Acute Retigabine Administration Reduces Level of Glutamate in Rat Hippocampus

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Introduction

Many neurological disorders, such as epilepsy, migraine or chronic pain are characterized by neuronal hyperexcitability. Kv7 activators like retigabine, reduce neuronal excitability and have been shown reduce neurotransmitter release in the nervous system [1]. However it is not known if suppression of neurotransmitter release via Kv7 opening leads to global reduction of neurotransmitter levels. The Kv7 class of potassium channels provides an important target for developing novel therapies to target diseases which are thought to be caused by neuronal hyperactivity. The success of a new drug development can be greatly improved with the existence of robust translatable biomarker to assess efficacious drug exposure or mechanism of action in preclinical studies and in clinical trials. Here we present the utility of glutamate (GLU) measured with magnetic resonance spectroscopy (MRS) as a noninvasive, potentially translatable mechanistic biomarker for various nervous system disorders. Retigabine was used as a reference agent in this study and is currently in clinical trials being assessed for efficacy as a treatment of epilepsy.

Methods

Animal handling and MRI/MRS procedures were approved by our local IACUC. Sixteen male SD rats (Charles River Laboratories, Wilmington, MA, 206 ± 28 g) were housed in polycarbonate isolators with Sani-chips® 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 12 cm ID gradient insert (200 mT/m) and 38 mm litzcage transmit-receive volume RF coil (Doty Scientific, Columbia, SC) under the isoflurane general anesthesia at controlled rat core temperature (37.1 ± 0.6°C). Subcutaneous catheter was also inserted for the drug delivery. 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 10-14 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 retigabine (10 mg/kg, N = 8) was administered, followed immediately by 9 MRS scans (25 min each, 1-2 min apart). One water non-suppressed spectrum with the same parameters but NS = 8 was also acquired for each animal for water scaling and eddy current baseline correction in post-process. Resultant spectra were analyzed using LCModel [2]. Time series data were analyzed using two way repeated measures ANOVA with SNK post-hoc tests (SigmaStat, Point Richmond, CA). Data were normalized to the first basal spectrum and are presented as % mean ± S.E.M.

Results

LCModel output has consistently showed the SNR of 16 and %SD below 20 for estimation of creatine+phosphocreatine, GABA, GLU, glutamine (GLN), myo-inositol, NAA, choline and taurine in all spectra. Particularly, the %SD value for GLU was around 5% all the time. The effect of acute retigabine administration on GLU level over time was significant ($F = 5.172$, $P < 0.001$) and is shown in fig. 1. The total amount of GLU decreased significantly 1 hour after retigabine injection and reached a minimal value at 2 to 3 hours showing about 20% decrease compared to baseline and vehicle group. No other metabolite in the proton spectra showed significant response to the tested compound.

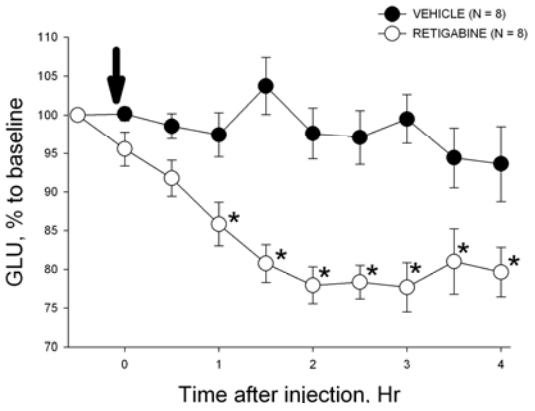

Figure 1. Effect of retigabine on the GLU concentration in rat hippocampus. Rats were injected with vehicle (2 ml/kg, closed circles) or retigabine (10 mg/kg, open circles) at time 0 (black arrow). Data were normalized to the first spectrum and expressed as percent to baseline. Data are mean ± S.E.M.
* denotes the statistically significant differences between groups (SNK post-hoc tests after two-way RM ANOVA)

Discussion

Increased cholinergic activity in vivo inhibits the M-current comprised of Kv7(2-5) potassium channel subunits, and increases the resting membrane potential of neurons thus reducing the excitation threshold required for generation of action potentials. At the synapse action potentials facilitate synaptically mediated neurotransmitter release. Retigabine shifts the voltage of activation for Kv7 channels to more hyperpolarized potentials thus decreasing the chance for action potential generation and in turn decreasing neurotransmitter release. GLU is one of the major excitatory neurotransmitters in CNS and has been shown to be elevated in neuropsychiatric disorders such as epilepsy [3]. GLU can be measured non-invasively with MRS in both humans and animals [4], which affords us with the opportunity to use it as a translatable mechanistic or potentially a disease biomarker for drug research in this area. This study provides proof of concept and a first step towards the establishing such a biomarker. Further validation and translational studies are warranted.

References

1. Sills GJ et al (2000). Pharmacol Res, 42: 553-7.
2. Provencher SW (2001). NMR Biomed, 14: 260-4.
3. van Gelder NM and Sherwin AL (2003). Neurochem Res, 28: 353-65.
4. Yang S, et al. (2008). Magn Reson Med, 59: 236-44.