Pathophysiological effects of a well-controlled exposure to intermittent hypoxia in mice: validation of a sleep apnea model.

S. Conotte, A. Tassin, K. Zouaoui Boudjeltia and A. Legrand

Introduction & Methods

Obstructive sleep apnea (OSA) is one of the most frequent sleeping disorders and is characterized by recurrent episodes of airway collapse leading to hypoxia-reoxygenation cycles [1]. We have previously described a standardized device to mimic intermittent hypoxia (IH) in mice (Figures 1 and 2; [2]). Parameters validated to evaluate our IH murin model are described in table 1, in parallel with corresponding OSA characteristics. Here, we present results related to polycythemia and heart hypertrophy in C57BL/6J mice exposed to cyclic hypoxia (ranging 5% to 21% FIO₂) during 35 days. Sham animals were exposed to 21% FIO₂. After exposure, blood was collected and the heart removed, weighed, cutted at the apex (Figure 3, black line), and frozen in OCT. Histochimical analysis was then performed on 10 µm cryosections (Figure 3, blue lines, L0-L5) for morphometric analysis.

Results

A. Polycythemia on Intermittent Hypoxia

IH mice exhibit an increased haematocrit from day 8 up to 35 (Figure 4). This polycythemia, concomitant to a decreased in PaO₂, is likely due to HIFs (hypoxia inducible factor) activation (Figure 5).

B. Heart Hypertrophy

In the present study, we showed that IH mice exhibited a cardiac hypertrophy and an increased haematocrit indicating a polycythemia throughout HIF1 activation. These characteristics being commonly observed in OSA [3-5], this study constitutes a validation step for the model, highlighting its usefulness in the field of respiratory diseases.

References


Acknowledgements

The ISPPC (CHU Charleroi, Belgium) provides funding for the conception of intermittent hypoxia chamber.

Cryosections and macroscopic analyses were performed in collaboration with the Laboratory of Histology, UMONS (Prof. D. Nonclercq).

Acknowledgements

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Table 1: Parameters evaluated to validate our system.

<table>
<thead>
<tr>
<th>OSA features</th>
<th>IH mice - analysis</th>
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<tbody>
<tr>
<td>Polycythemia</td>
<td>Haematocrit</td>
</tr>
<tr>
<td>Heart hypertrophy</td>
<td>Morphometric analysis</td>
</tr>
<tr>
<td>Respiratory muscle dysfunction</td>
<td>Organ bath (rigidity)</td>
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<td>Switch to anaerobic metabolism</td>
<td>Metabolomic (RMN - urine)</td>
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Table 2: Parameters evaluated to validate our system.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measurement</th>
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<tbody>
<tr>
<td>Heart weight</td>
<td>Body weight</td>
</tr>
<tr>
<td>Polycythemia</td>
<td></td>
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<tr>
<td>Hypoxia (IH) effect</td>
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Figure 1: (A) Schematic representation of the gas flow in the cage. The device allow a homogenous gas concentration (in dark blue) in all individual boxes. (B) Representative record of SaO₂ (haemoglobin oxygen saturation, in black) during intermittent hypoxia phases from 21% to 6% FIO₂. (fraction inspirated Oxygen, in red) During the 4 consecutive cycles, the hypoxic phase (FIO₂, 6%) was progressively lengthened (15, 20, 30 and 45 s) ([Chobut et al., 2012]).

Figure 2: Technical scheme of the device allowing to expose mice to hypoxia-normoxia cycles.

Figure 3: Representative sections realized for heart morphometrical analysis in IH and sham animals. Microscopical: Masson’s Trichrome histological sections of heart. Frozen sections (10 µm) were prepared at 200 µm intervals and fixed in 4% paraformaldehyde. (L0) Bud formation; (L1 - L2) Ventricular myocardium; (L3) Septum; (L4) Aorta; (L5) aorta.

Figure 4: Evolution of intermittent hypoxia (IH) effect on haematocrit.

Figure 5: Mechanisms of HIF1α regulation in normoxia and hypoxia. (Modified from Maes et al. (2012 Nationale Nederlandse Fysiotechnieke 15(2): 202). HIF-1α (subunit) is submitted to hydroxylation by a prolyl hydroxylase (PHD) and is directed to the proteasomal degradation pathway. In hypoxia, HIF activity is inhibited. This allows HIF1α to be translocated in the nucleus, form a transcription complex in association with a HIF-1β subunit and interact with the hypoxia response element (HRE) present in the promoter regions of hypoxia-responsive genes. The HIF-1 activity leads to the West inhibition and endothelial cell proliferation.

Figure 6: Measure of heart thickness after 35 days of intermittent hypoxia (IH) (one way ANOVA, p = 0.001).

Figure 7: Measure of septum thickness in different cardiac sections (L0-L5) after 35 days of Intermittent Hypoxia (IH) (T-test; p = 0.01).

Figure 8: Measure of right ventricle thickness in different cardiac sections (L0-L3) after 35 days of Intermittent Hypoxia (IH) (T-test; NS).

Figure 9: Measure of right ventricle thickness in different cardiac sections (L0-L3) after 35 days of Intermittent Hypoxia (IH) (T-test; NS).

Figure 10: Measure of right ventricle thickness in different cardiac sections (L0-L3) after 35 days of Intermittent Hypoxia (IH) (T-test; NS).

Figure 11: Measure of right ventricle thickness in different cardiac sections (L0-L3) after 35 days of Intermittent Hypoxia (IH) (T-test; NS).

Figure 12: Measure of right ventricle thickness in different cardiac sections (L0-L3) after 35 days of Intermittent Hypoxia (IH) (T-test; NS).

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