrifuged at 8000 ×*g* for 10 min to sediment the mitochondria. The mitochondrial pellet was washed twice with IS and the last one with suspension solution (IS without EGTA) at 8000 ×*g* for 5 min each. The residue was washed and re-suspended in a cold solution containing mannitol and sucrose. The mitochondrial protein concentration was evaluated by the Bradford method (1976) using bovine serum albumin as standard. The purity of preparation was determined by immunodetection of the mitochondrial outer membrane voltage dependent anion channel (VDAC) and by the absence of the cytosolic glyceraldehyde phosphate dehydrogenase (GAPDH) (data not shown).

2.3.1. mPTP resistance to opening Ca^{2+}-mediated

The mPTP resistance to opening was assessed by incubating 0.3 mg/mL of isolated mitochondria from WKY and SHR in a buffer containing (in mmol/L): 120 KCl, 20 MOPS, 10 Tris HCl, and 5 KH_2PO4 adjusted to pH = 7.4. After 5-min preincubation, in the mitochondria energized with the addition of 5 mmol/L succinate was assessed the resistance to opening of mPTP with 20, 50, 100, 200, 500 and 1000 μmol/L $CaCl_2$. If mPTP is open, solutes will be free to enter the inner matrix, causing the mitochondria to swell. These changes are observed as decreases of light scattering and followed using a temperature-controlled Hitachi F4500 spectrofluorometer operating with continuous stirring at excitation and emission wavelengths of 520 nm (Baines et al., 2003). Light scattering decrease (LSD) was calculated for each sample by taking the difference of scattered light between before and after the addition of $CaCl_2$.

2.3.2. Calcium retention capacity

Calcium retention capacity (CRC) was defined here as the amount of Ca^{2+} required triggering a massive Ca^{2+} release by isolated cardiac mitochondria (Obame et al., 2008). It is used as an indicator of the resistance of the mPTP to opening after matrix Ca^{2+} accumulation and expressed as nmol $CaCl_2$ per mg of mitochondrial proteins. Extramitochondrial Ca^{2+} concentration was recorded with 0.5 μmol/L calcium green-5N (Invitrogen, Carlsbad, CA, USA) with excitation and emission wavelengths set at 506 and 532 nm, respectively. Isolated mitochondria from WKY and SHR (0.5 mg/ml) were suspended in 2 mL buffer (150 mmol/L sucrose, 50 mmol/L KCl, 2 mmol/L KH_2PO4, and 5 mmol/L succinate in 20 mmol/L Tris/HCl, pH 7.4). At the end of the preincubation period (300 s), successive pulses of 10 μM Ca^{2+} were added. When mitochondria cannot hold more Ca^{2+}, the mPTP is opened and the concentration of this ion increases in the incubation medium. After sufficient calcium loading, extramitochondrial calcium concentration abruptly increased, indicating a massive release of Ca^{2+} by mitochondria as a result of mPTP opening as previously described (Tang et al., 2007). Number of pulses, total time (TT, sec), pulse time (PT, sec) and velocity of Ca^{2+} efflux after mPTP opening were also measured.

2.3.3. Mitochondrial membrane potential

Mitochondrial potential changes were evaluated by measuring rhodamine-123 (RH-123) fluorescence quenching under the buffer described above containing RH-123 0.1 μM. As suggested by Emaus et al. (1986), the experimental work has been performed by exciting RH-123 at 503 nm and detecting the fluorescence emission at 527 nm. During the measurements, the reaction medium containing mitochondria (0.1 mg/ml) was continuously stirred. Mitochondrial membrane potential ($\Delta\Psi m$) was calculated following the instructions previously detailed (Scaduto and Grotyohann, 1999) using the Nernst–Guggenheim equation. According to those authors, RH-123 uptake is in proportion to $\Delta\Psi m$, therefore the rate of fluorescence quenching has also to be a function of $\Delta\Psi m$, as well as the steady-state level of fluorescence decrease. Changes of $\Delta\Psi m$ produced by Ca^{2+} 20 and 200 μM addition with and without cyclosporine A (CsA) were also calculated.

2.4. Statistical analysis

Data were expressed as means ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Newman–Keul's post-test used for multiple comparisons among groups. Values of $p < 0.05$ were considered to indicate statistical significance.

3. Results

Fig. 1 shows a typical trace of pulse and pressure used for systolic blood pressure (SBP) measurement in WKY (A) and SHR (B). Mean data of SBP, plus the values of body weight (BW, g), heart weight (HW, mg) and hypertrophic index (HI) calculated as HW and BW ratio of both rat strains are displayed in Table 1. SBP and HI were significantly higher in SHR than WKY, indicating the presence of hypertrophy associated with high pressure as one recognized characteristic of hypertensive animals.

In Fig. 2 the mitochondrial swelling, measured as light scattering decrease (LSD), after different Ca^{2+} concentrations in samples derived from WKY and SHR hearts were depicted. The LSD produced by the addition of Ca^{2+} 20 μM was similar in both rat strains whereas that at higher Ca^{2+} concentrations significant differences between WKY and SHR were found. Thus, mPTP of mitochondria from SHR hearts shows a lesser response to Ca^{2+} than WKY. Additionally, the number of Ca^{2+} 10 μM pulses to produce the cessation of mitochondrial Ca^{2+} uptake and mPTP opening was significantly lower in hypertensive than normotensive rats (Fig. 3). Consequently, Ca^{2+} retention capacity (CRC) and total time (TT, sec) were significantly lesser whereas the time of each pulse (PT) was higher for SHR compared to WKY mitochondria. Thus, CRC acquired values of 180 ± 14 vs. 520 ± 40 nmol Ca^{2+}/mg prot,

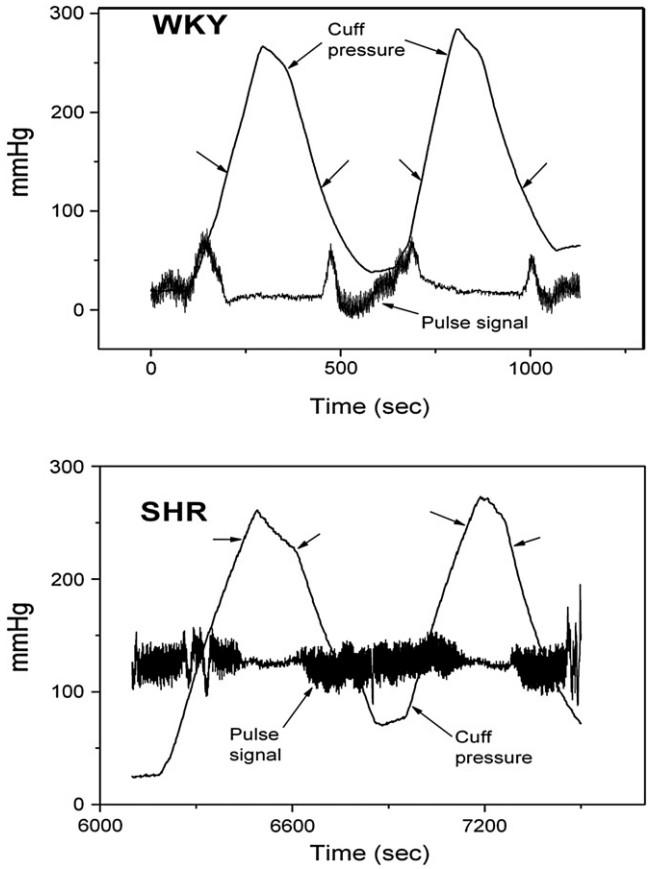

Fig. 1. Typical trace of pulse and pressure used for SBP measurement in WKY and SHR.

TT of 337 ± 49 vs. 566 ± 25 s and PT of 44 ± 4 vs. 77 ± 12 s for SHR and WKY, respectively (Fig. 4).

Examining the fluorescence change produced by mitochondria Ca^{2+} massive release observed in CRC measurement we found that it was significantly lower in SHR compared to WKY. This change was characterized by the slope at origin which was significantly lesser for SHR than WKY (0.013 ± 0.002 vs. 0.080 ± 0.007 a.u./s) (Fig. 5).

Fig. 6 shows typical traces of rhodamine fluorescence changes – expressed in arbitrary units – used for the membrane potential ($\Delta\Psi m$) determination. Mean value of $\Delta\Psi m$ – expressed in mV – observed in SHR mitochondria was significantly less negative than WKY, indicating that a more depolarized state predominates in organelles from hypertensive rats. The addition of Ca^{2+} 20 μM to mitochondrial suspension did not modify $\Delta\Psi m$ whereas that Ca^{2+} 200 μM produced a change significantly lower in SHR than WKY mitochondria (38 ± 3 vs 53 ± 4 mV). These changes were not modified by the presence of cyclosporine A (CsA), indicating that mPTP is not involved in the effects on $\Delta\Psi m$ Ca^{2+}-mediated.

Fig. 7 shows the correlation between LSD and $\Delta\Psi m$ (r = 0.99), evidencing that a low mitochondrial swelling produced by Ca^{2+} addition – situation observed in SHR – was associated with a less

Table 1
Data of systolic blood pressure (SBP), body weight (BW), heart weight (HW) and hypertrophy index (HI) in WKY and SHR.

	WKY	SHR
SBP (mm Hg)	124 ± 1	235 ± 6**
BW (g)	372 ± 6	368 ± 8
HW (mg)	920 ± 40	1380 ± 50**
HI	2.48 ± 0.13	3.75 ± 0.11**

** $p < 0.01$.

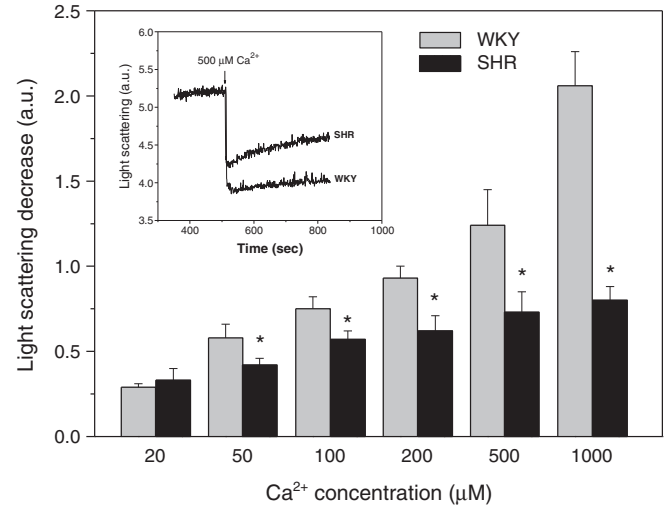

Fig. 2. Light scattering decrease (LSD) in arbitrary units (a.u.) after the addition of different Ca^{2+} concentrations (20, 50, 100, 200, 500 and 1000 μM) to samples of mitochondria isolated from WKY and SHR (n = 6 for each one). Inset: Typical trace of light scattering for SHR and WKY at Ca^{2+} 500 μM. Note that from Ca^{2+} 50 μM LSD is significantly lesser in SHR compared to WKY. *$p < 0.05$ with respect to WKY.

electronegative $\Delta\Psi m$. Opposite changes were detected in mitochondria isolated from the hearts of normotensive rats.

CRC/$\Delta\Psi m$ ratio vs SBP correlation is depicted in Fig. 8. These parameters follow a significant positive correlation (r = 0.95; $p < 0.0001$) indicating that the high values of SBP found in SHR are accompanied by high values of CRC/$\Delta\Psi m$ ratio.

4. Discussion

Our results show that mitochondria from SHR possess an abnormal Ca^{2+} handling, displaying a lesser mPTP opening Ca^{2+} mediated, Ca^{2+} retention capacity and velocities of Ca^{2+} influx and efflux and a lower $\Delta\Psi m$ than its normotensive control, WKY.

Increased opening of mPTP is usually triggered by stressors such as oxidative stress and mitochondrial Ca^{2+} overload, leading to mitochondrial dysfunction and eventually cell death (Halestrap, 2009). Our data show that, from Ca^{2+} 50 μM, the mitochondrial swelling – assessed as light scattering decrease – was significantly lesser in SHR than age-matched normotensive rats. That is, in our experimental conditions,

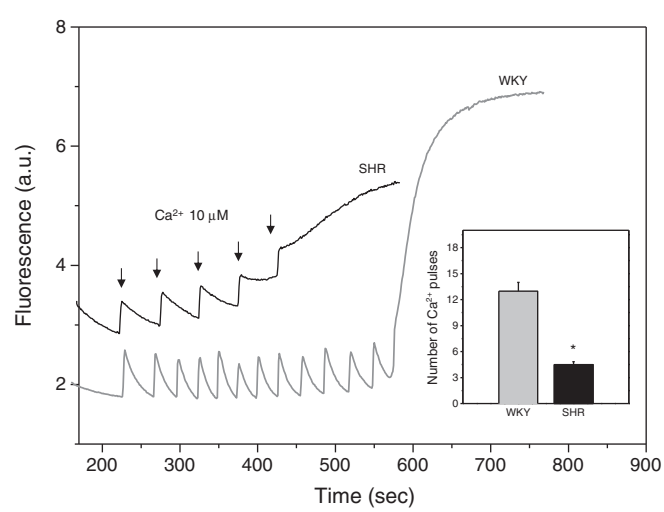

Fig. 3. Typical traces of changes of calcium green fluorescence after Ca^{2+} addition in samples of mitochondria from WKY and SHR. Inset: Number of Ca^{2+} pulses (NP) in both rat strains (n = 6 for each one). NP is significantly higher in WKY than SHR mitochondria. *$p < 0.05$.

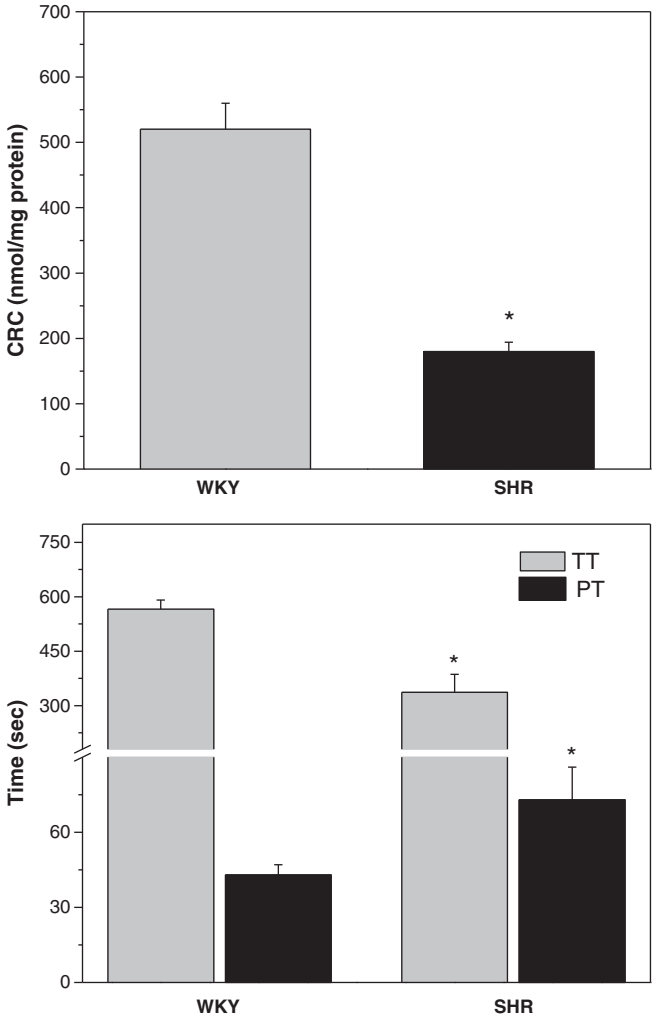

Fig. 4. Values of Ca^{2+} retention capacity (CRC), total time (TT) and time of each pulse (PT) in mitochondria isolated from SHR and WKY hearts (n = 6 for each one). Note that CRC and TT are significantly lesser whereas PT is higher in SHR in comparison to WKY. *p < 0.05.

the mPTP of cardiac mitochondria from hypertensive rats possesses a decreased sensitivity or response to Ca^{2+} compared to those organelles of age-matched normotensive rats. These results contrast with those

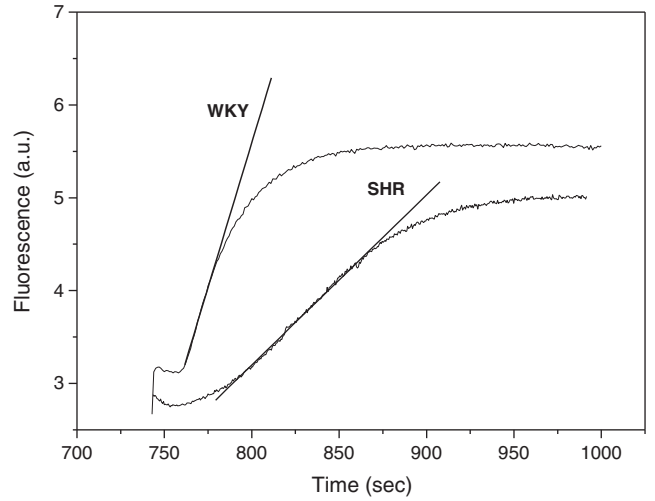

Fig. 5. Time course of Ca^{2+} efflux from mitochondria from SHR and WKY hearts. This Ca^{2+} movement is slower in SHR than WKY, characterized by a lesser slope at origin in hypertensive rats (0.013 ± 0.002 vs. 0.080 ± 0.007 a.u./s, *p < 0.05).

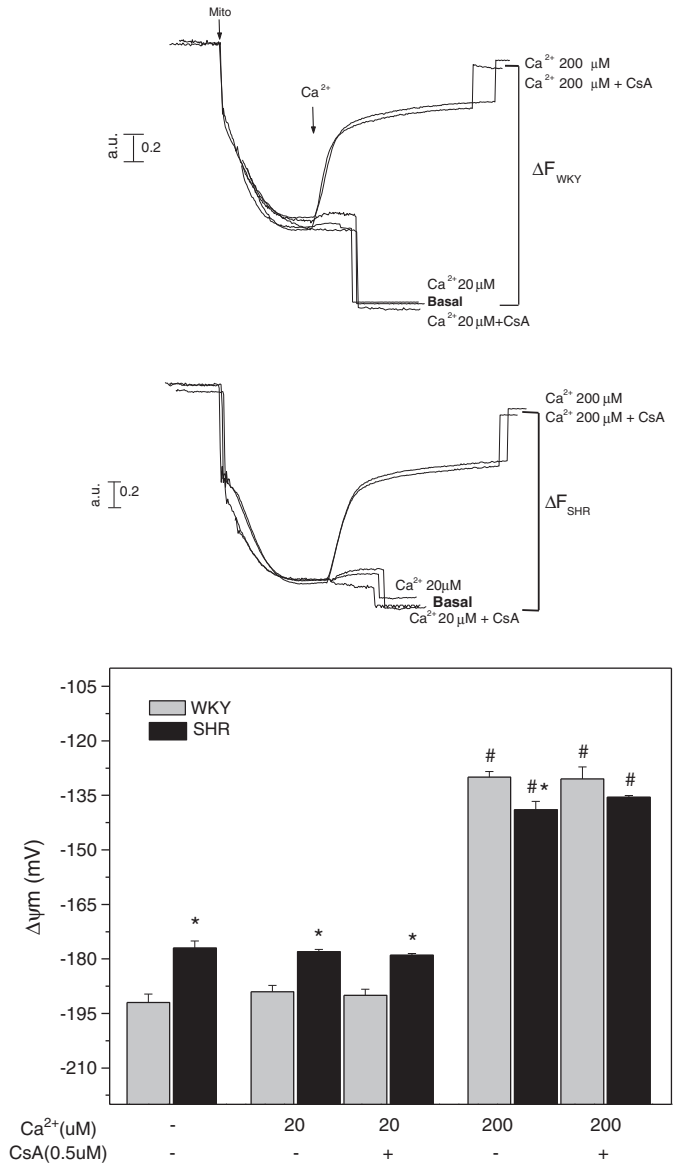

Fig. 6. Upper panel: Typical fluorescence changes (ΔF), expressed in arbitrary units (a.u.) observed for mitochondrial membrane potential (ΔΨm, mV) measurement in mitochondria isolated from SHR and WKY hearts (n = 6 for each one). Lower panel: Basal ΔΨm and the changes detected after addition of Ca^{2+} 20 and 200 μM with and without cyclosporine A (CsA). Ca^{2+} 20 μM did not modify the ΔΨm whereas that Ca^{2+} 200 μM decreased ΔΨm in both rat strains being the change significantly lesser in SHR than WKY. These changes were not affected by CsA treatment. *p < 0.05 with respect to WKY; #p < 0.05 with respect to basal ΔΨm.

previously reported by Strutyns'ka et al. (2012), who showed that mPTP of SHR mitochondria has an increased sensitivity to Ca^{2+}.

Now, the question to be solved is: What are the reasons for the observed differences between SHR and WKY? Homeostasis of mitochondrial Ca^{2+} is well maintained by a balance of Ca^{2+} uptake, sequestration and release mechanisms (Griffiths, 2009; Gunter and Sheu, 2009; Santo-Domingo and Demaurex, 2010). Heart mitochondria exposed to a continuous Ca^{2+} challenge are able to buffer the infused Ca^{2+} resulting in a steady state extra-mitochondrial Ca^{2+} concentration. The latter lasted until a threshold where retained Ca^{2+} is released and extra-mitochondrial Ca^{2+} concentration increases. Data of calcium green method shows that the Ca^{2+} threshold concentration required for mPTP opening in mitochondria isolated from SHR was approximately 3 orders of magnitude lesser to that observed in those organelles from WKY and similar to that obtained in normotensive animals after ischemia and reperfusion (Gedik et al., 2013). In other words,

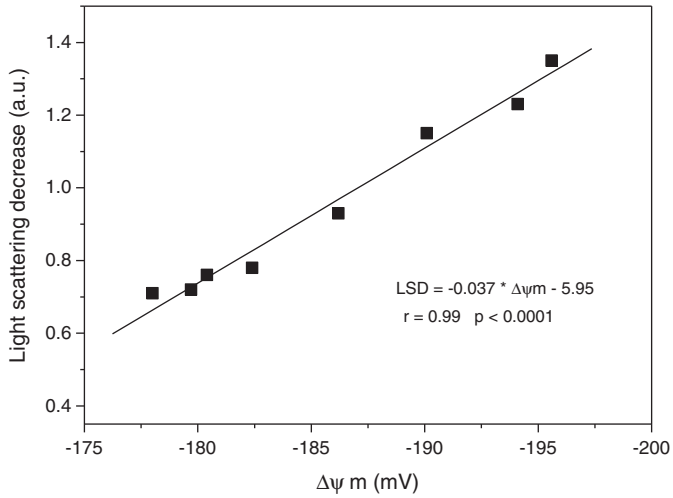

Fig. 7. Correlation between light scattering decrease (LSD), expressed in arbitrary units (a.u.) and mitochondrial membrane potential (ΔΨm).

mitochondria of SHR showed a significant decrease in the matrix Ca^{2+} buffering capacity compared to WKY. This difference could be attributed to the differences of mitochondrial ultrastructure or related to Ca^{2+} basal content. By electronic microscopy abnormalities in the ultrastructure of cardiac mitochondria of SHR – lesser cristae per micrometer and less organized – compared with organelles of WKY were previously described (Hickey et al., 2009; Postnov et al., 2000). With relation to Ca^{2+} level, it has been proposed that the content of that ion in mitochondria from hypertensive is higher than normotensive rats (Postnov et al., 2007). Some defects of mitochondrial ATP synthase detected in cardiomyocytes from SHR could explain the abnormally high intramitochondrial Ca^{2+} levels found in those rats (Das and Harris, 1990). Therefore, both factors (ultrastructure and Ca^{2+} upload) could be involved in the lesser tolerance of mitochondria from SHR to Ca^{2+} addition. On the other hand, the slightest Ca^{2+} retention capacity (CRC) – given by the lowest Ca^{2+} pulses number – detected in cardiac mitochondria from SHR was accompanied by higher duration of each pulse and lesser total time than mitochondria isolated from WKY hearts. These results are indirect evidence of lower velocities of Ca^{2+} uptake and release in mitochondria of hearts from hypertensive in comparison to normotensive rats.

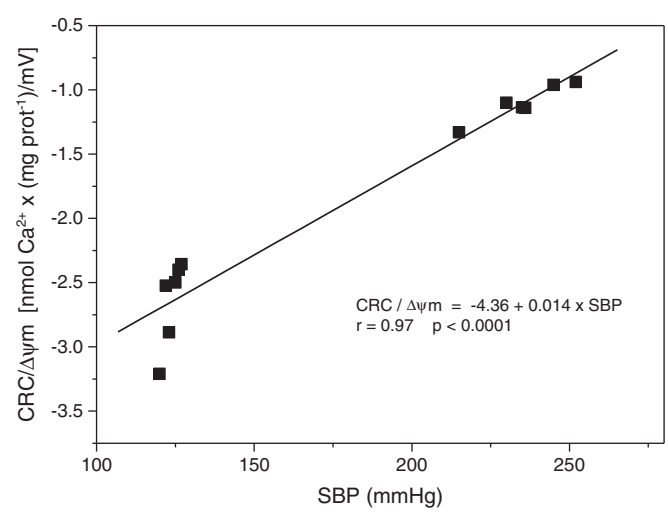

Fig. 8. Correlation between Ca^{2+} retention capacity (CRC)/mitochondrial membrane potential (ΔΨm) ratio and systolic blood pressure (SBP).

Which are the possible explanations for these differences? Indeed, the crucial points are the mechanisms implicated in the mitochondrial Ca^{2+} uptake and release. Ca^{2+} uptake principally occurs through a highly selective channel — the Ca^{2+} uniporter. A recent study (Fieni et al., 2012) showed that heart tissue presents a lower mitochondrial Ca^{2+} uniporter current density than other tissues. Therefore, a further slower Ca^{2+} uniporter current in SHR than WKY mitochondria could explain the lower Ca^{2+} influx detected in that rat strain. However, differences in other mechanisms of Ca^{2+} uptake, such as mitochondrial ryanodine receptor, mitochondrial uncoupling proteins, Ca^{2+}/H^{+} exchanger cannot be discarded (Pan et al., 2011). Moreover, the mitochondrial potential (ΔΨm) play an important role to provide the bioenergetics driving force for ATP production and the charge gradient required for mitochondrial Ca^{2+} uptake. So that if mitochondria are depolarized before they have accumulated significant amounts of Ca^{2+}, they are protected from subsequent Ca^{2+} uptake and mPTP opening (Korge et al., 2011). However, if mitochondria are already loaded with Ca^{2+}, ΔΨm dissipation triggers mPTP formation/opening. Our data show that SHR mitochondria have a lesser electronegative ΔΨm than WKY which would be consistent with a high Ca^{2+} upload in the cardiac organelles of that rat strain. This fact could also be another possible explanation of the smaller Ca^{2+} influx velocity – evidenced by a greater time of Ca^{2+} disappearance from the extracellular medium by calcium green method – to the cardiac mitochondria from hypertensive in comparison to normotensive rats. Furthermore, the addition of Ca^{2+} produces a lesser change of ΔΨm in SHR than WKY, indicating that the driven force to Ca^{2+} uptake is decreased in that rat strain. However, this small Ca^{2+} entry was sufficient to promote mPTP opening, characterized by a lower mitochondrial swelling (Fig. 2) and kinetics of opening (Fig. 4) in SHR than WKY. Indeed this is evidenced by the high correlation between light scattering decreases and ΔΨm values (Fig. 7). Thus, a lower electronegative ΔΨm is able to promote lesser mitochondrial swelling indicating that mPTP of mitochondria from SHR hearts offers some resistance to open. This was characterized by the kinetic of Ca^{2+} release and mPTP opening (slope at origin), which was lower for SHR than WKY. This fact could be explained considering that mitochondria of SHR are – in a basal state – more open to the cytosol than WKY due to mitochondrial morphology alterations in hypertensive than normotensive rats (Sang-Bing and Hausenloy, 2010). A reduction of sirtuin protein expression – recently demonstrated (Tang et al., 2014) – and a concomitant deacetylation of CyP-D could also be contributing to the behavior of SHR. Additionally, and taking into account that the uniporter is now recognized as a complex of proteins that include a Ca^{2+} pore forming component and accessory proteins (Foskett and Philipson, 2014), changes of these proteins could be involved in the abnormalities of Ca^{2+} handling found in mitochondria from SHR hearts.

Respect to Ca^{2+} efflux, it has been established that a Na^{+}/Ca^{2+} exchanger (NCLX) localized to cristae is the principal pathway (Boyman et al., 2013; Gunter and Pfeifer, 1990; Palty et al., 2010). Therefore, alterations in NCLX could explain the lesser Ca^{2+} release velocity detected in SHR than WKY mitochondria. However, although it has been recognized defects in plasma membrane NCX expression/activity in SHR (Anderson et al., 1999; Ward et al., 2010), it is known whether there are alterations of NCLX in that rat strain. The slower Ca^{2+} efflux velocity in mitochondria from hypertensive than normotensive rats could also be attributed to the differences of ΔΨm. This hypothesis is in agreement with a previous work which demonstrates that when ΔΨm is depolarized, the energetic cost of removing excess Ca^{2+} from the matrix is huge, and both Na^{+}/Ca^{2+} and H^{+}/Ca^{2+} exchanges are kinetically slow (Andrienko et al., 2009). On the other hand, a previous investigation shows that the mPTP opening allows accumulated matrix Ca^{2+} to flow rapidly out of the matrix down its concentration gradient and equilibrate with cytoplasmic free Ca^{2+} (Bernardi and Petronilli, 1996) at a much faster rate than possible via the exchangers. However, in our case, CsA does not modify the response of ΔΨm to Ca^{2+} in both rat strains indicating that mPTP is not the preferred

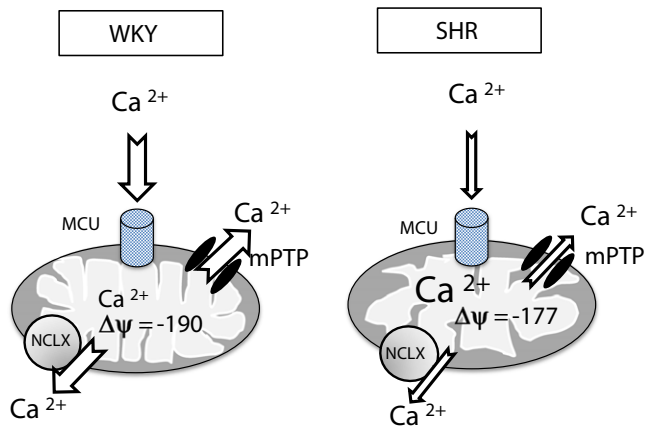

Fig. 9. Schematic representation of the possible mechanisms of Ca^{2+} handling operating in mitochondria from SHR and WKY hearts.

pathway of Ca^{2+} input or output of mitochondria and/or the presence of CsA-insensitive component of the mPTP.

The relationship between oxidative stress and increased blood pressure has been demonstrated in many models of experimental hypertension. Increased ROS formation precedes development of hypertension in SHR, suggesting that ROS participate in the development and maintenance of hypertension (Kitiyakara and Wilcox, 1998). Markers of oxidative stress, such as thiobarbituric acid reactive substances and F2α-isoprostanes, tissue concentrations of $\bullet O_2^-$ and H_2O_2, and activation of NAD(P)H oxidase and xanthine oxidase, are increased, whereas levels of NO and antioxidant enzymes are reduced in experimental hypertension (Redon et al., 2003; Touyz and Schiffrin, 2004).

Taking into account the association between oxidative stress and hypertension previously demonstrated, the presence of an oxidant-regulated mPTP (Morota et al., 2013) could be other possible explanation of the altered response to Ca^{2+} detected in mitochondria from SHR.

5. Conclusions

This study provides evidence about the abnormal Ca^{2+} handling by mitochondria from SHR hearts, characterized by a lower $\Delta\Psi m$, lesser response of mPTP to Ca^{2+} and lower velocities of Ca^{2+} uptake and release in comparison to mitochondria from age-matched normotensive WKY hearts (Fig. 9). These differences could be the cause and/or the consequence of the high systolic blood pressure and associated hypertrophy.

Furthermore, the acknowledgment of the Ca^{2+} homeostasis disturbances in SHR – which might mimic what is happening in essential hypertension – , could contribute to develop effective strategies to attenuate the deleterious effects of the chronic high pressure.

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