Effects of SHISA7 on $GABA_A$ Receptors and Learning

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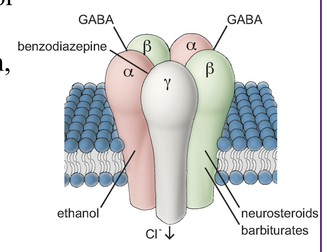
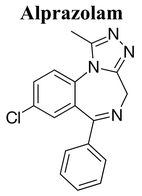
Introduction

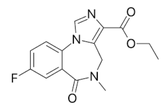
The γ-aminobutyric acid type-A ($GABA_A$) receptor is a ligand-gated chloride ion channel. Its endogenous ligand is GABA, which is the major inhibitory neurotransmitter of the central nervous system. In addition, the $GABA_A$ receptor complex has allosteric binding sites for several other types of ligands 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.



Alprazolam



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.



Flumazenil

Previous experiments have shown that genetically modifying the proteins around the $GABA_A$ receptor can also allosterically modify the effects of drugs that bind to this receptor complex, and thereby alter learning and memory. More specifically, recent data has shown that the deletion of SHISA7, a transmembrane protein that regulates the conformational activity of the $GABA_A$ receptor complex, leads to a decrease in the effects of benzodiazepines in mice.[1]

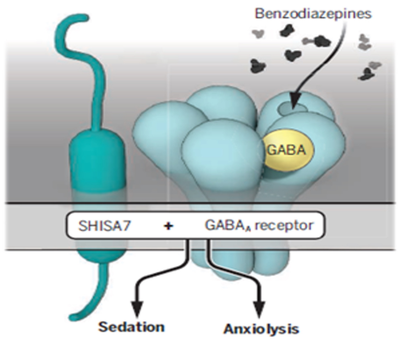
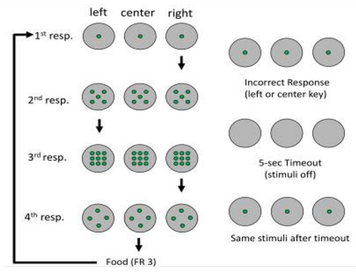


Figure 1. Model suggesting that SHISA7 is required for benzodiazepines to exert sedative and anxiolytic effects through $GABA_A$ receptors; the precise mechanisms remain unknown.[1]

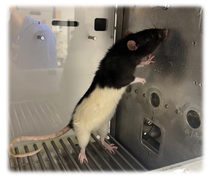
The Long-Evans rat is a common lab rat used in behavioral studies of drug-abuse related traits.

Methods

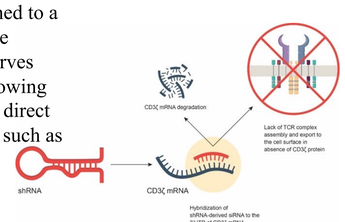
Sixteen mildly food-deprived rats were trained to respond under a repeated-acquisition baseline. Each rat acquire a four-response sequence on three keys under a second-order variable-ratio (VR) schedule of food reinforcement.



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

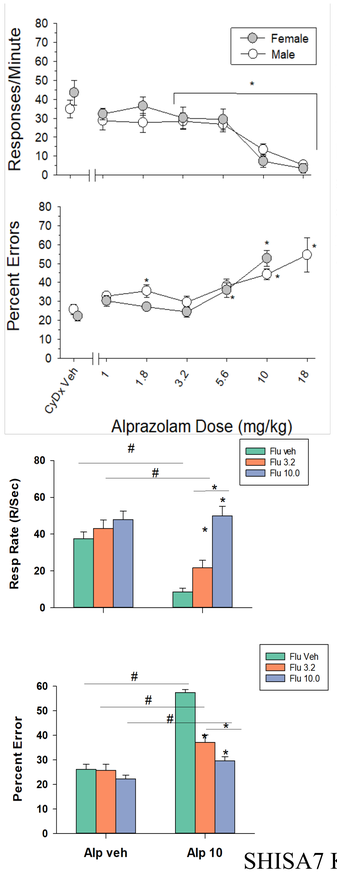
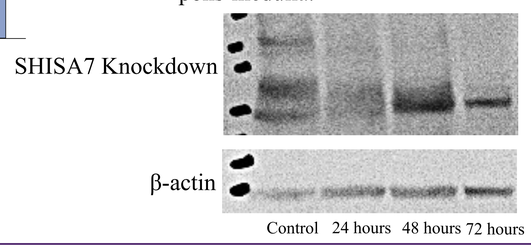


Figure 2. Effects of Alprazolam on overall response rate and percent error in 8 male Long-Evans rats and 8 female Long-Evans rats responding under the repeated-acquisition baseline. Points indicated with asterisks indicate a significant difference between drug dose and cyclodextrin vehicle as determined by two-way repeated measures ANOVA tests (CyD x 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 represents a significant difference between a given drug dose and 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

Figure 4. Representative immunoblots showing relative SHISA7 levels in the pons-medulla.



Effects of Alprazolam and Flumazenil on Acquisition Errors

Rat 1721

Alprazolam Vehicle & Flumazenil Vehicle (5a)

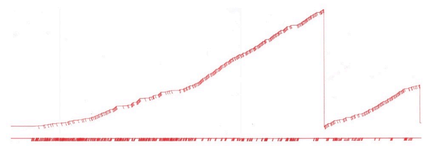


Figure 5a. Cumulative records showing the with-in sessions responding for rat 1721 during a vehicle (control) injection The response pen stepped up for each and was deflected downward for each completion of the four-response chain. Errors were indicated by the deflection of the event (lower) pen.

Flumazenil Vehicle & Alprazolam 10 mg/kg (5b)

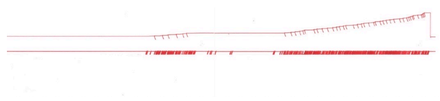


Figure 5b. Cumulative records showing the with-in sessions responding for rat 1721 during a Flumazenil vehicle & Alprazolam 10 mg/kg injection.

Flumazenil 10 mg/kg & Alprazolam 10 mg/kg (5c)

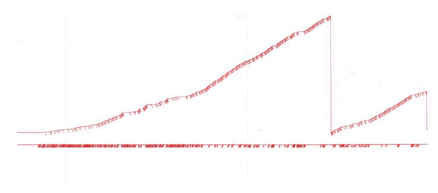


Figure 5c. Cumulative records showing the with-in sessions responding for rat 1721 during a Flumazenil 10 mg/kg & Alprazolam 10 mg/kg injection.

- Figure 5's results reflect those indicated in Figure 2 and Figure 3. Injection of Alp (10 mg/kg) shows significant reduction in response rate and an increase in percent error as compared to when rat 1721 was injected with vehicle.
- Injection of Flu (10 mg/kg) prior to Alp (10 mg/kg) shows no significant difference in response rate.

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 does-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

(1) Han W, Li J, Pelkey KA, Pandey S, Chen X, Wang YX, Wu K, Ge L, Li T, Castellano D, Liu C, Wu LG, Petralia RS, Lynch JW, McBain CJ, Lu W. Shisa7 is a $GABA_A$ receptor auxiliary subunit controlling benzodiazepine actions. Science. 2019 Oct 11;366(6462):246-250. doi: 10.1126/science.aax5719. PMID: 31601770; PMCID: PMC7382361.