

Pulsed arterial spin labeling of hypo- and hyperventilated mice

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Introduction

Recent data in several neurological diseases [1] suggest that decreases in basal cerebral blood flow (CBF) may precede dementia. Arterial spin labeling methods have been widely used to study perfusion of the brain in humans, rats and, to some extent, in mice (review [2]). Rather than relying on baseline CBF alterations, the cerebral vascular response (CVR) under a hypercapnic challenge may be a more sensitive indicator. To study the CVR in animals, they are challenged with gas mixtures to induce hypercapnia or hypoxia. However, in free-breathing animals changes in respiratory response under general anesthesia differs, depending on the anesthetic agent, strain, age or the transgenic model that is studied [3-4]. In a ventilated setting, it would be possible to prevent this respiratory compensation and its confounding effect. We therefore investigated the feasibility to obtain hyper- and hypocapnia in ventilated mice by adjusting the ventilation rate and tidal volume. CBF was monitored using FAIR-ASL of the mouse brain during this hypo- and hyperventilation protocol.

Methods

Animals and surgical procedures:

Twelve week-old wild type C57Bl/6/J mice (n=12) were anesthetized with a mixture of urethane (1.2 g/kg) and alfa-chloralose (50 mg/kg) injected i.p., intubated and mechanically ventilated with oxygenated (Minivent 845; Hugo Sachs/Harvard Apparatus). Additionally 1mg/kg pancuroniumbromide was administered to assure optimal breathing control throughout the experimental protocol.

Mice were subjected to a standardized hypo-hyperventilation protocol (by adjusting ventilatory settings) with intermediate and final restitution of the initial settings. Rectal temperature was kept between 36.0 and 37.5°C and heart rate was monitored. A first arterial blood sample was taken during the first episode of normoventilation (normocapnia). Blood gases were analyzed using a ABLflex800 (Radiometer, Denmark) with 55 ul microcapillaries (readout of pH, pO₂, pCO₂). When pCO₂ levels were within a physiological range (30-40mmHg; else ventilator settings were slightly adjusted), mice were placed inside the MRI. Additional blood samples during hypoventilation and at the end of the protocol were obtained by reopening the tail artery wound. The total experimental time was about 2.5h (60 min imaging time; 10-15min /ventilation episode). The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the Institutional Animal Care Commission and Ethical Committee.

MRI acquisition and processing

MR images were recorded on a 9.4T Biospec system (Bruker, Ettlingen, GE) with a 7cm linearly polarized resonator for transmission and a 1 cm surface coil for receiving. For localization purposes 2-dimensional axial T2-weighted images (RARE; T_{eff}=48ms, TR=3500ms) with a slice thickness of 300 um were recorded. Perfusion maps were recorded (SLTH=1.5 mm) over the cortex, thalamus and hippocampus using the FAIR technique (6 inversion times: 100ms, 550ms, 1000ms, 1450ms, 1950ms, 2400ms; repetition time = 12s; inversion pulse was a hyperbolic secant of 14ms and SW 14.1kHz) with a spin echo epi readout (BW = 500kHz, TE=30.7ms, TET=2x1min12s). Three CBF-maps per episode were recorded. Maps were calculated according to Kwong et al. [5] and assuming a blood partition coefficient of 0.9mlg⁻¹.

Results & Discussion

A protocol for ventilation of mice inside the scanner was established which allows hypo- and hypercapnic challenges with CBF changes monitored by arterial spin labeling. Therefore, ventilation was started at a tidal volume (TV) of 3xbody weight+155 ul and respiratory rate (RR) of 135 strokes per minute (spm). During hypoventilation TV was reduced with 25% and RR was set at 70 spm, while during hyperventilation TV was increased with 20% and RR with 25% of the initial settings. Arterial blood samples obtained at the start of the ventilation protocol and during hypoventilation confirmed a significant hypercapnia (pCO₂ normoventilation 35±4 mmHg versus hypoventilation 68±16 mmHg, p<0.05) accompanied by a significant decrease in pH (hypoventilation: pH=7.35±0.07 vs. normoventilation: pH=7.11±0.11). The mean pCO₂ during hyperventilation was 17±8 mmHg. At the end of the protocol pCO₂ values remained higher under the initial ventilatory settings (50±11mmHg).

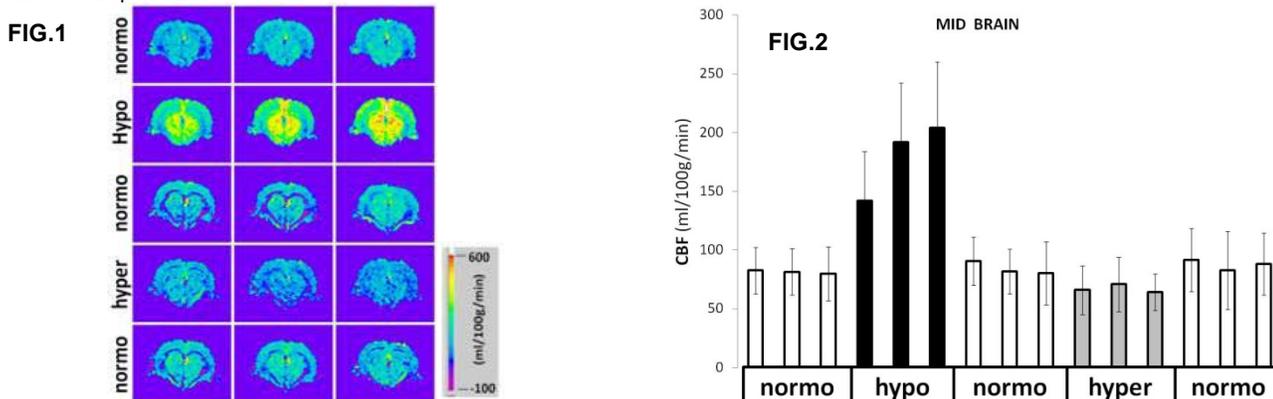
CBF was stable during normoventilation and increased shortly after onset of hypoventilation (average increase 100±40 ml/100g/min; fig.1 and 2). Switching back to normoventilation, CBF values quickly returned to the initial level. Hyperventilation resulted in a lower CBF although the decrease was less pronounced (20±8 ml/100g/min). Heart rate was stable (average 550±70bpm) except for a transient decline in rate at onset of hypoventilation.

The CVR may be a more sensitive indicator of vascular disorders, potentially underlying neurological diseases, than the baseline CBF values alone. Using this protocol CVR can be studied in different mice models under well-controlled conditions.

Fig. 1 Time-dependent perfusion maps during the total protocol. Switching of ventilation in order between normo- (top)/ hypo- / normo- / hyper-/normoventilation (bottom) (three CBF maps recorded per ventilation setting). Increases in flow during hypoventilation (= "hypo" or hypercapnia) and decreases during hyperventilation (= "hyper" or hypocapnia). Data are mean ± SD and reflect different pCO₂ levels during hypoventilation.

Fig. 2 Averaged CBF of the brain during the total ventilation protocol (n=12, error bars = SD; reflect differences in pCO₂ between animals).

Hypoventilation causes a typical hypercapnic response in arterial blood gasses and in CBF; opposite although less in magnitude hyperventilation causes a drop in CBF.



References

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