Differences in mitochondrial function between brain and heart of senile rats exposed to acute hypobaric hypoxia. Role of nitric oxide

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ABSTRACT

Rat brain and heart display different endogenous protective responses against hypobaric hypoxia in an age-dependent way. The aim of the present work was to evaluate the effects of acute hypobaric hypoxia (HH, 48 h) on brain and heart mitochondrial function as well as the participation of nitric oxide (NO) in old rats (22-month old).

Cortical mitochondria from rats exposed to HH decreased respiratory rates (37 %, state 3) and membrane potential (20 %), but NO and H_2O_2 production increased by 48 %, and 23 %, respectively. Hippocampal mitochondria preserved O_2 consumption and H_2O_2 production, decreased membrane potential (18 %) and increased NO production (46 %). By contrast, HH decreased NO production (53 %) in mitochondria from left heart ventricles associated with increased cytochrome oxidase activity (39 %) and decreased NADPH oxidase activity (31 %). Also, a tendency to increase complex I-III (24 %) and complex II-III (65 %) activity was observed.

In conclusion, after HH hippocampal and cortical mitochondria showed mild uncoupling and increased NO production. However, only the hippocampus preserved O_2 consumption and H_2O_2 levels. Interestingly, heart mitochondria showed a decreased ROS production through increased cytochrome oxidase activity associated with a decrease in NO production. This may be interpreted as a self-protective mechanism against hypoxia.

1. Introduction

Molecular O_2 availability is essential for aerobic organisms, and this necessity becomes more critical during old age (Yeo, 2019). However, O_2 involvement in oxidative metabolism generates potentially deleterious effects through reactive oxygen species (ROS) (Bunn and Poyton, 1996; Cadenas, 2018; Chance et al., 1979; Gerschman et al., 1954; Lee et al., 2020). Thus, organisms sense O_2 concentration and respond to hypoxia activating adaptive processes that will improve the likelihood of survival (Lee et al., 2020). Etiopathogenesis of several neurological (Panov, 2014) and cardiovascular diseases (Madamanchi and Runge, 2007) are associated with mitochondrial hypoxic dysfunction through respiratory impairments and increased ROS levels (Cadenas, 2018; Dröge, 2002a).

Brain is particularly sensitive to oxygen deficiency. It demands 20 % of resting O_2 consumption in humans to support the high rate of ATP formation necessary to maintain the ionic homeostasis for release and uptake of neurotransmitters associated with synaptic transmission (Alle et al., 2009; Berry and Kaeberlein, 2021). Depending on brain regions and cell types, organisms display different adaptive responses to hypoxia related to metabolic reprogramming and ROS removal (Van Elzen et al., 2010).

Brain mitochondria play an important role in the response to sustained hypobaric hypoxia (Costa and La Padula, 2020). Previous results from our laboratory suggested that the mechanisms responsible for acclimatization would be time-dependent, according to the physiological function of the brain area, as shown in hippocampal and cortical mitochondria of rats exposed to hypobaric hypoxia for 1 or 7 months and also involves the participation of the NO system (Czerniczyniec et al., 2015).

In myocardium, acclimatization to hypoxia was associated with several changes to protect the heart against an episode of hypoxia/reoxygenation (Costa and La Padula, 2020; La Padula et al., 2008; La Padula and Costa, 2005). In young and mature animals cardioprotection was associated with an increased mitochondrial NO production (La Padula et al., 2008; Zaobornyj et al., 2005). However, upon aging further increased NO production and decreased cytochrome oxidase activity by chronic hypoxia was not associated with cardioprotection (Czerniczyniec et al., 2015; Pourbagher-Shahri et al., 2021). In addition, we observed a shorter lifespan compared to normoxic animals (La Padula and Costa, 2005; Zaobornyj et al., 2005). According to this evidence, and studies of acute hypoxia in young (La Padula et al., 2018) and prepubertal (La Padula et al., 2021) rats, the age of the animals and the duration of the stimulus become critical factors determining whether moderate hypoxia could be harmful or paradoxically beneficial (La Padula et al., 2008; Manukhina et al., 2006; Zaobornyj et al., 2005).

The aim of the present work was to evaluate the differential effect of acute exposure to hypoxia on mitochondria of cerebral cortex and hippocampus and of the left ventricle of the heart of senile animals, particularly the involvement of NO. We propose a comparative hypoxic impact over these tissues, which are closely linked by their sensitivity to oxygen.

2. Materials and methods

2.1. Animals and hypobaric hypoxia exposure conditions

Twenty-two month old male Wistar rats of the CHbbTHOM albino strain were subjected to a simulated 4400 m altitude (58.7 kPa = 440 mmHg) during 48 h (HH) in a hypopressure chamber (La Padula and Costa, 2005). Same number of rats remained as normoxic group at sea level atmospheric pressure (101.3 kPa = 760 mmHg). Animals had ad-libitum access to water and food and were maintained on a scheduled 12 h light-dark cycle. The partial pressure of O_2 in the inspired air was 11.3 kPa = 85 mmHg and 21.2 kPa = 159 mmHg, for hypoxic and normoxic rats, respectively.

The study was carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Research and published by the National Institutes of Health (NIH Publications No. 8023, revised 1978). Also, animals received care in accordance with the 6344/96 regulation of the Argentinean National Drug, Food, and Medical Technology Administration (ANMAT).

2.2. Isolation of subcellular fractions

Brains and hearts were quickly removed after euthanasia. Dissection of cerebral cortex, hippocampus (Madison and Edson, 2001) and heart left ventricles was performed. Immediately, heart left ventricles were frozen at −20 °C.

Fresh cerebral cortex and hippocampus from 2 animals of each experimental group were pooled and homogenized in MSH buffer in a ratio of 1:5 w/v and centrifuged at 700*g* for 10 min. The supernatant was collected and centrifuged at 8000*g* for 10 min. The resulting pellet containing mitochondria was washed and resuspended in MSH buffer (Czerniczyniec et al., 2011) to a final concentration of 20–25 mg/ml protein. Oxygen consumption, mitochondrial membrane potential and hydrogen peroxide production were measured in the obtained fresh isolated mitochondria. This protocol was repeated 4 times.

Left ventricles from normoxic and hypobaric animals (HH) were thawed and homogenized individually (not pooled) using the same differential centrifugation procedure described above. Heart left ventricles mitochondria were submitted to two processes of freezing and thawing and were homogenized using a tuberculin syringe with a needle in order to obtain submitochondrial membranes (Boveris et al., 2002).

2.3. Measurements performed in fresh intact mitochondrial fraction

2.3.1. Mitochondrial oxygen uptake

An oxygraph for high-resolution respirometry (Hansatech Oxygraph, Hansatech Instruments Ltd., Norfolk, England) was used for measurements of oxygen consumption. Measurements using cortical or hippocampal fresh mitochondria (0.5–1 mg/ml) were performed in a buffer containing 70 mM sucrose, 230 mM mannitol, 5 mM phosphate, 4 mM MgCl₂, 20 mM Tris-HCl (pH 7.4) 1 mM EDTA, and 0.2 % BSA. Glutamate (6 mM) and malate (6 mM) were used as substrates to measure state 2 and 1 mM ADP was added to register state 3 oxygen consumption at 30 °C (Boveris et al., 1999; Nicholls and Ferguson, 2013).

2.3.2. Mitochondrial hydrogen peroxide production

Hydrogen peroxide (H_2O_2) generation was determined in fresh cortical and hippocampal mitochondria from normoxic and hypoxic animals using a fluorometric scopoletin-horseradish peroxidase (HRP) method (365 λ_{exc} - 450 λ_{em}) (Boveris, 1984). Mitochondrial samples (0.1–0.3 mg/ml) were placed in a reaction medium containing 70 mM sucrose, 230 mM mannitol, 20 mM Tris-HCl (pH 7.4), 0.3 μM SOD, 0.8 μM HRP, 1 μM scopoletin, 6 mM glutamate and 6 mM malate at 37 °C. H_2O_2 (0.05–0.35 μM) was used as standard.

2.3.3. Mitochondrial membrane potential

Mitochondrial membrane potential was determined by flow cytometry. Isolated intact mitochondria (25 μg/ml) were incubated for 20 min in MSH buffer supplemented with 5 mM malate, 5 mM glutamate, 4 mM MgCl₂, 1 mM phosphate and 500 nM TMRE (tetramethylrhodamineethyl ester) at 37 °C (Bustamante et al., 2000). For the analysis, a common marker (M_1) was fixed on the median value of the distribution of fluorescence events that represents approximately 50 % of the total fluorescent events. Quantification of TMRE fluorescence histogram differences was performed based on the number of events under the median value of the distribution and results were expressed as the percentage of TMRE fluorescence taking as 100 % the fluorescence events corresponding to control samples (Karadayian et al., 2014).

2.4. Enzymatic assays

2.4.1. Complex IV activity

Cytochrome oxidase activity was evaluated in submitochondrial membranes (0.5 mg protein/ml) by a spectrophotometric assay based on the measurement of the rate of oxidation of 50 μM ferrocytochrome *c* (Yonetani, 1967).

2.4.2. Nitric oxide synthase (NOS) activity

In order to measure NO production, oxidation of oxyhemoglobin to methemoglobin at 577–591 nm ($\varepsilon = 11.2$ mM^{-1}·cm^{-1}) was followed by a spectrophotometric method at 37 °C (Boveris et al., 2002). The reaction medium was composed of 50 mM phosphate buffer (pH 7.4), 50 μM L-arginine, 100 μM NADPH, 1 mM CaCl₂, 4 μM Cu,Zn-SOD, 10 μM dithiothreitol, 0.1 μM catalase, 25 μM oxyhemoglobin (expressed per heme group) and 0.5–1 mg protein/ml submitochondrial membranes.

2.4.3. Complexes I-III and II-III activity

Activity of NADH-cytochrome *c* reductase was measured in submitochondrial membranes by following spectrophotometrically the reduction of cytochrome *c* (550 nm; $\varepsilon = 19.6$ mM^{-1} cm^{-1}) at 30 °C. The reaction medium contained 100 mM phosphate buffer (pH = 7.4), 0.5 mM KCN, 0.2 mM NADH, 25 μM cytochrome *c* and 0.2 mg/ml submitochondrial membranes (Navarro et al., 2005). To determine succinate cytochrome *c* reductase activity, NADH was substituted by 20 mM succinate.

2.4.4. NADPH oxidase activity

NADPH oxidase activity was analyzed in mitochondrial membranes

(0.5 μg μL− 1) incubated in a reaction medium containing 50 mM Na_2HPO_4/NaH_2PO_4 (pH 7.4), 150 mM sucrose, 1 mM EGTA, 25 mM $NaHCO_3$, 0.75 μM lucigenin and 5 μM NADPH. Chemiluminescence was measured using a Varioskan® LUX microplate reader at 37 °C (Li et al., 1998). As a control, measurements were conducted in the presence of 1 μM SOD. Results were quantified as area under the curve (AUC).

2.5. Materials

ADP, arginine, catalase, cytochrome *c*, dithiothreitol, EDTA, EGTA, FCCP, free fatty acid BSA, folin reagent, glutamic acid, horseradish peroxidase, HEPES, lucigenin, malic acid, mannitol, NADPH, oxyhemoglobin, scopoletin, sucrose, succinate, SOD were purchased from Sigma Chemical Co (St. Louis, MO, USA) and tetramethylrhodamine ethyl ester (TMRE) from Calbiochem, San Diego, CA, USA. Other reagents were of analytical grade.

2.6. Statistical analysis

Results are presented as mean ± SEM. The analysis of the results was performed using unpaired Student *t*-test or ANOVA. Graph Pad Instat statistical software was used; differences were considered significant when $p < 0.05$.

3. Results

3.1. Effect of acute hypobaric hypoxia on cortical and hippocampal mitochondrial function

The malate-glutamate dependent oxygen consumption was measured in state 2 (controlled respiration prior to addition of ADP) and in state 3 (active respiration, the maximal physiological rate of O_2 uptake and ATP synthesis) (Nicholls and Ferguson, 2013). As shown in Table 1, cortical mitochondria from rats exposed to HH showed lower respiratory rates than normoxic ones in both states 2 (43 %) and 3 (37 %). In contrast, no significant changes were observed in oxygen consumption in either metabolic states in hippocampal mitochondria due to acute hypobaric hypoxia exposure.

3.1.1. Mitochondrial membrane potential

As observed in the representative histograms, TMRE fluorescence median decreased after HH in cortical mitochondria (median value: 77.0) as compared with the normoxic condition (median value: 127.5) (Fig. 1A iii, iv). Similar results were observed in hippocampal mitochondria (normoxic median value: 92.9; hypoxic median value: 78.4) (Fig. 1B iii, iv). Quantification of TMRE fluorescence is presented in Fig. 1C. Acute hypobaric hypoxia was able to induce cortical and hippocampal mitochondrial depolarization by 20 % and 18 %, respectively, as compared with normoxic conditions. As expected, high

depolarization was detected in mitochondria from both brain areas after 0.5 μM FCCP addition (Fig. 1C).

3.1.2. Hydrogen peroxide production

In normoxic conditions, cortical H_2O_2 production rates were 0.63 ± 0.04 nmol/min. mg protein, being significantly increased by 23 % in the hypobaric hypoxia condition (Fig. 2). On the other hand, no significant changes in H_2O_2 production were observed in hippocampal mitochondria after acute hypobaric hypoxia (Fig. 2).

3.1.3. Nitric oxide synthase activity

The effect of HH on cortical and hippocampal mitochondrial NOS activity is shown in Fig. 3. Acute exposure to hypobaric hypoxia significantly increased NO production by 48 % and 46 % in cortical and hippocampal submitochondrial membranes, respectively (Fig. 3).

3.2. Effect of acute hypobaric hypoxia on mitochondria left heart ventricles

Heart left ventricles mitochondria were frozen immediately after isolation. Enzyme activities measurements were performed in samples from normoxic and HH animals.

3.2.1. Cytochrome oxidase activity

Cytochrome oxidase activity was assayed in submitochondrial membranes obtained from the heart left ventricle as previously described. Acute hypobaric hypoxia increased cytochrome oxidase activity by 39 %, as compared with normoxic conditions (Fig. 4).

3.2.2. Nitric oxide synthase activity

Left ventricle submitochondrial membranes from rats exposed to HH showed an important decrease in NOS activity (53 %) as compared with normoxic condition (Fig. 5).

3.2.3. Complex I-III and II-III activities

The effect of HH on mitochondrial respiratory complexes activity was measured in the left ventricle heart of rats after 48 h HH and is presented in Table 2. Hypobaric hypoxia showed a strong tendency to increase complex II-III activity by 65 % as compared with normoxic conditions. Also, complex I-III activity presented a slight tendency to increase (24 %) after HH treatment.

3.2.4. NADPH oxidase activity

Fig. 6 presents NADPH oxidase activity in normoxic and HH animals. Signal quantification as AU showed a significant decrease of 31 % in hypoxic animals as compared with normoxic animals ($p < 0.05$).

4. Discussion

Taking into account that availability of oxygen become very critical for the organisms during old age (Lesnefsky et al., 2016; Nakamura et al., 2015), the aim of this work was to evaluate the differential effect of acute hypoxia on mitochondrial function in cerebral cortex, hippocampus and heart left ventricle of senile animals.

Previous studies from our laboratory showed that NO metabolism and mitochondrial membrane potential act as key physiological modulators of mitochondrial respiration during hypobaric hypoxia (Czerniczyniec et al., 2015; La Padula et al., 2021, 2018). It is nowadays widely accepted that NO modulates mitochondrial respiration through the reversible and O_2-competitive inhibition of cytochrome oxidase (Brown, 2001), slowing down substrate oxidation, electron flow and stores of chemical energy. Several groups propose that an inhibition in respiratory rates induces an increase in proton leakage and ROS levels and its accumulation in the matrix leads to the collapse of the mitochondrial membrane potential (Batandier et al., 2004; Skulachev, 2006; Weiss et al., 2003). However, mild uncoupling protects from oxidative damage
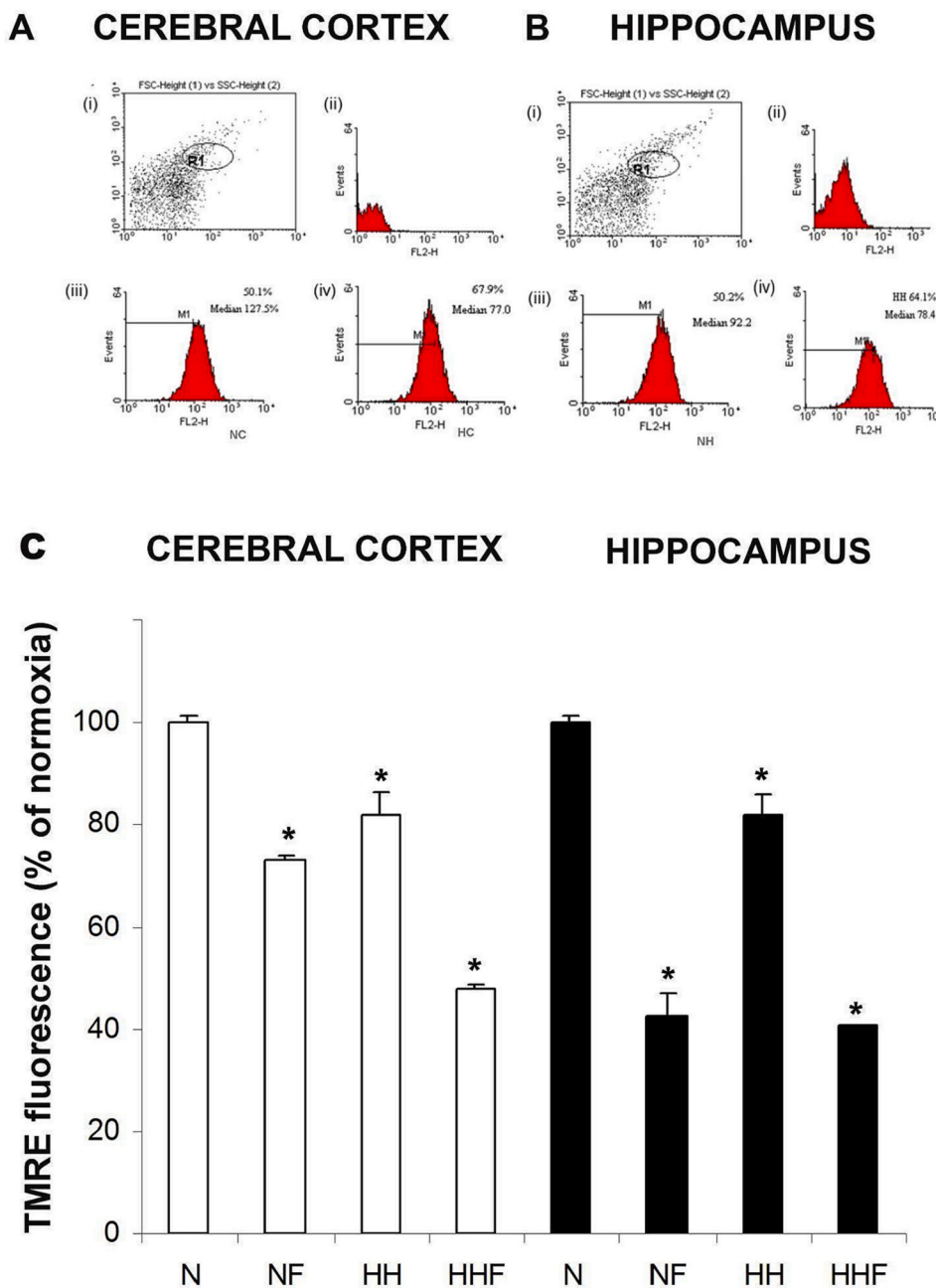
Table 1
Cortical and hippocampal mitochondrial O_2 consumption of rats submitted to 48 h of hypobaric hypoxia.

Condition	Oxygen consumption (ng-at O/min.mg protein)			
	Cerebral cortex		Hippocampus	
	N	HH	N	HH
State 2	10.4 ± 0.7	6.0 ± 0.4*	12.2 ± 0.7	11.0 ± 0.4
State 3	38 ± 3	24 ± 2#	43 ± 5	41 ± 4

N: normoxic, HH: 48 h hypobaric hypoxia. Values are mean ± SEM of 4 individual mitochondrial samples from cerebral cortex and 3 individual mitochondrial samples from hippocampus each obtained from a pool of cerebral cortex or hippocampus of 2 rats.
* $p < 0.05$.
\# $p < 0.01$ vs N.

A   CEREBRAL CORTEX

B   HIPPOCAMPUS

C   CEREBRAL CORTEX   HIPPOCAMPUS

Fig. 1. Mitochondrial membrane potential in cerebral cortex and hippocampus of rats submitted to 48 h hypobaric hypoxia. A–B. (i) Typical dot-blots of FSC-H vs. SSC-H indicating a gated mitochondrial population (R1) and (ii) the histograms of gated events versus relative fluorescence intensity (FL-1) corresponding to unloaded, (iii) normoxic or (iv) hypobaric hypoxia condition. Each histogram represents a typical experiment. C. Bars graph quantification of TMRE relative fluorescence intensity (r.f.i.). Fluorescence events were quantified as the number of events which drop under a common marker M1 taking control fluorescence events as 100 %. Bars represent the mean $\pm$ SEM of 3 individual mitochondria samples each obtained from a pool of cerebral cortex or hippocampus of 2 rats. *$p < 0.05$, compared with the N respective group. FCCP (F) was used as positive control membrane depolarization. HH: 48 h hypobaric hypoxia; N: normoxic.

by reducing ROS production due to decreased $\Delta\psi$ and local oxygen availability (Brand, 2000). The induction of proton leak through the UCPs is an important mechanism to control ROS production via adjustments in $\Delta\psi$ (Brand, 2000). Calcium handling is also associated to adaptation to hypoxic conditions. For example, calcium regulates mitochondrial permeability transition pore (Belosludtsev et al., 2020), and changes in NO levels modulates L Ca^{2+} and RyR Ca^{2+} channels at cytosol (Bers, 2002) decreasing calcium overload-damage.

In the present work, NO production increased by 48 % in the cerebral cortex after exposure to HH. Consequently, inhibition in state 3 oxygen consumption (37 %) and increase in H_2O_2 production (23 %) were observed, despite the mitochondrial membrane depolarization (20 %). We propose that being the cerebral cortex a highly vascularized and robust tissue (Shaw et al., 2021), it would be less sensitive to the lack of oxygen, allowing a decrease in oxygen consumption through increased NO. This situation would promote a better distribution of oxygen to nearby mitochondria and to the other brain areas (Fig. 7).

As shown in Fig. 3, the increase in NO production (46 %) observed in hippocampal tissue was not able to inhibit oxygen consumption after acute hypobaric hypoxia, probably due to compensatory effects on mitochondrial membrane potential that help to preserve H_2O_2 levels as senile normoxic animals. Thereby, the hippocampus of senile animals maintains an elevated availability of oxygen through the mitochondrial membrane depolarization. The hippocampus is less vascularized than other cortical areas and particularly susceptible to hypoxia. Due to a lower blood oxygenation and vascular density in the hippocampus, oxygen levels become limiting for ATP generation (Shaw et al., 2021). Therefore, the hippocampus must preserve the uptake of oxygen and H_2O_2 levels, reducing the membrane potential which in turn compensates the effects of the increased NO (Fig. 7). We consider mitochondrial ROS production as a balance between the stimulation of the electron flow through mitochondrial membrane depolarization (which tends to decrease ROS) and the opposite effect where NO inhibits electron flow at the respiratory chain (which tends to increase ROS). In the present
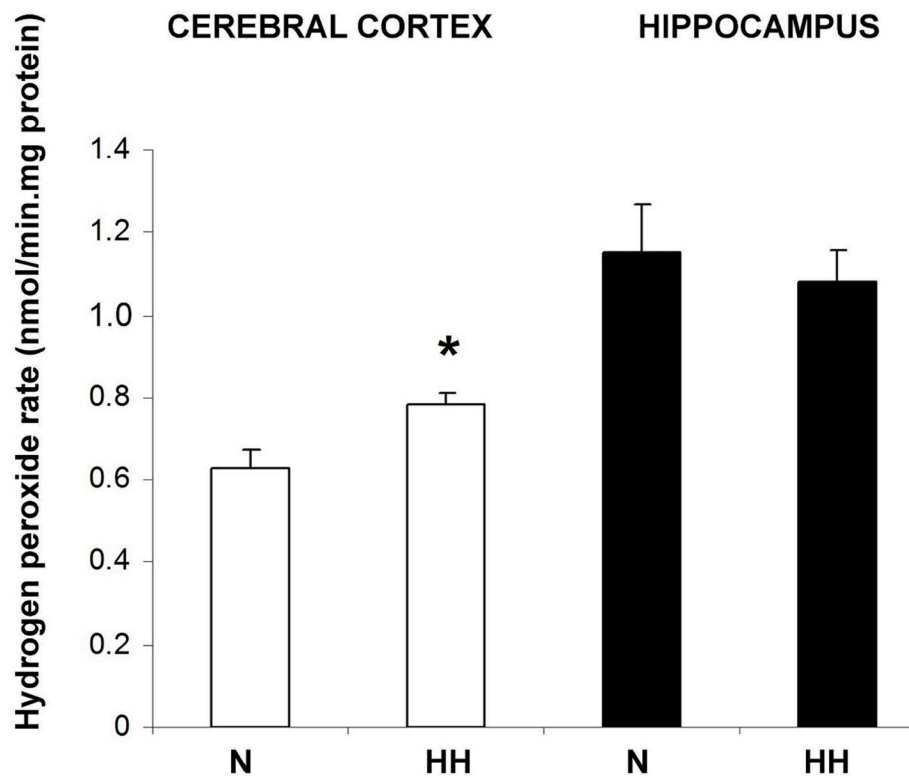
CEREBRAL CORTEX   HIPPOCAMPUS

Fig. 2. H_2O_2 production rates in cortical and hippocampal mitochondria of rats submitted to 48 h hypobaric hypoxia. HH: 48 h hypobaric hypoxia; N: normoxic. Values are mean ± SEM of 4 individual mitochondrial samples from cerebral cortex and 3 individual mitochondrial samples from hippocampus each obtained from a pool of cerebral cortex or hippocampus of 2 rats; * $p < 0.05$ vs. N.
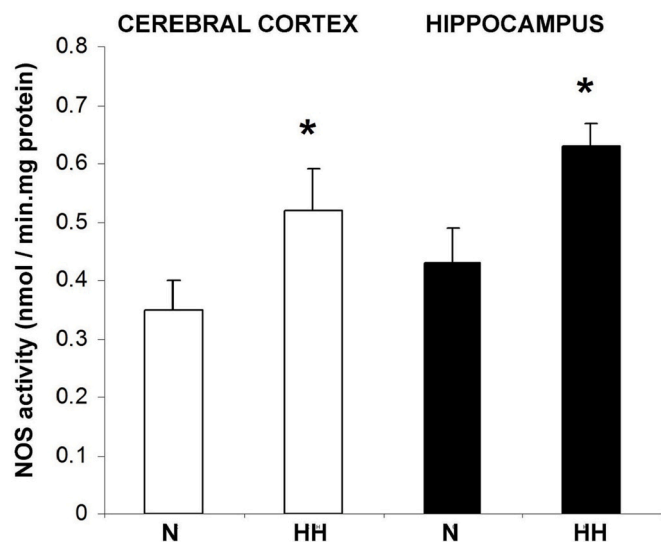

Fig. 3. NOS activity in brain mitochondria of rats submitted to 48 h hypobaric hypoxia. HH: 48 h hypobaric hypoxia; N: normoxic. Values are mean ± SEM of 4 individual mitochondrial samples from cerebral cortex and 3 individual mitochondrial samples from hippocampus each obtained from a pool of cerebral cortex or hippocampus of 2 rats; * $p < 0.05$ vs. N.

Fig. 4. Cytochrome oxidase activity of left ventricle mitochondria from rats submitted to 48 h hypobaric hypoxia. White column: normoxic group, Black column: hypobaric hypoxia group (HH).Values are mean ± SEM; * $p < 0.05$ vs. HH.

experimental model, membrane depolarization of the cortical mitochondria fails to reverse the effect of increased NO on the respiratory chain.

It is important to note that normoxic basal H_2O_2 production was higher in hippocampus (1.2 nmol/min.mg protein) than in cerebral cortex (0.63 nmol/min.mg protein), supporting the hypothesis that the hippocampus is more vulnerable to oxidative damage (Wang and
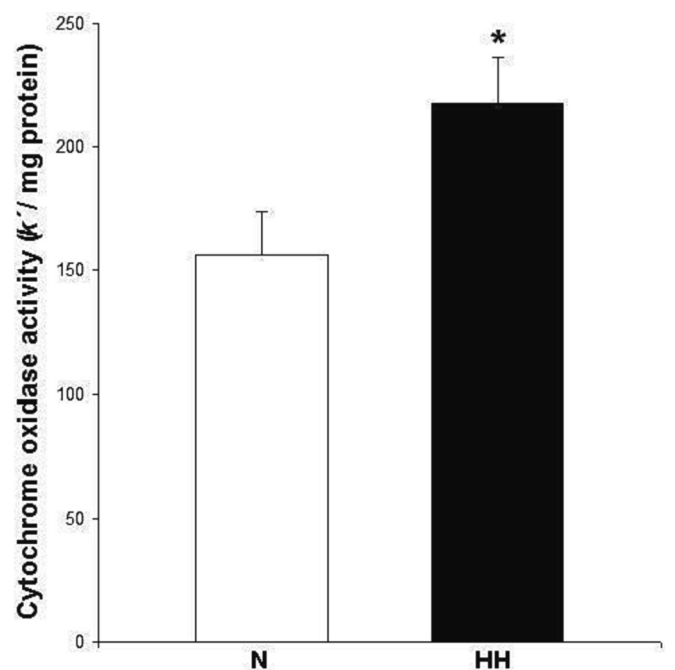
Michaelis, 2010).

Different responses between hippocampus and cerebral cortex have been observed after exposure to hypobaric hypoxia in several
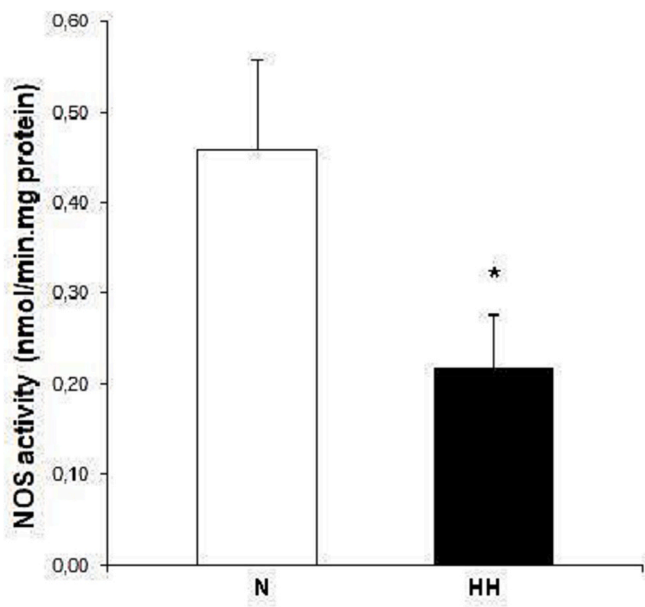
Fig. 5. NOS activity of left ventricle mitochondria rats submitted to 48 h hypobaric hypoxia. White column: normoxic group (N), Black column: 48 h hypobaric hypoxia group (HH). Values are mean ± SEM, * p < 0.05 vs. HH.

Table 2
Complex I-III and II-III activities of left ventricle mitochondria from rats submitted to 48 h hypobaric hypoxia.

Enzyme activity	N	HH
NADH-cytochrome *c* reductase (nmol/min.mg protein)	1.0 ± 0.1	1.2 ± 0.1
Succinate-cytochrome *c* reductase (nmol/min.mg protein)	1.6 ± 0.2	2.7 ± 0.6

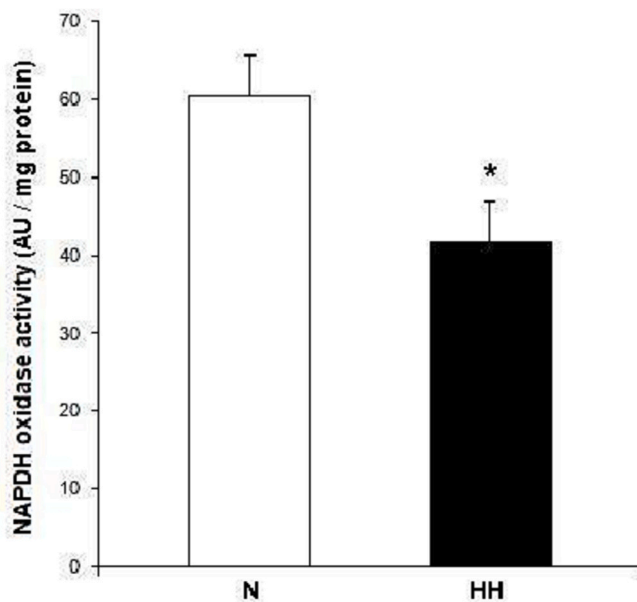Values represent the mean ± SEM. N: normoxic group, HH: hypobaric hypoxia group.

Fig. 6. NADPH oxidase activity in left ventricle mitochondria from rats submitted to 48 h hypobaric hypoxia. White column: normoxic group (N), Black column: 48 h hypobaric hypoxia group (HH). Values are mean ± SEM, * p < 0.05 vs. HH.

experimental models. Sharma et al. (2013) observed that the hippocampus of rats exposed to 7600 m simulated altitude was more vulnerable to hypoxia than the cerebral cortex (Sharma et al., 2013). In addition, a higher susceptibility to hypoxia was reported for hippocampus and striatum compared to cerebral cortex, evidenced by an increase in ROS production and NO levels and a decrease in the antioxidant defence system in animals exposed to 6100 m (Maiti et al., 2006). Also, Hota et al. (2007) described that oxidative stress levels are time-dependent. Exposure during 14 days to hypobaric hypoxia (6100 m) was able to reduce the oxidative stress in rat hippocampus, as compared to 7 days of exposure (Hota et al., 2007). As described above, our results showed that exposure to acute hypobaric hypoxia of senile animals displayed different responses than those previously observed in young and mature animals (Czerniczyniec et al., 2015).

Regarding cardiac left ventricles, in the present work we observed a significant decrease in NO production (47 %) after exposure to HH. This result was opposite to that observed in hippocampal and cerebral cortex mitochondria and correlated with other reports indicating that the brain is more sensitive to hypoxia than the heart (Kim et al., 2016). The mentioned NO decrease allows a greater oxygen uptake for respiratory activity which has been evidenced through an increase in cytochrome oxidase activity (39 %) in the left heart ventricle after exposure to HH (Fig. 7). This effect differs with the already reported age-related decrease in the activity of mitochondrial complex IV during chronic hypoxia (Dröge, 2002b; La Padula and Costa, 2005). The differences observed in oxygen consumption between the heart and brain areas could be due to structural differences at the respiratory chain level (Kim et al., 2016), mainly complex I (Stevic et al., 2022; Yoval-Sánchez et al., 2022). It has been proposed that the integrity of the respiratory chain would be preserved in the heart due to the lower impact of NO as a protein nitrosylator (Nakamura et al., 2015).

Mitochondria are the main source of ROS in the cardiovascular system (Murphy and Steenbergen, 2007). In physiological conditions, electron transport to O_2 is closely related to ATP synthesis. In pathophysiological situations of cardiovascular disorders, a decreased or compromised electron transport chain function increases ROS production. In our study, the decrease in NO production promotes oxygen consumption through the activation of respiratory complexes, decreasing electron leakage and ROS generation.

Hormones, hemodynamic forces, and local metabolic changes are capable of inducing cardiovascular NADPH oxidase isoforms activity (Dröge, 2002a). In the present work, we observed a decrease (31 %) in NADPH oxidase activity in mitochondria from left heart ventricle after hypobaric hypoxia exposure, indicating a probable adaptation to acute hypobaric hypoxic conditions as part of the mechanisms of redox homeostasis. In accordance, Giorgi et al. (2018) suggested that a decrease in NADPH oxidase activity could be due to a negative feedback mediated by an excess of ROS at the systemic level due to an increase in eNOS activity in endothelial cells (Giorgi et al., 2018). During acute hypoxia there is an early large vascular production of NO by eNOS (Molina et al., 2013) that could be responsible via negative feedback of the inhibition of the mitochondrial NO production, preserving normal O_2 consumption and mitochondrial ATP production necessary to maintain the mechanical activity of the heart.

5. Conclusions

Summing up, in the presence of the same hypoxic stimulus, the tissues respond specifically according to their ability to handle and distribute oxygen relative to their redox environment. In the brain, the modulation of NO levels, mitochondrial membrane potential, and ROS production would form an integrated mechanism to respond against hypoxia.

Left ventricle was the only tissue that presented a decrease in ROS production, which could be considered a positive adaptation to the low acute oxygen pressure through changes in the NO system. These
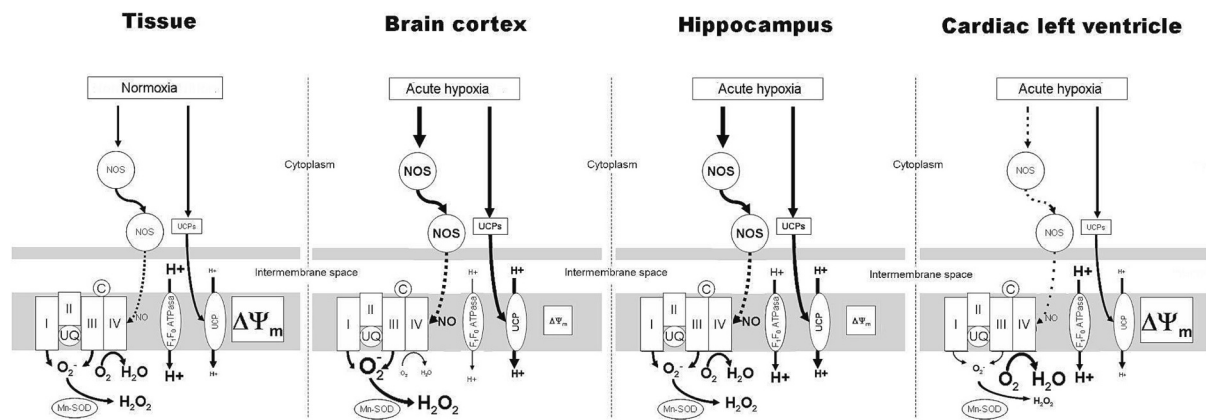
Fig. 7. General scheme for mitochondrial response to acute hypobaric hypoxia.
Cerebral cortex is a more irrigated and robust tissue, and therefore would be less sensitive to the lack of oxygen, allowing a decrease in oxygen consumption through increased NO. In this way it ensures a better diffusion of oxygen to other mitochondria. Hippocampus is a tissue very sensitive to hypoxia due to its less irrigation and access to oxygen. Therefore, it must preserve the uptake of oxygen, reducing mitochondrial membrane potential which in turn compensates for the increased NO. Left ventricle is a tissue with a high energy demand, and under hypoxic conditions must increase its access to oxygen by decreasing the production of NO, thus increasing its respiratory activity, and decreasing ROS. Dotted lines: inhibition; continuous lines: stimulation. The thickness of the letters and lines indicates the degree of activation.

observations are consistent with reports indicating a better adaptive response of heart to hypoxia than brain. Our results suggest that different experimental protocols and therapeutic strategies should be used according to the age and the affected tissue.

Funding

Ethics approval statement

The present study had the legal ethical accreditation from the 6344/96 regulation of the Argentinean National Drug, Food, and Medical Technology Administration (ANMAT).

Credit authorship contribution statement

PLP, LEC, AK, SLA, AC contributed to the conceptualization, design, and methodology of the study; PLP and AC conducted the statistical analysis and prepared the original draft; all authors critically reviewed the article and approved the final version.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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