Fully non-invasive multiparametric NMR exploration of muscle function in anesthetized rat in a single experiment

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Introduction Dynamic multi-parametric functional (mpf) NMR is a powerful approach which consists in confronting rapidly interleaved NMRS and NMRI data (on the order of seconds) to explore multiple facets of system regulations (on the order of minutes) following a single stimulus. Compared to separate acquisitions of data following successive stimuli, this considerably improves the relevance of the acquired information by reducing data dispersion due to intrinsic and irreducible variability of exercise or stress test.

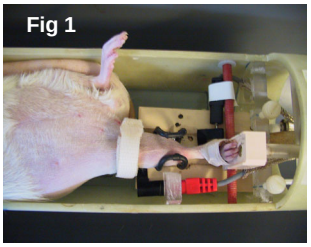
In recent years, this approach has been developed in view of clinical applications [1,2,3], has been succesfully applied to the study of metabolism in physiology and pathology of human muscle [4,5], and is arousing interest of other research groups [6]. Yet, though it was pioneered on animals [7] it has never since been used in pre-clinical experimentation, despite the obvious pertinence of using a similar approach in animal models, and despite the increasing use of animal models to test novel therapeutics in diseases of major clinical importance, such as atherosclerosis, diabetes and their complications.

Here we present the methodology and preliminary data of an mpf-NMR protocol specifically designed for simultaneous exploration of muscle perfusion by ASL-NMRI and high energy phosphate metabolism by ^{31}P NMRS in the hind-leg of anesthetized rat following totally non-invasive ischemic exercise.

Methodology

Animals Zucker male rats weighing 105.6+/-7.2 g at 5 weeks (Charles River Laboratories/GMI, L'arbresle, France.) were studied at 5 (n=8) and 7 (n=6) weeks of age. Experiments on animals complied with guidelines for the care and use of laboratory animals. For experiments, anesthesia was induced at 4% isoflurane, and maintained at 1.5 % of isoflurane in medical air delivered at 1.5l. min^{-1}, and body temperature was maintained by a heating water bed.

Electro-stimulation and force measurement Electro-stimulation was performed by percutaneous stimulation of the calf muscles using a Compex II (Compex, Ecublens, Switzerland) electro-stimulator connected to carbon electrodes coated with conducting gel placed at the popliteal fossa and Achilles tendon. We used bipolar electrical pulses of 100 Hz frequency and 0.2 ms width, at supramaximal intensity (14 to 18 mA), for 1 min at a frequency of 0.5 Hz. The custom-built ergometer was equipped with an amagnetic force-sensitive resistance and exerted force was recorded on a PC through an analog/digital card [8]. To reinforce hypoxia already induced by maximal isometric contraction, an air-cuff (custom made by Hokanson Inc., Bellevue, WA, USA) was placed around the upper hind-limb of the rat and inflated to 240mm Hg.

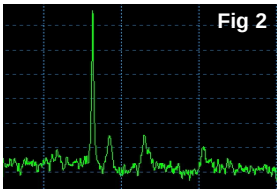


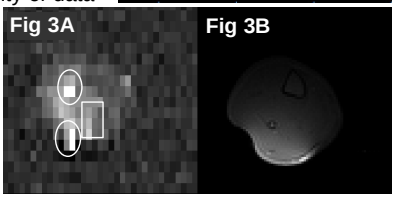
NMR Experiments were performed in a 4T magnet (Magnex, Abingdon, UK), fitted with a 200 mT shielded gradient insert and interfaced to Biospec Avance (Bruker, Karlsruhe, D) console and electronics. A custom-built linear RF discrete cosine volume coil, (9-cm inner diameter, 16-cm length) was used for ^{1}H transmission. The ^{1}H signal was collected through an orthogonal passively decoupled 9-mm diameter surface coil. The ^{31}P signal was excited and collected through a saddle-shaped coil (2.5cm diameter, 2cm length) placed around the rat calf, with it's B1 field orthogonal to the surface ^{1}H receiver coil. The setup (except for volume coil) in Fig. 1. shows the rat positionned within the bed, on the heating pad, with ergometer and ^{1}H receiver and ^{31}P coils.

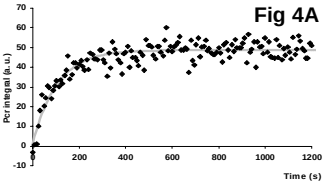
Muscle perfusion and PCr resynthesis rates were measured simultaneously in rat hind-leg during 25min of recovery from a 1 min ischemic exercise. This was repeated twice in the course of one experiment, the whole experiment including setup lasting just over an hour. The interleaved acquisitions consisted of a single-shot fast spin echo image (matrix 128*32, FOV 8*4cm, inter echo spacing 2.9ms) with a pulsed ASL module of perfusion[9] interleaved with a four scan ^{31}P spectrum, TR 2 s, providing 8s of recovery for ^{1}H arterial water spins in the meantime. The two modules were readily combined using the MultiScanControl tool developed by Bruker to facilitate such multiple explorations [3]

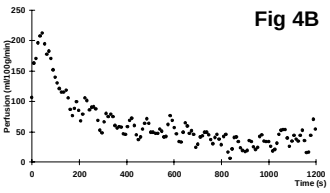
Results

Fig. 2A. shows an 8s ^{31}P spectrum acquired at rest. Fig. 3A shows the difference image between positively and negatively labeled perfusion-weighted images for a 5 week old rat. Perfusion is measured in the rectangular ROI excluding inflow effects (circled on the image). Fig. 3B is the correspondfing anatomical image. Fig 4A and B show the time courses of Cr rephosphorylation and of reactive hyperemic hyperperfusion for one rat following one exercise bout, displaying the quality of data that are obtained within a single experiment in these very young rats.









The PCr recovery time constant corrected for pH was 92±16s for rats at 5 weeks, not different from 101±20s for rats at 7 weeks of age. Pooled perfusion data for all rats are shown in Fig.5 for rats at 5 weeks (A) and rats at 7 weeks (B).

Conclusion Our ongoing interest in pathologies affecting muscle function and regulation has lead us to construct similar tools for the rat as those developed for multiparametric exploration of human muscle function and oxygenation. Because this tool is destined to provide repeated studies on a routine basis for a same individual, all procedures are fully non invasive. Preliminary results show good quality data even on very small animals.

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