Cardiac energetics of an anoxia-tolerant heart: In vivo 31P-NMR studies of the freshwater turtle Trachemys scripta

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Introduction
The relationship between cardiac energy status and the depression of myocardial performance during oxygen deprivation functioning has been an issue in cardiovascular research for years. The time course of changes in collapsing mammalian systems is fast such that the mechanistic underpinning of the loss in performance has remained elusive (Allen and Orchard, 1987; Wu et al., 2008). A number of anoxia-tolerant ectothermic vertebrates (reptiles and fishes) exist, which overwinter for months without oxygen and display slow-motion changes in cardiac functioning. These intrinsic cardiac performance and energy status parameters when compared to mammalian systems. The aim of this study was to use the anoxia-tolerant freshwater turtle (Trachemys scripta) as an animal model to investigate the changes in energy status of the anoxic working heart which supports overwintering a compostate state (e.g. Stecyk et al., 2007). Moreover, autonomic cardiovascular control is blunted in cold turtles (Hicks and Farrell, 2000; Stecyk et al., 2004a), rendering cold anoxic turtles unique for an in vivo examination of a temporal relation between intrinsic cardiac performance and high-energy phosphate metabolism.

Materials and methods
We utilized in vivo 31P-nuclear magnetic resonance (NMR) spectroscopy for direct and repeated measurements of cardiac high-energy phosphates and intracellular pH (pHi) of unanaesthetized turtles (Trachemys scripta) during prolonged anoxia at 21°C and 5°C. Trachemys scripta (body mass: 630 ± 76g) turtles were placed individually into closed, water-containing plastic chamber and restrained by two Velcro straps, 24 h prior to MR experiments. MR measurements were carried out initially under normoxic conditions and then at regular intervals during anoxia exposure for up to 11 days. All MR measurements were conducted in a Bruker 47/40 Biospec DBX system (40 cm bore, gradient (50 mT/m)). A 5 cm 31P-1H-surface coil was placed directly under the heart for in vivo 31P-NMR spectroscopy. Anoxia was initiated by flooding the chamber completely with water and by continuous bubbling with N2. At 21°C 31P-NMR spectra were acquired once every 10–15 min over 2.85 hrs of anoxia. At 5°C anoxia exposure lasted 11 days. 31P-NMR spectra were acquired once every 15 min for the first 18 h and then on days 3, 7 and 11. Between analyses, anoxic turtles were returned to a cold room, and continuously exposed to a N2 atmosphere at 5°C. In vivo 31P Nuclear magnetic resonance parameters: SW: 4000 Hz; flip angle: 60º (bp32; 200 μs); TR: 1 s; scans: 512. Data were related to the functional parameters of cardiac performance obtained in a parallel imaging study (Bock et al. 2009). Cardiac energy status (free energy change of ATP hydrolysis) was evaluated following the principles outlined by Pörtner et al. (1996).

Results and Discussion
In vivo 31P-NMR spectroscopy led to reproducible results during 11 days of anoxia exposure, with data under normoxic conditions similar to previous studies on isolated turtle hearts (e.g. Wasser et al., 1990). Figure 1 represents examples of in vivo 31P-NMR spectra of the turtle heart under normoxic and anoxic conditions at the respective temperatures. A clear reduction and decrease of dG/dδ and a doubling of dP/dβ. None of these cellular changes correlated with the reduction in ATP levels are already reduced under normoxic conditions (visible from the reduced β-ATP signal in Spectrum C).

Figure 1: Examples of in vivo 31P-NMR spectra of the turtle heart under normoxic and anoxic conditions (A, C) and after 2.85 hrs (B) and 11 days of anoxia (D) at the respective temperatures. A clear reduction in P i and ATP can be observed under anoxia at both temperatures. This reduction is more pronounced at 5°C, where ATP levels are already reduced under normoxic conditions (visible from the reduced β-ATP signal in Spectrum C).

References