

Sex difference in the sensitivity of cardiac mitochondrial permeability transition pore to calcium load

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Abstract Most of the experimental studies have revealed that female heart is more tolerant to ischemia/reperfusion (I/R) injury as compared with the male myocardium. It is widely accepted that mitochondrial dysfunction, and particularly mitochondrial permeability transition pore (MPTP) opening, plays a major role in determining the extent of cardiac I/R injury. The aim of the present study was, therefore, to analyze (i) whether calcium-induced swelling of cardiac mitochondria is sex-dependent and related to the degree of cardiac tolerance to I/R injury and (ii) whether changes in MPTP components—cyclophilin D (CypD) and ATP synthase—can be involved in this process. We have observed that in mitochondria isolated from rat male and female hearts the MPTP has different sensitivity to the calcium load. Female mitochondria are more resistant both in the extent and in the rate of the mitochondrial swelling at higher calcium concentration (200 μ M). At low calcium concentration (50 μ M) no differences were observed. Our data further suggest that sex-dependent specificity of the MPTP is not the result of different amounts of ATP synthase and CypD, or their respective ratio in mitochondria isolated from male and female hearts. Our results indicate that male and female rat hearts contain comparable content of MPTP and its regulatory protein CypD; parallel immunodetection revealed also the same contents of adenine nucleotide translocator or voltage-dependent anion channel. Increased resistance of female heart mitochondria thus cannot be explained by

changes in putative components of MPTP, and rather reflects regulation of MPTP function.

Keywords Heart · Mitochondrial permeability transition pore · Sex difference · Calcium-induced swelling

Introduction

Ischemic heart disease (IHD) is the leading cause of morbidity and mortality in both men and women in the developed countries. Epidemiological studies have clearly shown that in pre-menopausal women, the onset of IHD occurs on average 10 years later than in men, with myocardial infarction occurring 20 years later. Menopause thus plays a decisive role in the increase in cardiovascular risk: there was a 10-fold increase in IHD after menopause compared with only 4.6-fold increase in the same age group in men [1, 2]. Most of the experimental studies have confirmed the epidemiological observations (for a review see [3–5]). Although sex-related difference in cardiac tolerance to oxygen deficiency was first described in the 1980s [6], detailed investigation of this issue started only in recent years. Increased resistance of the female myocardium to ischemia/reperfusion (IR) injury was shown in dogs, rats, mice, and rabbits [7–10]. It follows that female sex favorably influences cardiac sensitivity to I/R; however, further analyses are required to clarify the responsible mechanisms.

Sex differences can be observed already in the normal heart, even at the molecular level. Among the most important ones are undoubtedly the differences in myocardial calcium metabolism [11]: the female rat heart has significantly higher levels of the L-type calcium channels (responsible for Ca^{2+} release from the

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sarcoplasmic reticulum) and Na^+ – Ca^{2+} exchange protein (responsible for removal of Ca^{2+} from the cardiac cell). Moreover, significant sex differences were also found in the uptake of Ca^{2+} by cardiac mitochondria; mitochondria from female hearts have lower Ca^{2+} uptake rates and improved recovery of mitochondrial membrane potential from Ca^{2+} -induced depolarization [12]. The female heart has lower mitochondria content; they generate less H_2O_2 than in males [13]. Recently, significant sex-related differences were observed in cardiac expression of genes associated with energy metabolism; they may indicate a likely involvement of mitochondria in susceptibility to IHD [14]. All these findings could help understand why female myocardium suffers less I/R injury.

It has been known for more than 60 years that mitochondria become leaky, uncoupled and massively swollen if they are exposed to high calcium concentrations, especially in the presence of phosphate and when accompanied by oxidative stress (for a review see [15, 16]). This phenomenon became known as the permeability transition. Mitochondrial permeability transition pore (MPTP) opening is traditionally linked to mitochondrial depolarization, cessation of ATP synthesis, Ca^{2+} release, pyridine nucleotide depletion, inhibition of respiration and, *in vitro*, matrix swelling. It is now widely accepted that mitochondrial dysfunction and particularly MPTP opening plays a major role in determining the extent of cardiac I/R injury [17].

Detail mechanisms of the pore regulation as well as its molecular structure are not yet fully elucidated. Up to now there is a common agreement in the literature that calcium ions are the inducer of the pore opening and that phosphate ions and ROS can potentiate its effect [17]. An important regulatory role has mitochondrial protein cyclophilin D (CypD) localized in mitochondrial matrix; its interaction with the pore facilitates calcium to open the pore [18]. CypD binding can be prevented by cyclosporine A and sanglifehrin A [19, 20]; in this situation MPTP is insensitive to calcium action and remains closed. However, the molecular structure of MPTP is far from to be solved. The original concept of multicomponent MPTP-forming high conductance megachannel at contact sites of inner and outer mitochondrial membrane, consisting of adenine nucleotide translocator (ANT), voltage-dependent anion channel/porin (VDAC), hexokinase type II, creatine kinase and other proteins was ruled out by genetic analysis (for review see [21]). Instead, recent studies support the view that MPTP is created by the dimers of F_0F_1 -ATP synthase and the pore is formed at the interface of membranous F_0 parts of ATP synthase complexes. The regulatory CypD interacts with lateral stalk of ATP synthase complex and the inhibitor of MPTP, cyclosporin A releases CypD from ATP synthase [22–24]. Another possibility is that the

MPTP is formed by subunit c oligomer of ATP synthase [25, 26].

In our previous paper, we have tested the hypothesis that the role of cardiac MPTP in I/R injury differs in neonatal (highly hypoxic tolerant) and adult myocardium [27]. We have observed that the extent of Ca-induced swelling in mitochondria from neonatal rats was significantly lower than that from the adult animals; mitochondria from neonatal rats were more resistant at higher concentrations of calcium. Not only the extent but also the rate of calcium-induced swelling was about twice higher in adult than in neonatal mitochondria. These results support the idea that lower sensitivity of the neonatal MPTP to opening may be involved in the mechanism of the higher tolerance of the neonatal heart to IR injury.

The aim of the present study was, therefore, to analyze (i) whether calcium-induced swelling of cardiac mitochondria is sex-dependent and related to the degree of cardiac tolerance to IR injury and (ii) whether changes in MPTP components—CypD and ATP synthase can be involved in this process.

Materials and methods

Animals

For all experiments Wistar male and female adult 3-month-old rats were used. Animals had free access to water and standard laboratory diet. They were maintained on 12-h light/12-h dark cycle. All investigations conform to the “Guide for the Care and the Use of Laboratory Animals”, published by the US National Institutes of Health.

Chemicals

All chemicals were of highest commercially available purity and were purchased from Sigma Aldrich Co. Germany.

Isolation of mitochondria from rat heart

The animals were anesthetized by CO_2 and sacrificed by cervical dislocation (http://oacu.od.nih.gov/ARAC/documents/Rodent_Euthanasia_Adult.pdf). The hearts were dissected and both ventricles and septum were separated, cut and homogenized at 0 °C by a Teflon-glass homogenizer as 10 % homogenate in a medium containing 0.25 M sucrose, 10 mM Tris–HCl, 2 mM EGTA, and bovine serum albumin fatty acid free 0.5 mg/ml, pH 7.2. The homogenate was centrifuged for 10 min at $600\times g$. Resulting supernatant was centrifuged for 10 min at $10,000\times g$. The mitochondrial sediment was washed twice

in a sucrose medium without EGTA and BSA by centrifugation for 10 min at $10,000\times g$. Sediment of washed mitochondria was suspended in 0.5 ml of 0.25 M sucrose, 10 mM Tris–HCl, pH 7.2. Protein concentration was determined by the Bradford method [28]. Integrity of each mitochondrial preparation was tested by determination of the respiratory control index (RCI) using OROBOROS-K2 high-resolution oxygraphy [29].

Measurement of mitochondrial swelling

Parameters of the mitochondrial swelling process were measured as described in our previous papers [27, 30] with minor modifications. Mitochondrial swelling was estimated from the decrease of absorbance at 520 nm in a Perkin Elmer Lambda spectrophotometer at 30 °C in swelling medium containing 125 mM sucrose, 65 mM KCl, 10 mM HEPES (pH 7.2), 5 mM succinate and 1 mM K-phosphate [31]. Mitochondria were added to 1 ml of incubation medium to provide absorbance about 1 (about 0.4 mg protein). After 1 min of preincubation of mitochondrial suspension CaCl_2 solution was added and the absorbance changes at 520 nm were detected in 0.1 min intervals for further 5 min. Two parameters of the swelling process were evaluated: (a) the extent of swelling as absorbance change during 5 min ($\Delta A_{520}/5$ min), (b) the maximum swelling rate obtained after derivation of the swelling curve and expressed as absorbance change during 0.1 min ($\Delta A_{520}/0.1$ min).

Polarographic measurements

Respiration of isolated rat heart mitochondria was determined by OROBOROS-K2 oxygraph (Austria) at 30 °C as previously [29]. Incubation medium contained 80 mM KCl, 10 mM Tris–HCl, 3 mM MgCl_2 , 4 mM K-phosphate, and 1 mM EDTA, pH 7.2. To the medium were subsequently added mitochondria (0.1 mg protein/ml), 2.5 mM malate, 10 mM oxoglutarate, 1 mM ADP, 5 μM cytochrome *c* and 10 mM succinate. Respiratory rates were calculated as well as respiratory control index and activation effect of added cytochrome *c*.

Electrophoresis and Western blot analysis

Tricine SDS polyacrylamide gel electrophoresis (SDS-PAGE) [32] was performed on 10 % (w/v) polyacrylamide slab minigels (Mini Protean, Bio-Rad). The samples were incubated for 20 min at 40 °C in 2 % (v/v) mercaptoethanol, 4 % SDS (w/v), 10 mM Tris–HCl, and 10 % (v/v) glycerol. For analysis 4 μg protein aliquots of isolated mitochondria were used. The separated proteins were blotted onto PVDF membranes (Immobilon-P, Millipore)

by semi-dry electro transfer for 1 h at $0.8 \text{ mA}/\text{cm}^2$. The membranes were blocked with 5 % (w/v) not-fat milk in TBS, 0.1 % (v/v) Tween-20 and then incubated for 2 h or overnight with subunit-specific antibodies. We used antibodies from Abcam against respiratory chain complex I (NDUFA9, ab14713), complex II (SDH70, ab14715), complex III (Core2, ab14745), complex IV (Cox4, ab14744), complex V—ATP synthase (F_1a , ab110273), cyclophilin D (F) (CypD, ab110324), citrate synthase (CS, ab129095), VDAC/porin (ab14734) and rabbit antibody against rat heart adenine nucleotide translocator (ANT [33]). Quantitative detection was performed using infrared dye-labeled secondary antibodies (goat anti-mouse IgG, Alexa Fluor 680—A21058 or goat anti-rabbit IgG, Alexa Fluor 680—A21109 from Life Technologies) and Odyssey Infrared Imager (Li-Cor); the signal was quantified by AIDA 3.21 Image Analyzer software (Raytest).

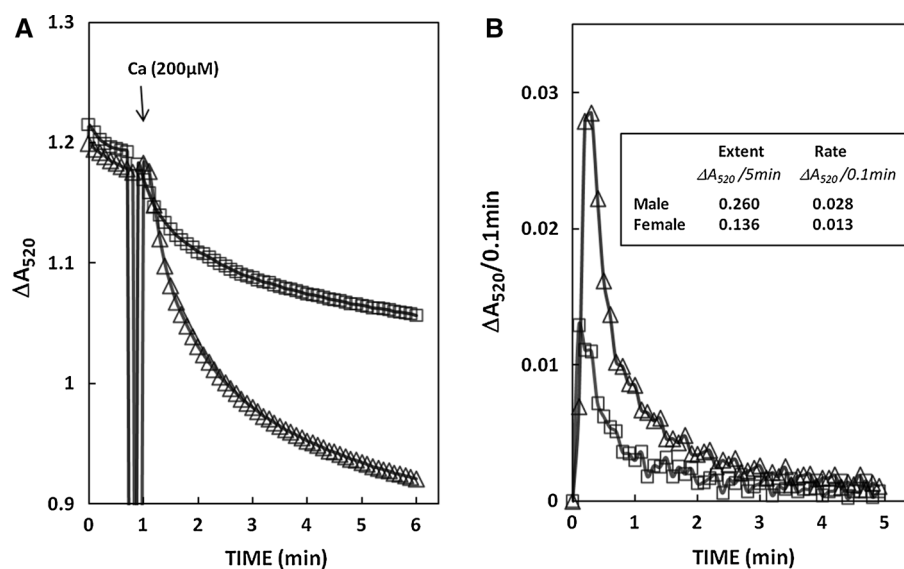
Statistical analysis

The data were statistically evaluated by unpaired Student *t* test. Values lower than 0.05 were considered as statistically significant.

Results

Sensitivity of male and female mitochondria to calcium load was tested by measurements of mitochondrial permeability transition pore (MPTP) function. Its opening is activated by increased free calcium in cytosol of cardiomyocytes. These changes may be tested also in vitro on isolated mitochondria. When calcium ions are added to suspension of mitochondria the pore is opened, water enters into mitochondria and mitochondrial swelling can be evaluated as decreased absorbance of mitochondrial suspension. Figure 1 illustrates conditions of our experiments. Isolated mitochondria were added to the incubation medium and after 1 min CaCl_2 (200 μM) was added. Opening of the MPTP is indicated by the decrease in absorbance for next 5 min of incubation, when the extent of swelling is reaching its maximum values. It is evident that the same amounts of mitochondria from male and female heart give the same values of initial absorbance and after addition of CaCl_2 they have different extent and different rate of swelling (Fig. 1A). From these classical curves, we can obtain in a digital value the extent of swelling. The maximum rate of swelling is more difficult to calculate from these curves. However, when these curves are transferred by simple derivatization of the original data (Fig. 1A), new curves (Fig. 1B) are obtained from which digital values of the maximum swelling rates can be easily evaluated as shown in the inset of Fig. 1B. The extent of swelling of

Fig. 1 Determination of calcium-induced swelling by rat heart mitochondria from male (open triangle) and female (open square) rats. Extent of swelling was calculated from the swelling curves (A) and expressed as the decrease of absorbance at 520 nm during 5 min after addition of 200 μ M CaCl_2 . Maximum rate of swelling (B) was calculated from curves obtained after derivatization of curves presented in A. Inset in B indicates digital values obtained from particular curves. Experimental conditions are described in methods



female mitochondria represents 52 % of male samples, similarly, as the maximum rate of swelling (46 %).

Table 1 summarizes the data obtained from a group of 8 samples of male and female mitochondria isolated from rat hearts. We tested two concentrations of added CaCl_2 , 200 μ M and 50 μ M. According to our previous experiments on mitochondria from male adult rats, 200 μ M CaCl_2 gives maximum values of swelling extent that can be obtained by mitochondrial permeabilization by alamethicin [34, 35]. With 50 μ M concentration both the extent and the rate of swelling were lower (Table 1). From Table 1 it is evident that with the 50 μ M CaCl_2 as swelling inducer, we do not see any differences in the extent and rate of swelling between mitochondria from male and female rat heart. However, at higher CaCl_2 concentrations, we observed that female mitochondria are more resistant to calcium-induced swelling. Both the extent of swelling and the maximal rate of swelling are lower in mitochondria from female heart than in mitochondria from male heart. When an additional swelling activating agent phenylarsine oxide [36] was added to the lower CaCl_2 (50 μ M), the extent and the rate of the swelling were increased in both male and female

mitochondria; however, then there were no differences between mitochondria from male and female hearts (Table 1).

To assess how the observed changes in mitochondrial swelling depend on the components of mitochondrial oxidative phosphorylation system (OXPHOS), we compared also respiratory chain activities in mitochondria from male and female hearts. In Table 2 are summarized the data from 5 male and female rats. We did not observe any difference in all parameters tested. Maximum respiration rate with complex I and complex II substrates, State 3 and State 4 respiration and Respiratory Control Index were the same. Also the activating effect of cytochrome *c*, which indicates the damage of outer mitochondrial membrane during isolation procedure was the same. Quantification of respiratory chain complexes by Western blotting using specific antibodies (Fig. 2) further showed the same amounts of respiratory chain complexes I–IV (AU/mg protein) in male and female heart mitochondria (Table 3).

In addition, we have tested whether the differences in the mitochondrial swelling cannot be explained by different amount of MPTP-forming ATP synthase or cyclophilin

Table 1 Calcium-induced swelling of isolated mitochondria from male and female rat hearts. AsOX—phenylarsine oxide

Additions	Extent of swelling			Maximum rate of swelling		
	Male ($\Delta A_{520}/5\text{ min}$)	Female	Female/male	Male ($\Delta A_{520}/0.1\text{ min}$)	Female	Female/male
50 μ M CaCl_2	0.085 \pm 0.010	0.089 \pm 0.015	1.05	0.0068 \pm 0.0013	0.0064 \pm 0.0013	0.94
200 μ M CaCl_2	0.259 \pm 0.029	0.159 \pm 0.028 ^a	0.61	0.0211 \pm 0.0026	0.0136 \pm 0.0012 ^a	0.64
50 μ M CaCl_2 + 25 μ M AsOX	0.245 \pm 0.029	0.225 \pm 0.007	0.92	0.0132 \pm 0.0030	0.0100 \pm 0.0010	0.76

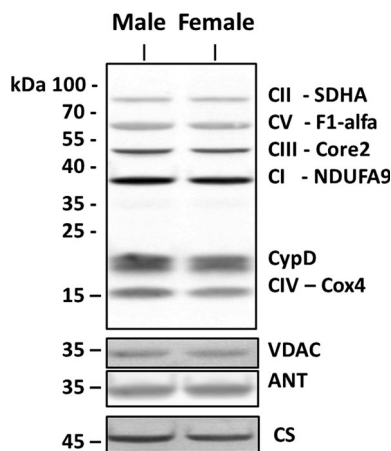
Experimental conditions are described in “Materials and methods”. Values are mean \pm SD

^a Significant difference ($p \leq 0.05$) between female and male group, $n = 8$

Table 2 Respiration with indicated substrates of isolated mitochondria (pmole oxygen/s/mg protein) from male and female rat hearts

	Male	Female	Female/male
	pmole oxygen/s/mg protein		
2.5 mM malate + 10 mM α -ketoglutarate	444 \pm 76	533 \pm 86	1.20
+1.5 mM ADP	2631 \pm 317	2589 \pm 278	0.98
+10 μ M cytochrome <i>c</i>	4137 \pm 511	4102 \pm 435	0.99
+10 mM succinate	5401 \pm 686	5991 \pm 776	1.11
	Male	Female	Female/male
Respiratory control index (RCI)	11 \pm 1.4	9 \pm 0.7	0.82
Activation by cytochrome <i>c</i> (%)	37 \pm 1.5	39 \pm 2.2	1.05

Experimental conditions are described in “Materials and methods”. Values are mean \pm SD, *n* = 4

**Fig. 2** Immunodetection of respiratory chain complexes I–V, cyclophilin D (CypD), and voltage-dependent anion channel (VDAC), adenine nucleotide translocator (ANT) and citrate synthase (CS) in male and female rat heart mitochondria. SDS-PAGE/Western blot analysis was performed using 4 μ g protein aliquots of isolated mitochondria. Experimental conditions are described in methods

D (CypD), which plays an important role in gating of the MPTP. As demonstrated in Table 3, also the amount of ATP synthase and CypD per mg protein was the same in

Table 3 Specific content of respiratory chain complexes I–V, cyclophilin D and cyclophilin D content relative to ATP synthase content determined by SDS-PAGE and Western blotting in mitochondria isolated from male and female rat hearts

	Male	Female	Female/male
	AU/mg protein		
Complex I (NADH dehydrogenase)	31.07 \pm 2.1	28.13 \pm 2.66	0.91 \pm 0.12
Complex II (Succinate dehydrogenase)	5.66 \pm 0.8	5.75 \pm 0.96	1.05 \pm 0.29
Complex III (<i>bc1</i> complex)	16.25 \pm 1.66	14.19 \pm 2.04	0.88 \pm 0.18
Complex IV (cytochrome <i>c</i> oxidase)	21.89 \pm 2.40	19.95 \pm 3.93	0.92 \pm 0.21
Complex V (ATP synthase)	11.14 \pm 1.08	10.43 \pm 1.87	0.94 \pm 0.21
Cyclophilin D (CypD)	48.42 \pm 3.05	45.18 \pm 6.63	0.93 \pm 0.13
	Male	Female	Female/male
Cyclophilin D/ATP synthase	4.36 \pm 1.96	4.35 \pm 1.95	1 \pm 0.09

Experimental conditions are described in “Materials and methods”. Values are mean \pm SD, *n* = 4

mitochondria from male and female hearts, the same was also the ratio between CypD and ATP synthase. ANT and VDAC, formerly believed structural components of MPTP are dispensable for MPTP formation [37, 38] but may be still involved in its regulation [39]. Interestingly, their specific contents were the same in male and female heart mitochondria, as well as the content of matrix marker, citrate synthase (Table 4).

We have also tested (not shown) the amount of CypD in heart mitochondria from newborn and adult rat hearts that differ in sensitivity of the MPTP to calcium as it has been shown previously [27]. Similarly as in the above experiments with adult male and female mitochondria, we did not find any difference. Mitochondria from newborn rats that have lower sensitivity to calcium load had the same amount of ATP synthase and CypD as mitochondria from adult hearts.

Discussion

It is now widely accepted that mitochondrial dysfunction, and particularly MPTP opening, plays a major role in determining the extent of injury the heart suffers during

Table 4 Specific content of adenine nucleotide translocator, voltage-dependent anion channel/porin and citrate synthase determined by SDS-PAGE and Western blotting in mitochondria isolated from male and female rat hearts

	Male AU/mg protein	Female	Female/male
Adenine nucleotide translocator (ANT)	23.49 ± 3.06	21.63 ± 3.64	0.92 ± 0.06
Voltage-dependent anion channel (VDAC)	2.77 ± 1.06	2.36 ± 1.06	0.87 ± 0.35
Citrate synthase (CS)	11.17 ± 3.22	9.93 ± 3.74	0.87 ± 0.12

Experimental conditions are described in “Materials and methods”. Values are mean ± SD, $n = 4$

reperfusion after a prolonged period of ischemia [15, 40]. The conditions that occur following ischemia and reperfusion are exactly those that would induce MPTP opening; in particular, the heart experiences calcium overload, high Pi and low adenine nucleotide concentrations during ischemia. These prime the MPTP for opening when reperfusion causes oxidative stress and accumulation of calcium by the reenergized mitochondria; the pore remains closed during ischemia but opens early in reperfusion [41]. This occurs as the pH returns to normal from the low values of ischemia which inhibit the MPTP opening. Under such conditions it seems to us that MPTP function may be at least partly responsible for sex-dependent differences in cardiac sensitivity to IR injury.

The major result of our study revealed sex differences in calcium-induced swelling of cardiac mitochondria. Direct measurements in isolated mitochondria from male and female rat hearts as a measure of MPTP function demonstrated that female heart mitochondria are more resistant to calcium-induced swelling at high, 200 μ M CaCl₂. Both the extent and the maximal rate of swelling are lower in mitochondria from female heart than in mitochondria from male heart. In contrast, phenylarsine oxide activation of swelling resulted in comparable swelling rates and extents in male and female mitochondria indicating comparable capacity of MPTP.

Functional measurements of respiratory chain activities further showed that there are no differences between male and female mitochondria in substrate oxidation and coupled ATP generation, indicating analogous specific content of respiratory chain enzymes. This was fully confirmed by quantitative immunodetection of all respiratory chain complexes. Quantitative immunodetection analysis to evaluate possible differences in MPTP content showed that male and female mitochondria contain comparable amounts of ATP synthase, the protein complex most likely representing the core MPTP structure, as well as of MPTP regulatory protein CypD. The recent key discovery that the MPTP is formed by the F₀F₁-ATP synthase is redirecting research towards the mechanisms that switch this vital enzyme from an energy-conserving to an energy-dissipating device. These hold great promise to improve our

understanding of the pathophysiological events that trigger the transition in heart diseases and to set a logical frame for therapeutic strategies [42].

Observed resistance of female heart mitochondria thus cannot be explained by changes in putative components of MPTP, and rather reflects regulation of MPTP function. One of possible explanations could be a difference in calcium interaction with CypD, an MPTP interacting regulator sensitizing MPTP opening to calcium, pointing to modified calcium binding and sensing in accordance with observed regulation of CypD by posttranslational modification (for review see [43]). Thus, Ser/Thr phosphorylation [44], Cys oxidation/nitrosylation [45, 46], and Lys acetylation and Sirt3-mediated deacetylation, in particular, [47, 48] have been shown to modulate MPTP function [49]. CypD remains, however, a viable target and the current hurdles between preclinical studies and clinical application of MPTP-inhibitory strategies will be overcome by more specific inhibitors of cyclophilin isoforms by drugs targeting the MPTP sites other than CypD and their combinatorial use [42].

Another possibility could be a change in calcium transport to mitochondria resulting in lower intramitochondrial calcium level in female heart mitochondria. Interestingly the latter mechanism is supported by direct measurements of calcium uptake by Arieli et al. [12], who demonstrated that female rat heart mitochondria cope more successfully with external calcium load by decreasing the rate of calcium influx by the calcium uniporter (MCU). The interactions between MCU and calcium uptake regulatory proteins MICU1, MICU2, MCUR1, SLC25A23, and EMRE may be here of crucial importance (see [50]).

The importance of MPTP opening in mediating IR injury was confirmed by demonstrating that inhibition of MPTP opening by cyclosporine A and sanglifehrin A is protective in a wide range of models, including a small proof of principle clinical trial [15, 51]. Further confirmation was provided by showing the hearts of CypD knockout mice are greatly protected against IR injury [52]. Nevertheless, the effects are modest and not observed in all species. One reason for this may be that CypD only facilitates MPTP opening which can occur in its absence when the stimulus is sufficient. Furthermore, CypD-mediated

MPTP formation is not the only trigger for I/R injury and MPTP-independent necrotic pathways also contribute to damage. We have observed that the protective effect is age-dependent: sangliferin A had no protective effect on the highly hypoxic tolerant neonatal heart [27]. It would be, therefore, of great interest to know whether the degree of protection may be also sex-dependent.

Clearly, a major priority must be to clarify the molecular identity of the proteins that make up the MPTP and how they interact. Since the front runners, the ANT, PiC, and F_0F_1 -ATP synthase, all have essential roles in oxidative phosphorylation, it is possible that knock-down or knock-out experiments will not be a suitable approach to provide unequivocal evidence for their role. This is especially true if the pore is formed from a novel conformation of one or more of these proteins or at the interface between them [17]. To analyze the possible age and sex differences in the structure and function of MPTP should be the subject of further experiments aimed at elucidation of molecular mechanisms enabling male–female differences in calcium homeostasis [53].

We may conclude from our data that in mitochondria isolated from rat male and female hearts the MPTP has different sensitivity to the calcium load. Female mitochondria are more resistant both in the extent and in the rate of the mitochondrial swelling at higher calcium concentration (200 μ M). At low calcium concentration (50 μ M) no differences were observed. Our data further suggest that sex-dependent specificity of the MPTP is not the result of different amounts of ATP synthase and CypD, or their respective ratio in mitochondria isolated from male and female hearts. These results indicate that male and female rat hearts contain comparable content of MPTP and its regulatory protein CypD; parallel immunodetection revealed also the same contents of ANT and VDAC in male and female rat heart mitochondria.

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