Murine Orthostatic Response During Prolonged Vertical Studies: Effect on Cerebral Blood Flow Measured by Arterial Spin-Labeled MRI

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Introduction

High-field MRI scanners are, in principle, well suited to study mice; however, many high-field magnets employ a vertical design which may influence the physiological state of the rodent. The purpose of this study was to investigate the orthostatic response in mice, in particular on cerebral blood flow (CBF) and its regulation, during a prolonged MR experiment in a head-up vertical position.

Methods

C57Black/6J mice (25-30g) were assigned into one of four groups: horizontally positioned (n = 6), vertically positioned (n = 11), vertically positioned with intravenous phenylephrine administration (n = 3), and vertically positioned with a 1.5 mL bolus i.v. injection of saline (n = 4). Mice were anesthetized with 1% isoflurane and N_2O:O_2 (1:1), endotracheally intubated and mechanically ventilated. Phenylephrine was administered i.v. at the dose of 1 μg kg^{-1} min^{-1} after the acquisition of the T_2-weighted images and maintained for the duration of the experiment. Physiological parameters such as mean arterial blood pressure (MABP), heart rate, and arterial CO_2 tension ($PaCO_2$), were monitored. Body temperature, measured with a rectal thermometer, was maintained at 37 ± 0.5˚C using a warm air heating system (SA Instruments, New York, USA). Mice were placed into a custom-built cradle in the prone position. The cradle was designed such that the mice could be placed in either a horizontal or vertical position without further manipulation.

MR studies were performed on a 4.7-Tesla, 40cm bore Bruker AVANCE-DBX system, equipped with a 15 cm diameter shielded gradient insert and a home-built saddle-type RF coil. Each side of the coil has a 3 cm single turn and the two sides were separated by approximately 3 cm. The coil was designed to allow for open access on three sides such that the mouse head and cradle could be centered in the coil in either a horizontal or vertical position. For all imaging experiments, an FOV = 4 cm and slice thickness = 2 mm were used. Maps of T_{1obs} [1] were generated from a three-parameter exponential fit to a series of spin-echo images with variable *TR* (*TR* = 8000, 4300, 2300, 1200, 650, 350, 185, 100 msec, 2 averages, 128 x 70 matrix). Perfusion spin-echo images were acquired in duplicate using the arterial spin-labeling technique [2] (*TR*/*TE* = 2000/10, 20, 30, summation of 3 echoes, 2 averages, 128 x 70 matrix) with labeling applied ± 2 cm from the imaging plane. CBF maps were generated from: $CBF = \lambda \cdot (T_{1obs} \cdot 2\alpha)^{-1} \cdot (M_C - M_L) \cdot (M_C)^{-1}$, where M_C and M_L are the magnetization intensities from the control and labeled images, respectively. A spatially constant value of 0.9 mL · g^{-1} was assumed for the blood brain partition coefficient for water (λ). The spin labeling efficieny (α) [3] was determined in each study with gradient echo images with spin-labeling applied at ± 6 mm (*TR*/*TE* = 100/9.6 msec, 45˚ flip angle, 8 averages, 256 x 256 matrix).

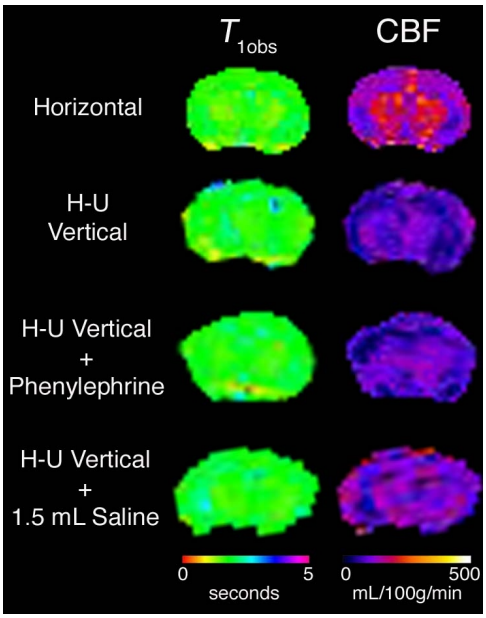

Figure 1: Representative T_{1obs} maps (left column) and CBF maps (right column), from mice positioned horizontally, head-up vertically, head-up vertically with i.v. phenylephrine administration and head-up vertically with i.v. injection of 1.5 mL of saline.

Results

Representative T_{1obs} maps obtained in studies are also shown in Figure 1. T_{1obs} values were relatively similar for most regions of the brain, approximately 1.75 ± 0.14 sec with the exception of the thalamus (1.57 ± 0.12 sec) and the hippocampus (1.89 ± 0.17 sec). Since α and T_{1obs} were similar across all groups, differences observed in CBF are due solely to perfusion changes.

Representative CBF maps are shown in Figure 1 (right column). CBF was consistently found to be ~40% lower in mice placed in the vertical position as compared to those in the horizontal position ($p < 0.05$ for vertical vs horizontal) When MABP was increased with phenylephrine in mice in the vertical position, CBF remained significantly lower than observed in mice positioned horizontally. In contrast, the i.v. administration of 1.5 mL of saline increased CBF to a value not significantly different from that observed in horizontally positioned mice.

Discussion

We report an important reduction of CBF in vertically vs horizontally positioned mice. This study appears to contradict the classical theory of cerebral autoregulation in that a decrease in CBF is observed while MABP remains relatively constant between the prone and erect positions in mice. However, our results show that the CBF values for vertical mice treated with phenylephrine remain relatively constant as MABP was increased from 75 mmHg to 135 mmHg; supporting a conclusion that cerebral autoregulation is intact in the expected range, albeit the CBF autoregulatory curve for blood pressure is shifted downward.

Although gravitational effects in mice are small, our data support the hypothesis that a prolonged vertical position results in a reduction in central venous pressure from venous pooling in the lower extremities, leading to increased sympathetic tone and vasoconstriction of larger proximal vessels. This results in a vertical downwand shift of the autoregulatory curve and reduced CBF. Our protocol does not induce ischemia, nor is there hypotension, which removes the stimulus that would trigger the normal autoregulatory distal vasodilation to maintain CBF [4]. Therefore CBF is lowered in the vertical position, but also regulated at this lower level by the distal vasculature. This hypothesis is strongly supported by our findings that 1) despite reduced CBF in the vertical position, blood pressure autoregulation is intact as tested with phenylephrine infusion, and 2) prophylactic saline administration to compensate for venous pooling mitigates the reduction in CBF. However, taken together, our observations do not permit distinction of sympathetic involvement or some other mechanism such as neurohumoral responses, changes in cerebral metabolism, or anesthetic effects. In conclusion, implications of using a vertical position for studies of mice must be recognized when interpreting results from MRI that rely on CBF such as BOLD and fMRI studies.

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References

1. Hendrich KS, Kochanek PM, Williams DS, Schiding JK, Marion DW, Ho C. *Magn. Reson, Med.* **42**, 673-681 (1999).
2. Detre JA, Leigh JS, Williams DS, Koretsky AP. *Magn. Reson. Med.* **23**, 37-45 (1992).
3. Zhang W, Williams DS, Koretsky AP. *Magn, Reson, Med.* **29**, 416-421 (1993).
4. Giller CA, Levine BD, Meyer Y, Buckey JC, Lane LD, Borchers DJ. *J. Neurosurg.* **76**, 961-966 (1992).