Teasing Apart the Desired Effects of Anesthetics from Unwanted Side Effects at $GABA_A$ Receptors

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Background: GABA receptors are important molecular targets for anesthetics, but it remains unclear how these receptors are involved in producing unconsciousness or contributing to unwanted side effects. Our group has developed new anesthetic agents that specifically target $GABA_A$ slow receptors. Using *in silico* docking-based screening algorithms, a novel molecular core was designed to target the same binding site as etomidate and propofol. Structures were designed to eliminate etomidate's side effect causing adrenal suppression. A series of compounds was generated by the program and the top ten compounds were first tested on tadpoles, then in rat brain slice electrophysiology experiments. Finally, *in vivo* experiments were conducted with propofol and the most promising experimental drug of the series, BB, to compare their hemodynamic effects.

Methods: For the *in vivo* studies, the compounds where pipetted into the amphibians' water and rats were administrated drugs by intravenous bolus. For electrophysiology, rat brain slices were incubated for at least two hours prior to experiment, and submerged in artificial cerebrospinal fluid (ACSF). In the hippocampal CA1 area, bipolar tungsten stimulating electrodes were placed to evoke field potentials via Schaffer-collateral fiber inputs. Paired-pulse population spike (PS) responses were recorded using a microelectrode placed near the pyramidal cell body. Control recordings were acquired with brain slices before and after a test compound was added to the ACSF. Additionally, we compared the experimental compound's actions with those produced by propofol and etomidate, agents known to selectively increase $GABA_AR$-mediated inhibition. We used picrotoxin, a chloride channel blocker to probe $GABA_AR$ involvement in effects.

Results: When exposed to BB, tadpoles quickly lost consciousness then fully recovered. When BB was injected into rats, the rodents also lost consciousness and recovered fully. Also, their heart rate and blood pressure were considerably more stable compared to propofol effects. Our electrophysiology results show that etomidate and BB produced a reversible enhancement of GABAAR-mediated slow inhibition that was more selective than propofol. All of BB's effects occurred by acting specifically on $GABA_A$-slow receptors, like etomidate. Propofol, in contrast, clearly enhanced other forms of $GABA_AR$-mediated inhibition (e.g. fast and tonic receptors). The effects of all agents were fully reversed by picrotoxin.

Conclusions: The experimental compound BB had an anesthetic effect via $GABA_AR$s as predicted. It was more selective than propofol, which acted mainly on $GABA_A$-fast, followed by -tonic and slow receptors, instead of just the $GABA_A$-slow receptors that were most sensitive to our experimental compounds. Thus, both etomidate and our experimental compound BB demonstrated the same $GABA_AR$ selectivity, which correlates with the observed decreased in undesirable hemodynamic side effects for both compounds compared to propofol.