Role of Estradiol Receptor Beta (ERβ) on Arterial Pressure, Respiratory Chemoreflex and Mitochondrial Function in Young and Aged Female Mice

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Abstract

We tested the hypothesis that ERβ is involved in respiratory control in female mice. We used young adult (5–6 months-old) and aged (17–18 months-old) ERβKO or wild-type controls (WT) female mice to assess arterial blood pressure (via a tail-cuff sensor) and indices of respiratory pattern (sighs and apneas – recorded by whole body plethysmography at rest). We also measured respiratory parameters at rest and in response to brief (<10 min) exposure to hypoxia (12% O_2) or hypercapnia (5% CO_2). Because ERβ is localized in mitochondria, and because estradiol and ERβ agonist increase mitochondrial O_2 consumption, we assessed the mitochondrial respiration (with a high-resolution oxygraph system) and the *in vitro* activity of the complex I of the electron transfer chain in samples of brain cortex in aged wild-type and ERβKO female mice. Compared to young WT mice, young ERβKO mice had elevated arterial blood pressure, but similar ventilatory responses to hypoxia and hypercapnia. In old ERβKO female mice compared to old WT mice, the arterial blood pressure was lower, the frequency of sighs was higher and the frequency of apneas was lower, and the hypoxic and hypercapnic ventilatory responses were reduced. In old ERβKO mice mitochondrial respiration and complex I activities in the brain cortex were lower than in WT mice. We conclude that ERβ has age-specific effects on vascular and respiratory functions in female mice.

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15.1 Introduction

Through their effects in the central nervous system, steroid hormones such as estradiol or progesterone play an essential role in the regulation of sexual behavior (Brinton et al. 2008; Micevych et al. 2015). These hormones are also involved in processes governing mood or cognition (Marrocco and McEwen 2016), and regulate homeostatic functions such as thermoregulation (Charkoudian et al. 2017) or respiratory control (Boukari et al. 2016, 2017; Behan and Wenninger 2008). Because the occurrence of sleep apnea is higher in men than in women and increases after menopause in women (Block et al. 1979; Bixler et al. 2001), it is of clinical relevance to better understand the effects of ovarian hormones on respiratory regulation. Earlier studies performed in cats suggested that the main effect of estradiol was limited to increase the expression of the progesterone receptor, thereby increasing the respiratory effect of progesterone (Bayliss et al. 1990). Thus, the effects of estradiol, and the role of the classical estradiol receptors (ERα and ERβ) in respiratory control remain poorly investigated. Nonetheless, we have reported that in newborn rats, long-term treatment with estradiol slightly reduced respiratory frequency and increased the frequency and length of apnea under resting conditions (Lefter et al. 2008). During hypoxia, estradiol treatment suppressed the normal decrease of body temperature, without altering the ventilatory response (Lefter et al. 2008). Furthermore, we showed recently that estradiol protects against the increased chemoreflex activity and respiratory instabilities induced by exposure to intermittent hypoxia (Laouafa et al. 2017b), conceivably due to protective effects of estradiol against the oxidative stress induced by intermittent hypoxia (Laouafa et al. 2017b), and likely involve effect of estradiol on mitochondrial respiration and production of reactive oxygen species in female rats exposed to intermittent hypoxia (Laouafa et al. 2017a). These results indicate that estradiol could have important roles on respiratory regulation that remains poorly understood. The effects of estradiol could be more important under specific pathophysiological conditions, and/or may be specific to sex- or age.

Classical ERs belong to the superfamily of steroid receptors, acting as transcription factors able to modulate the expression of target genes that have an "Estrogen Response Element" in their promoter region (Harris 2007). Two ERs have been described, ERα and ERβ, that are coded by different genes (Kuiper et al. 1996). We previously reported by immunohistochemistry that ERβ is expressed in the peripheral chemoreceptors of adult rats (Joseph et al. 2006), and ERβ mRNA and protein are densely expressed in central areas involved in respiratory control including the nucleus tractus solitarius, locus coeruleus, and the parabrachial nucleus (Shughrue et al. 1997; Shughrue and Merchenthaler 2001).

In the present study, we tested the hypothesis that ERβ is involved in respiratory control in female mice. We used young adult (5–6 months-old) and aged (17–18 months-old) ERβKO female mice to assess respiratory parameters at rest and in response to brief (<10 min) exposure to hypoxia (12% O_2) or hypercapnia (5% CO_2). Because previous studies showed elevated arterial pressure in young ERβKO female mice (Zhu et al. 2002), we also recorded arterial pressure in young and aged mice. Finally, because ERβ is localized to mitochondria, and because ERβ agonist and estradiol increase mitochondrial O_2 consumption (Irwin et al. 2012), we assessed the mitochondrial respiration and the *in vitro* activity of the complex I of the electron transfer chain in samples of brain cortex in aged wild-type and ERβKO female mice.

15.2 Material and Methods

We used female ERβKO mice (Krege et al. 1998) (B6.129P2-Esr2tm1Unc/J; n = 9) and their recommended wild-type control (WT: C57BL/6 J; n = 7) from The Jackson laboratory. The mice were kept in our animal care facility under standard conditions with food and water available ad-libitum. All experiments were approved by our local ethic committee (Ref: 2014–156) and strictly adhered to the rules of the Canadian

Council on Animal Care. We used the same mice at the age of 5–6 months (young) and 17–18 months (old) to perform measurement of arterial pressure and respiratory recordings. Old mice were sacrificed a few days after the last measurements and brain samples were used to assess mitochondrial respiration and in vitro activities of mitochondrial complex I.

15.2.1 Measurements of Arterial Pressure

Arterial blood pressure was measured as described previously in female rats (Laouafa et al. 2017b) by the tail cuff method and volume-pressure recording (CODA system – Kent Scientific, Torrington, CT, USA). Conscious mice were placed in a restrainer tube over a warmed blanket. After 30 min of habituation, several recordings were performed, separated at least by 5 min. We report the mean of the 3 lowest values for systolic, diastolic, and mean arterial pressures.

15.2.2 Respiratory and Metabolic Recordings

All recordings were performed as previously described for adult mice (Boukari et al. 2016; Marcouiller et al. 2014). Briefly, animals were placed in a whole-body plethysmograph chamber (Emka Technologies – Paris, France), flushed with fresh room air. For each animal, the flow rate was adjusted to avoid excessive built-up of CO_2 within the recording chamber. Flow rate fluctuated between 160 and 250 ml/min, CO_2 was measured in the outflowing air and was between 0.4 and 0.6%. Mice were left undisturbed for 4 h under room air to ensure a period of normoxic recordings representative of a resting state, during which mice spend much of their time asleep (Marcouiller et al. 2014; Bastianini et al. 2017). A subsampling pump was connected to the chamber for analysis of $O_2\%$, $CO_2\%$, and water pressure using dedicated gas analyzers (FC-10, CA-10 and RH-300 respectively, Sable Systems International, Las vegas, NV, USA). During recordings, values of $O_2\%$, $CO_2\%$ and water pressure in the inflowing gas line were periodically measured and later used for calculation of O_2 consumption and CO_2 production using standard equations (Boukari et al. 2016). Rectal temperature was measured at the end of the normoxic recording period. Following the 4 h periods of recordings, the animals were exposed to brief periods of hypoxia (12% O_2–10 mins) and hypercapnia (5% CO_2–10 min) in randomized order, with 10–15 minutes between each test.

15.2.3 Analysis of Respiratory and Metabolic Rate Variables

All respiratory and metabolic variables were analyzed as performed previously in adult rats or mice (Laouafa et al. 2017b; Boukari et al. 2016; Marcouiller et al. 2014), to report respiratory frequency, tidal volume, minute ventilation, O_2 consumption rate, and CO_2 production under normoxic conditions and in response to hypoxia or hypercapnia. Because exposure to hypoxia was short (<10 min) and measurements are typically taken around the 5th minute of exposure, we estimated that rectal temperature and metabolic rate were similar in hypoxia and normoxia. We used standard criteria to report sigh, post-sigh apnea, and spontaneous apnea frequency under resting conditions (Laouafa et al. 2017b; Boukari et al. 2016; Marcouiller et al. 2014).

15.2.4 Mitochondrial Respiration Recordings on Permeabilized Brain Samples

In old mice, a few days after the respiratory measurements, the animals were sacrificed with an overdose of anesthetic (ketamine/xylasine), the brain cortex was rapidly dissected and immediately used to perform measurements of mitochondrial respiratory functions using an O2K Oroboros respirometer (Oroboros Instruments, Innsbruck, Austria).

After calibration of the Oroboros chambers, cortex samples were weighted (2–3 mg), and

recordings of O_2 consumption performed at 37 °C in a respiration buffer (0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM potassium lactobionate, 10 mM KH_2PO_4, 20 mM Hepes, 110 mM sucrose, 1 g/l BSA). Based on a previous study establishing the optimal conditions for measurements of mitochondrial respiration in permeabilized brain samples (Herbst and Holloway 2015), the samples are incubated in the recording chamber for 20 min with saponin (50 μg/ml). For each sample, we have differentiated mitochondrial respiration linked to NADH oxidation through the mitochondrial respiratory complex I (using pyruvate 5 mM, malate 0.2 mM, and glutamate 10 mM as substrates), and mitochondrial respiration linked to $FADH_2$ oxidation through the mitochondrial respiratory complex II (succinate 5 mM and rotenone 4 μM to block complex I activity). After equilibration with the substrates, ADP (500 μM) is added to the chambers to measure O_2 consumption under normal phosphorylating state (ATP synthesis – mitochondrial respiratory state 3). We then add cytochrome-c to assess the integrity of mitochondrial membranes, then oligomycin (2 μg/mg tissue) was added to block ATP synthesis and measure O_2 consumption due to leakage of protons in non-phosphorylating state (no ATP synthesis – state 4). Uncoupling is further exaggerated by adding graded bolus of carbonyl cyanide m-chlorophenylhydrazone (CCCP – 0.5 μM in 1 μl/bolus) until reaching stable O_2 consumption. We then blocked the activity of the complex 3 with antimycin A (2.5 μM) to measure non-mitochondrial O_2 consumption due to cytosolic oxidases. Finally, the maximum activity of the complex 4 (cytochrome c oxidase) is measured by using ascorbate (2 mM) and tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD; 0.5 mM).

15.2.5 *In vitro* Activity of the Mitochondrial Complex I

Frozen cortex (20 mg/ml) were homogenized in 100 mM phosphate buffer KPI (100 mM K_2HP0_4, 100 mM KH_2PO_4, PH7,4) + EDTA 2 mM. Then, the tissues were centrifuged for 10 min at 1500 g, and the supernatant was collected and kept frozen at −80 °C. The concentration of proteins was determined by a standard colorimetric BCA assay kit (Thermo Scientific, catalogue # 23225) and complex I activity was normalized to protein concentration.

Complex I activity was assessed by measuring the disappearance rate of NADH by spectrophotometry. Briefly, the day of measurement, a solution containing 50 mM KPI buffer, 1 mM EDTA, 100 μM decylubiquinone, and 3.75 mg/ml BSA (10%) was prepared. A similar solution was prepared with rotenone (10 μM: inhibitor of complex I activity). 4 μl of each sample was added in two different wells. In the first well, we added 250 μl of solution containing rotenone, and in the second well we added 250 μl of cocktail without rotenone. We then added 3 mM of NADH into each well, and read the absorbance at 340 nm every 15 s for 3 min to monitor the rate of NADH disappearance. Since NADH can be consumed by complex I of the mitochondria and by cytochrome b5 reductase, the activity of the complex I was obtained by subtracting the slope of the well with rotenone (activity without complex I) from the slope of the well without rotenone (total activity).

15.2.6 Statistics

For arterial blood pressure, we used a student's t-test to assess if significant differences appeared between WT and ERβKO mice at each age. For respiratory variables at rest, we used a one-way ANOVA to assess if significant differences appear between groups (Young WT, young ERβKO, old WT, old ERβKO). When the ANOVA reported a significant effect ($P < 0.05$) we used a Fisher's LSD test to assess differences between WT and ERβKO mice with each age, and differences between old and young mice for each genotype. For the hypoxic or hypercapnic ventilatory response, we used 2 way-ANOVA for repeated measures, with genotype and gas exposure as grouping variables, followed by a post-hoc Fisher LSD test if significant effect of

genotype or significant interaction between genotype and gas exposure appeared. All analyses were performed using GraphPad Prism 7. All values are reported as mean ± sem.

15.3 Results

15.3.1 Arterial Pressure in Young and Old WT and ERβKO Female Mice

As previously reported in ERβKO mice (Zhu et al. 2002), the systolic, mean, and diastolic arterial pressures were elevated in young ERβKO compared to wild-type (Fig. 15.1 – upper panels).

However, in older mice there was an opposite effect, with ERβKO mice showing reduced diastolic and mean arterial pressures than wild-type (Fig. 15.1 – lower panels).

15.3.2 Body Weight, Respiratory and Metabolic Variables at Rest in Young and Old WT and ERβKO Female Mice

Body weight was higher in ERβKO vs wild-type mice at both ages (Table 15.1), the mean of the difference between the WT and KO mice was 1.8 ± 0.8 g in young mice, and 14.2 ± 2.7 g in older mice, thus indicating a gradual increase in
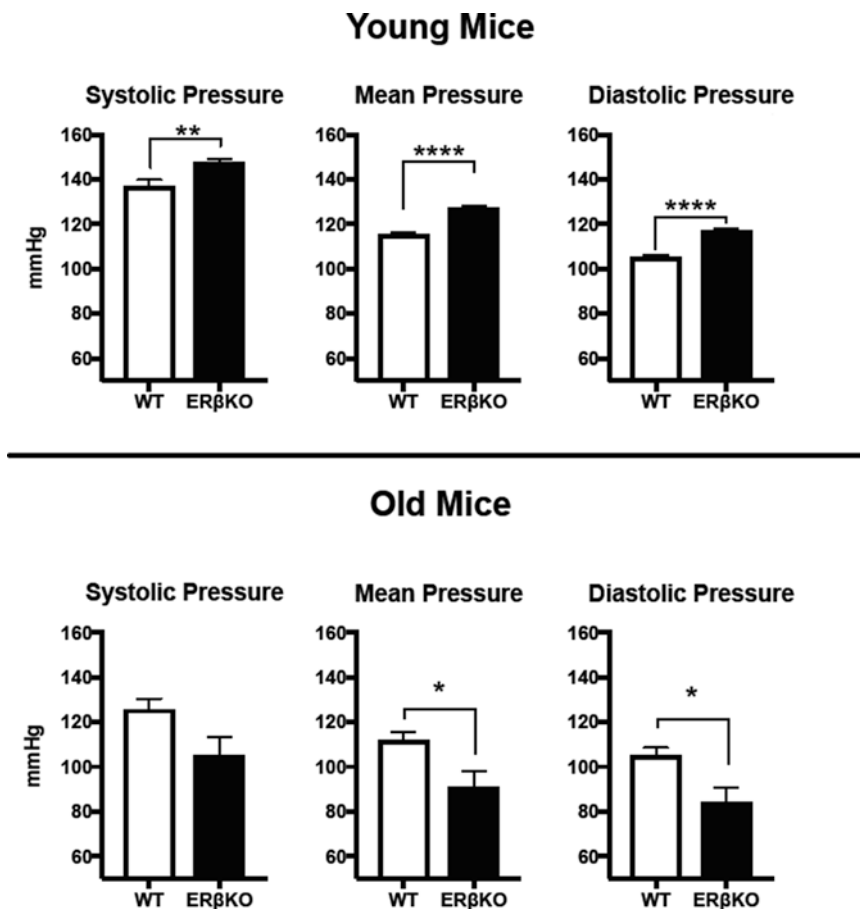

Fig. 15.1 Systolic, mean, and diastolic arterial pressure in young and old wild-type (WT) and ERβKO female mice. *, **, ****: $p < 0.05$, $p < 0.01$, and $p < 0.0001$ ERβKO vs WT

Table 15.1 Respiratory and metabolic variables recorded at rest in young (5–6 months) and old (17–18 months) wild-type (WT) and ERβKO female mice

		WT	βERKO
BW	Young	19.8 ± 0.3	21.5 ± 0.7*
	Old	28.4 ± 1.2°°°	42.5 ± 2.2***/°°°°
Tr	Young	36.0 ± 0.5	37.0 ± 0.2
	Old	36.3 ± 0.3	36.3 ± 0.3
fR	Young	163 ± 7	149 ± 6
	Old	162 ± 9	184 ± 9*/°°
V_T ml/100 g	Young	0.50 ± 0.10	0.52 ± 0.08
	Old	0.65 ± 0.07	0.58 ± 0.08
V_E ml/min/100 g	Young	83.5 ± 19.7	80.8 ± 14.9
	Old	105 ± 14	107 ± 20
VO_2 ml/min/100 g	Young	5.0 ± 0.6	5.3 ± 0.7
	Old	4.3 ± 0.5	4.1 ± 0.4
VCO_2 ml/min	Young	3.7 ± 0.4	3.8 ± 0.4
	Old	2.9 ± 0.2	2.7 ± 0.7°
VCO_2/VO_2	Young	0.772 ± 0.06	0.733 ± 0.02
	Old	0.703 ± 0.059	0.670 ± 0.028

Number of animals into each group: Young (7 WT and 8 KO), Old (6 WT and 9 KO)
BW body weight (grams), *Tr* rectal temperature (°C), *fR* respiratory frequency (breaths/min), V_T tidal volume (ml/100 g), V_E minute ventilation (=fR x V_T, in ml/min/100 g), VO_2 O_2 consumption rate (ml/min/100 g), VCO_2 CO_2 production rate (ml/min/100 g), VCO_2/VO_2 respiratory exchange ratio
*, ***$p < 0.05$, and $p < 0.001$ ERβKO vs WT (same age)
°°, °°°, °°°°$p < 0.01$, $p < 0.001$, and $p < 0.0001$ old vs young (same genotype)

body weight during aging in ERβKO mice. Previous studies reported an increased body weight in ERβKO mice, which was associated with increased fat mass when mice reached 10–12 months of age (Seidlova-Wuttke et al. 2012) but not in younger (4 months-old) animals (Ohlsson et al. 2000). This suggests an important role of ERβ to avoid excessive adipose tissue accumulation during aging in female mice, but this remains poorly documented (Davis et al. 2013).

All respiratory and metabolic parameters measured at rest were similar between WT and ERβKO mice. Compared to young ERβKO, in old KO mice respiratory frequency was higher, and CO_2 production rate was lower (Table 15.1). Interestingly, in old ERβKO mice the frequency of sigh was more elevated than in WT mice and in young KO mice (Fig. 15.2). The frequency of post-sigh apnea was reduced by aging, and in aged ERβKO mice the frequency of post-sigh and spontaneous apnea were drastically reduced compared to aged WT mice.

15.3.3 Hypoxic and Hypercapnic Ventilatory Responses in Young and Old WT and ERβKO Female Mice

During the exposures to hypoxia and hypercapnia in young mice, there were no differences between wild-type and ERβKO (Fig. 15.3). In aged mice, compared to wild-type controls ERβKO aged mice had a reduced ventilatory response to hypoxia due to a lower tidal volume (Fig. 15.4 – upper rows). In response to CO_2 exposure, minute ventilation was 39% lower in ERβKO compared to wild-type mice (Fig. 15.4 lower rows), again due to a lower elevation of tidal volume in response to the CO_2 exposure.
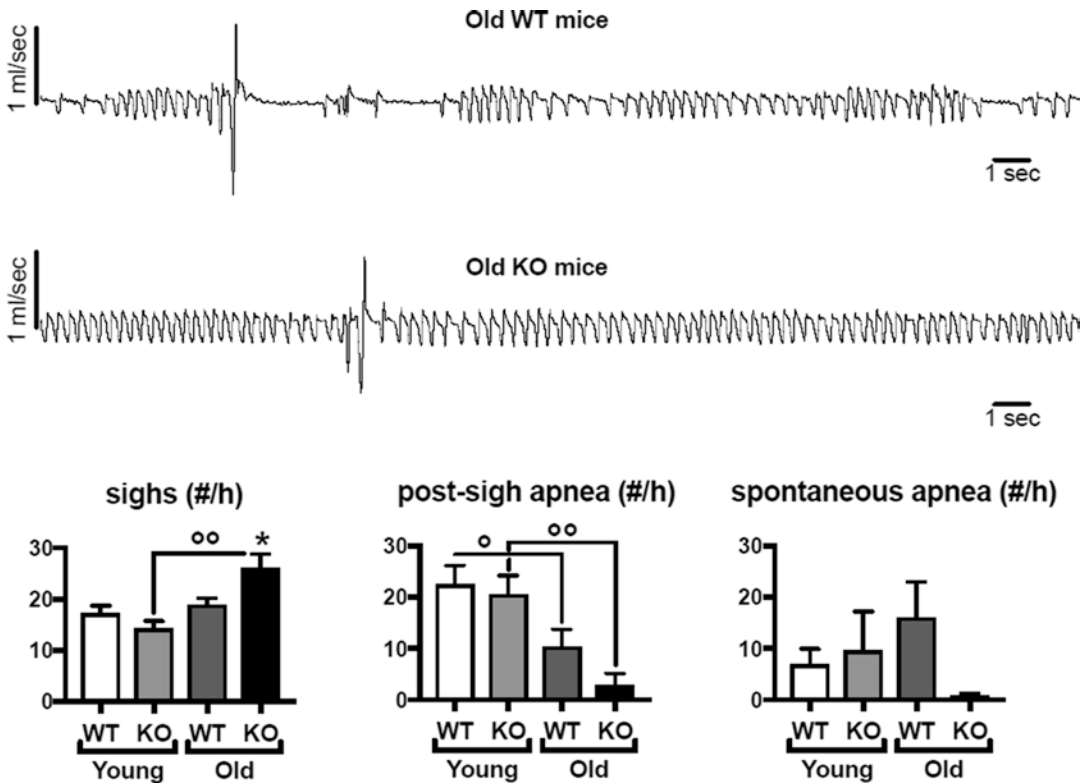
Fig. 15.2 Typical respiratory recordings from an old WT and an old ERβKO mice showing the occurrence of a sigh followed by apneas in the WT but not in the ERβKO mice. Lower panels: frequency of sigh, post-sigh apnea, and spontaneous apnea recorded at rest in young and old WT and ERβKO mice. *p < 0.05 ERβKO vs WT (same age). °, °°p < 0.05 and p < 0.01 old vs young (same genotype)

15.3.4 Mitochondrial Respiration and *In vitro* Activity of Electron Transport Chain Complexes in Young and Old WT and ERβKO Female Mice

Mitochondrial respiration was measured only in old WT and ERβKO mice using permeabilized cortical brain samples. When the respiratory activity was activated by substrates of complex I, the mitochondrial O_2 consumption was lower in ERβKO mice compared to WT control (Fig. 15.5). This was not observed with substrates of complex II, and there was no significant difference for the maximum activity of complex IV (cytochrome oxidase) between wild-type and ERβKO mice.

The activity of complex I measured *in vitro* by the disappearance rate of NADH was lower in ERβKO mice compared to WT (Fig. 15.5).

15.4 Discussion

The present studies show that deletion of ERβ in female mice leads to age-specific alterations of the cardio-vascular and respiratory systems. Interestingly, while elevated blood pressure was present in young ERβKO female mice, our results indicate that this effect does not persist in older ERβKO mice. In fact, in old ERβKO mice arterial blood pressure was lower than in old WT. In contrast, alterations in the respiratory pattern and chemoreflex functions appeared in old, but not in
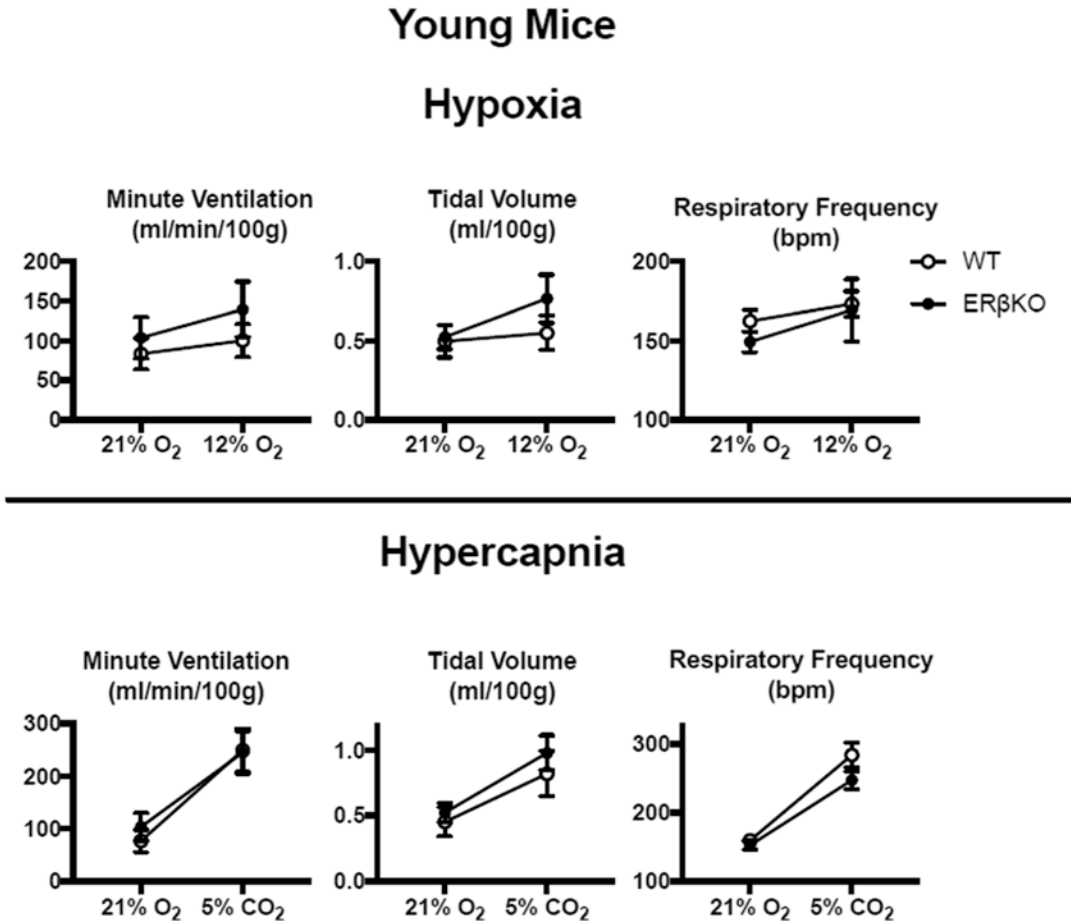
Young Mice

Hypoxia

Hypercapnia

Fig. 15.3 Ventilatory responses to hypoxia (upper panels) and hypercapnia (lower panels) in young (5–6 months) wild-type (WT) and ERβKO female mice

young ERβKO female mice. In older mice, our results also show a reduced mitochondrial respiration in brain cortex through alteration of complex I activity.

15.4.1 Role of ERβ on the Cardiovascular System in Old Vs Young Female Mice

A recent systematic review based on the analysis of 88 studies (mostly on animal models or tissues) provides a comprehensive overview on the role of ERβ in the female cardiovascular system (Muka et al. 2016). ERβ is expressed in endothelial cells, and has an important role by a direct, vasodilatory action on blood vessels. ERβ regulates nitric oxide (NO) bio-availability and inflammatory markers in arterial walls, thus contributing to the regulation of arterial pressure (Muka et al. 2016). Our results showing elevated blood pressure in young female ERβKO mice are in line with previous studies, showing similar results (Zhu et al. 2002). We've been surprised to observe that arterial pressure is reduced in old ERβKO mice compared to their wild-type controls, and we are not aware of previous published report showing this effect. While it has been concluded that ERβ plays a vasodilatory role (Muka et al. 2016), some studies have reported that vasodilation mediated by an ERβ agonist does not require NO signaling, and that ERβ might

Old Mice
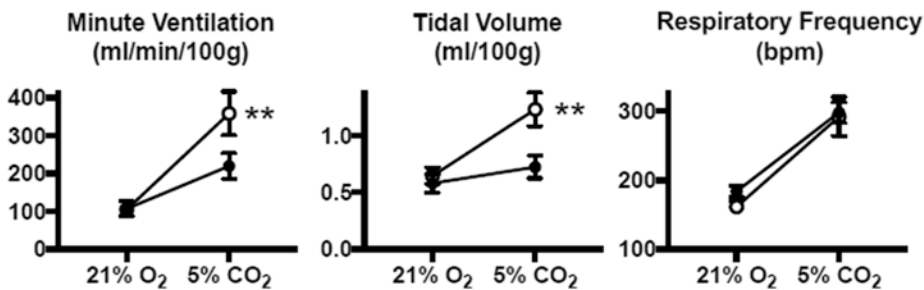
Hypoxia
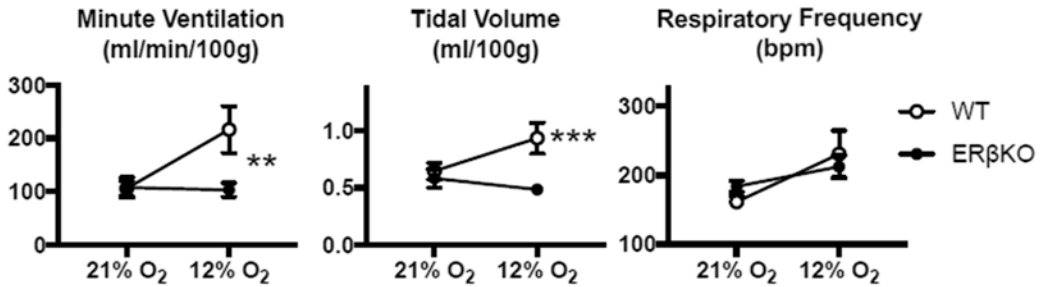
Hypercapnia

Fig. 15.4 Ventilatory responses to hypoxia (upper panels) and hypercapnia (lower panels) in old (17–18 months) wild-type (WT) and ERβKO female mice. **, ***p < 0.01, and p < 0.001 ERβKO vs WT

downregulate ERα- and NO-mediated vasodilation (Cruz et al. 2006). Furthermore, in a mouse model of accelerated senescence, age profoundly impacts the effect of estrogen-mediated regulation of NO and oxidative stress systems. In young females, estrogen increases the expression of endothelial NO synthase (eNOS) and decreases oxidative stress, but in aged mice estradiol treatment does not modulate eNOS expression. In isolated aorta of aged mice estradiol increases the expression of the NADPH oxidase subunit NOX1, and increases generation of superoxide anion (O_2^-, one of the major reactive oxygen species) by NADPH oxidase (Novensa et al. 2011). Interestingly, such reversal of estrogen action on

the vascular system in aged mice was associated to increased expression level of ERβ and reduced expression level of ERα, leading to a high ratio of ERβ/ERα expression, (Novensa et al. 2011). It has been reported that ERβ inhibits ERα-dependent gene expression, and might thus opposes the vascular effects of ERα (Matthews and Gustafsson 2003). Since ERα is a potent modulator of vasodilation and NO synthesis (Traupe et al. 2007), we might therefore hypothesize that in aged ERβKO mice ERα signaling in blood vessels is increased, leading to vasodilation and reduced blood pressure. This scenario remains speculative, but we believe that it helps understanding our results.
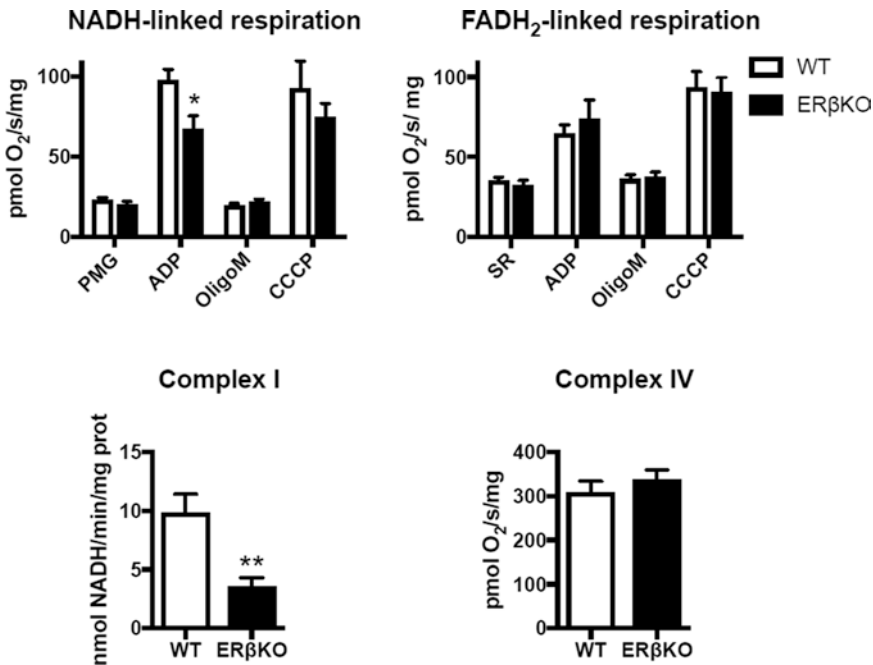
Fig. 15.5 Mitochondrial function in samples of permeabilized brain cortex in old (17–18 months) WT and ERβKO female mice. Upper panels: O_2 consumption rate (pmol O_2/second/mg tissue) with substrates of complex I (left – NADH-linked respiration), and substrates of complex II (right – FADH$_2$- linked respiration). PMG: Pyruvate, malate, glutamate (substrates of complex I), *SR* succinate, rotenone (substrate of complex II and blockade of complex I), *ADP* adenosine di-phosphate, *OligoM* Oligomycin A, *CCCP* carbonyl cyanide m-chlorophenylhydrazone. See text for further details. Lower panels: activity of complex I and IV of the electron transport chain (see text). *, **$p < 0.05$, and $p < 0.01$ ERβKO vs WT

15.4.2 Role of ERβ on the Respiratory Control System in Old Vs Young Female Mice

Contrasting with data showing higher blood pressure in young ERβKO mice, there was no evidence of changes of the respiratory control system in these animals. However, compared to their wild-type controls, old ERβKO had several alterations, including higher frequency of sighs recorded during sleep, but a much lower occurrence of post-sigh and spontaneous apneas, and reduced hypoxic and hypercapnic ventilatory responses. As mentioned in the introduction, ERβ mRNA and protein are both expressed in brainstem areas involved in respiratory control (such as the nucleus tractus solitarius, locus coeruleus, parabrachial nucleus), in hypothalamic nuclei (Shughrue and Merchenthaler 2001; Shughrue et al. 1997), and in peripheral chemoreceptors (Joseph et al. 2006). The fact that ERβ is present in the peripheral chemoreceptors and in the nucleus tractus solitarius, the main projection site of the peripheral chemoreceptors in the brainstem (Finley and Katz 1992), lend support to the hypothesis that ERβ modulates responses elicited by activation of the peripheral chemoreceptor, which is in line with the drastically attenuated hypoxic response in ERβKO female mice. Interestingly, the hypercapnic ventilatory response was not completely abolished but reduced by about 50–55% in ERβKO mice compared to wild-type controls. Furthermore, responses to hypoxia and hypercapnia were both reduced owing to a lower tidal volume, and the magnitude of the difference observed between WT and ERβKO for both hypoxic and hypercapnic ventilatory responses were similar. These

findings suggest a common mechanism might be responsible for the altered hypoxic and hypercapnic ventilatory responses, possibly involving a reduction of the peripheral chemoreceptor drive in response to hypoxia and hypercapnia. Alternatively, a central inhibition and/or altered integrative processes common to respiratory responses to hypoxia and hypercapnia could also explain these results.

Other interesting aspects of our data are the differences of sigh and apnea frequency in normoxia between WT and ERβKO mice. As reported for alterations of the chemoreflex responses, these effects are observed only in old mice. Sighs are very common, and play an important, but often overlooked, role to regulate the respiratory control system, and could possibly have functions extending well beyond this system (Ramirez 2014). Because they are readily observable in brainstem slices, sighs are likely an intrinsic property of the respiratory control system, and in most cases, *in vitro* and *in vivo* sighs are followed by a cessation of breathing, referred to as "post-sigh apneas" (Ramirez 2014). Interestingly, while sigh frequency was increased in ERβKO mice compared to WT controls, the frequency of post-sigh apnea was drastically reduced in ERβKO mice. While we could speculate on the significance of these findings in light of our most recent understanding of sigh functions and underlying neurobiology (Ramirez 2014), we prefer to remain cautious, and simply report this intriguing phenotype observed in aged ERβKO female mice.

15.4.3 Role of ERβ on Mitochondrial Respiration in Old Female Mice

Finally, our data shows that ERβ deletion reduces mitochondrial respiratory activity and activity of electron transfer chain complex I in brain cortex samples. These results are intriguing because at the level of the peripheral chemoreceptors recent data support the hypothesis that the mitochondrial activity contributes to oxygen sensing (Fernandez-Aguera et al. 2015; Chang 2017;

McElroy and Chandel 2017). Therefore, if similar effects of ERβ deletion are present in the peripheral chemoreceptors of ERβKO mice, this may likely contribute to the reduced hypoxic ventilatory response in older mice. These data are in line with the effect of estradiol on mitochondrial function, which are mediated by ERα and ERβ (Yang et al. 2004; Irwin et al. 2012; Razmara et al. 2008). The modulations of mitochondrial function by E_2 and ERs are thought to contribute to cardio-vascular and neurological protective effects in women before menopause, and to explain the longer life expectancy of women compared to men (Nilsen and Brinton 2004; Arnold et al. 2012; Irwin et al. 2012; Razmara et al. 2008).

15.5 Conclusion

We conclude that KO mice are a reliable model to assess the role of specific estradiol receptor on respiratory regulation. We started our studies with young ERβKO mice, and because there was no evidence for altered respiratory control system, we decided to keep these animals until they reached an older age. Our data reveal that in 17–18 months-old mice, the deletion of ERβ leads to several alterations of the respiratory control system, including reductions of chemoreflex functions. Because this is associated with a lower mitochondrial respiratory activity in the central nervous system, our results show that ERβ acts at different levels of the oxygen transport cascade (from pulmonary ventilation to mitochondrial O_2 consumption). This may be relevant in pathological or physiological conditions in which O_2 homeostasis is compromised (sleep apnea, altitude, lung diseases), and research efforts to further understand the role of estradiol and ERs in these conditions should be further developed.

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