Metabolic Responses in Kanic Acid Induced Lesion in Rat

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Introduction

Intracranial micro-injection of kainic acid (KA), a drug producing neuronal loss and reactive gliosis [1,2], has been widely used as animal models for studying cellular processes in neurons as well as various neurological diseases and disorders such as brain injuries and epilepsy. Here we demonstrate metabolic changes reflecting neurochemical responses to the KA lesion in the rat hippocampus can be investigated quantitatively using magic spin angle (MAS) NMR at the high spectral resolution.

Materials and Methods

Kainic Acid Induce Lesion Unilateral intracerebroventricular injection of kainic acid, KA (0.5 μg/0.5 μl artificial CSF (aCSF) into the right lateral ventricle) was employed in order to produce neuronal loss and induce reactive gliosis in, but not exclusive to, the right hippocampi of adult male rats. Two control adult male rats received 0.5ul aCSF into the right lateral ventricle. After injection, rats were allowed to recover from anesthesia and were monitored for seizure activity. One week later rats were anesthetize with isoflurane and immediately decapitated. Sections containing the complete hippocampi were removed on ice and immediately frozen on dry ice and stored at -80 ^{o}C. Three coronal sections were taken bilaterally from CA1and CA3 regions of the hippocampus for NMR analysis.

MAS NMR experiments The intact tissue samples were taken from CA1 and CA3 from four rat brains (two with lesions and two normal controls). Each sample was weighed (ranging from 31 mg to 38 mg) and placed in the separated sample holder/rotor. All samples were measured three times during a 12 hour period at 4 ^{o}C and spectra were found unchanged during the course of our experiments, indicating there was no detectable sample degradation. Quantification of metabolites was obtained using either water as the internal reference or external reference. One dimensional NMR spectrum was collected using CPMG sequence. Two dimensional TOCSY spectra were collected for metabolite/resonance assignment.

Results and Discussions

Figure 1 shows a set of NMR spectra from the control and KA-treated samples. The key observations include: 1) NAA levels decreased substantially in the KA-treated hippocampi samples compared to the normal controls. 2) Cho derivatives levels, particularly, phosphate-Cho, were increased in the KA-treated samples compared to the normal controls 3) Cho increases and NAA decreases were more pronounced in the sites ipsilateral to the injection (CA1* and CA3*) compared to the sites in the contralateral hemisphere. It is known that kainic acid (KA) injections produced neuronal loss as well as reactive gliosis in both CA1 and CA3 on the injected side relative to the control side, and relative to the same hippocampal regions in intact and untreated control rats. Thus, our results from MAS NMR analysis support the notion that a decrease in NAA as an indication of neuronal loss. An increase in Cho, often associated with high membrane turnover rate and cell breakdown, can be also correlated. It was suggested that an increase in mI as an indication of enhanced glial activity in treated animals compared to controls. However, our data did not show significant changes in the level of mI as we had anticipated. This may be due to that development of reactive gliosis may take more than 1 week. To assess the MAS NMR's ability to quantify the hormonal induced neurochemical changes, we semi-quantified the changes of several metabolites of interest, e.g., NAA, Cr, total Cho (PCho+Cho derivatives) and mI when compared to the control sample using external reference. The concentration was normalized to the level of Cr in the control sample since Cr level is least varied in the sample. The change of Cho, NAA and the unknown resonances are most noticeable and can be quantitatively compared to the control. For instances, the ratio of NAA/Cr decreased from 0.92 in the control sample to 0.15 in the CA1* (KA-treated side), while the CA3* and CA3 also exhibited the decreased of NAA with ratio of NAA/Cr at 0.12 and 0.73, respectively (vs. 0.92 in control). The ratio of Cho/Cr increased remarkably from 1.32 in the control sample to 2.40 in the CA1* of treated brains.

In conclusion, these data show that MAS NMR is able to detect even subtle changes in NAA such as those between lesioned and unleasioned sides of the hippocampus in KA-treated rats.

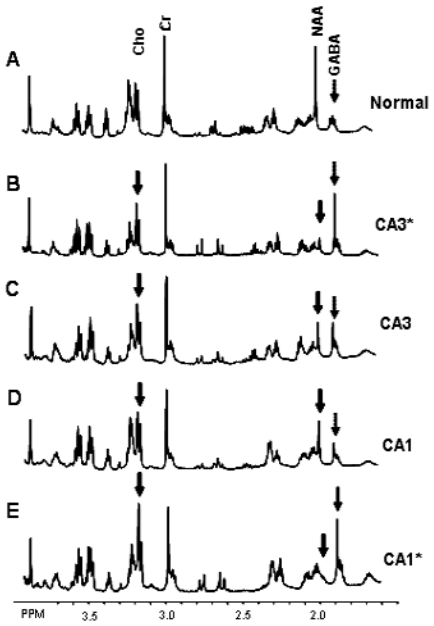

Figure 1. NMR spectra of tissue samples from the hippocampi (CA1) of a non-treated rat (**A**) and a kainic acid-treated rat (**B-E**). * indicates the samples were from the treated hemisphere. Phosphate-Cho and NAA signals are labeled with arrows (solid) as their intensity changes in the treated samples. A possible GABA signal (dashed arrow) appeared in the treated samples.

Reference: [1]. Smith BN, Dudek FE. *J Neurophysiol.* 2002; 87:1655-8. [2]. Abraham H, Losonczy A, et al. *Brain Res.* 2001;906 :115-26.