Respiratory Physiology & Neurobiology 266 (2019) 156–162

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

NADH-linked mitochondrial respiration in the developing mouse brain is sex-, age- and tissue-dependent

C. Arias-Reyes[a,b], K. Losantos-Ramos[a], M. Gonzales[c], D. Furrer[d], J. Soliz[a,b,*]

[a] Institut universitaire de cardiologie et de pneumologie de Québec, Centre Hospitalier Universitaire de Québec (CHUQ), Faculty of Medicine, Université Laval, Québec, QC, Canada
[b] Instituto de Biología Molecular y Biotecnología, Facultad de Ciencias Puras y Naturales, Universidad Mayor de San Andrés, La Paz, Bolivia
[c] Instituto Boliviano de Biología de la Altura, Facultad de Medicina, Universidad Mayor de San Andrés, La Paz, Bolivia
[d] Oncology Axis, CHU of Quebec Research Center, Laval University, Quebec City, Canada

ARTICLE INFO

Keywords:
Mice
Mitochondria
Sex dimorphism
Brain development
Brainstem
cortex

ABSTRACT

Mitochondria play a major role in the brain. Apart from energy production, mitochondria regulate key factors in the activation of cell signaling pathways such as survival, proliferation, and differentiation. While all these processes occur during the physiological development of the brain, it is surprising that the mitochondrial functions and functioning in the brain during the postnatal development remain poorly explored. In this work, we collected samples of brainstem and cortex of mice at postnatal ages 3 (P3), 21 (P21), and at adulthood (3 months old) and evaluated the mitochondrial oxygen consumption after complex I activation. To do so, we used our oxygraph-2 K system (OROBOROS) that measures the mitochondrial bioenergetics in saponin-permeabilized tissue punches of 2 mg weight. Furthermore, as sex dimorphism in the brain occurs since very early stages of development, we performed experiments in brain samples of male and female mice. Accordingly, the mitochondrial oxygen consumption rate (OCR) was evaluated under activation of complex I (NADH-linked respiration – mitochondrial state 3), and during the inhibition of the complex V (ATP synthase) with oligomycin (mitochondrial state 4). In following, the respiratory control ratio (RCR – state 3/state4) was calculated as an index of mitochondrial oxidative-phosphorylation coupling. Our results show that the activity of the mitochondrial complex I in the brain increases along with the postnatal development in a sex- and tissue-dependent manner, with males showing higher activity than females, and with brainstem tissue showing higher activity than cortex. Our data may contribute to a better understanding of the sex-dependent maturation of the cortex and the cardiorespiratory network located in the brainstem.

1. Introduction

Mitochondria have an extensive role in the neural tissue. Mitochondrial functions are required to cell growth and reproduction, to regulate neurogenesis and differentiation, to promote effective neurotransmission, and to maintain cell membrane ionic gradients (Hara et al., 2014). Furthermore, mitochondria promote the development of synaptic pruning (Xavier et al., 2016), induce angiogenesis, and regulate key mechanisms of apoptosis, inflammation, oxidation, and cytotoxicity (Hamanaka and Chandel, 2010). Such functions are especially important at postnatal ages when the neural tissue is in full process of development and maturation. As such, mitochondria in the cortex need to be involved in learning, perception and coordination tasks (Picard and McEwen, 2014). In the brainstem, mitochondria might be required in the maturation of the neural network controlling the cardiorespiratory function (Giuffrida et al., 1979). Furthermore, compared to other tissues, the brain is more vulnerable to oxidative stress. This particular susceptibility is the consequence of 1) the brain tissue holding a lower antioxidant enzyme activity (mainly catalase is weak in the brain (Brannan, 1981), 2) high quantity of lipids (which are targets of lipid peroxidation)(Milder and Patel, 2012; Souza et al., 2013), 3) very large amount of metal ions (able to react with H_2O_2 to form the hydroxyl radical through the Fenton reaction)(Connor, 1992), 4) consumption of large amount of oxygen that promotes the production of ROS (Halliwell, 2001; Lagranha et al., 2017), and 5) the use of large amounts of glutamate as an excitatory neurotransmitter leading to an elevation of intracellular Ca^{2+} that activates pro-oxidant systems (such as phospholipase A2 and neuronal nitric oxide synthase - nNOS) (Lagranha et al., 2017). Supplementary complexity of mitochondria in the brain occurs by the fact that the cerebral mitochondrial functioning

* Corresponding author at: Institut Universitaire de Cardiologie et de Pneumologie de Québec (IUCPQ), 2725, chemin Sainte-Foy, Québec, QC, G1V 4G5, Canada.
E-mail address: jorge.soliz@crchuq.ulaval.ca (J. Soliz).

https://doi.org/10.1016/j.resp.2019.05.011
Received 22 March 2019; Received in revised form 9 May 2019; Accepted 20 May 2019
Available online 22 May 2019
1569-9048/

is sex-dependent. Mitochondria play an essential role in sex steroid hormone biosynthesis (Gaignard et al., 2017a). On the other way around, sex steroid hormones (estradiol, progesterone, and testosterone) are able to modulate the mitochondrial energy production, cell signaling, and control of oxidative stress (Gaignard et al., 2017a). These hormones play a crucial role during the postnatal brain development, as they promote the development of brain areas (such cortex) involved in masculine behavior (a process called "masculinization") and repressing the development of feminine behavior (or "de-feminization") (Hsu et al., 2001; MacLusky and Naftolin, 1981; Toran-Allerand, 1984). Similarly, the incidence of respiratory diseases of neural origin at early ages is higher in males than in females (Mage and Donner, 2006), and our understanding of such heterogeneity remains limited (Fournier et al., 2011). Accordingly, with this background, it is reasonable to hypothesize that the mitochondrial respiration in the brain during the postnatal development is region- age- and sex-dependent. Keeping in mind that (from the five-multi-protein mitochondrial complexes) the complex I is the largest and most complicated (associated with devastating neural disorders with onset in early childhood) (Distelmaier et al., 2009), in this work we evaluated the mitochondrial oxygen consumption rate (OCR) after complex I activation in male and female C57Bl6 mice at postnatal days 3 and 21, and adulthood (3 months). Our results suggest that differences in the OCR after activation of the complex I may be associated with a quicker development of the brain of females compared to males, and with earlier development of cortex tissue than brainstem in both sexes.

2. Material and methods

2.1. Animals

C57Bl6 male and female mice at postnatal (P) ages P3 (n = 5–6) and P21 (n = 8–9), and adulthood (at 3-months-old, n = 8–9) were used in this work. These ages were chosen because 1) at P3 the brain of mice is still immature and, compared to females, the brain of males undergoes into a first stage of masculinization and de-feminization (MacLusky and Naftolin, 1981), 2) at P21 the brain of mice are mature but not yet exposed to pubertal hormonal secretion, and 3) adult animals are sexually mature and thus, brains are already shaped (masculinized and feminized) by the pubertal hormonal secretion. Animals were purchased from Jackson laboratory and kept and reproduced in our animal care facility under standard conditions with food and water available ad-libitum. All experimental procedures were approved at Laval University, Animal Care Committee, Québec, Canada, and the protocols were in accordance with the guidelines detailed by the Canadian Council on Animal Care.

2.2. Tissue sampling

At corresponding experimental ages animals were killed by dislocation, the brainstem and cortex (approximately 2 mg) were rapidly dissected and immersed in cold (4 °C) respirometry medium MiR05 (0.5 mM EGTA, 3 mM MgCl2*6H2O, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH2P04, 20 mM HEPES, 110 mM sucrose, and 1 g/l bovine serum albumin pH = 7). The remaining brainstem and cortex tissues collected from the same animals were stored (at −80 °C) for protein content determination.

2.3. Protein content

Frozen samples of cortex and brainstem were thawed, weighed and placed into pre-chilled Eppendorf tubes containing a 10% w/v solution of PBS 1X supplemented with EDTA (0.5 mM). The samples were homogenized and centrifuged at 1500 g for 8 mins at 4 °C. The supernatant was recovered and the protein concentration was evaluated by colorimetric methods using the DC™ Protein Assay kit from BIORAD.

2.4. Evaluation of mitochondrial respiration

The Oxygraph-2k (OROBOROS Instruments, Innsbruck, Austria) was used to measure mitochondrial bioenergetics. Calibration of the oxygraph was performed prior to every experiment, in accordance with the manufacturer standardized procedures (Gnaiger, 2008). Based on previous studies establishing the optimal conditions for measurements of mitochondrial respiration brain samples (Herbst and Holloway, 2015; Laouafa et al., 2019), tissues were incubated (15 min; 37 °C; inside the oxygraph recording chamber) in MiR05 medium supplemented with saponin (50 µg/ml - used to facilitate substrates and oxygen diffusion) for allowing permeabilization and thermal stabilization. Of note, the concentration of gases in the incubation solution (MiR05 - inside the respiratory chambers) is determined by the environmental PO_2 and PCO_2, the temperature (37 °C), and the O_2 solubility factor. As such, the O_2 concentration in the MiR05 ranges between 150 and 200 nmol/ml at the beginning of the experiment. Such O_2 is consumed by mitochondria and will allow evaluating the oxygen consumption rate (OCR). Data of O_2 consumption was obtained by following the substrate-uncoupler-inhibitor titration (SUIT) protocol (Gnaiger, 2007). The mitochondrial oxygen consumption was evaluated

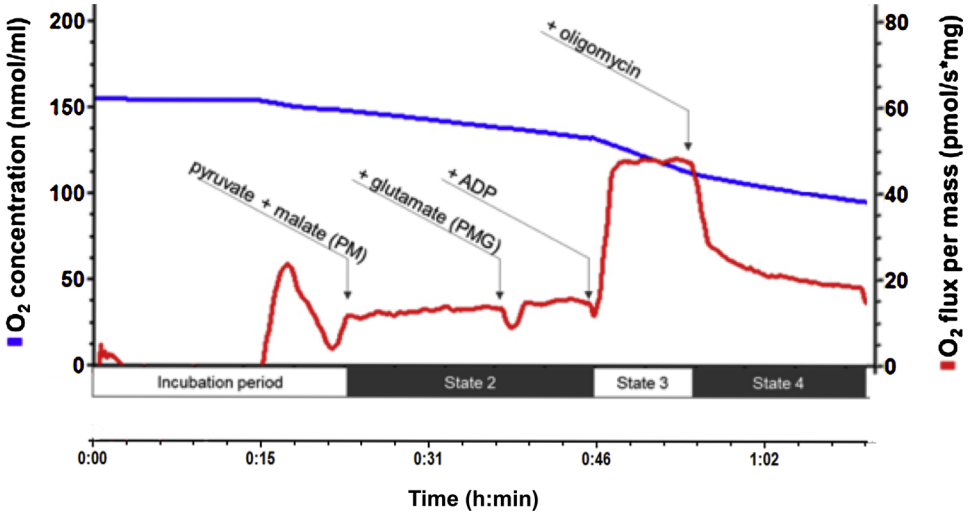

Fig. 1. Schematic representation of the mitochondrial oxygen consumption protocol (SUIT) used in this work. High-resolution Oxygraph-2k was used to evaluate the mitochondrial respiration after activation of the complex I in brain samples. Classical recording of mitochondrial O_2 consumption (red line) derived from the variation of O_2 concentration (blue line) inside the analysis chamber was performed. Mitochondrial states were reached by the titration of different substrates: pyruvate, malate (state 2-PM); + glutamate (state 2-PMG); + ADP (state 3), and oligomycin (state 4).

under different states (schematized in Fig. 1) described in the following:

State 3: is the mitochondrial oxygen consumption rate when the respiratory substrates to fuel up the mitochondrial complex I (5 mM of pyruvate, 2 mM of malate, and 10 mM of glutamate) and ADP (2.5 mM) are added to the medium. This procedure allows for evaluating the OCR of the mitochondrial respiratory chain.

State 4: is the mitochondrial oxygen consumption when oligomycin (2.5 μM – an inhibitor of ATP synthase) is added to the medium. As the ATP synthase is inhibited, this procedure allows evaluating the OCR of the mitochondrial respiratory chain due to a membrane proton leak.

Respiratory control ratio (RCR): The RCR is the ratio between the state 3 over the state 4. As such, the RCR is a parameter that indicates the level of coupling between oxidation and phosphorylation process in the mitochondria. Such coupling may be affected by two parameters: 1) the activity of the ATP synthase that determines the maximal OCR (state 3) of the mitochondrial respiratory chain, and 2) the flow of protons through the inner mitochondrial membrane that can be affected by the integrity of the membrane itself, or by the presence of uncoupling molecules (UCPs) (Brand and Nicholls, 2011).

Finally, as part of our verification protocol for this type of experiments, the evaluation of the mitochondrial membrane integrity was performed on a couple of experiments per group by adding cytochrome c to the incubation medium. As expected, no changes were observed (data not shown).

2.5. Statistical analysis

Data on protein concentration were evaluated for each tissue by separate by 2-way ANOVA with age and tissue as the grouping variables. Mitochondrial oxygen consumption rates were adjusted by subtracting the non-mitochondrial residual oxygen consumption from the total oxygen consumption at each respiratory state. Data on oxygen consumption were normalized by the protein content and analyzed by RM ANOVA tests using age (P3, P21 or adult) and sex (male or female), or age and tissue (cortex or brainstem) as grouping variables. When significant differences in treatments or interactions were found, a post-hoc Fischer's multiple comparison test was performed. All analyses were done in Prism v. 6.01 software (GraphPad Software, Inc.). All experiments have n = 6 to 10. Values are reported as mean ± s.e.m. and the significance p was set at 0.05.

3. Results

3.1. Age influences total protein concentration in brain samples of male and female mice

Total protein concentration was evaluated in brainstem and cortex samples of male and female mice at three different ages, postnatal day 3 (P3), postnatal day 21 (P21) and adulthood (at about 3 months old). As shown in Fig. 2, a significant effect of age was observed in both tissues of male and female rats. In the brainstem, however, while P21 and adult females have sustained significantly elevated levels of protein, males show a peak of protein concentration at P21 and then return to P3 levels at adulthood. In the cortex, equally higher protein concentrations are present at P21 and adulthood in comparison to P3 in male and female animals. No sex-dependent differences were found at any evaluated age neither in the brainstem (Fig. 2a) nor the cortex (Fig. 2b).

3.2. Mitochondrial state 3 in the brainstem increases only in adult males

Mitochondrial respiration under activation of the complex I was evaluated in male and female brainstem samples of mice at P3, P21, and adulthood. Sex-related comparisons in the respiratory activity showed higher OCR in adult males compared to adult females at the mitochondrial state 3 (Fig. 3a). However, no sex-related differences in the OCR were found at the mitochondrial state 4 (Fig. 3b) and the RCR (Fig. 3c).

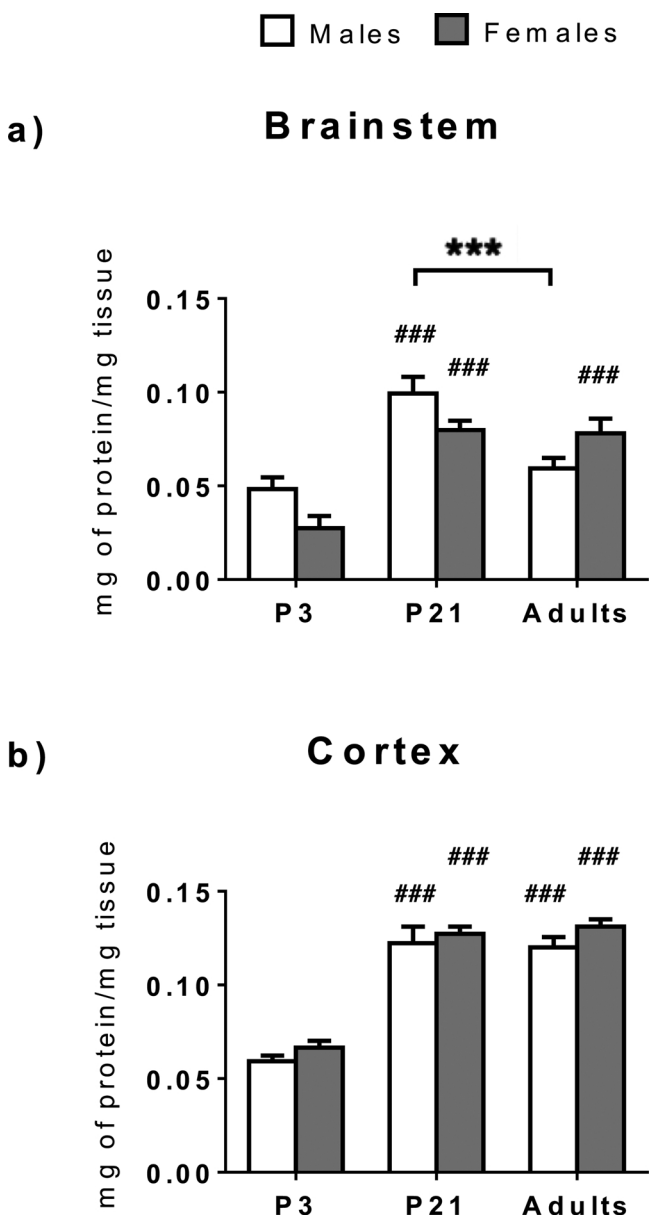

Fig. 2. Total protein quantification in the brainstem and cortical samples. Total protein content increases from P3 to P21 and from there on it remains constant until adulthood. No sex-related changes were observed at any tested age. ***: $p < 0.001$; ### and $p < 0.001$ versus P3 same sex.

Age-related assessments showed increased OCR in adult males compared to P3 and P21, but not in females (Fig. 3a). On the other hand, no differences in the respiratory activity of brainstem samples between ages were found at the mitochondrial state 4 (Fig. 3b) and the RCR (Fig. 3c).

3.3. Mitochondrial OCR in the cortex is sex- and age-dependent

Mitochondrial respiration under activation of the complex I was also evaluated in male and female cortex samples. Sex-related comparisons revealed higher OCR in state 3 in males compared to females at P21 and adulthood but not at P3 (Fig. 4a). The RCR was significantly higher in males than females only at P21 (Fig. 4c). No sex-related differences were found in the mitochondrial OCR at state 4 (Fig. 4b).

Age-related assessments in cortex samples showed a significant increase in OCR starting at P21 ages continuing to adulthood in males and

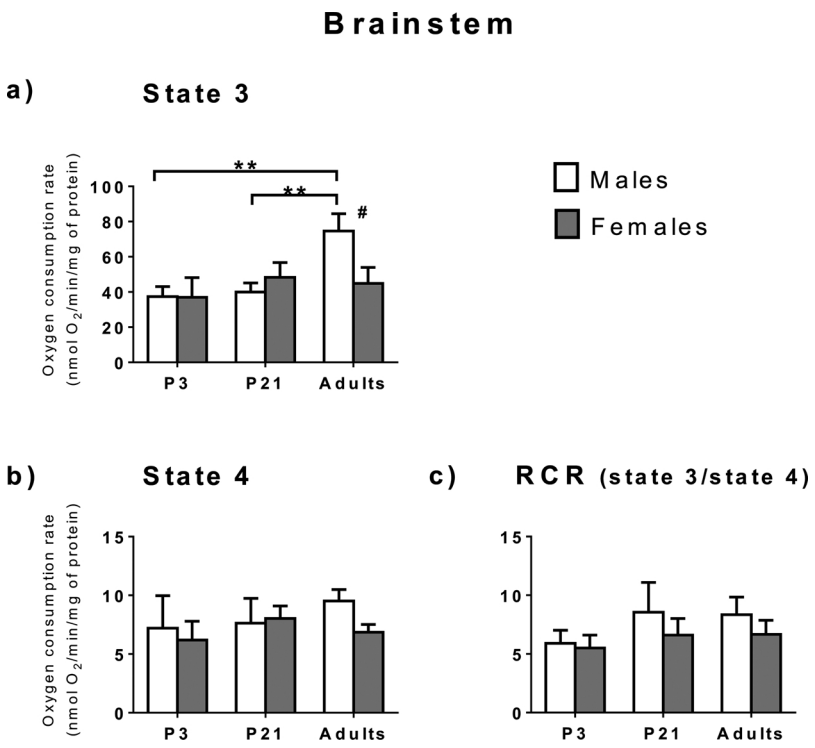

Fig. 3. Mitochondrial respiration after the activation of the complex I in permeabilized brainstem samples of male and female mice. Oxygen consumption rate with substrates of (a) complex I (pyruvate, malate, glutamate, and ADP) (State 3) and (b) oligomycin (state 4). (c) RCR: respiratory control ratio (state 3/state 4). **: p,0.01; #: $p < 0.05$, same age.

females at the mitochondrial state 3 (Fig. 4a) and state 4 (Fig. 4b). No age-related differences were found in RCR (Fig. 4c).

3.4. Mitochondrial respiration in mice is brain-region-dependent

Comparison between brain regions of male animals evidenced that the mitochondrial OCR under activation of the complex I is higher in the brainstem than cortex at P3 and adulthood at the mitochondrial states 3 and 4 (Fig. 5a and b). However, no tissue-related differences were found in the RCR (Fig. 5c). In females, significant increased mitochondrial OCR state 3 and state 4 was found in the brainstem compared to the cortex at all ages (Fig. 6a and b). However, no differences were found in the RCR (Fig. 6c).

4. Discussion

In the present study, we have investigated the respiratory activity with substrates of the mitochondrial complex I in the brain (brainstem

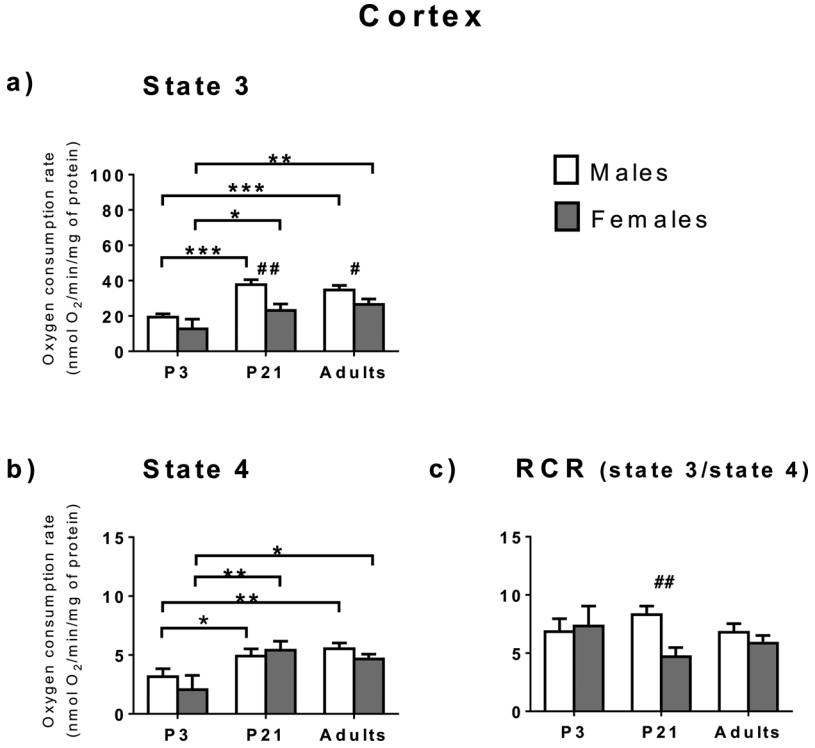

Fig. 4. Mitochondrial respiration after the activation of the complex I in permeabilized cortex samples of male and female mice. Oxygen consumption rate with substrates of (a) complex I (pyruvate, malate, glutamate, and ADP (State 3) and (b) oligomycin (state 4). (c) RCR: respiratory control ratio (state 3/state 4). *, **, ***: $p < 0.05$, p,0.01, and $p < 0.001$; #, ##: p,0.05, and p,0.01, same age.

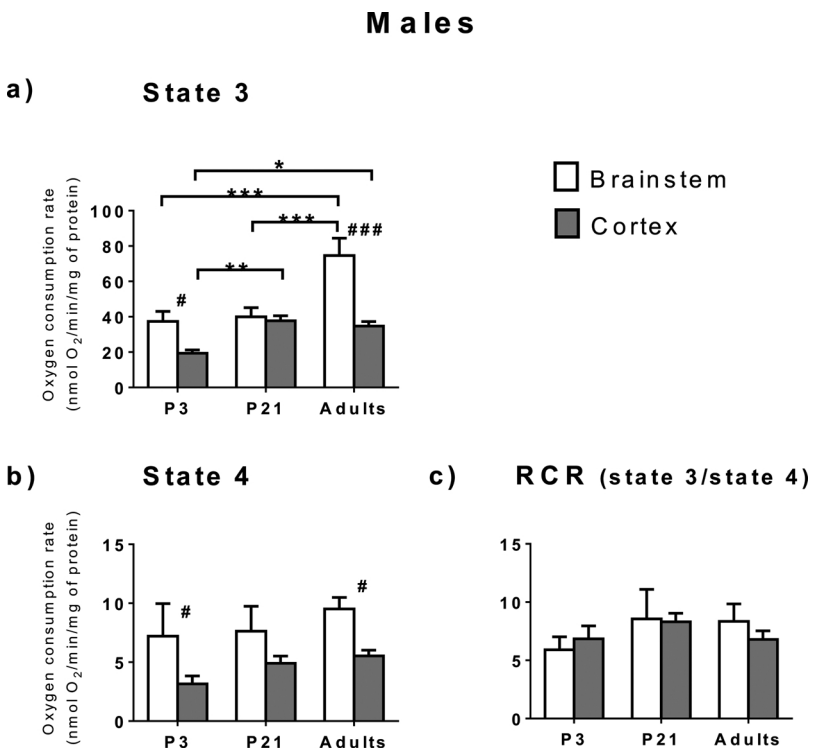

Fig. 5. Mitochondrial respiration after the activation of the complex I in permeabilized brainstem and cortex samples of male mice. Oxygen consumption rate with substrates of (a) complex I (pyruvate, malate, glutamate, and ADP (State 3) and (b) oligomycin (state 4). (c) RCR: respiratory control ratio (state 3/ state 4). *,**,***: p,0.05, p,0.01, and $p < 0.001$; #, ###, ###: $p < 0.05$, and $p < 0.001$, same age.

and cortex) of male and female mice at postnatal ages (P3, P21) and adulthood. The main findings of the present report are: 1) the mitochondrial OCR under complex I activation (state 3) in the brainstem is sex-dependent only at adult ages, with males showing higher mitochondrial respiration than females; 2) OCR with substrates of the complex I (state 3) in the cortex is sex-dependent at P21 and adulthood, with males showing higher mitochondrial respiration than females; 3) significant increase of the OCR under complex I activation (state 3) in the brainstem occurs at adulthood and in male animals only. While a significant increase of the maximal OCR (state 3) in the cortex starts at P21 and remains high at adulthood in male and female animals; 4) finally, compared to the brainstem, increased OCR with substrates of the complex I (state 3) occurs in the cortex of male and female animals. Keeping in mind that at postnatal ages brain tissues are in full process of development and maturation, and that the OCR with substrates of complex I can be used as a tracer of developmental activity, our results suggest that females reach brain maturation earlier than males, and that the cortex tissue of male and female animals matures earlier than the brainstem region.

The brain has a very high metabolic rate. In fact, despite accounting

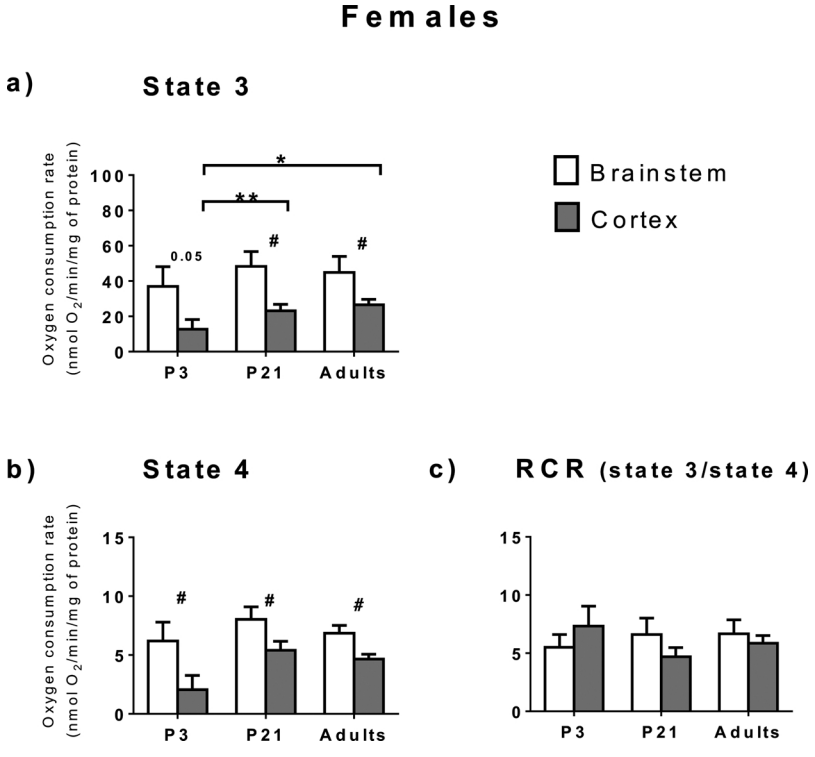

Fig. 6. Mitochondrial respiration after the activation of the complex I in permeabilized brainstem and cortex samples of female mice. Oxygen consumption rate with substrates of (a) complex I (pyruvate, malate, glutamate, and ADP (State 3) and (b) oligomycin (state 4). (c) RCR: respiratory control ratio (state 3/ state 4). *,**: p,0.05, and p,0.01; #: $p < 0.05$, same age.

for only 0.5–2% of the body weight, the brain consumes between 10% and 20% of the total O_2 inspired at rest (Silver and Erecińska, 1998). Such highly metabolic demand reflects the fact that neurons are highly differentiated cells needing a large amount of ATP for maintenance of ionic gradients across the cell membranes and for neurotransmission. Moreover, paralleling bioenergetics requirements, apoptosis regulation, cell signaling, and control of oxidative stress processes are also accomplished by the mitochondrion. The role of mitochondria in neural tissue and the impact of sex were extensively investigated at adult ages (Gaignard et al., 2017a, b; Gaignard et al., 2015). These studies have been designed to analyze the consequences of aging (Gaignard et al., 2017a). However, studies determining the mitochondrial respiration in the brain at postnatal ages are yet scarce. Furthermore, in contrast to studies performed at adult ages, the impact of sex- and age in the mitochondrial function during the postnatal development of the brain remains poorly investigated. In this work, we investigated the respiratory rate after activation of the complex I in the brainstem and the cortex of male and female mice. Our experimental protocol was adapted from previous ones (Laouafa et al., 2018, 2019). The measurement of the mitochondrial OCR was performed in 2 mg of tissue punches always collected from the same brain region. Although we did not perform further experiments to test it, the low standard deviation of the mean in our data suggests that the number and type of cells in punches, as well as the mitochondrial volume per punch is similar. Furthermore, apart from classical anatomical and molecular (in terms of pathways) studies, the aim of this work is to investigate brain development from a more bioenergetic point of view. It is clear however that the convergence of these different, but complementary, visions needs to be explored.

4.1. Adult mitochondrial NADH-linked respiration is sex- and brain region-dependent

Sex differences studies at adulthood in mice and rats show that compared to male, females have higher protection against oxidative stress, and produce less free radical species (Gaignard et al., 2017a, 2015). These experiments were performed in 3-months old C57Bl6 mice, in which the whole right hemisphere was used to record the mitochondrial oxygen consumption in isolated and enriched mitochondrial fractions (Gaignard et al., 2015). Results of this study showed that in comparison to males, female brain mitochondria had 1) higher NADH-linked respiration rate; 2) increased pyruvate dehydrogenase complex (PDHc); and 3) higher mitochondrial reduced glutathione (GSH). Experiments in humans showed that women had higher overall cerebral glucose metabolism compared to men (Yoshizawa et al., 2013), suggesting that glucose-dependent mitochondrial production of energy is higher in females compared to males as a result of an increase of PDHc activity rather than an increase of oxidative phosphorylation (Gaignard et al., 2017a). In line to these data, our results show also that the mitochondrial functioning in the adult brain of mice is sex-dependent. Furthermore, our results show that mitochondrial respiration is brain region-dependent. More specifically, our results show that the NADH-linked respiration rate in male mice is higher than in females in brainstem and cortex regions. However, our results show also no sex differences in the RCR, which indicates that the oxidative-phosphorylation coupling is similar in males and females brain mitochondria, thus suggesting that it may exist mayor production of free radicals in males. Under physiological conditions, the production of free radicals is not associated with oxidative stress but cell signaling. As the animals in this study are healthy young adults, it is tempting to suggest that higher respiratory mitochondrial activity in males covers also the stimulation of an additional number of molecular pathways regulated by reactive oxygen species. In line, it is also known that higher metabolic activity by mitochondria in the brain may lead to slow and cumulative increase production of reactive oxygen species, oxidative damage, and cell aging (Starkov, 2008). This circumstance matches with the fact that female mammalian species are known to live longer than males, and are less susceptible to old age-related neurodegenerative diseases. (Harman, 1956). Apart from these differences between our results and those of previous works, it is also necessary to consider that experiments in our laboratory were performed by using permeabilized tissue samples and not isolated mitochondria. In fact, the respiratory mitochondrial activity can be drastically altered when it is outside its cellular environment due to the lack of stimulation of the different mitochondrial substrates, whose concentrations in vivo might also depend on sex.

4.2. Mitochondrial NADH-linked respiration during development is sex-, age-, and brain region-dependent

Mitochondrial respiration during developmental ages extends to important functions, including mitochondrial biogenesis, mitophagy, migration, morphogenesis, and Ca^{2+} balancing, and synaptic pruning and regulation of synaptic transmission (Hagberg et al., 2014; Leaw et al., 2017; Picard and McEwen, 2014). Current data demonstrate that respiratory mitochondrial activity in the injured neonatal brain is sex-specific. Using a rat model of neonatal hypoxia-ischemia (P7), it was reported that maximal respiratory mitochondrial activity and GPx activity were significantly impaired in males. In addition, a 3- to 4-fold increase in oxidative protein carbonylation (a marker of oxidative stress) was observed in the cortex and hippocampus of injured males but not in that of females (Demarest et al., 2016). In line with this finding, in a rat model of pediatric traumatic brain injury (P17-P21), males showed lower mitochondrial glutathione (GSH; an important antioxidant) content than females (Robertson and Saraswati, 2015). Sex-based differences were also found in young mice (6–8 weeks); male brains exhibited a lower respiratory mitochondrial activity, lower reserve capacity, higher H_2O_2 production, and higher NOX production than female brains (Khalifa et al., 2017). Despite these interesting findings showing that under injury conditions females are better protected than male in terms of oxidative stress-induced damages, our knowledge of respiratory mitochondrial activity under physiological conditions is yet poor. In fact, control (or shame) data in the above-described works show increased NADH-linked respiration (state 3) in cortex of males compared to females at the postnatal age 7 (Demarest et al., 2016), but no differences in the cortex at postnatal day 17–21 (Robertson and Saraswati, 2015). Our results clearly show that mitochondrial respiration after the activation of the complex I at neonatal ages is sex and age-specific, depending on the brain region. Compared to the brainstem region, the respiratory activity of the mitochondrial complex I (state 3) in the cortex at P3 is sex-specific, with males having higher activity than females, and region-specific, with double activity in the brainstem than the cortex. In fact, these results are in line with the fact that the development/maturation of the respiratory system is one major task at neonatal ages. On the other hand, it is interesting to observe that our data show that the mitochondrial NADH-linked respiration in the cortex of male and female mice increases at P21. It is tempting to suggest that this finding is in line with the concept that tasks associated with the cortex region (such as memory, attention, perception, cognition, awareness, and languages) are mainly developed during the childhood until youth. Similarly, it is interesting to observe that compared to male mice, females show decreased NADH-linked respiration rate at P3 and P21. This data suggest that the maturation of cortical tasks may start (and also finish) earlier in females compared to males. Finally, results in female animals show also that at P21 the RCR is lower in the cortex of females compared to males. These results may also be associated with higher production and action of free radical species in cell signaling in males, however, more studies are required to determine its physiological meaning. Moreover, such differences should correlate with the impact of sex hormones during development (and at adult ages). In fact, neonatal surges of testosterone promote the development of brain areas involved in masculine behavior (a process

called "masculinization") and repress the development of feminine behavior (or "de-feminization") (Hsu et al., 2001; MacLusky and Naftolin, 1981; Toran-Allerand, 1984). These effects are mediated by a dual mechanism that involves both a direct activation of the androgen receptor by testosterone (or its active metabolite dihydrotestosterone) and the aromatization of testosterone to estradiol within target neurons and the subsequent activation of estradiol receptors (Bodo and Rissman, 2008; Ivanova and Beyer, 2000; Kudwa et al., 2006; Toran-Allerand, 1984). Keeping in mind that testosterone regulates mitochondrial functions by activating nuclear receptors (while estradiol and progesterone do it throughout the activation of nuclear and mitochondrial receptors), the age-dependent mitochondrial role by sex results intricate and challenging.

In conclusion, the present study shows that the respiratory mitochondrial activity with substrates that fuel up the complex I during development and at young adult ages changes in life with brains-region maturation and tasks development. Furthermore, accordingly to differences in the physiology of males and females, our results also show that the mitochondrial respiration after activation of the complex I is sex-dependent. Our data establish the base platform for better interpret sex-related differences in mitochondrial and oxidative stress under pathological conditions, as well as in the sex-based neural therapies, as mitochondria start to be important targets in the protection of the brain.

Acknowledgments

The authors wish to thank the Canadian Institutes of Health Research (SVB-158607), the IUCPQ Foundation, along with the Respiratory Disease Funds Foundation. The salary of Jorge Soliz is supported by the “Fonds de recherche du Quebec-Santé” (FRQ-S; FQ121919). The authors have no actual or potential conflict of interest to disclose.

References

Bodo, C., Rissman, E.F., 2008. The androgen receptor is selectively involved in organization of sexually dimorphic social behaviors in mice. Endocrinology 149, 4142–4150.
Brand, M.D., Nicholls, D.G., 2011. Assessing mitochondrial dysfunction in cells. Biochem. J. 435, 297–312.
Brannan, T.S., Maker, H.S., Raes, I.P., 1981. Regional distribution of catalase in the adult rat brain. J. Neurochem. 36, 307–309.
Connor, J.R., Benkovic, S.A., 1992. Iron regulation in the brain: histochemical, biochemical, and molecular considerations. Ann. Neurol. 32, 551–561.
Demarest, T.G., Schuh, R.A., Waddell, J., McKenna, M.C., Fiskum, G., 2016. Sex-dependent mitochondrial respiratory impairment and oxidative stress in a rat model of neonatal hypoxic-ischemic encephalopathy. J. Neurochem. 137, 714–729.
Distelmaier, F., Koopman, W.J., van den Heuvel, L.P., Rodenburg, R.J., Mayatepek, E., Willems, P.H., Smeitink, J.A., 2009. Mitochondrial complex I deficiency: from organelle dysfunction to clinical disease. Brain 132, 833–842.
Fournier, S., Joseph, V., Kinkead, R., 2011. Influence of juvenile housing conditions on the ventilatory, thermoregulatory, and endocrine responses to hypoxia of adult male rats. J. Appl. Physiol. 111, 516–523.
Gaignard, P., Frechou, M., Liere, P., Therond, P., Schumacher, M., Slama, A., Guennoun, R., 2017a. Sex differences in brain mitochondrial metabolism: influence of endogenous steroids and stroke. J. Neuroendocrinol. 30.
Gaignard, P., Liere, P., Thérond, P., Schumacher, M., Slama, A., Guennoun, R., 2017b. Role of sex hormones on brain mitochondrial function, with special reference to aging and neurodegenerative diseases. Front. Aging Neurosci. 9, 406.
Gaignard, P., Savouroux, S., Liere, P., Pianos, A., Therond, P., Schumacher, M., Slama, A., Guennoun, R., 2015. Effect of sex differences on brain mitochondrial function and its suppression by Ovariectomy and in aged mice. Endocrinology 156, 2893–2904.
Giuffrida, A., Gadaleta, M., Serra, I., Renis, M., Geremia, E., Del Prete, G., Saccone, C., 1979. Mitochondrial DNA, RNA, and protein synthesis in different regions of developing rat brain. Neurochem. Res. 4, 37–52.
Gnaiger, E., 2007. Mitochondrial Pathways and Respiratory Control. Oroboros MiPNet Publications, Innsbruck 96 pp.
Gnaiger, E., 2008. Polarographic oxygen sensors, the oxygraph, and High-Resolution respirometry to assess mitochondrial function. Drug-Induced Mitochondrial Dysfunction. John Wiley & Sons, Inc., Hoboken, NJ, USA), pp. 325–352.
Hagberg, H., Mallard, C., Rousset, C.I., Thornton, C., 2014. Mitochondria: hub of injury responses in the developing brain. Lancet Neurol. 13, 217–232.
Halliwell, B., 2001. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging 18, 685–716.
Hamanaka, R.B., Chandel, N.S., 2010. Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. Trends Biochem. Sci. 35, 505–513.
Hara, Y., Yuk, F., Puri, R., Janssen, W., Rapp, P., Morrison, J., 2014. Presynaptic mitochondrial morphology in monkey prefrontal cortex correlates with working memory and is improved with estrogen treatment. Proceedings of the National Academy of Sciences of the United States of America 111, 486–491.
Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.
Herbst, E.A., Holloway, G.P., 2015. Permeabilization of brain tissue in situ enables multiregion analysis of mitochondrial function in a single mouse brain. J. Physiol. (Paris) 593, 787–801.
Hsu, H.K., Yang, R.C., Shih, H.C., Hsieh, Y.L., Chen, U.Y., Hsu, C., 2001. Prenatal exposure of testosterone prevents SDN-POA neurons of postnatal male rats from apoptosis through NMDA receptor. J. Neurophysiol. 86, 2374–2380.
Ivanova, T., Beyer, C., 2000. Ontogenetic expression and sex differences of aromatase and estrogen receptor-alpha/beta mRNA in the mouse hippocampus. Cell Tissue Res. 300, 231–237.
Khalifa, A.R., Abdel-Rahman, E.A., Mahmoud, A.M., Ali, M.H., Noureldin, M., Saber, S.H., Mohsen, M., Ali, S.S., 2017. Sex-specific differences in mitochondria biogenesis, morphology, respiratory function, and ROS homeostasis in young mouse heart and brain. Physiol. Rep. 5.
Kudwa, A.E., Michopoulos, V., Gatewood, J.D., Rissman, E.F., 2006. Roles of estrogen receptors alpha and beta in differentiation of mouse sexual behavior. Neuroscience 138, 921–928.
Lagranha, C.J., Silva, T.L.A., Silva, S.C.A., Braz, G.R.F., da Silva, A.I., Fernandes, M.P., Sellitti, D.F., 2017. Protective effects of estrogen against cardiovascular disease mediated via oxidative stress in the brain. Life Sci.
Laouafa, S., Roussel, D., Marcouiller, F., Soliz, J., Bairam, A., Joseph, V., 2018. Role of Estradiol Receptor Beta (ERβ) on Arterial Pressure, Respiratory Chemoreflex and Mitochondrial Function in Young and Aged Female. Mice. Adv. Exp. Med Biol. 1071, 115–127.
Laouafa, S., Roussel, D., Marcouiller, F., Soliz, J., Gozal, D., Bairam, A., Joseph, V., 2019. Roles of oestradiol receptor alpha and beta against hypertension and brain mitochondrial dysfunction under intermittent hypoxia in female rats. Acta Physiol. Oxf. (Oxf), e13255.
Leaw, B., Nair, S., Lim, R., Thornton, C., Mallard, C., Hagberg, H., 2017. Mitochondria, bioenergetics and excitotoxicity: new therapeutic targets in perinatal brain injury. Front. Cell. Neurosci. 11, 199.
MacLusky, N.J., Naftolin, F., 1981. Sexual differentiation of the central nervous system. Science 211 (1294-), 1303.
Mage, D.T., Donner, M., 2006. Female resistance to hypoxia: does it explain the sex difference in mortality rates? J. Womens Health (Larchmt) 15, 786–794.
Milder, J., Patel, M., 2012. Modulation of oxidative stress and mitochondrial function by the ketogenic diet. Epilepsy Res. 100, 295–303.
Picard, M., McEwen, B.S., 2014. Mitochondria impact brain function and cognition. Proc. Natl. Acad. Sci. U. S. A 111, 7–8.
Robertson, C.L., Saraswati, M., 2015. Progesterone protects mitochondrial function in a rat model of pediatric traumatic brain injury. J. Bioenerg. Biomembr. 47, 43–51.
Silver, I., Erecińska, M., 1998. Oxygen and ion concentrations in normoxic and hypoxic brain cells. Adv. Exp. Med. Biol. 454, 7–16.
Souza, M.A., Mota, B.C., Gerbatin, R.R., Rodrigues, F.S., Castro, M., Fighera, M.R., Royes, L.F., 2013. Antioxidant activity elicited by low dose of caffeine attenuates pentylenetetrazol-induced seizures and oxidative damage in rats. Neurochem. Int. 62, 821–830.
Starkov, A.A., 2008. The role of mitochondria in reactive oxygen species metabolism and signaling. Ann. N. Y. Acad. Sci. 1147, 37–52.
Toran-Allerand, C.D., 1984. Gonadal hormones and brain development: implications for the genesis of sexual differentiation. Ann. N. Y. Acad. Sci. 435, 101–111.
Xavier, J.M., Rodrigues, C.M., Sola, S., 2016. Mitochondria: major regulators of neural development. Neuroscientist 22, 346–358.
Yoshizawa, H., Gazes, Y., Stern, Y., Miyata, Y., Uchiyama, S., 2013. Characterizing the normative profile of 18F-FDG PET brain imaging: sex difference, aging effect, and cognitive reserve. Psychiatry Res. 22, 78–85.