Magnetic Resonance analysis of the effects of acute ammonia intoxication on a rat brain

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Introduction

Increased ammonia levels play a central role in the cerebral alterations in acute liver failure. Acute intoxication with large doses of ammonium salts leads to rapid death of animals. Acute ammonia intoxication induces activation of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptors in brain *in vivo*[1]. Moreover, ammonia induced death of mice and rats is prevented by blocking NMDA receptors with antagonists acting on different sites of the receptor[2,3]. These data support the idea that acute ammonia intoxication leads to increased activation of NMDA receptors in brain, which play a main role in the mechanisms leading to ammonia-induced death. The subsequent events by which activation of NMDA receptors results in animal death remain unclear. Astrocytes swelling and associated brain edema seem important steps in the pathogenic mechanisms in acute liver failure and ammonia intoxication[4,5]. The aim of this work was to study the effects of acute ammonia intoxication on different cerebral parameters *in vivo* using magnetic resonance and to assess which effects are dependent or independent of NMDA receptors activation.

Methods

All experiments were performed in a Bruker PharmaScan® systems using a 7.0 T horizontal-bore superconducting magnet, equipped with a ^{1}H selective birdcage resonator of 38 mm and a Bruker gradient insert with 90 mm of diameter (maximum intensity 300 mT/m. Data were analyzed in a Linux platform with software written in house in IDL. The different brain regions were identified according to the stereotaxic coordinates of the rat brain atlas[6]. The data were analyzed by one-way ANOVA followed Dunnett T-test. p values lower than 0.05 were considered statistically significant. Statistical analysis was performed using the Graph Pad Prism 4 software (GraphPad Software Inc. San Diego, CA, USA). We measured the apparent diffusion coefficient (ADC), T1, T2 and T2* maps in 16 brain areas of the rats treated with acute ammonium acetate (5mmol/kg i.p.). *In vivo* ^{1}H MR spectroscopy was performed in the cerebellum and in the substantia nigra. Moreover we assessed whether these effects on NMR imaging are prevented by blocking NMDA receptors using the selective antagonist MK-801.

Results and Discussion

The effects of acute ammonia intoxication are not the same in different brain areas. T1 relaxation time is reduced in 8 brain areas but not in the other 8. T2 relaxation time is reduced in ventral thalamus and globus pallidus, but not in the other areas. ADC values increased in hippocampus, caudate-putamen, substantia nigra and cerebellar cortex, reflecting vasogenic edema. ADC value decreased in hypothalamus, reflecting cytotoxic edema due to astrocytes swelling. ADC values were not affected in the other areas. *Myo*-inositol increased in cerebellum and substantia nigra, reflecting edema. Injection of ammonia did not induce any significant change in T2* map compared to vehicle administration in any of the 16 brain regions studied (not shown).

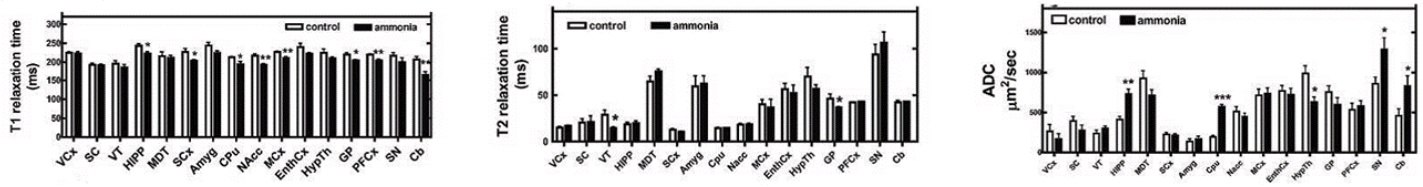

Figure 1: T1, T2 and ADC values for 16 brain regions in control rats versus rats with hyperammonemia induced by acute ammonia intoxication

N-acetyl-aspartate decreased in cerebellum, reflecting neuronal damage, but was not affected in substantia nigra. Myo-inositol levels increased significantly both in cerebellum and in substantia nigra. Injection of MK-801 alone did not induce any significant change in T1, T2 or ADC maps as compared to vehicle injection. Injecting MK-801 ten minutes before ammonia completely prevented the decrease in T1 relaxation time induced by ammonia in all the 8 areas affected. MK-801 also prevented the ammonia-induced reduction in T2 relaxation time in ventral thalamus and globus pallidus. Blocking NMDA receptors with MK-801 did not prevent ammonia-induced changes in ADC in any of the 5 brain areas affected. ADC values were similar for rats injected with ammonia or with MK-801 plus ammonia. Blocking NMDA receptors with MK-801 completely prevented ammonia-induced reduction in *N*-acetyl-aspartate in cerebellum. Previous injection of MK-801 did not affect the ammonia-induced increase in *myo*-inositol.

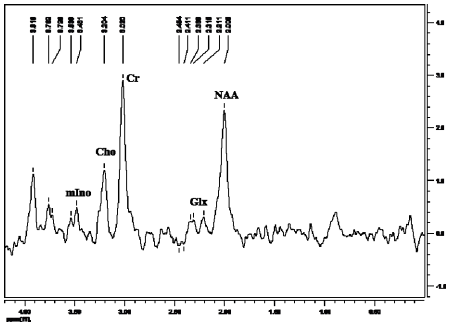

Figure 2: Cerebellum spectrum after acute ammonia intoxication (NAA: *N*-acetyl aspartate, Glx: glutamate and/or glutamine, Cr: creatine, Cho: choline containing compounds, mIno: *myo*-Inositol

Conclusions

The results show that acute ammonia intoxication induces regional selective alterations in T1, T2 and ADC maps. Neuronal damage (decrease in N-acetyl-aspartate) and changes in T1 and T2 are prevented by blocking NMDA receptors with MK-801 while changes in ADC or *myo*-inositol (induction of edema) are not prevented by MK-801.

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