Electrophysiological stimulation of excised rat muscle elicits a measurable change in tissue sodium concentration using 23Na-MRI

Frank Riemer[1], Joshua Kaggie[1], Cormac O'Neill[2], Mary McLean[3], James Grist[1], Myfanwy Hill[3], Joe Guy[3], Rolf Schulte[4], Martin Graves[1,2], James Fraser[5], and Ferdia Gallagher[1]

[1]Department of Radiology, University of Cambridge, Cambridge, United Kingdom, [2]Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom, [3]Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, United Kingdom, [4]GE Healthcare, Munich, Germany, [5]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom

Introduction

Changes in the tissue sodium gradient play an important role in cell signalling such as at the neuromuscular junction and as part of neuronal action potentials. ^{23}Na-MRI has the ability to measure the macroscopic sodium distribution. In this study we investigated the changes in tissue sodium in an electrically stimulated and freshly excised rat leg muscle.

Methods

The rat (Lister, age: 14 weeks) was sacrificed according to local ethical rules. Rat hind leg muscles were obtained within 1 hour of imaging. Muscles were placed in a container filled to 1 mm depth with buffer solution[1]. The container was placed on top of a custom ^{23}Na T/R coil of 15 cm diameter in a clinical 3T MRI (GE MR750, GE Healthcare, Waukesha, WI). A 3D-Cones sequence[2] was used for ^{23}Na-imaging (resolution = 3.8x3.8x3.8 mm^3, FOV = 20 cm, TE/TR = 0.5/50 ms, flip angle = 90°, readout length = 30 ms, 197 readouts, duration of one scan = 10 seconds). A waveform generator was placed outside of the room, and the cable connected to an MRI pass panel to stimulate the muscle with prongs attached to the muscle for field excitation (20 ms pulses, 1 second between pulses). A trap circuit at 33 MHz (>30 dB attenuation) was placed on the cables to remove noise at the imaging frequency for sodium. Images were corrected for B_1 sensitivity using the double angle method (FA = 30° and 60°)[3]. 20 and 85 mM NaCl in 4% agar phantoms were placed in the FOV for quantification[4]. Imaging was performed in 1 minute blocks (6 volumes per block), 4 blocks in total (2 blocks of stimulation interleaved with 2 rest blocks). SPM12 (UCL, London, UK) was used to find voxels undergoing significant sodium signal changes in the FOV. No response modelling function (such as hemodynamic response) was used due to the instantaneous nature of the sodium flux and low temporal resolution (10 s).

Results

Figure 1 shows a ^{1}H image of the set up. The short ^{23}Na-MRI scan (10 s per volume) yielded sufficient SNR (≈ 22 a.u. ; fig. 2). The average total sodium concentration in the muscle tissue at the beginning and end of the experiment was 37 mM. A change in sodium concentration between +1 to + 4 mM was detected during stimulation ($p < 0.001$, FWE corrected), fig.3. Muscle tetany during stimulation was confirmed visually. No signal changes were detected in the control muscle that was not stimulated.

Discussion

The results demonstrate for the first time that rapid sodium signal changes can be detected within excited skeletal muscle using ^{23}Na-MRI. In this experiment an alteration in tissue sodium was detected which corresponds to the sodium flux during activation of the neuromuscular junction. As the muscle was imaged *ex vivo*, contribution of blood flow (also a carrier of sodium ions) should not have an effect.

Conclusion

Here we have demonstrated that non-invasive ^{23}Na-MRI has the sensitivity and temporal resolution to be used to study functional changes in sodium concentration in muscle tissue during stimulation. This method could be applied in the future to investigate neuromuscular diseases where alterations in sodium transport are known to occur e.g. myopathies. This observation in muscle provides further evidence that ^{23}Na-MRI can be used as a functional measure of action potential activity within the central nervous system, which would complement traditional proton functional MRI measurements within the brain[5].

Acknowledgements

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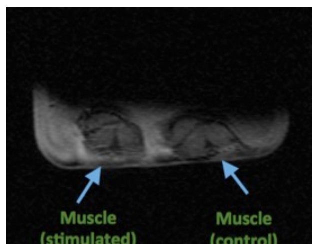

Fig. 1: Coronal 1H images of the muscle experiment set

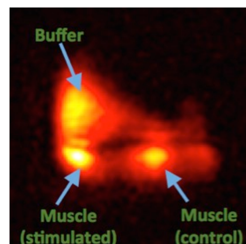

Fig. 2: One time-point coronal 23Na image of the muscle experiment set up.

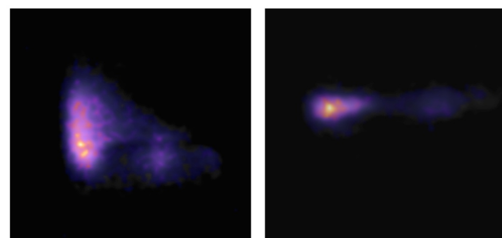

Fig. 3: One time-point coronal and axial 23Na image (purple) of the muscle experiment set up with superimposed activation clusters (yellow/orange, $p < 0.001$, corrected).