Acute Flupirtine administration reduces glutamate/glutamine ratio in rat hippocampus

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Introduction

Potassium channel dysfunction has been correlated with numerous neurological as well as peripheral disorders like chronic pain, ataxia, epilepsy, Parkinson's disease, neuromyotonia, congenital deafness and long QT syndrome. The common underlying theme is related to neuronal hyperexcitability due to uncontrolled depolarization. The Kv7 class of potassium channels provides an important approach for developing novel therapies to target diseases which are thought to be caused by abnormal neuronal excitability. Endogenous neurotransmitters can serve as biomarkers of mechanism in this case, and could strengthen the confidence in rationale for such drug programs.

Presented here is a measure of glutamate to glutamine ratio (Glu/Gln) using magnetic resonance spectroscopy (MRS) as a noninvasive potentially translatable mechanistic biomarker for voltage gated potassium channel activators with the reference agent, flupirtine.

Methods

Animal handling and MRI/MRS procedures were approved by our local IACUC. Fourteen male SD rats (Charles River Laboratories, Wilmington, MA, 192 ± 21 g) were housed in plastic isolators with organic cellulose bedding. Animals received food and water ad lib and maintained on a 12 hr/12 hr light/dark cycle, with testing during the light phase. MRS was conducted on a BioSpec 7T/210AS MRI system (Bruker BioSpin, Billerica, MA) with 12 cm ID gradient insert (200 mT/m) and 38 mm litzcage transmit-receive volume RF coil (Doty Scientific, Columbia, SC) under general isoflurane anesthesia at a controlled rat core temperature (36.7 ± 0.9°C). The spectroscopic voxel (8×4×2 mm) was carefully positioned at hippocampus on a high-resolution fast spin echo (RARE) scout brain images. The magnetic field homogeneity in this voxel was manually adjusted to yield a FWHM of 10-16 Hz. Proton MRS was performed using PRESS localized sequence with VAPOR water suppression with the following parameters: TE = 16.2 ms, TR = 3 s, NS = 512. After the first base MRS scan, either vehicle (2 ml/kg, N = 8) or flupirtine (30 mg/kg, N = 6) was administered via a subcutaneous catheter, followed by dynamic scanning for 4 hours. Resultant spectra were analyzed using LCModel (1) and water was used as an internal reference. Time series data were analyzed using two way repeated measures ANOVA with SNK post-hoc tests (SigmaStat, Point Richmond, CA).

Results

LCModel output has consistently shown a SNR of 16. In particular, the %SD value for Glu and Gln was approximately 4% and 6%, respectively. There was a concomitant decrease in Glu (15%) and increase in Gln by 27%. The net decrease in Glu/Gln was 28% with a statistical significance of $p<0.05$, shown in figure 1. The ratio decreased significantly an hour after flupirtine injection and peaked during 2 - 3 hours showing about 28% decrease compared to baseline and vehicle group. Other metabolites, NAA, taurine and myo-inositol did not show significant differences in response to the drug.

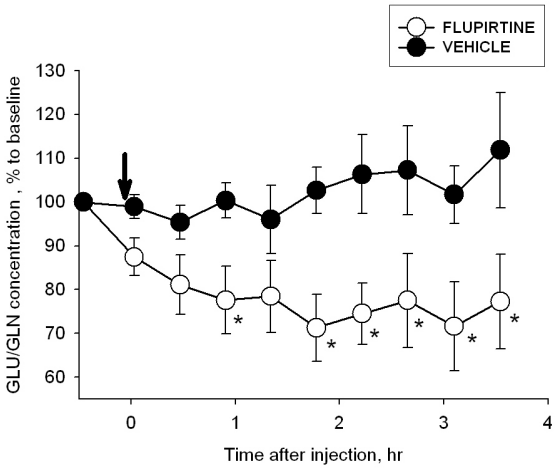

Figure 1. Effect of flupirtine on the GLU/GLN ratio in rat hippocampus. Rats were injected with vehicle (2 ml/kg, Vehicle (closed circles) or flupirtine (30 mg/kg, open circles) at a time 0 (black arrow). Data were normalized to the first spectrum and expressed as percent to baseline. Data are mean ± S.E.M.
* denotes the statistically significant differences between groups at $p<0.05$ (SNK post-hoc tests after two-way RM ANOVA)

Discussion

Flupirtine reduces neuronal excitability by hyperpolarizing the neuronal membrane thus decreasing the chance of neurotransmitter release (2). This is captured as a global reduction in Glu levels as measured by MRS. Glu, the major excitatory neurotransmitter, once released into the synaptic cleft by the neurons is rapidly taken up by the astrocytes and converted to Gln or oxidized. Since intra-astrocytic Glu concentration depends strongly on the extracellular Glu availability (3), inhibition of the release of Glu provides an explanation for the observed shift in equilibrium from Glu to Gln and provides for a robust mechanistic biomarker. The current study provides a proof of concept for the use of MRS to detect mechanism based changes in Glu-Gln cycling and this approach has the added advantage of being clinically translatable. Further validation and translational studies are warranted.

References

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