NMR Investigations of Neuronal and Astroglial Metabolism in Nicotine Addiction

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INTODUCTION: Nicotine addiction is a psychiatric disorder underlies the widespread use of tobacco products and contributes to the large number of chronic illnesses and leading cause of death globally and considerable health care costs associated with their use[1]. An understanding of how nicotine produces addiction and influences smoking behavior provides a necessary basis for optimal smoking cessation intervention. Glutamate and GABA are major excitatory and inhibitory neurotransmitter in the central nervous system. These neurotransmitters, play major roles in glucose and energy metabolism, cortical excitability and cognitive function, and are involved in many functions such as motor, behavior, cognition and emotion. It is well established that a neuronal astrocytic substrate cycle exists in the brain[2]. Dysfunction in these pathways is associated with many neurological and neuropsychiatric disorders. Thus the glutamate-GABA-glutamine axis is of major importance to brain function and cerebral well being. In this study we have used a novel approach of co-infusion of [U-$^{13}C_6$]glucose and [2-^{13}C]acetate to investigate neuronal and astroglial metabolism during nicotine addiction.

MATERIALS AND METHODS: All animal experiments were performed under approved protocols by the CCMB Animal Ethics Committee. One month old C57BL6 mice were treated with nicotine 0.5 (n=5), 1.0 (n=3) and 2.0 mg/kg (n=3) three times a day for a month. Control mice (n=4) were injected with normal saline for the same period. Metabolic measurements were carried out 2 days after the last treatment. Overnight fasted mice were anesthetized with urethane (1.5 g/kg) and tail vein was cannulated for the infusion of ^{13}C labeled substrates. Mice were infused with [U-$^{13}C_6$]glucose and [2-^{13}C]acetate for 20 min as a bolus-variable rate infusion[3]. At the end of the experiment, brain was frozen *in situ* in liquid nitrogen. Different brain regions (cortex, cerebellum, sub cortex and olfactory bulb) were dissected under frozen conditions. Metabolites were extracted from frozen tissues[4]. The concentrations and percent ^{13}C enrichment of metabolites were determined from the ^{1}H-[^{13}C]-NMR spectrum of the extract acquired at 14T (Bruker AVANCE spectrometer)[5] . The percentage ^{13}C enrichment of plasma glucose-C1 and acetate-C2 was measured using ^{1}H NMR peak of glucose-C1 at 5.2ppm and acetate 1.91 ppm, respectively. The contribution of [U-$^{13}C_6$]glucose and [2-^{13}C]acetate for the total measured ^{13}C labeling of amino acids from ^{1}H-[^{13}C]-NMR spectrum was determined using isotopomer analysis of ^{13}C-[^{1}H]-NMR of tissue extract.

RESULTS AND DISCUSSION: Cortical metabolites level did not change after chronic 0.5mg/kg nicotine treatment. However, the level of glutamate (16.4±0.6 vs. 14.9±0.5 μmol/g), GABA (3.2±0.6 vs. 2.2±0.1 μmol/g), glutamine (6.1±0.5 vs. 5.1±0.5 μmol/g) and NAA (8.7±0.3 vs. 7.5±0.2 μmol/g) was reduced significantly (P<0.01) with 1.0 mg/kg nicotine. Level of metabolites did not change significantly in other brain regions with chronic nicotine treatment. Results of labeling of cerebral amino acids form [U-$^{13}C_6$]glucose and [2-^{13}C]acetate is depicted in Fig. 1. It is evident from the graph that cortical Glu_{C4} and $GABA_{C2}$ labeling from [U-$^{13}C_6$]glucose was increased significantly after treatment of 0.5 mg/kg nicotine and also persisted in mice treated with 1.0 mg/kg nicotine, indicating increase in glutamatergic and GABAergic activity during nicotine addiction (Fig. 1A). Increased labeling of Gln_{C4} suggests an increase in neurotransmission with chronic nicotine treatment. Further, labeling of Glu_{C4} and $GABA_{C2}$ from [2-^{13}C]acetate also increased indicating an increase in glutamatergic and GABAergic neurotransmission after chronic nicotine addiction. These data suggest nicotine addiction leads to an increase in glutamatergic and GABAergic energy consumption and neurotransmission in cortex. These results are in consistence with increased activity in pre-frontal cortex, observed in functional studies during nicotine treatments[6].

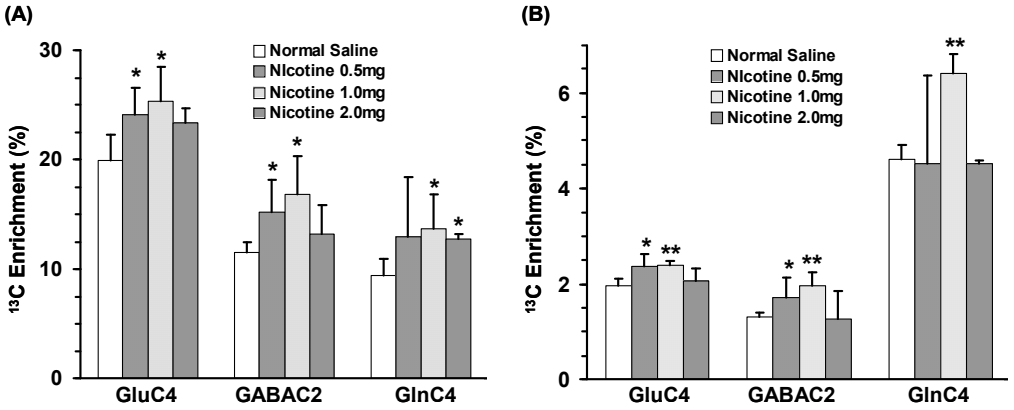

Fig. 1 ^{13}C Labeling of cortical amino acids from (A) [U-$^{13}C_6$]glucose, (B) [2-^{13}C]acetate; *p<0.05, **p<0.01

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ACKNOWLEDGEMENTS: This study was supported by funding from CCMB and CSIR.