Creatine deficiency, uptake and breakdown studied in brain and muscle of Arginine:Glycine Amidinotransferase deficient mice

C. Nabuurs1, M. Romeijn1, A. Veltien1, H. Kan1, D. Ibraaidi2, and A. Heerschap1

1Radiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, 2Institute for signal transmission, Hamburg, Germany

Introduction: The importance of creatine (Cr) for normal brain function is demonstrated by the severe symptoms of mental and muscular abnormalities in patients with Cr deficiency syndromes (CDS) [1]. Oral Cr administration is used successfully to replenish Cr levels in brain of patients with inborn errors of Cr biosynthesis enzymes (arginine-glycine amidino transferase AGAT, and guanidino acetate methyl transferase GAMT) [2,3]. Cr uptake in patients with AGAT deficiency was suggested to be faster, which was ascribed to toxic effects of guanidino acetate (Gua) accumulation in GAMT deficient patients and the competition between Cr and Gua uptake. As a result, lower Cr doses are prescribed for AGAT-/- than for GAMT-/- patients [2,3]. This difference could only be investigated in a few patients. The recent generation of a mouse model for AGAT-/- enables further investigation on the effects of AGAT-/- on brain and muscle metabolites and the response to Cr treatment without interference of accumulated Gua levels. Here we present a longitudinal metabolic study using in vivo 1H and 31P MR Spectroscopy (MRS).

Methods: MR spectra were obtained from muscle and brain of AGAT-/- and wild type (WT) mice on a 71 spectrometer (MR Solutions). For comparison all procedures were similar to previous studies on GAMT-/- mice using 1.0-1.8% isoflurane [4,5]. ISIS localized 31P MR spectra were acquired from brain (n=4,160-220 μL voxel, TR = 6750 ms, 512 ave). 31P spectra of hind limb muscle were measured without localisation (TR=7s, 128 ave). Localized 1H MR spectra were obtained in brain (STEAM, 8.8 μL voxel, TE = 15 ms, TM = 10 ms, TR = 5 sec, 256 ave) and muscle (12.3μL voxel). Cr uptake in brain and muscle were studied with identical 1H MRS measurements during >35 days of suppletion and 120 days of break down. The Cr was administered ad libitum via the drinking water (5.32 g/500ml with additional glucose (4.32 g/500ml). LCModel was used to obtain metabolite concentrations from the 1H MR spectra using water signals as an internal reference. 31P signals of Pcr, Pi and PME were analyzed with AMARES and normalized to ATP signals. Breakdown rates of Cr were determined by fitting the depleting metabolite curves to a mono exponential curve.

Results: The 1H MR and 31P MR spectra demonstrate an almost complete absence of total Cr and Pcr in AGAT-/- brain (fig 1-2) and muscle (spectra not shown here). In muscle, significantly high Pi levels were found (table 1), whereas in brain phospho mono esters (PME) were elevated. AGAT-/- mice showed a gradual Cr uptake in brain during 45 days of Cr suppletion, reaching normal levels at ~20 days, which is comparable to Cr uptake in brain of GAMT-/- mice (fig 2). In contrast, skeletal muscle shows a very fast uptake response, that was not seen in AGAT-/- mice [5]. Note the high Cr concentrations at the first two days of Cr suppletion in AGAT-/- muscle(fig.2). Breakdown of Cr was similar in both tissues (fig.3: brain: 2.1 ± 0.4 % day⁻¹; muscle: 1.0 ± 0.2 % day⁻¹). Interestingly, tauirine levels in muscle show additional changes upon Cr suppletion and depletion.

Discussion: This in vivo MRS study demonstrates that AGAT-/- brain and muscle tissue do not have an alternative high energy phosphor compound such as phosphorylated Gua in GAMT-/- mice [2,4]. To compensate for the lack of the creatine kinase system. However, the elevated Pi levels in AGAT-/- muscle indicate a change in the equilibrium of the phosphate homeostasis. In brain, Cr uptake is very slow when compared to the immediate replenishment of Cr in muscle. This suggests that the blood brain barrier delays transport of Cr into the brain. Moreover, the increased Pi levels in AGAT-/- brain indicate a change in the equilibrium of the phosphate homeostasis. This mouse model for CDS enabled the determination of Cr breakdown rates in muscle and brain accurately over a 0 to 100 range, which are in good agreement with the outcome of labeling studies which only focus at a 100-115% range [6]. In combination with non-invasive MRS techniques this mouse model, that can be switched to and from Cr depleted conditions, provides us with a unique opportunity to study Cr uptake and metabolic effects in CDS without interfering postnatal developmental adaptations or toxic Gua accumulations.