Effects of SHISA7 on GABA$_A$ Receptors and Learning

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Introduction

The $\gamma$-aminobutyric acid type-A (GABA$_A$) receptor is a ligand-gated chloride ion channel. Its endogenous ligand is GABA, which is the major inhibitory neurotransmitter of the central nervous system. In addition, the GABA$_A$ receptor complex has allosteric binding sites for several other types of ligands that include drugs from classes such as the benzodiazepines and barbiturates. These drugs are positive GABA$_A$ modulators that can be used as pharmacotherapies for anxiety and insomnia.

The benzodiazepine alprazolam is a positive allosteric modulator of the GABA$_A$ receptor complex, because it promotes the binding of GABA at its binding site. Increased GABA binding produces increased neuronal inhibition and this inhibition, in turn, leads to anxiolytic/sedative effects. Alprazolam is one of the most prescribed benzodiazepines for treating anxiety. Flumazenil is a neutral modulator of the benzodiazepine binding site on the GABA$_A$ receptor complex and serves as an antagonist. Flumazenil can dose-dependently block the effects of positive allosteric modulators.

Methods

Sixteen mildly food-deprived rats were trained to respond under a repeated-acquisition baseline. Each rat acquired a four-response sequence on three keys under a second-order variable-ratio (VR) schedule of food reinforcement.

After responding under the repeated-acquisition baseline stabilized, the rats were administered varying doses of the benzodiazepine alprazolam to establish a dose-effect curve ranging from an ineffective to a maximally effective dose.

Using short hairpin RNA (shRNA) attached to a rabies virus glycoprotein, SHISA7 will be knocked down in the rats. Dose-effect curves will then be re-established in the rats, allowing us to determine whether SHISA7 plays a direct role in the effects of GABA$_A$ modulators such as the benzodiazepines on learning.

Results

Figure 2. Effects of Alprazolam on overall response rate and percent error in 8 male Long-Evans rats and 8 female Long-Evans rats responding under the repeated-acquisition baseline. Points indicated with asterisks indicate a significant difference between drug dose and vehicle as determined by two-way repeated measures ANOVA tests (CyD × Veh). Sex and dose were used as factors for the analysis.

Figure 3. Effects of Alprazolam (Alp) and pre-treatment Flumazenil (Flu) on overall response rate and percent error. Flumazenil vehicle, Flumazenil 3.2 mg/kg, or Flumazenil 10.0 mg/kg were administered 5 minutes prior to Alprazolam 10 mg/kg or Alprazolam vehicle. Statistical analysis was done via a 3-way ANOVA. Asterisks indicate a significant difference between drug dose and cycloexetrin vehicle as determined by two-way repeated measures ANOVA tests. No difference in sex, so we repeated the stats using a 2-way repeated measures ANOVA using pre-treatment Flumazenil and Alprazolam.

Conclusion and Future Directions

Prior to SHISA7 knockdown, Alprazolam (1-18 mg/kg) significantly and dose-dependently decreased response rate and increased percent error.

Using western blot analysis, we were also able to demonstrate that rats administered the SHISHA7 shRNA knockdown virus 24, 48, and 72 hours before sacrifice reduced the SHISA7 protein in the cerebellum, but there was little or no reduction of SHISHA7 in the frontal cortex compared to control.

Future research efforts will be focused on significantly reducing SHISA7 in the entire brain of the 16 Long-Evans rats, then re-administering the dose effect curves for those rats. If the dose-effect curves show significant changes after SHISA knockdown, these data would confirm the initial evidence indicating that this family of proteins is critical for the function of the GABA$_A$ receptor complex and its role in learning. Moreover, these results would that SHISA7 can alter the effects of drugs that bind to the GABA$_A$ receptor complex and can be used in future refinement of GABA$_A$ receptor therapeutics.

References