Simultaneous observations of hemolymph flow and ventilation in marine spider crabs at different temperatures with flow weighted MRI

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Introduction

Flow weighted gradient echo MRI techniques can be used for angiography. Furthermore, these techniques allow to visualise the ventilation of aquatic animals (1). Therefore using MRI, it should be possible to observe ventilation and blood flow in marine animals at the same time.

Biochemical investigations led to the hypothesis that thermal tolerance is limited by insufficient oxygen supply to tissues at extreme temperatures (2). To test this hypothesis we investigated ventilation as well as hemolymph flow in the spider crab *Maja squinado* at temperatures between 2 °C and 12 °C with flow weighted MRI. These results were compared with ultra sonic doppler flow measurements and ventilation rate analyses at different temperatures.

Material and methods

Spider crabs (n= 5, weight 450-650 g) were fixed in a plexiglas perfusion chamber (19.4 cm diameter, 40 cm length) inside the magnet. Sea water flow through the chamber was provided continuosly at a rate of 1.5 l/min from an aerated, thermostatted 50 l water reservoir. Water temperature was recorded in the chamber at ± 0.1 °C with a fiber optic thermometer (Luxtron). Animals were allowed to acclimate for about 12 h at 12 °C inside the magnet before starting experimentation. Temperature was reduced from 12 °C to 2 °C within 8 h. All MR experiments were performed with a Bruker Biospec 4.7 T DBX system with actively shielded gradient coils (50 mT/m). Signal transduction and perception occurred with an ^{1}H cylindrical resonator (20 cm diameter) adapted for high conductivity samples. After optimization of the chamber position via scout images in all three directions, flow weighted gradient echo images were sampled in coronal and axial directions. Parameters were as follows: matrix size: 256*192, TR: 100 ms, TE: 10 ms, FOV: 12 cm, 6 slices (Sl-th of 2.2 mm) and a flip angle of 80° resulting in a scan time of 1.5 min. Water flow through the system was reduced during data aquisition to minimize imaging artefacts originating from sea water movements outside of the animal.

Results and Discussion

It is well known that crabs have an open circulatory system with a single chambered heart and well developed arteries, while venes are absent (3). Nonetheless, arterial (Fig 1, A+B) as well as venous flow (C) are clearly visible in coronal flow weighted images. Furthermore, water flowing through the gill chambers could be observed to be correlated with the ventilatory activity of the animal. During cooling ventilation stopped at 3.9 °C and signal intensities decreased indicating a drop of flow (Fig. 2). Further cooling caused flow to fall below detectable levels. Returning to control temperature reactivated ventilation and hemolymph flow. 12 h were sufficient for full recovery. These observations were in excellent agreement with ultra sonic measurements of hemolymph flow in single arteries as well as analyses of the ventilation rate in parallel experiments.

It appears that a reduction in ventilation as well as hemolymph flow go hand in hand, indicating a correlated drop in ventilatory and cardiac activity. Gill arteries (Fig. 1, A) showed a higher signal intensity at lower temperatures than other arteries (Fig 2), indicating a lower thermal sensitivity of flow through these organs.

In conclusion, it is possible to investigate ventilation and blood flow simultaneously with MRI in marine animals. Furthermore, decreasing temperature induced a decrease in ventilation as well as in hemolymph flow resulting in an oxygen limitation in the cold. The mechanisms responsible for the drop in ventilation and hemolymph flow remain to be investigated.

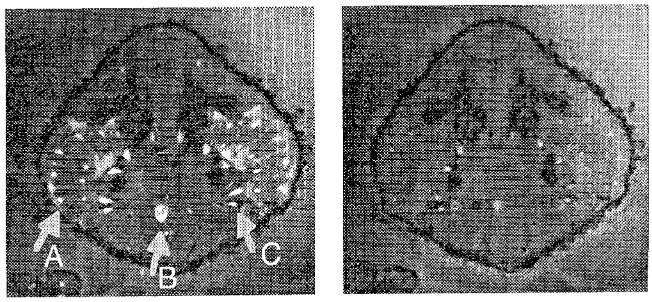

Figure 1+2: Coronal flow weighted images of the spider crab *Maja squinado* at different temperatures (left 9.2°C, right 3.9°C). The arrows indicate: A) gill arteries, B) aorta posterior, C) venous backflow. The diffusive signal intensity in the gill chambers resulted from ventilatory activity. Finally, ventilation stopped and flow vanished in the cold.

Literature:

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