Pharmacological Characterization of a Primary Nicotinic Receptor from the Neuromuscular Junction

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Andrew Cook[1], Muthukrishnan Renganathan, Ph.D.[1], Dazhi Xiong, Ph.D.[1], Cassidy Kilpack[1], Ali Yehia Ph.D.[2], Bryan Koci[1]
[1]Eurofins Panlabs I St. Charles, MO 63304, [2]Fluxion Biosciences I Alameda, CA 94502

Abstract

Nicotinic receptors at the neuromuscular junction are important targets in the study of autoimmune disorders such as myasthenia gravis. Historic efforts targeting nAChR drug discovery have focused on fluorescence-based calcium flux assays, along with low-throughput manual patch clamp techniques due to their dependence on complete liquid exchange.

With the advent of next-generation automated patch champ instruments with integrated solution control and rapid-solution change, extensive pharmacological characterization of the neuromuscular nAChR ((α1)2β1γδ) is now possible. This study aims to develop a sensitive and stable screening assay in order to profile the activities of new chemical entities against the main nAChR involved in myasthenia gravis. Data from several known agonists and antagonists will be presented.

Methods

We utilized a stable recombinant HEK293 cell line expressing human nAChR α1/β1/δ/ε ion channels, to investigate the pharmacological profile on the IonFlux HT automated patch clamp platform.

Agonist and antagonist assays were both performed at -60 mV holding potential. All antagonist compounds were pre-incubated, then co-applied with acetylcholine EC_{80} up to a maximum of three concentrations per experimental pattern. The agonist assay allows six additions per experimental pattern. Applications or co-applications of acetylcholine were standardized, using a 2- second duration across both assay modalities.

Agonist Activity for Two Compounds on nAChR α1/β1/δ/ε

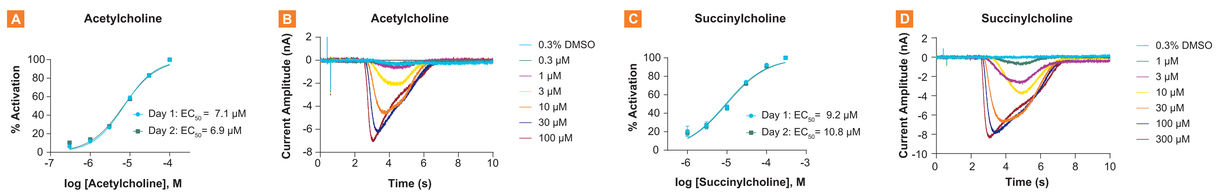

Figure 1. Agonist Assay reproducibility on 2 experimental days. A. Concentration response curves generated for the reference compound acetylcholine on 2 different experimental days. Error bars represent SEM. B. Sample current traces illustrating the a concentration response for Acetylcholine. C. Concentration response curves generated for the reference compound succinylcholine on 2 different experimental days. Error bars represent SEM. D. Sample current traces illustrating the concentration response for succinylcholine.

Inhibition of nAChR α1/β1/δ/ε Using Toxin Compounds

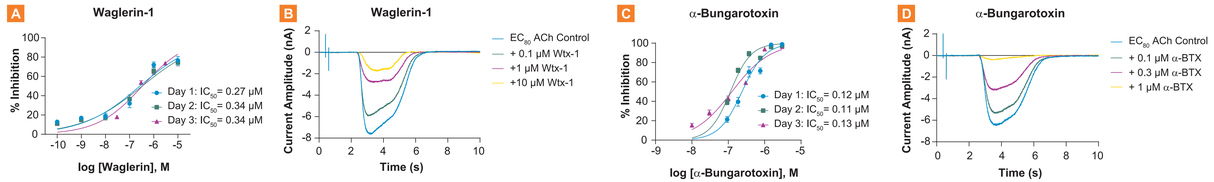

Figure 2. Inhibition of Current using toxin reference controls. A. Concentration response curves generated for Waglerin (Wtx-1) over 3 experimental days. Error bars represent SEM. B. Sample current traces illustrating concentration-dependent current inhibition by Wtx-1. C. Concentration response curves generated for α-Bungarotoxin (α-BTX) over 3 experimental days. Error bars represent SEM D. Sample current traces illustrating concentration-dependent current inhibition by α-BTX.

Antagonist Data for Muscle Relaxant Compounds

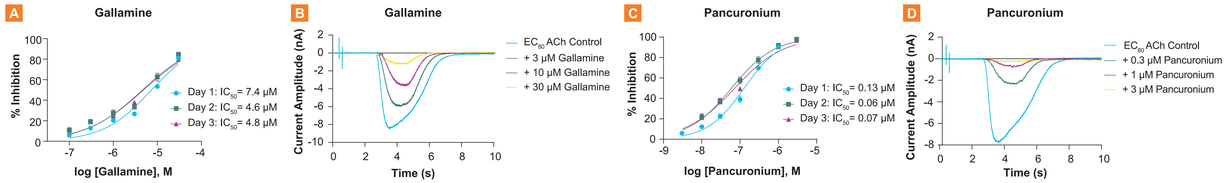

Figure 3. Inhibition of nAChR α1/β1/δ/ε current using muscle relaxant reference compounds. A. Concentration response curve generated for the reference compound Gallamine over 3 experimental days. Error bars represent SEM. B. Sample current traces illustrating the concentration dependent current inhibition by Gallamine. C. Concentration response curves generated for the reference compound pancuronium over 3 experimental days. Error bars represent SEM. D. Sample current traces illustrating the concentration dependent current inhibition by gallamine.

Antagonist Data for Two Additional Compounds

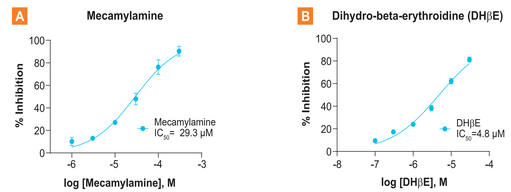

Figure 4. Antagonist effects of two compounds. A. Concentration response curve generated for the reference compound mecamylamine. Error bars represent SEM. B. Concentration response curve generated for the reference compound dihydro-beta-erythroidine (DHβE). Error bars represent SEM.

Results Table

Compound	Experimental Mode	Observed EC_{50}/IC_{50} Range
Acetylcholine	Agonist	6.9 µM to 7 µM
Succinylcholine	Agonist	9.2 µM to 10.8 µM
Waglerin-1	Antagonist	270 nM to 340 nM
α-bungarotoxin	Antagonist	110 nM to 120 nM
Gallamine	Antagonist	4.6 µM to 7.4 µM
Pancuronium	Antagonist	60 nM to 130 nM
Mecamylamine	Antagonist	29 µM
DHβE	Antagonist	4.8 µM

Summary

By utilizing the IonFlux HT instrumentation, we developed and validated multiple high-throughput assays for compound screening on human nAChR α1/β1/δ/ε ligand-gated ion channels in both agonist and antagonist modes. The microfluidic rapid solution exchange, ligand wash capabilities and reproducibility of pharmacology over multiple days, make the IonFlux an ideal tool to screen compound libraries for putative drug candidates on nAChR α1/β1/δ/ε receptors.

References

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