Acute Administration of Nicotine Reduced myo-Inositol and GABA levels in nucleus accumbens in rats

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Introduction

The increased levels of myo-inositol (MI), a glial membrane-bound protein-carbohydrate conjugate, are associated with elevated glial activity. Glial-neuronal interactions are one mechanism by which the rewarding properties of addictive compounds may be modulated within the biological reinforcement pathway. MI concentration could be assessed relatively easily with noninvasive proton MRS in different parts of the CNS, which could provide a useful biomarker for drug addiction research in the area of pharmaceutical drug development. In this study, we evaluate MI response to acute nicotine challenge using localized high field proton MRS in normal rats.

Materials and Methods

Animal handling and MRI/MRS procedures were approved by our local IACUC. Fifteen male CD rats (Charles River Laboratories, Wilmington, MA, 308 ± 8g) were housed in plastic isolators with organic cellulose bedding. They received food and water *ad lib* and a 12 hr/12 hr light/dark cycle, with testing during the light phase. MRS was conducted in the BioSpec 7T/210AS MRI system (Bruker BioSpin, Billerica, MA) with 38 mm litzcage transmit-receive volume RF coil (Doty Scientific, Columbia, SC) under the isoflurane general anesthesia at controlled rat core temperature (37 ± 1°C). The spectroscopic voxel (4×4×4 mm^3) was carefully positioned at *n. accumbens* using *a. commisure* as a landmark on a high-resolution scout spin-echo brain images. The magnetic field homogeneity in this voxel was adjusted using FASTMAP to the water line width of less than 12 Hz. Proton MRS was performed using PRESS localized sequence with CHESS water suppression with the following parameters: TE = 16 ms, TR = 3 s, 256 averages, resulting in a total acquisition time ~13 min/spectrum. At least 12 spectra per animal were acquired. Rats were injected with nicotine (1 mg/kg, N = 10) or saline (controls, 1 ml/kg, N = 5) subcutaneously before they were positioned inside the magnet. The first MR spectrum was acquired 44 ± 9 min after injection. Resultant spectra were processed using LCModel [1]. MI, N-acetylaspartate (NAA), γ-aminobutiric acid (GABA), glutamate (GLU), and glutamine (GLN) were measured and their amounts expressed as ratios to creatine. Spectra obtained in the period of 40 to 120 min after nicotine administration were also summed to produce cumulative time-averaged spectra, which underwent the standard LCModel analysis. Time series data were analyzed using repeated measures ANOVA and time-averaged values – using non-paired t-test (SigmaStat, Point Richmond, CA). Data are presented as mean ± S.E.M.

Results

LCModel calculated the concentrations of MI, NAA, GLU, and GLN from our spectra successfully with a very high level of confidence (SD < 9%). The concentration of GABA was estimated with smaller but acceptable confidence (SD < 35%). Averaging of the spectra improved the confidence level of LCModel estimates to 5-7% for major metabolites and < 20% for GABA due to significantly increased SNR. The time course of MI and GABA during first 120 min after nicotine administration is shown in Fig.1. Repeated measures ANOVA revealed the significant decrease in both MI and GABA due to nicotine during this period. After 2 hours time point no significant differences in any metabolite were detectable between groups. The summary of time-averaged metabolite changes after nicotine challenge is presented in Table 1. Non-paired *t*-test indicated the significant differences between control and treated groups for MI and GABA.

Discussion

Clinical work has shown that MI levels within the anterior cingulate, as measured by MRS, decrease markedly in response to acute nicotine administration (unpublished observation). Current preclinical study also showed a significant drop in MI levels in the rats' *n.accumbens*, a structure chosen to be analogous to the anterior cingulate region in human. MI is an end product of the PI cycle and increased cellular activity leads to an accumulation of inositol monophosphates and subsequent depletion of MI [2]. We found an unexpectedly high amount of GABA in *n.accumbens* – about 10 times of what has been reported for the other parts of the brain in rats [3] and human [4]. Therefore it was possible for LCModel to estimate GABA concentration with relatively high confidence (SD < 20%). Moreover, we detected a reliable decrease in GABA levels due to nicotine, which also could be associated with rewarding properties of addictive compounds. However, the confidence level of GABA concentration estimates with LCModel is marginal, therefore these data should be treated with caution. The additional studies are needed to better understand the mechanism of MI and GABA involvement in the nicotine addiction and to validate the use of MI and possibly GABA as a potential addiction biomarker.

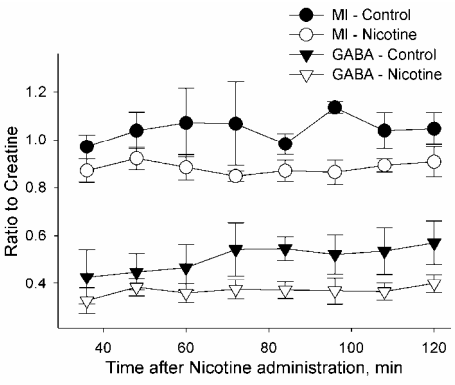

Figure 1. The time course of myo-inositol and GABA concentration in *n.accumbens* of rats subjected to single nicotine injection (1 mg/kg, SC).

References

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Table 1. The metabolite ratios to creatine from time-averaged spectra.

	MI	GABA	NAA	GLU	GLN
Control	1.04 ± 0.05	0.57 ± 0.08	0.98 ± 0.08	1.17 ± 0.04	0.75 ± 0.05
Nicotine	0.88 ± 0.03	0.39 ± 0.02	0.88 ± 0.01	1.12 ± 0.03	0.67 ± 0.02
P_{ANOVA}	0.037	0.043	0.104	0.496	0.257
P_T	0.0105	0.0122	0.0962	0.2596	0.1304

P_{ANOVA} – statistical significance between groups with repeated measures ANOVA
P_T – statistical significance between groups with *t*-test (time-averaged spectra)