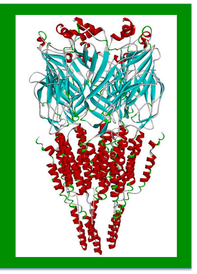
# Characterization of ibogaine analogs on the h$\alpha_3\beta_4$ nicotinic acetylcholine receptor.

Ross, Jordan
University of Alaska Fairbanks, Department of Chemistry and Biochemistry



## Introduction

Nicotine addiction is a global health problem that affects nearly one-third of the population. Animal models have shown that the beta-4 subunit of nicotinic acetylcholine receptors (nAChR) expressed in the habenulo-interpeduncular pathway plays a particularly important role in modulating many of the symptoms of nicotine withdrawal in mice. Ibogaine, a naturally occurring compound extracted from the root bark of a West African shrub, has been shown to reduce drug self administration in animal models of addiction. Ibogaine is considered to be a dirty drug due to its nonspecific interaction at a variety of receptor subtypes. This "nonspecificity" contributes to its hallucinogenic, tremorigenic, and cardiovascular compromising properties. It is thought that the anti-addictive effects of ibogaine are due to its antagonism of the $\alpha_3\beta_4$ nAChR. In this study we explore the interaction of ibogaine on the $\alpha_3\beta_4$ nAChR with the hope of developing more selective, more effective therapeutics in the treatment of addiction.

## Materials/Methods

*Xenopus Laevis* oocytes were injected with mRNA coding for the human $\alpha_3\beta_4$ nicotinic acetylcholine receptor, and incubated for 24-36 hours. Electrophysiological recordings were done using a technique known as two electrode voltage clamp. Compounds were classified according to their IC/$EC_{50}$ values, the concentrations required to inhibit a response by 50%, or elicit a 50% of maximum response.

Definitions:

Agonist: A compound that elicits a physiological response when it binds to its specific receptor.

Antagonist: A compound that interferes with the physiological action of an agonist.

## Results

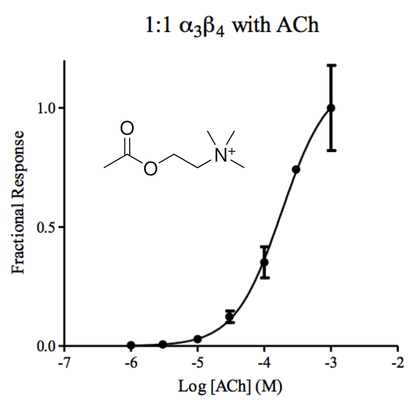



**Fig 1. ACh dose response curve.** The endogenous agonist acetylcholine was determined to have an $EC_{50}$ of 182±1.5 µM at the h$\alpha_3\beta_4$ nAChR. Each point represents N=4 replicates.

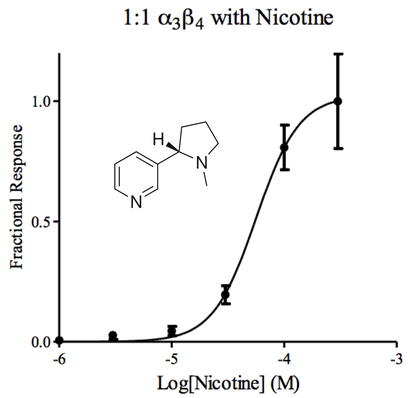



**Fig 2. Nicotine dose response curve.** The partial agonist nicotine was determined to have an $EC_{50}$ of 56.2±1.2 µM at the h$\alpha_3\beta_4$ nAChR. Each point represents N=4 replicates.

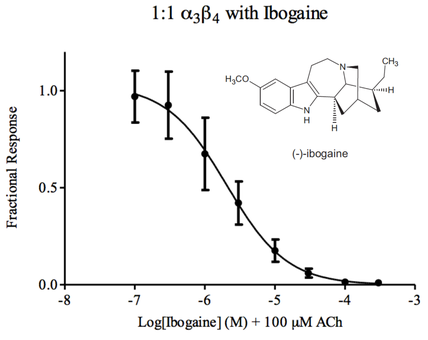



**Fig 3. Ibogaine inhibition curve.** Increasing ibogaine concentrations inhibit 100% of 100 µM ACh induced ion flux. Ibogaine was determined to have an $IC_{50}$ of 2.0±1.1 µM at the h$\alpha_3\beta_4$ nAChR. Each point represents N=4 replicates.

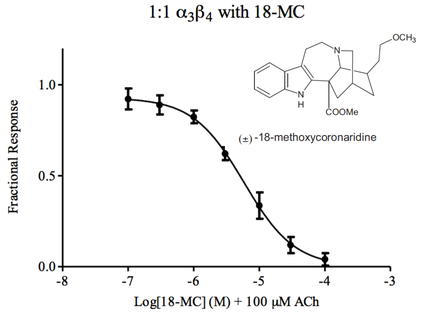



**Fig 4. 18-MC inhibition curve.** Increasing 18-MC concentrations inhibit 100% of 100 µM ACh induced ion flux. 18-MC was determined to have an $IC_{50}$ of 5.8±1.1 µM at the h$\alpha_3\beta_4$ nAChR. Each point represents N=4 replicates.

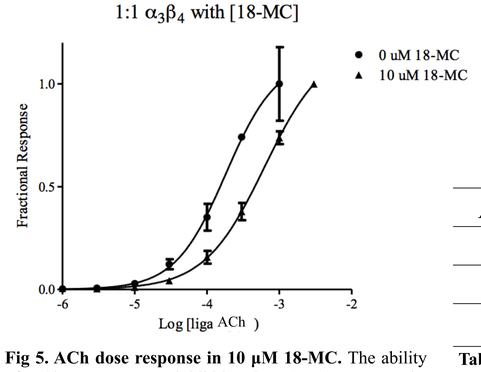



**Fig 5. ACh dose response in 10 µM 18-MC.** The ability of ACh to overcome inhibition by 18-MC suggest the possibility of a competitive mode of inhibition. In the presence of 10 µM 18-MC ACh $EC_{50}$ value is ~ 3.45 times larger (625±1.2 µM) than in the absence. Each point represents N=4 replicates.

| Ligand | | |
|---|---|---|
| Acetylcholine | $EC_{50}$ µM | 181.7 |
| Nicotine | | 56.2 |
| Ibogaine | $IC_{50}$ µM | 2.0 |
| 18-MC | | 5.8 |

**Table 1.** Summary of Results

## Discussion

$EC/IC_{50}$ values obtained are in accordance with those found in the literature. Ibogaine and its derivative 18-MC were both found to inhibit 100% of acetylcholine induced ion flux through the h$\alpha_3\beta_4$ nAChR. Evidence suggest that antagonism of the h$\alpha_3\beta_4$ receptor plays a particularly important role in mediating withdrawal symptoms in the habenulo-interpeduncular pathway. 18-MC retains the same high affinity for this receptor subtype that is required to be an effective therapeutic.

It is currently thought that ibogaine inhibits the h$\alpha_3\beta_4$ nAChR in a noncompetitive manner. Preliminary evidence shows that increasing ACh concentrations is able to overcome 18-MC inhibition (Fig 5), suggesting a competitive mode of inhibition. This data is not conclusive however, further experiments must be conducted to determine the voltage dependence of ibogaine binding.

Future Work:

- Determine Ibogaine/18-MC binding site
- Explore the voltage dependence of Ibogaine and 18-MC so as to determine channel block.

## Major Findings

- Ibogaine and 18-MC inhibit 100% of agonist induced ion flux.
- Ibogaine has an $IC_{50}$ of 2.0±1.1 µM
- 18-MC has an $IC_{50}$ of 5.8±1.1 µM
- High affinity at the $\alpha_3\beta_4$ nAChR suggests a particularly important role for this receptor in addiction.

## Acknowledgements

This research was supported by the IdEA Network of Biomedical Research Excellence (INBRE) through an undergraduate student project support (USPS) fellowship. Thanks to Dr. Marvin Schulte for his wisdom and guidance as I continue to work on this project.