Discovery of Novel Anesthetic Compounds using Zebrafish Larvae

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Background

Novel general anesthetic compounds for use in medicine can provide increased treatment options for patients undergoing invasive medical procedures such as extensive surgeries, cardiac catheterization, or organ biopsy. Both side effects of current anesthesia including vomiting, malignant hyperthermia, hypotension, and cognitive dysfunction as well as co-morbid patient health conditions including diabetes mellitus, hypertension, and obesity affect the choice of anesthetics used and considerably impact treatment outcomes. Recently, zebrafish larvae have emerged as a platform for high throughput screening of novel neuroactive compounds. Compared to the standard method of frog tadpole loss-of-righting-reflex assays, zebrafish experiments have many advantages: smaller anesthetic drug dosages, less required lab facility space, and faster drug screening ability.

Research Question

The purpose of this study is to conduct a high throughput screen of over 2,000 uncharacterized drug compounds for potential anesthetic properties.

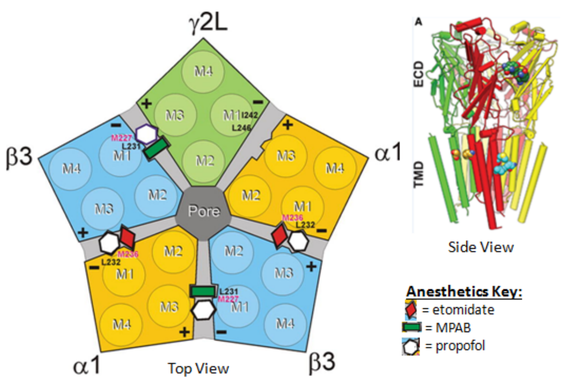

Fig 1: Anesthetic binding sites on GABA-A Receptor. Top view with select binding pockets for etomidate, MPAB, and propofol (left). MPAB = R-5-allyl-1-methyl-5-(m-trifluoromethyl-diazirinylphenyl) barbituric acid. Side view of receptor transmembrane section (top right). ECD = extracellular domain; TMD = transmembrane domain.

Materials/Methods

The photomotor response (PMR) of dark-adapted 7-day-old post-fertilization wild-type Tubingen strain zebrafish larvae exposed to a 0.2 second bright white light stimulus was analyzed using a specialized motion tracking video system (ViewPoint Zebralab). This bright light stimulus startles the zebrafish into a brief burst of movement. Each 96-well plate of zebrafish larvae was tested 10 times over a period of 30 minutes.

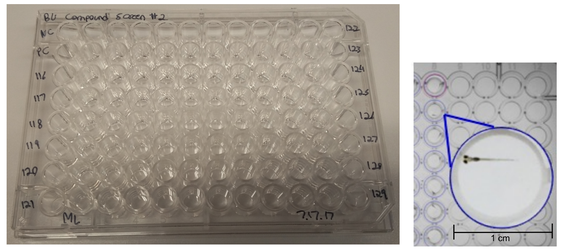

Fig 2: Prepared 96-well plate (left). One zebrafish is placed into each well of a 96-well plate and six zebrafish are initially tested with a small concentration of novel compound. Zebrafish size measurement (right). Zebrafish easily fit into the 1 cm well, spanning only about 0.5 cm in length as 7-day-old larvae.

Anesthetic effects of new compounds were quantified by calculating PMR inhibition, the decrease in movement after the stimulus. Sedative effects were quantified by comparing pre-stimulus basal activity inhibition, the normal zebrafish movement during periods of no stimulus, to anesthetized zebrafish basal activity.

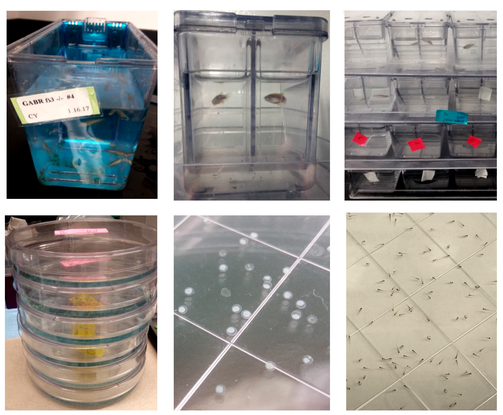

Fig 3: Steps to preparing a zebrafish assay. Top (left to right): A) Select a zebrafish tank for breeding; B) Separate male and female zebrafish into individual breeding tanks with dividers; C) Leave fish overnight with dividers and then remove dividers in the morning. Bottom (left to right): D) Zebrafish embryos from tanksare incubated at 30 degree Celsius; E) Fertilized zebrafish embryo plate is cleaned daily; F) 7-day-old zebrafish larvae are used for novel compound screens.

Results

Several prospective novel anesthetic compounds have been identified and more screens to assess the reversibility and potency of each drug are being performed. Reversibility is quantified by observing the ability of zebrafish to recover from the drug PMR inhibition overnight. Potency is measured by the IC50 of the novel compound compared to that of known anesthetics, such as etomidate.

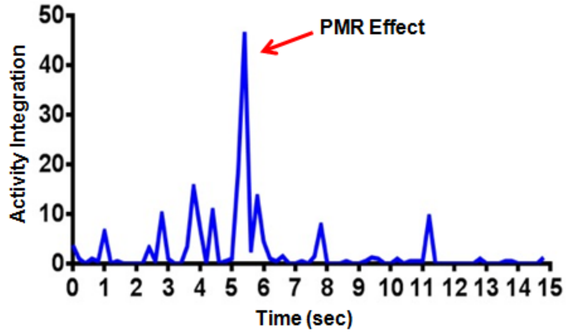

Fig 4: Photomotor response (PMR) inhibition. Basal activity shown throughout 15 seconds of Zebralab recording with increased movement surpassing threshold level due to a bright burst of light in un-anesthesized zebrafish shown at 5 seconds.

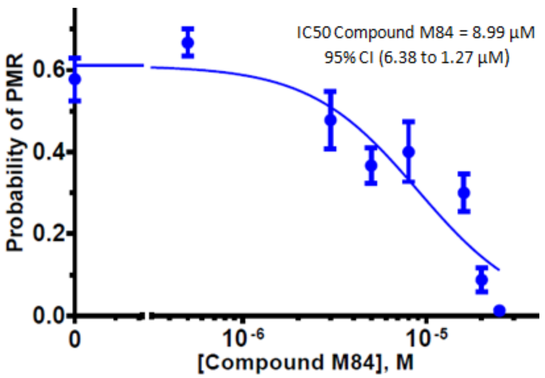

Fig 5: Compound 84 potency curve. As concentrations of Compound 84 increased, the level of PMR inhibition increased, as shown by the downward trend of the curve. Reversibility was also tested to rule out any toxicity effects.

Specifically, we discovered Compound 84 which shows a high potency (IC50 = 8.99 μM, 95% IC: 6.38 to 12.7 μM) and full reversibility. All other tested compounds did not demonstrate significant anesthetic ability compared to an etomidate control.

Results (Cont.)

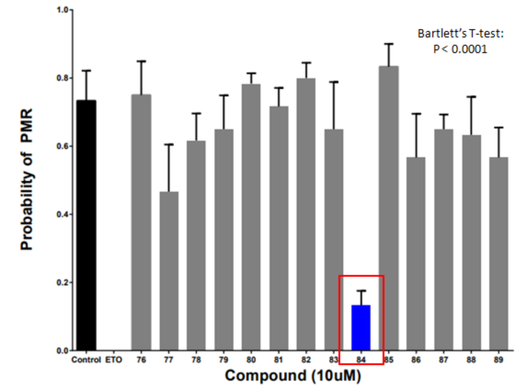

Fig 6: Compound 76-89 potency screen. Compound 84 demonstrates a much lower PMR level compared to other unknown compounds tested. Negative control (labeled "control") was tested with only DMSO and positive control (labeled "ETO") was tested with etomidate, a known anesthetic.

Conclusion

Zebrafish photomotor response is a promising method for high throughput identification of novel anesthetics. Overall, we aim to reduce toxic side effects of various drug combinations and to exploit the beneficial properties of anesthetic drugs to improve treatment efficacy.

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