Application Note

Activation of nAChR receptors expressed in TE671 cells on the Patchliner^{e}

Ion Channels:
nAChR $\alpha 1\beta 1\gamma\delta$

Cells:
TE671

Tools:
Patchliner^{e}

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Summary

Nicotinic acetylcholine receptors (nAChR) are acetylcholine (ACh) and nicotine-gated cation permeable ion channels, which mediate fast synaptic transmission at central synapses and neuromuscular junctions. Neuromuscular nAChR form heteromeric proteins composed of four subunits: α, β, γ (or ε) and δ. Depending on the developmental stage, the AChR subunit stoichiometry changes from α1β1γδ (embryonic) to α1β1εδ (adult)[1]. Several inherited and acquired diseases are associated with nAChR dysfunction, most of which lead to impaired neuromuscular transmission and muscle weakness. The acquired autoimmune disease myasthenia gravis (MG) is caused by autoantibodies targeting muscle nAChRs[2] that disrupts nerve-muscle communication resulting in muscle weakness and fatigue [3,4]. Inherited diseases called congenital myasthenic syndromes (CMS) are associated with several abnormalities affecting ACh-release, acetylcholinesterase activity, nAChR function and/or nAChR number [5,6,7]. Treatment has been limited to nonselective, chronic immunosuppressive therapies which have long-term toxicities. More selective and targeted therapies are now under development [6].

Here we present data collected on the Patchliner^{e} showing activation of nAChRα1β1γδ expressed in human TE671 cells with rapid application of ligand. We found that ACh activates nAChRα1β1γδ receptors with an EC_{50} value similar to those reported in the literature[8]. Data was acquired using the PatchlinerControl software developed for the Patchliner^{e} and analyzed using DataControl PL, the analysis software for the Patchliner^{e}. This software enables concentration response curves to be constructed at the click of a button and data to be pooled and averaged from multiple NPC-16 chips.

Results

We used TE671 cells that endogenously express nAChR α1β1γδ subunit combination. Acetylcholine (ACh) was used to activate the current responses. Solutions were stacked inside the pipette of the Patchliner^{e} to minimize exposure time to the ligand, thus making multiple additions of ligand to each well possible. In addition, multi-hole chips with 4 holes per well were used to increase current amplitudes and maximize success rate. The pipetting speed of application can be adjusted on the Patchliner^{e} along with the ligand volume. In these experiments, exposure time was 175 ms. Using DataControl PL, concentration response curves can be constructed with just a few mouse clicks and data can be pooled from multiple chips and days. The individual concentration response curves (CRC) for each well are constructed and displayed, allowing manual inspection of raw data traces and CRCs (Figure 1). An average

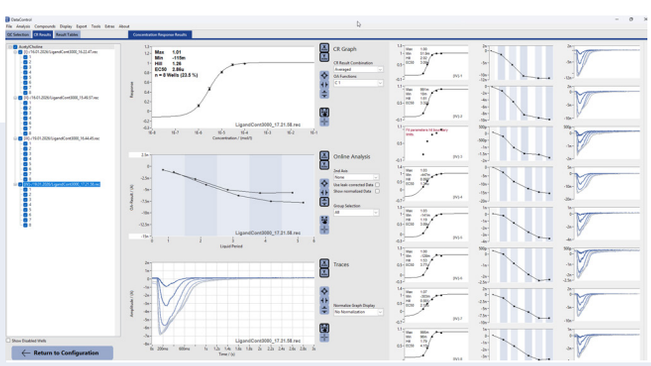

Figure 1: *The analysis software for the Patchliner^{e}, DataControl PL. Data can be loaded and pooled from multiple chips. Concentration response curves are constructed and displayed and data can be exported as graph files and data spreadsheets.*

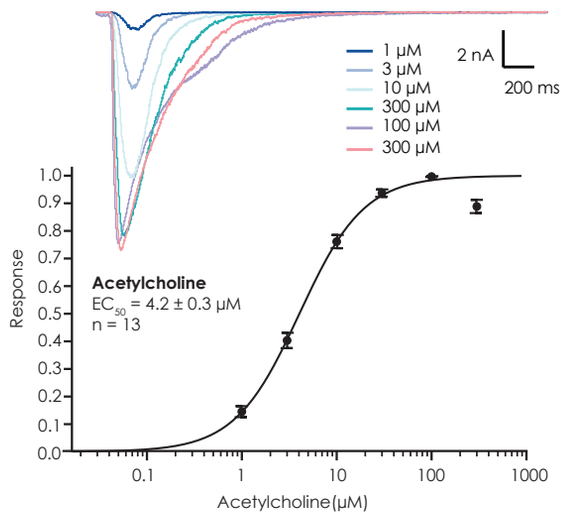

Figure 2: *nAChR expressed in TE671 cells was activated by increasing concentrations of ACh. Shown is the CRC for ACh for an average of 13 wells and the corresponding traces from an example cell.*

CRC is also constructed and can be exported in various file formats.

The average concentration response curve for ACh activation of nAChR α1β1γδ is shown in Figure 2 with example traces also displayed in the inset. The average EC_{50} for ACh was 4.2 ± 0.3 µM (n = 13 wells) in excellent agreement with the literature [8].

In summary, the Patchliner® is an ideal tool to study ligand-gated ion channels such as nAChR expressed in TE671 cells. Small volumes, precise timing of solution addition and high success rates ensure efficient investigation of ligand-gated ion channels (LGIC) modulators including activators, potentiators and inhibitors.

References

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Methods

Cells

TE671 cells endogenously expressing nAChR α1β1γδ (CLS; #300355) were used.

Electrophysiology measurements

Cells were cultured and harvested according to Nanion's standard protocols. Cells were resuspended in Nanion's external recording solution (#08 3001) and stored in the CellHotel of the Patchliner® before being dispensed into each well of the NPC-16 chip. Nanion internal (#08 3008) and external solution (#08 3001 or #08 3004) compositions are available upon request. Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner®. To activate nAChR, ACh was applied using the stacked solution approach and rapidly applied to the cells at a constant holding potential of -100 mV. Data was acquired using PatchlinerControl and analyzed using DataControl PL.

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