SOPHION BIOSCIENCE

Application Report

Large molecules on QPatch: IgG antibodies against α-cobratoxin as novel antivenom

Evaluation of engineered antibodies neutralizing α-cobratoxin-mediated inhibition of the nicotinic acetylcholine receptors using automated patch-clamp

Summary

To determine if engineered IgG anti-α-cobratoxin antibodies would prevent the blocking of the nicotinic acetylcholine receptor, a protocol was set up using QPatch II. Here we show that:

- QPatch II is well suited for efficiently characterizing both peptide toxins and antibody function.
- Current induced by adding acetylcholine could be abolished completely using α-cobratoxin, and that this effect could be reversed by preincubating the toxin with different IgGs before injection.
- Prevention of current inhibition by the IgGs was dose-dependent, and the potency of different IgGs could be determined using this method.
- Increased affinity between the IgGs and α-cobratoxin resulted in higher neutralization potency.

Introduction

Large molecules in Automated patch clamp

Large molecules have gained attention due to their mode of action, often achieving greater target specificity and potency than small molecule drugs. This, however, comes at a cost: often, they are expensive and scarce and can be subject to unwanted polyreactivity ("stickiness"). In addition, they are more sensitive to their environment, as their three-dimensional structure is key to their function and relies on other, weaker interactions than covalent bonds.

While automated patch clamp (APC) systems have been used for small molecule drug discovery and characterization for the past 20 years, the use of APC systems for large molecule characterization has been limited.

Novel antivenom: human monoclonal IgG antibodies against α-cobratoxin

Every year 2.7 million people are bitten by venomous snakes resulting in hundreds of thousands of permanent disabilities and deaths (1). The only available treatment for snakebite envenoming is traditional antivenom, based on animal-derived serum.

A novel type of antivenoms currently under development is based on recombinant human antibodies, engineered to bind neurotoxins with high affinity and thereby neutralize the toxic effects.

The purpose of this study was to investigate the in vitro neutralization potential of engineered antibodies targeting 8KDa α-neurotoxins, one of the most medically relevant groups of snake toxins. α-neurotoxins are antagonists of the nicotinic acetylcholine receptor (nAChR) and prevent acetylcholine-based signal transduction (2, 3).

Here we evaluated if the engineered increase in affinity between the recombinant antibodies and α-cobratoxin (an α-neurotoxin from Naja kaouthia) would translate to improved neutralization potential in an electrophysiological nAChR assay, run on QPatch II.

Results and discussion

Setting up the assay

First, the appropriate concentration of Ach required to activate 80% (EC_{80} value) of the nAChR channels in the cell line used for the study was determined using 3-fold dilutions ranging from 10mM to 50nM. The recorded currents are shown in Fig. 1 A, and a dose-response curve was generated based on the peak current (see Fig. 1 B). From the dose-response curve, an EC_{80} value of 69.4 µM (±9.2 µM) was determined; hence, 70 µM was used for the following experiments. Six repetitions of antagonist addition confirmed the stability of activation using this concentration. The resulting sweeps and average peak currents are shown in Fig.1 C and D.

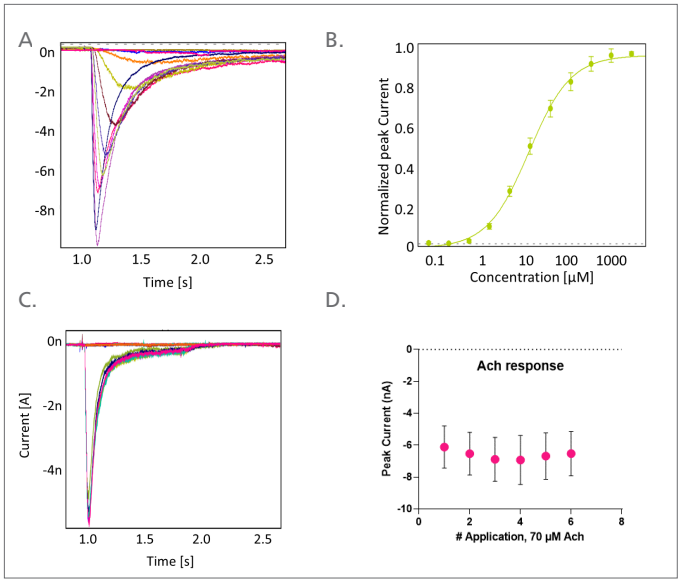


Fig. 1: 12-point acetylcholine concentration-response: (A) Sweep plot and (B) dose-response curve showing how increasing concentrations of ACh increases the measured current running across the cell membrane. 70 µM ACh was used throughout the rest of the experiments. (C) Sweep plot and (D) average of six ACh additions, demonstrating the stability of the assay (100% success rate, no run-down or desensitization).

Validating the target and determining toxin concentration

Next, we needed to determine if we could inhibit the nAChR using α-cobratoxin. We added 10-fold dilutions of α-cobratoxin ranging from 10 µM to 10 nM. The results showed that α-cobratoxin did indeed completely prevent the ACh response at the highest tested concentrations. The sweep plot and resulting dose-response curve are shown in Fig. 2A and Fig. 2B, respectively. The IC_{80} value was determined from the dose-response plot to be 3.6 nM (± 0.2 nM) α-cobratoxin. Hence a concentration of 4 nM was used for all following experiments.

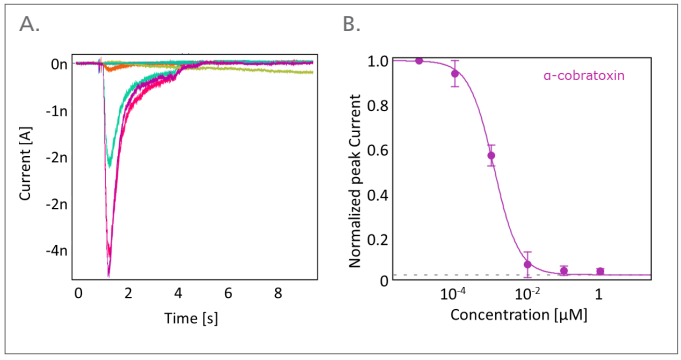


Fig. 2: Concentration-response for α-cobratoxin: (A) Sweep plot and (B) dose-response curve showing how increasing concentrations of α-cobratoxin result in a decrease in the current measured. 4 nM α-cobratoxin was used, resulting in approximately 80% current inhibition.

Evaluation of the neutralizing effect of eight IgG antibodies

A range of different IgGs was tested for their ability to neutralize the current-inhibiting effect of α-cobratoxin. Different concentrations of the IgG candidates were preincubated with α-cobratoxin before addition to determine their resulting dose-responses.

As seen in Fig. 3 C and D, the IgGs successfully prevented the current-inhibiting effect of α-cobratoxin in varying degrees. As a control, an irrelevant IgG, not binding α-cobratoxin, was included and showed no prevention of the current-inhibiting effect of α-cobratoxin ("Sham" in Fig. 3D).

The IgGs' neutralizing effect was concentration-dependent, and the IC_{50} values for each IgG could be determined. This made it possible to rank the IgGs according to their neutralizing effect (see Table 1).

Previously, the binding kinetics and affinity of the antibodies to α-cobratoxin were determined using Surface Plasmon Resonance. A correlation between the affinity and the IC_{50} value determined in the electrophysiological evaluation was seen (data not shown).

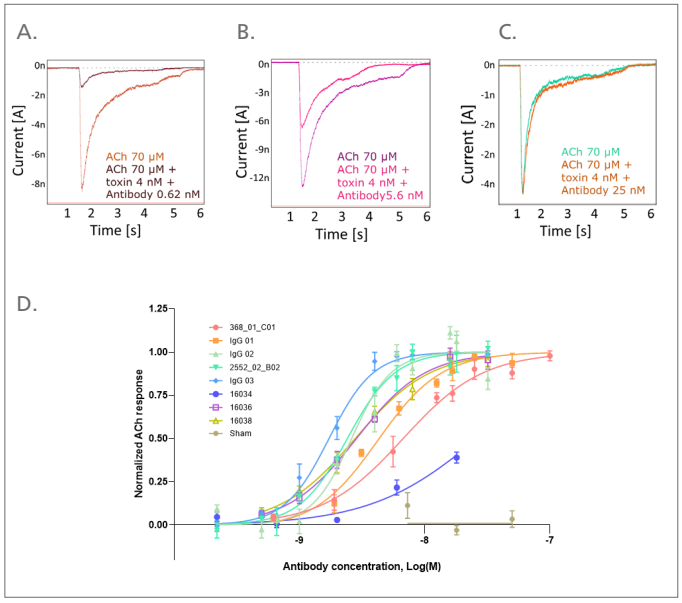
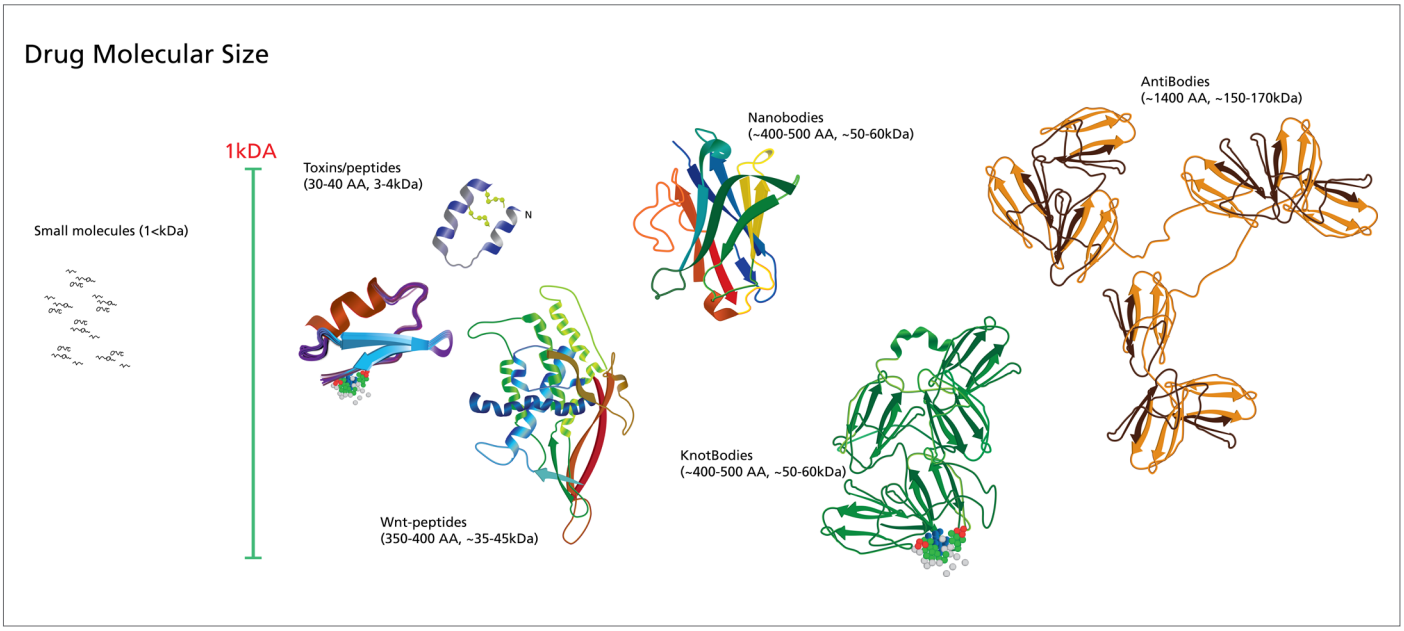


Fig. 3: (A-C) Sweep plots of representative traces showing the effect of increasing concentrations of the 368_01_C01 antibody (D) dose-response curve showing how increasing concentrations of the 8 IgGs preincubated with α-cobratoxin result in increased prevention in the loss of current mediated by α-cobratoxin. An irrelevant IgG was used as a control ("Sham"), which did not prevent the inhibitory effects of α-cobratoxin.

Table 1: Ranking of the eight IgGs according to their neutralizing potential. The neutralizing potential is reflected in the IC_{50} value, the antibody concentration needed to inhibit the blocking effect of 4 nM α-cobratoxin. 95% confidence intervals for the IC_{50} value are reported in the last column.

IgG	IC_{50}	95% CI
IgG_03	1.7 nM	(1.4 to 2.0)
2552_02_B02	2.4 nM	(2.1 to 2.7)
IgG_02	2.6 nM	(2.2 to 3.1)
16038	2.8 nM	(2.0 to 3.8)
16036	2.9 nM	(2.2 to 3.6)
IgG_01	4.2 nM	(3.5 to 5.1)
368_01_C01	6.7 nM	(4.8 to 9.3)
16034	26 nM	(18 to 37)



Methods

Cells

- TE/RD cells endogenously expressing nAChR were cultured at 37°C and 5% CO2 in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and streptomycin, and two mM sodium pyruvate. Cells were purchased from ATCC.
- On the day of the experiment, cells were harvested using detachin and kept in serum-free media until further use. The QPatch automated cell preparation unit was used to resuspend cells in saline just before the start of the experiment.

Pharmacology

- nAChR mediated current was elicited by adding 70 µM acetylcholine (ACh) (Sigma-Aldrich), approximately the EC_{80} value.
- After compound wash-out, 2 U acetylcholinesterase (Sigma-Aldrich) was added to ensure complete ACh removal.
- The ACh response was allowed to stabilize over three additions. Third addition was used as a baseline recording, and the fourth addition to evaluate the toxin: antibody effect.
- The antibodies were incubated with 4 nM α-cobratoxin for at least 30 min before addition.
- Cells were preincubated with 4 nM α-cobratoxin ± antibody for at least five min before ACh addition.

Electrophysiology

- Whole-cell access was gained using a single pressure pulse to -250 mbar for 1 s. Cells were afterwards clamped at V_{hold} = -90 mV and -10 mbar.
- All experiments were performed using multihole QPlates (10 holes/well).
- Data were analyzed using Sophion's Analyzer and Prism (GraphPad) software. Data are represented as mean ± SD.

References

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