Phosphorylated Guanidinoacetate in muscle of GAMT deficient mice only partly compensates for PCr as assessed by 31P MRS and functional measurements

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Introduction

The enzyme guanidinoacetate methyltransferase (GAMT) is a key enzyme in the biosynthesis of creatine (Cr), an important compound in the energy metabolism of skeletal muscle and brain. In the past decade, a number of patients suffering GAMT deficiency, and therefore lacking Cr, were described showing several clinical symptoms including muscle hypotonia and dyskinesia [1]. To enable in depth study of the disorder, GAMT deficient knockout mice (GAMT-/-) were generated [2]. Surprisingly, these mice are viable and are able to walk normally, suggesting adaptations in energy metabolism. Therefore, the aim of this study was to investigate force characteristics and energy metabolism of hindleg muscle of GAMT -/- mice by monitoring ischemia with ^{31}P MRS and by high intensity functional muscle measurements.

Methods

Force measurements of medial gastrocnemius muscles were performed as described previously [3]. After determination of optimum length, the force-frequency relationship was studied by comparing a single tetanus with the same tetanus after a brief pre-conditioning tetanus (CT). The tetani had a stimulation duration of 250 ms and stimulation frequency was varied (80-200 Hz), the CT consisted of a tetanus with a fixed duration and stimulation frequency. In a separate experiment, the resistance to fatigue was examined by applying a series of 30 repeated isometric contractions (duration 55 ms, stimulation frequency 150 Hz) within 7.5s.

^{31}P-MRS measurements were performed at 7.0T and a three-turn solenoid coil was used for ^{31}P measurements, together with an Alderman-Grant type of ^{1}H coil for shimming. Mice were anaesthetized with 1,5 % Isoflurane and body temperature was maintained using a warm water blanket.

Ischemia measurements were carried out on wild type (WT, n=7) mice, GAMT-/- mice without Cr supplementation (GAMT-/-$_{,0}$, n=7), and GAMT -/- mice supplemented 48 hours with 1 % Cr (GAMT-/-$_{,Cr}$ n=4). A diaphragm plate that allowed reversible and reproducible obstruction of blood flow through the hind limb was used to apply ischemia. Spectra were recorded with a pulse-acquire sequence (TR=1400 ms, 76 averages) for 7 minutes prior to ischemia, during the 25 minutes of ischemia and 16 minutes of recovery.

Saturation transfer (ST) measurements were performed on 6 WT and 6 GAMT-/- mice. The γ-ATP signal was selectively saturated (500–5000 ms) prior to acquisition (TR=7000 ms, 64 averages). All data were processed using MRUI [4].

Results

Force measurements showed that absolute force was lower in GAMT-/- animals, as was absolute muscle mass ($p<0.05$). Therefore, relative force (normalized to muscle mass) did not differ between groups. The force frequency relationship showed a minor reduction in the force of the second tetanus at low stimulation frequencies (60 and 80 Hz). Repeated isometric contractions showed a reduced ability to maintain force in GAMT -/- animals (figure 1).

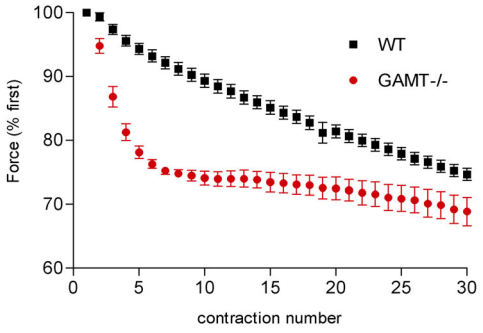

Figure 1. Force characteristics of WT (n=7, black squares) and GAMT-/- (n=4, red circles) during repeated stimulation. Force signals are normalised to the first contraction and shown as mean ± SEM.

The ^{31}P measurements showed that GAMT-/-$_{,0}$ display a twofold lower SNR of the β-ATP peak. Prior to ischemia, GAMT -/-$_{,0}$ mice showed a negligible phosphocreatine (PCr) content along with a new signal which was assigned to phosphorylated guanidinoacetate (PGua), the immediate precursor of Cr, as has also been observed in patients [1]. Supplementation of Cr resulted in the appearance of a PCr signal which was already visible after one day of Cr supplementaton (figure 2). During ischemia in GAMT -/-$_{,0}$ mice, the decrease of the PGua signal was comparable to the decrease of PCr observed in WT mice while its recovery after ischemia was significantly delayed ($p<0.05$). The recovery after ischemia of PGua in GAMT-/-$_{,Cr}$ mice did not differ from the non-supplemented group. ST measurements revealed a non-detectable flux from PGua to γ-ATP.

Conclusion and discussion

Since the lower force production of GAMT-/- mice during high intensity stimulation was caused by a lower absolute muscular mass, it is unlikely to expect dramatic changes in ATP concentrations between mouse types. Therefore, the decreased SNR of the ATP peak found by ^{31}P MRS in GAMT -/- hind leg muscle is probably caused by a lower muscle mass. Interestingly, ^{31}P spectra show that instead of PCr, GAMT -/- animals have PGua and that, during ischemia, this compound is used for high-energy phosphoryl transfer at a rate comparable to PCr. Since Cr supplementation did not result in an increase of the recovery rate of PGua, the inability of mitochondrial creatine kinase to phosphorylate Gua [5] apparently is not the limiting factor for the rate of PGua recovery. In combination with the results of the ST experiment this suggests that the delay in the recovery of the PGua signal after ischemia is caused by reduced enzyme kinetics.

These reduced enzyme kinetics could cause the impaired ability to maintain force during repeated isometric contractions by a decrease in local ATP concentrations. A reduced excitation-contraction coupling as was found previously in cytosolic creatine kinase deficient mice [6] is unlikely to cause this reduction as the force frequency relationship was normal above 100 Hz.

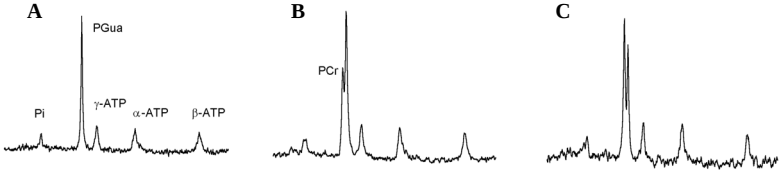

Figure 2. Resting spectra (Tr=7000 ms, 128 averages) showing the increase in signal intensity of PCr in hind leg muscle of GAMT -/- mice before (A) and after 1 day (B) and 2 days (C) of Cr supplementation.

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