$GABA_A$ α4 Receptors Mediate the Amnestic Effect of Isoflurane on Hippocampal-Dependent Learning

V. Rau[1], I. Oh[1], G. Homanics[2], M. S. Fanselow[3], E. I. Eger[1]

[1]Dept. of Anesthesia and Perioperative Care, University of California San Francisco, San Francisco, CA; [2]Depts. of Pharmacology & Chemical Biology and Anesthesiology, University of Pittsburgh, Pittsburgh, PA; [3]Dept. Of Psychology, University of California Los Angeles, Los Angeles, CA

INTRODUCTION

❖ Anesthesia blocks the formation of memories during surgery. $GABA_A$-Rs in the hippocampus have been shown to play a role in mediating the amnestic effect of inhaled anesthetics (Caraiscos et al., 2004; Sonner et al., 2005).

❖ Using Pavlovian fear conditioning, we assessed the amnestic effect of the inhaled anesthetic isoflurane on two mouse strains with genetically engineered mutations of the α2 (α2 knockin) and α4 (α4 knockout) subunit of the $GABA_A$-R.

- ❖The α2 knockin was genetically engineered to express two point mutations in the GABRA2 locus changing serine (S) to histidine (H) at position 270 and leucine (L) to alanine (A) at position 277 in the α2 subunit.
- ❖The α4 knockout strain was engineered to lack the α4 subunit by targeted disruption of the Gabra4 gene.

❖ Conditional freezing to context and conditional freezing to tone were evaluated in each strain. An intact hippocampus is necessary for fear to context but not for fear to tone. By observing whether the mutant strains show reduced suppression of fear to context but not fear to tone, we can assess the contribution of hippocampal $GABA_A$ α2 and α4 receptors to isoflurane-induced amnesia.

METHOD

❖ Mice were generated as previously described (Chandra et al., 2006; Sonner et al., 2007). Groups of mice (in each genotype and concentration) consisted of 8-13 animals.

❖ Training - Day 1

Prior to fear conditioning, mice were equilibrated to the desired concentration of isoflurane. After 30 min of equilibration, mice were quickly placed in the training chambers, which had been previously equilibrated to the respective concentration of isoflurane. After a 3-min baseline exploratory period in the chambers, mice received three (α2 knockin mice) or six (α4 knockout mice) pairings of tone (2000 Hz, 90 db) and shock (0.6 mA for α2 knockin mice, 1.0 mA for α4 knockout mice; 2 s) separated by 1 min. Different protocols were used to ensure that mutants and wildtypes had statistically similar freezing levels at 0% isoflurane.

❖ Testing - Days 2 and 3

The following two days, mice were tested for fear to context and fear to tone. For fear to context, mice were returned to the chamber in which they were trained for an 8 min test. For the tone test, mice were tested in a second context. They were given a 3-min exploratory period, then 6 30-s tone presentations (2000 Hz, 90 db), separated by 60 s, with no shock. The order of the context and tone tests was counterbalanced, such that half of each genotype was tested to context first and tone second and vice versa.

❖ Each animal's behavior was scored every 8 s during the observation period, and a percentage was calculated by dividing the number of freezing observations a mouse had by the total number possible during the observation period.

❖ ANOVA was used to analyze freezing scores, and Fisher's PLSD was used for preplanned group comparisons.

RESULTS and CONCLUSIONS

❖ Isoflurane significantly suppressed fear to context and tone in all mice (results of ANOVA shown in figure captions).

❖ There was no effect of genotype on either fear to context (Fig 1) or tone (Fig 2) for the α2 knockin mice. The α2 knockin mice were statistically higher than wildtypes at 0.2% isoflurane on the measure of fear to tone, but this is not likely a meaningful finding, as this trend did not continue for the other concentrations.

❖ α4 knockout mice showed resistance to the effect of isoflurane on context conditioning (Fig 3) compared to their wildtype counterparts, but did not show any difference in anesthetic sensitivity on cued fear conditioning (Fig 4).

❖ These results suggest that $GABA_A$-Rs containing the α4 subunit play a critical role in mediating the effect of inhaled anesthetics on hippocampal-dependent learning.

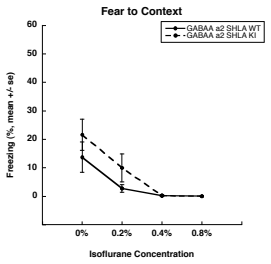

Fig. 1. Fear to context in α2 knockin mice. Isoflurane significantly suppressed context freezing, as shown by the ANOVA results, $F(3,56) = 10.95$, $P < 0.0001$.

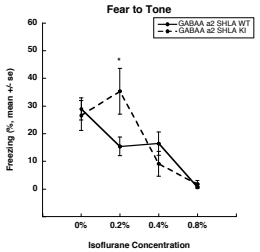

Fig. 2. Fear to tone in α2 knockin mice. The ANOVA revealed a main effect of isoflurane, $F(3,56) = 15.57$, $P < 0.0001$, and an isoflurane x genotype interaction, $F(3,56) = 3.70$, $P < .05$. Knockin and wildtype mice were statistically different at 0.2% isoflurane, $P < 0.0.5\%$.

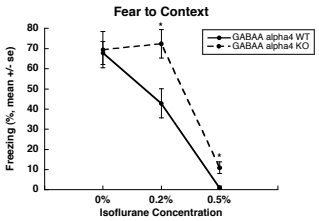

Fig. 3. Fear to context in α4 knockout mice. Isoflurane significantly suppressed context freezing, $F(2,48) = 47.65$, $P < 0.0001$, and the α4 knockout mice showed resistance to this suppression compared to wildtype mice $F(2, 48) = 6.41$, $P < 0.05$.

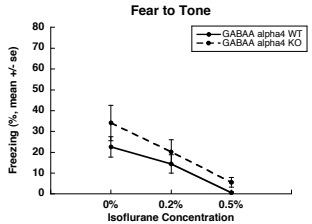

Fig. 4. Fear to tone in α4 knockin mice. The ANOVA revealed a main effect of isoflurane, $F(2,48) = 11.04$, $P < 0.0001$.

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Supported in part by NIH Grant 1P01GM47818.