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Gender-dependence of hyperthermia-induced changes in respiration of rat liver mitochondria

Rasa Žūkienė,

Zita Naučienė,

Laima Degutytė-Fomins,

Vida Mildažienė*

Centre of Environmental Research, Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

The effect of hyperthermia on the respiration of liver mitochondria derived from animals of different genders was estimated. We compared the effect of heating in the febrile (40 °C) and supraphysiological (41–46 °C) temperature range on the respiration rate in metabolic state 2 (V_2) and state 3 (V_3) of isolated mitochondria derived from male and female rat liver. Our results indicate that an increase of temperature in the fever range activates female but inhibits male liver mitochondrial respiration in state 3. Female mitochondria are less sensitive to supraphysiological temperature hyperthermia (41–43 °C). However, a severe hyperthermia (44–46 °C) strongly inhibits and uncouples oxidative phosphorylation in mitochondria, but the temperature-induced increase in inner membrane permeability is stronger in female mitochondria. At 46 °C, independently of gender, mitochondria are completely uncoupled ($V_2 = V_3$) and are unable to phosphorylate ADP. The increased respiration rate in the uncoupled state proves that febrile temperature (40 °C) activates oxidation processes in liver mitochondria isolated from female rats. For this reason, at 40 °C the respiratory control index (RCI) decreases to a much lower extent in mitochondria derived from female liver.

Key words: liver mitochondria, hyperthermia, gender (sexual) dimorphism

INTRODUCTION

Gender (sexual) dimorphism is observed for numerous functions of different tissues [1–3] and is recognized as an important factor in pathophysiology [4]. A recent study has shown a striking gender dimorphic gene expression in mouse somatic tissues, especially in the liver; approximately 70% of the genes were differently expressed among sexes, thus implying that liver function may include extensive gender dimorphism [1]. Most of the studies demonstrated the female liver to be more tolerant under stressful conditions during liver surgery (ischemia / reperfusion [5], hemorrhage / resuscitation [4], hepatectomy, portal branch ligation, endotoxemia) than the male liver. Female sex hormone estrogen [6], thyroid hormones [7] or hormone-independent genetic factors ("genetic sex") [8] could contribute to sex differences and the consequent predisposition to liver cancer or hepatic pathologies.

Supra-physiological hyperthermia (usually in the range 42–45 °C) is clinically applied for cancer treatment [9–10]. The events following the exposure of cells to moderate heating involve an interplay of multiple factors operating at different regulatory levels and inducing changes in the metabolic activities, signal transduction, and gene expression. Very little is known about differences in cellular response to heating under fever compared to more severe hyperthermic conditions. Mitochondria are the key players in cellular response to heating and hyperthermia-induced cell death [11]; however, the response of mitochondria from different tissues to heating was investigated only by a few research groups [11–14]. Data on sexual dimorphism in oxidative capacity for liver mitochondria are rather controversial [15–18], and so far there has been no study on the effect of hyperthermia on isolated liver mitochondria derived from animals of different gender.

* Corresponding author: E-mail: v.mildaziene@gmf.vdu.lt

The aim of this study was to compare the effect of febrile (40 °C) and supraphysiological (41–46 °C) heating on classical functional parameters (state 2 (V_2) and 3 (V_3) respiration, RCI, respiration rate in uncoupled state) of isolated mitochondria derived from male and female rat liver. The obtained results show a significant gender dependence in mitochondrial response to hyperthermic treatment in the febrile range, but very minor differences in the range of supraphysiological temperatures that provoke a strong uncoupling and the entire loss of mitochondrial energy supplying function in liver mitochondria for both genders.

MATERIALS AND METHODS

Isolation of mitochondria. Mitochondria were isolated from livers of 3 months old Wistar rats (250–300 g) as described elsewhere [19], using the isolation medium containing 160 mM KCl, 20 mM Tris,10 mM NaCl, 5 mM EGTA, 1 mg/ml BSA (pH 7.7). After isolation, the mitochondria were suspended in a suspension buffer (SB) containing 180 mM KCl, 20 mM Tris, 3 mM EGTA (pH 7.3) and stored on ice. The concentration of the mitochondrial preparation was approximately 50 mg of mitochondrial protein per ml of stored suspension. Protein content was determined by the modified buret method [20].

Determination of the dissolved molecular oxygen concentration. The concentration of molecular oxygen dissolved in the assay medium at different temperatures (37–47 ± 0.1 °C) was determined polarographically using the glucose oxidase catalyzed reaction between D-glucose and O_2 while the pH of the medium was strictly controlled at each temperature (pH 7.2). The molar ratio coefficient of the reaction between D-glucose and O_2, was defined in the medium with a known concentration of dissolved oxygen at 37 °C.

Measurement of mitochondrial respiration. Mitochondrial respiration at different temperatures (37–46 ± 0.1 °C) was measured in a closed, stirred and thermostated 1.5 ml glass vessel equipped with a Clark-type oxygen electrode. The assay medium (AM) contained 20 mM Tris, 5 mM KH_2PO_4, 110 mM KCl, 50 mM creatine, 2.3 mM $MgCl_2$, pH 7.2. Excess of creatine kinase (0.1 mg/ml) was added to maintain the steady-state respiration. The experiments were performed using 5 mM pyruvate plus 5 mM malate as the oxidizable substrate. Mitochondria (1 mg protein / ml) had been incubated in the assay medium with the respiratory substrate (state 2) for 3 min at 37, 40, 41, 42, 43, 44, 45 or 46 °C before the state 3 respiration was initiated by addition of 1 mM ATP. The respiration rate in uncoupled state was induced by addition of 50 μM 2, 4-dinitrophenol in the presence of oligomycin (2 μg/mg protein) and 1 mM ATP. The rate of mitochondrial respiration in state 2 (V_2), state 3 (V_3) and the respiratory control index (RCI = V_3 / V_2) are defined according to the conventional terminology [21].

RESULTS AND DISCUSSION

Liver tumour is a wide spread disease, and only 10–15% of patients can be treated by radical surgery [22]. Radiofrequency and laser ablation is a local tumour destruction method, creating a high-temperature (coagulation) necrosis zone and a hyperthermic transition zone with a temperature gradient in the liver tissue [23]. The transition zone is supposed to be responsible for tumour recurrence [24]. Mitochondria as suppliers of cellular energy, conveyers of apoptotic signal and sites of increased ROS production are potentially important for the response of liver tissue to therapeutic hyperthermia and fever. More detailed knowledge on the perturbations of mitochondrial functions induced by hyperthermic treatment in healthy liver tissue is important for a better understanding of the hyperthermic killing mechanism and, possibly, for revealing the potential molecular factors useful for a more selective destruction of tumour and maintaining viable the healthy tissue. Data on the gender-specific differences in the activities and amount of oxidative phosphorylation components in liver mitochondria are rather controversial [15–18, 25].

In this study, the rate of 5 mM pyruvate + 5 mM malate oxidation in states 2 and 3 was measured at 37, 41, 40, 42, 43, 45, 46 °C in mitochondria isolated from female and male rat liver. The value of V_2 at 37 °C was low and similar for females and males (24.1 ± 1.3 and 27.0 ± 3.0 nmol O · min^{-1} · mg $protein^{-1}$, respectively) (Fig. 1). An increase in V_2 at 40 °C was significant both in females (by 15%) and in males (by 14%). With the further increase of the temperature, V_2 progressively increased as compared to that at 37–44 °C – by 87% in females and by 36% in males. At 45–46 °C, a significantly higher V_2 was also registered for females.

At 37 °C, V_3 was significantly lower in mitochondria from the female (109.9 ± 3.8 nmol O · min^{-1} · mg $protein^{-1}$) than from the male liver (129.5 ± 5.0 nmol O · min^{-1} · mg $protein^{-1}$). An increase of temperature to 40 °C caused an opposite change of V_3 in females and males: V_3 increased significantly (by 8%) in females, but decreased by 14% in males. Thus, under fever conditions, mitochondria from female liver respired faster than those from male, whereas *vice versa* was true at a normal body temperature (37 °C). The rise of temperature to 41–43 °C inhibited respiration in state 3 only in males. At 44 °C, however, V_3 was lower than at 37 °C by 17% in females and by 41% in males. At 46 °C, V_3 was equal to V_2 for both genders.

The respiratory control index (RCI), calculated as the V_3 / V_2 ratio, is an important functional parameter of mi-

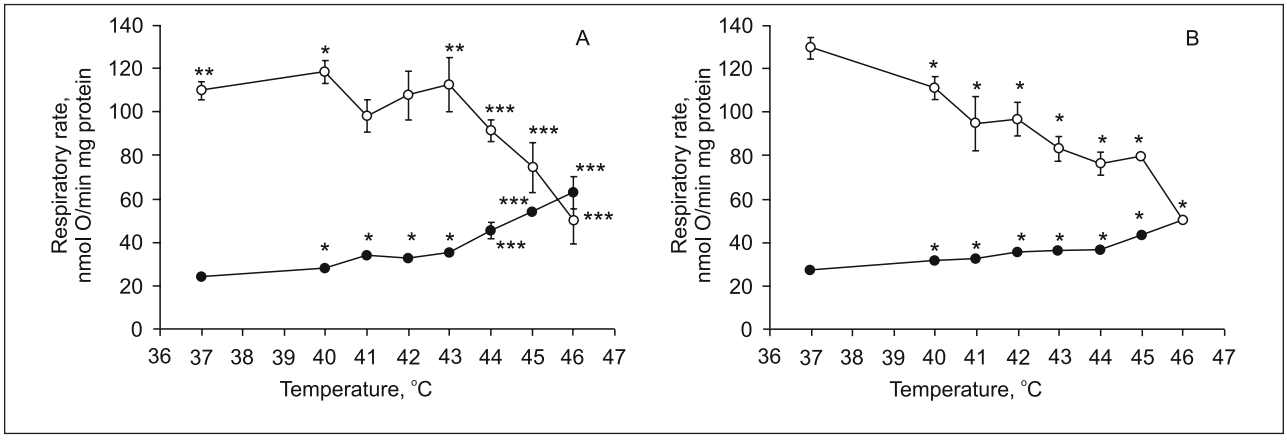

Fig. 1. Dependence of states 2 and 3 respiration rate in isolated female (A) and male (B) rat liver mitochondria on incubation temperature. Averages from n = 4 independent experiments ± SEM. ● – state 2, ○ – state 3, * – statistically significant difference as compared to 37 °C ($p < 0.05$), ** – statistically significant gender effect ($p < 0.05$)

tochondrial quality or injury, characterizing the degree of coupling of oxidative phosphorylation. The ratio of oxygen consumption flux in state 3 to the flux of membrane leak usually decreases when the barrier function of the inner mitochondrial membrane is compromised due to a damage or increase of membrane permeability because of some other reasons (e. g., temperature induced the rearrangement of membrane structure). The values of RCI at different incubation temperatures of mitochondria isolated form rat male and female liver are presented in Table.

One can see that RCI at 40 °C statistically significantly decreased in mitochondria from both genders; however, the effect of temperature was much greater in mitochondria derived from male liver. The reason may be a very similar increase in state 2 respiration rate (Fig. 1) and small, but opposite, effects on V_3 (activation for female and inhibition for male).

However, the further increase in temperature up to 45 °C leads to a more prominent decrease in RCI: in female mitochondria RCI decreased more significantly (by 70%, $p < 0.05$) than in male mitochondria (by 55%, $p < 0.05$) as compared with 37 °C. These results show that heating at supraphysiological temperatures has a higher impact on membrane leak in female mitochondria as compared with male.

A lower RCI decrease at 40 °C in female mitochondria compared with that of male (Table) and the activation of state 3 respiration (Fig. 1) indicate that substrate oxidation may be activated in the febrile range. To prove this assumption, the respiration rate was measured in the conditions where substrate oxidation was uncoupled from phosphorylation (uncoupled state). The respiration in the uncoupled state (V_{DNP}), induced by adding DNP, was measured at 37 and 40 °C (Fig. 2). In these conditions, mitochondria did not synthesize ATP, regardless of an active electron transfer along the respiratory chain and a high rate of oxygen consumption. The results show no difference at 37 °C between V_3 and V_{DNP} (Fig. 2). At 40 °C, V_{DNP} was significantly (by 21%) higher compared with V_{DNP} at

Table. **Dependence of RCI on the incubation temperature and gender**

Temperature, °C	RCI and RCI difference (%) compared to 37 °C	
	Male	Female
37	4.90 ± 0.35	4.52 ± 0.15
	–	–
40	3.28 ± 0.16*	4.15 ± 0.16* **
	–33%	–8%
42	2.63 ± 0.20*	3.28 ± 0.13* **
	–46%	–27 %
45	2.19 ± 0.21*	1.38 ± 0.22* **
	–55%	–70%

Average from n = 4 independent experiments ± SEM. * – statistically significant temperature effect compared to 37 °C, ** – statistically significant effect of gender ($p < 0.05$).

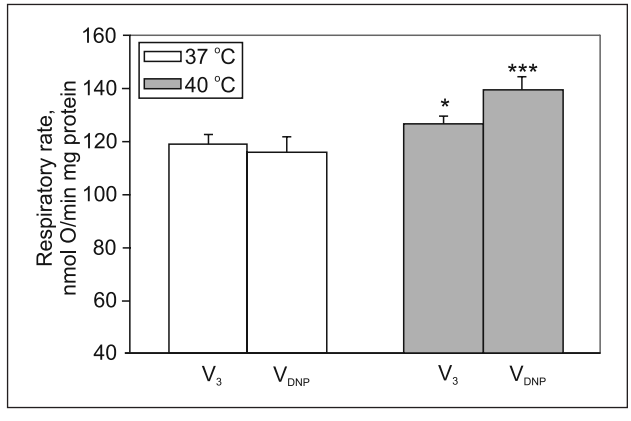

Fig. 2. Respiration rate in state 3 and in uncoupled state (VDNP) in female liver mitochondria at 37 and 40 °C. Averages from n = 4 independent experiments ± SEM, * – statistically significant temperature effect ($p < 0.05$), ** – statistically significant uncoupling effect ($p < 0.05$)

37 °C and by 10% higher compared with V_3 at 40 °C. These results confirm that a febrile temperature (40 °C) activates oxidation processes in liver mitochondria isolated from female rats.

Our study has revealed (for the first time) that the response profile of liver mitochondria to hyperthermia is gender-dependent. In the fever-range, the respiration of female mitochondria in state 3 is slightly activated, whereas in male mitochondria it is inhibited. Female mitochondria were less sensitive to the supraphysiological range of hyperthermia (41–43 °C) as compared with male mitochondria. However, more severe hyperthermia (44–46 °C) strongly inhibited and uncoupled oxidative phosphorylation both in female and male mitochondria. A considerably higher effect of severe hyperthermia (44–46 °C) on the inner membrane permeability in female mitochondria as compared with those of males may be also denoted as a gender-dependent feature. At 46 °C, mitochondria are totally uncoupled ($V_2 = V_3$) in both genders and are not able to phosphorylate ADP. Thus, the above results lead to the conclusion that, due to the activated oxidative subsystem, under fever conditions the mitochondrial energy supplying functions are performed more efficiently in the liver of females than in males. Taking into account that liver is involved in numerous important metabolic and detoxification processes, this should be beneficial for successfully combating infectious diseases in females. In the range of supraphysiological heating which is usually used in hyperthermic therapy (43–46 °C), female liver mitochondria exhibit a lower V_3 sensitivity under mild heating (41–43 °C) but a higher increase in membrane leakage (V_2 increases and RCI decreases at 43–46 °C) in comparison with male mitochondria. It remains to be determined how important are these differences for the survival of healthy hepatic tissue during tumour thermoablation in different zones located at a different distance from the ablation electrode and the coagulation necrosis area in the affected liver tissue.

It is difficult to compare these results with data of other studies since the information on gender-specific differences in the activities and the amount of oxidative phosphorylation components in liver mitochondria is rather controversial [15–18]. Valle et al. investigated the dependence of the activity of respiratory chain components on gender and determined that the activity of complexes I and III was higher in females than in males, and the activity of citochrome c oxidase (COX) was gender-independent [18]. Other investigators have shown that COX activity is by 144% higher in male than in female liver mitochondria. In male liver, a higher content of the COX subunit COX II, encoded in mitochondria, and of the subunit COX IV, encoded in the nucleus, was detected [24]. Electron microscopy data showed that the mitochondrial size distribution was higher in the female than male rat liver [17]. We have demonstrated that a febrile temperature (40 °C) activates oxidation processes not only in liver mitochondria isolated from female rats (Fig. 1), but also in mitochondria isolated from the heart of both gender rats [26]. It remains to be established what are the molecular reasons for the gender dependence of mitochondrial response to hyperthermia; anyway, most possibly this is the sex hormone that determines the differences in protein expression and membrane structure components important for the mitochondrial oxidative phosphorylation system.

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HIPERTERMIJOS SUKELTŲ ŽIURKĖS KEPENŲ MITOCHONDRIJŲ KVĖPAVIMO GREIČIO POKYČIŲ PRIKLAUSOMYBĖ NUO LYTIES

Santrauka

Šiame darbe siekta įvertinti hipertermijos poveikį iš skirtingos lyties žiurkių kepenų išskirtų mitochondrijų kvėpavimui. Palyginome karščiavimo temperatūros (40 °C) ir suprafiziologinio kaitinimo poveikį (41–46 °C) mitochondrijų, išskirtų iš žiurkių patinų ir patelių, kvėpavimo greičiui esant antrai (V_2) ir trečiai metabolinei būsenai (V_3). Gautais duomenimis, karščiavimo temperatūra aktyvino patelių ir slopino patinų mitochondrijų kvėpavimą esant trečiai metabolinei būsenai. Patelių mitochondrijos taip pat yra mažiau jautrios ribotai suprafiziologinei temperatūrai (41–43 °C), lyginant su patinų mitochondrijomis. Grubesnė hipertermija (44–46 °C) stipriai slopina ir atskiria iš abiejų lyčių gyvūnų išskirtų mitochondrijų oksidacinį fosforilinimą, tačiau sukelia didesnį patelių vidinės mitochondrijų membranos pralaidumą jonams, lyginant su patinų mitochondrijomis. Esant 46 °C temperatūrai, nepriklausomai nuo lyties mitochondrijos yra visai atskirtos ($V_2 = V_3$) ir praradusios gebėjimą fosforilinti ADP. Karščiavimo temperatūra (40 °C) didina patelių kepenų mitochondrijų kvėpavimo greitį, kai šios yra atskirtos. Tai įrodo, kad karščiavimas aktyvina oksidacinius procesus patelių mitochondrijose, todėl, esant 40 °C temperatūrai, patelių kepenų mitochondrijų kvėpavimo kontrolės indeksas (RCI) mažėja ne taip stipriai kaip patinų.

Raktažodžiai: kepenų mitochondrijos, hipertermija