Metabolic Effects of Methamphetamine on the Young Mouse Brain

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Introduction

Methamphetamine (Meth) abuse in adolescents is a health and social problem in the U.S. The neurotoxicity associated with Meth has been primarily studied in adult subjects, both humans and animals. Little is known about the effects of this drug on the developing brain, especially in adolescence where a number of circuits are established and refined. We studied the effects of Meth on young mice under different dosing regimens to establish a new animal model for Meth neurotoxicity in the adolescent brain. Meth-induced changes in behavior were measured. Meth-induced metabolic effects were assessed using proton spectroscopy to measure cerebral metabolite levels in brain extracts. Our studies revealed dose-dependent effects of Meth on a number of brain metabolites. The significance of the observed changes is discussed.

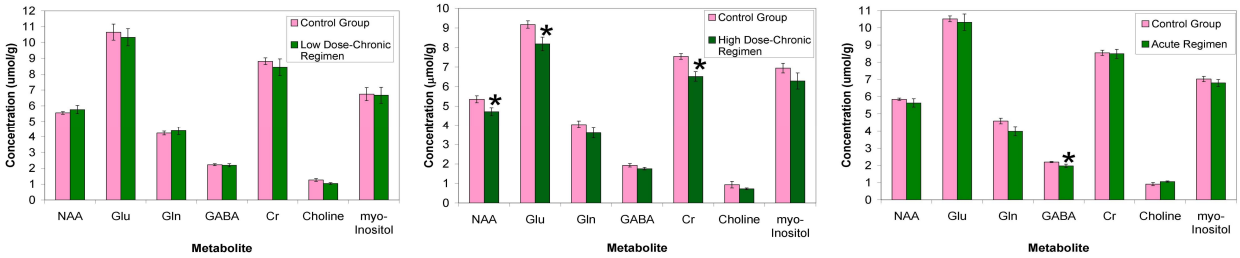
Methods

Male C57 BL/6J mice at 4 weeks of age were used. D-Methamphetamine HCl (Sigma, St. Louis, MO) was administered in either an acute regimen or in chronic regimen. The acute regimen consisted of a single injection of Meth at 30mg/kg body weight. Two doses were studied in the chronic regimen: a low dose of 8mg/kg or a high dose of 15mg/kg injected daily for 10 successive days. Age-matched control groups of mice received saline injections. During Meth administration, body surface temperature was measured; locomotor and stereotypy behaviors were observed and scored. 24 hours after the last injection, heads were flash frozen, brains excised and whole brains extracted using a chloroform/methanol procedure optimized in our laboratory for mouse brains. Aqueous phase of the extract was lyophilized and dissolved in D_2O for proton spectroscopy on a 600MHz Bruker Avance spectrometer. Fully relaxed 1D proton spectra of brain extracts were acquired using a pre-saturation pulse sequence, spectral width 7300Hz and 64K data points. Cerebral metabolite concentrations, expressed as µmol/g wet weight of brain, were calculated from integrals of well-resolved resonances relative to a standard TSP of known concentration.

Results

Mice that were given the acute regimen and high dose-chronic regimen of Meth demonstrated very strong locomotor behaviors such as running and climbing, as well as stereotypy activities such as chewing, licking, repetitive head movements and rearing as compared to control group animals. Behavioral changes such as those observed are characteristic Meth effects [1]. Increased behavioral activity began 15min after each injection and lasted 3 to 4 hours. Body surface temperature of mice in these groups was 1.2°F higher than in control mice. In contrast, temperature of mice given the low dose-chronic regimen did not increase. Nor did they show any increase in stereotypy and locomotor activities.

Resonances from the following major cerebral metabolites in the brain extract spectra were analyzed to assess neurotoxicity of the administered Meth regimens: N-acetyl aspartate (NAA), glutamate (Glu), glutamine (Gln), γ-amino butyric acid (GABA), creatine (Cr), choline compounds (Cho), and myo-inositol (mI). Resonances from lactate, alanine, aspartate, acetate and taurine were also seen in the aliphatic region of the spectra. Concentrations of cerebral metabolites in mice given the low dose-chronic regimen of Meth (n=6) were statistically the same as that in control mice. In contrast, concentrations of several metabolites decreased significantly in mice given the high dose-chronic regimen of Meth (n=6): NAA -12% ($p<0.05$), Glu -11% ($p<0.05$), and Cr -14% ($p<0.05$). Concentration of GABA also decreased (-9%), although it did not reach statistical significance. Only the concentration of GABA decreased (-11%; $p<0.02$) in mice that received a single dose of acute Meth treatment (n=6).



Cerebral metabolic changes in Meth-treated mice. Left: Chronic regimen-low dose; Middle: Chronic regimen-high dose; Right: Acute regimen

Discussion

Our results clearly demonstrate a regimen- and dose-dependent Meth effect on the young mouse brain. Reduction of only GABA in mice subjected to the acute treatment shows that neurotoxicity of this Meth regimen is limited to GABAergic neurons. Metabolic abnormalities associated with Meth are prominent in the striatum. Nearly 90% of the neurons in the striatum are GABAergic and a single large dose of Meth as given in the acute regimen probably causes degeneration of a large fraction of striatal neurons. High dose-chronic regimen of Meth is toxic to both glutamatergic and GABAergic neurons in the mouse brain as seen by lower Glu and GABA levels. NAA is a neuronal marker and the decrease in NAA level may reflect neuronal dysfunction caused by Meth. Creatine is synthesized in the endoplasmic reticulum (ER) and phosphorylated in the mitochondria and NAA is synthesized in the mitochondria. Thus lower levels of NAA and Cr detected in mice treated with high dose-chronic regimen of Meth may be indicative of ER and mitochondrial dysfunction. Studies have shown that Meth disrupted mitochondrial function selectively in the striatum by decreasing ATP levels [2,3]. *(Acknowledgement: U54 NS39406, S10 RR13880)*

References

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