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Application Report

High throughput electrophysiological screen of antibody-based antivenom on Qube 384

Large molecules: evaluating the neutralization potential of recombinant antibodies against snake neurotoxins

Summary

- Qube 384 is capable of performing pharmacology measurements with large molecules.
- Qube 384 provides high throughput and high-quality recordings, enabling the evaluation of neurotoxins and neutralizing antibodies in an assay with a success rate of 95%.

Introduction

Large molecules

Currently, more than 90% of approved drugs are small molecules. Nevertheless, large molecules (> 1.000 Da), also known as biologics, are rapidly rising in prominence and importance in drug discovery. They already constitute the lion's share of the top 10 selling drugs worldwide.

Large molecules are notorious for being challenging to handle and study. However, here we show a high-throughput electrophysiological screen of antibody-based antivenom. Using the Qube 384, we have evaluated two groups of α-neurotoxins (7-8 kDa) and IgG antibodies (150-170 kDa).

Neutralizing antibodies

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.

One of the most medically relevant groups of snake toxins is α-neurotoxins. α-neurotoxins are antagonists of the nicotinic acetylcholine receptor (nAChR)[2, 3], preventing action potential firing and thereby muscle contraction.

To develop novel antivenom, human monoclonal IgG antibodies binding a range of α-neurotoxins from different snake species had been discovered, and affinity matured using chain-shuffling.

The purpose of this study was to investigate the cross-neutralizing potential of 15 IgGs, nanobodies and Quads (nanobody derivates[4]) against ten α-neurotoxins (six natural and four recombinantly expressed).

Fig. 1: Adult monocled cobra snake aka *Naja kaouthia* in defence position

Results and discussion

Neurotoxin evaluation

Ten α-neurotoxins (six natural and four recombinantly expressed) were evaluated for their ability to inhibit the current elicited by acetylcholine (see Figure 2). The α-neurotoxins used were more specifically: α-cobratoxin from *Naja kaouthia*, α-elapitoxin and short neurotoxin 1 (Dp4) from *Dendroaspis polylepis*, α-bungarotoxin from *Bungarus multicinctus*, and a long and short α-neurotoxin from *Naja melanoleuca* (Nm8 and Nm3, respectively). Moreover, four recombinantly produced α-neurotoxins were included (STX2, 10, 17, and 19) in this evaluation to investigate whether their function was retained through recombinant expression, as opposed to isolation from natural venom.

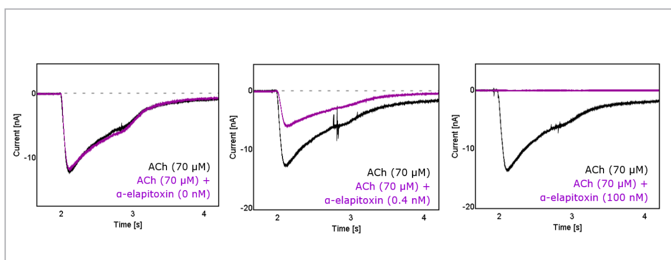

Fig. 2: Sweep plots showing how increasing concentrations of α-elapitoxin blocks the nAChR mediated current. The currents were elicited by 70 µM ACh.

From different toxin titrations, IC_{80} values were determined for each of the toxins (see Figure 3 and Table 1). These concentrations were used for the following experiments.

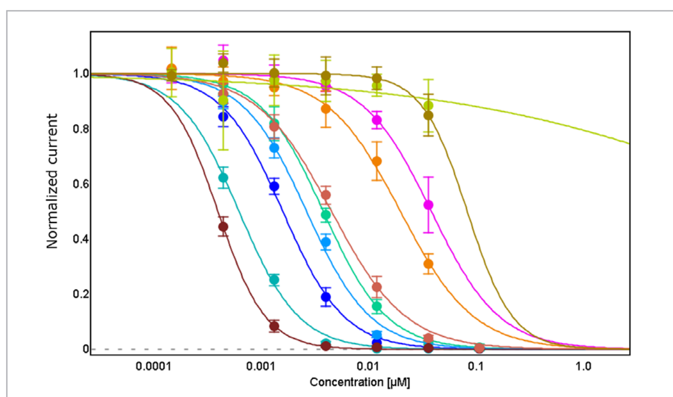

Fig. 3: Non-cumulative concentration-response curves showing how increasing concentrations of the ten toxins result in a decrease in the current measured. From the hill fit, both IC_{50} and IC_{80} were estimated (See Table 1).

Table 1: IC_{50} and IC_{80} values estimated for each of the 10 toxins: The IC_{50} was used to rank the toxins. The six natural toxins was used in the following experiment, where a concentration corresponding to the IC_{80} value was used. For this experiment, three QChips were used in total.

Toxin	IC_{50} (nM)	IC_{80} (nM)	Hill Slope	n
α-elapitoxin	0.40	0.81	2.0	96
α-cobratoxin	0.63	1.52	1.6	96
Dp4	1.13	3.60	1.2	94
α-bungarotoxin	2.74	7.00	1.7	96
Nm3	3.82	9.53	1.2	93
Nm8	4.56	13.7	1.3	95
STX19	18.0	57.3	1.5	86
STX17	38.1	121	1.2	95
STX2	>100	>100	N/A	91
STX10	>>100	>>100	N/A	91
BSA	-	-		94

Cross-screen of antibodies against neurotoxins

To develop novel antivenom, human monoclonal IgG antibodies binding a range of α-neurotoxins from different snake species had been discovered, and affinity matured using chain-shuffling. The purpose of this study was to investigate the cross-neutralizing potential of 15 antibodies against six α-neurotoxins.

To this end, 15 antibodies (seven IgGs, one nanobody, and seven Quads) were screened against the six natural α-neurotoxins (the four recombinantly produced toxins were not included). The antibodies were only tested in one concentration. Antibodies and toxins were mixed using the liquid handling ability of the Qube, preincubated for a minimum of 30 minutes and incubated with the cells for five minutes before acetylcholine addition. The current was then recorded and normalized to the current before adding the toxin:antibody mixture (see Figure 4). This experimental setup revealed neutralization patterns among the antibodies. It was used to determine antibodies with high specificity to a single α-neurotoxin and others with broader neutralization capacity (see Figure 4).

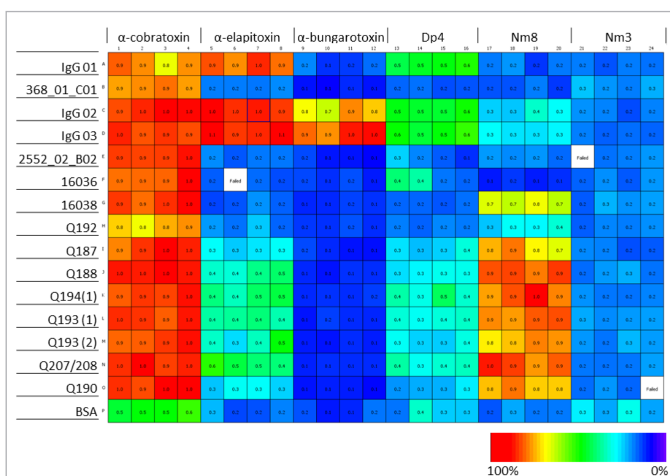

Fig. 4: QChip plate view displaying the size of the nAChR mediated current recorded after toxin: antibody addition. The values are normalised to the pure ACh response, with red (1) representing 100% toxin neutralisation. The plate view represents a heat map, showing the antibodies' neutralisation ability and clearly indicates whether an antibody has high specificity to a single α-neurotoxin or possesses a broader neutralisation capacity.

Methods

Cells

- TE/RD cells (from ATCC) endogenously expressing nAChR (α1β1δϒ) were cultured according to the guidelines of the manufacturer.
- On the day of the experiment, cells were harvested using detachine and kept in serum-free media until further use. The automated cell preparation unit of the Qube was used to resuspend cells in saline just before the start of the experiment.

Pharmacology

- nAChR mediated current was elicited by 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 antibodies were incubated with the toxins for at least 30 min before addition.
- Cells were preincubated with toxin + antibody for at least five min before ACh addition.

Electrophysiology

- Wholecell access was gained using a double pressure pulse to -250 mbar and -350 mbar, both for 2 s. Cells were afterwards clamped at V_{hold} = -60 mV and -10 mbar.
- All experiments were performed using multihole QChips (10 holes / well).
- Data was analyzed using Sophion's Analyzer. Data is represented as mean ± SD.

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