Automated Patch Clamp Evaluation of Snake Neurotoxins and Recombinant Antibody Antivenoms

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Background

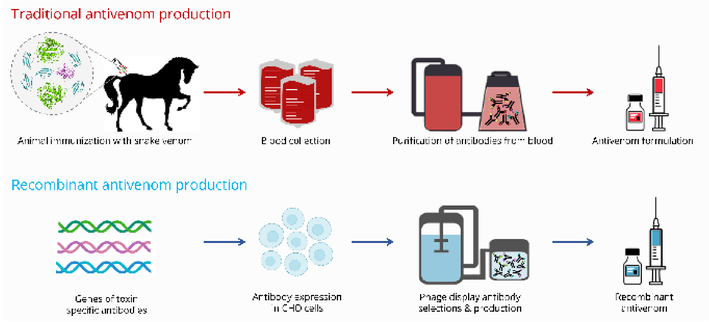
Snakebite was designated Neglected Tropical Disease (NTD) status by the WHO (2017), causing 100,000 yearly deaths and around 400,000 amputations. Each snake species has a unique venom, consisting of several dozen different toxins.

The century-old, traditional technique to generate snake antivenoms involved purifying antibodies from horse blood serum following immunization with snake venom. However, there are several drawbacks: equine-human immunoreactivity and side effects; batch-to-batch variation; specific to snake venom used.

In the last decade, advances in antibody engineering have made antibody discovery and development more efficient and specific, including creating recombinant antivenom antibodies to target and neutralize key toxin peptides. One of the most medically relevant groups of snake toxins are the α-neurotoxins, targeting the nicotinic acetylcholine receptor (nAChR).

For over two decades automated patch-clamp (APC) systems, have been used to advance our understanding of ion channel biophysics, pharmacology and their roles in physiology and disease.

Here, using QPatch II and Qube 384 APC, we functionally evaluate snake venom α-neurotoxins and anti-venom, toxin-neutralising IgG monoclonal antibodies (mAbs) on the muscle-type α1-nAChR.



Methods

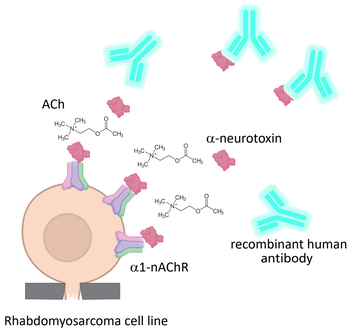
Evaluating the potential of recombinant IgG mAbs to neutralize α-cobratoxin (an α-neurotoxin from the monocled cobra, *Naja kaouthia*) and thereby prevent α-cobratoxin inhibiting of nAChR channels, planar whole-cell patch-clamp experiments were carried out on QPatch II, briefly:

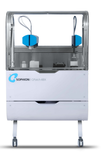
The human-derived Rhabdomyosarcoma RD (ATCC cat. #CCL-136) cell line, endogenously expressing the muscle-type α1-nAChR was patched, and an acetylcholine (ACh) concentration-response was generated (see Figure 1A).

The EC_{80} of ACh (70 µM) determined in Figure 1A was then used to determine the inhibitory effect of α-cobratoxin (see Figure 1B), followed by a study of the concentration-dependent neutralizing effect of eight different IgG mAbs (see Figure 2).

Next, the Qube 384 was employed for combinatory screening of both neutralizing potential and toxin cross-reactivity. In total, six different α-neurotoxins from four different elapid snake species were tested against 15 different IgG mAbs (see Figure 3).

Solutions: the composition of the intracellular solution was (mM): CsF 140, EGTA 1, HEPES 10, NaCl 10 (pH 7.2 with CsOH); the extracellular solution (mM): CaCl2 2, MgCl2 1, HEPES 10, KCl 4, NaCl 145, glucose 10 (pH 7.4 with NaOH).





QPatch II 48X

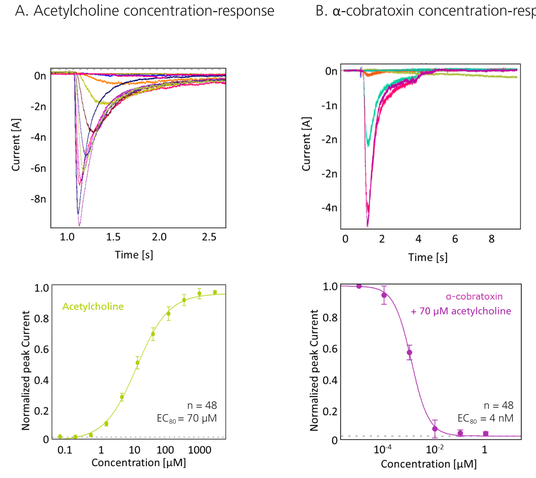


Fig. 1: **Inhibition of nAChR by α-cobratoxin.** (A) Current sweep plots (upper panel) and concentration-response curve (lower panel) showing the relationship between increased ACh concentration and the α1-nAChR current in Rhabdomyosarcoma RD cell line. A Hill fit to normalised current-ACh concentration plot determined the EC_{80} for ACh activation; 70 µM ACh was used throughout the rest of the experiments. (B) Current sweep plots (upper panel) and concentration-response curve (lower panel) showing increasing concentrations of α-cobratoxin result in a decrease in the current measured. A Hill fit to normalised current-α-cobratoxin concentration plot determined the IC_{80} for α-cobratoxin = 4 nM.

QPatch II 48X

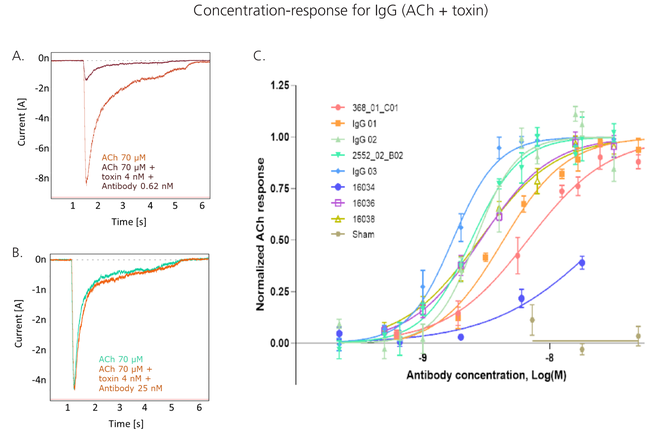


Fig. 2: **IgG mAbs neutralizing α-cobratoxin inhibition of nAChRs: functional IgG evaluation.** (A) Current sweep plots of ACh alone (70 uM, light brown) and in combination with α-cobratoxin (4 nM) and low concentration of IgG mAb (0.62 nM, dark brown). (B) Current sweep plots of ACh alone (70 uM, mint) and in combination with α-cobratoxin (4 nM) and high concentration of IgG mAb (25 nM, orange). (C) Concentration-dependent neutralization of ACh + α-cobratoxin (70 uM + 4 nM, respectively) by eight different IgG mAbs, with a non-specific IgG as a sham-control (olive green). The resultant normalized ACh response vs IgG mAb log concentration [M] curves determined the potency of each IgG mAb to neutralise the α-cobratoxin inhibition of nAChR currents.

Qube 384

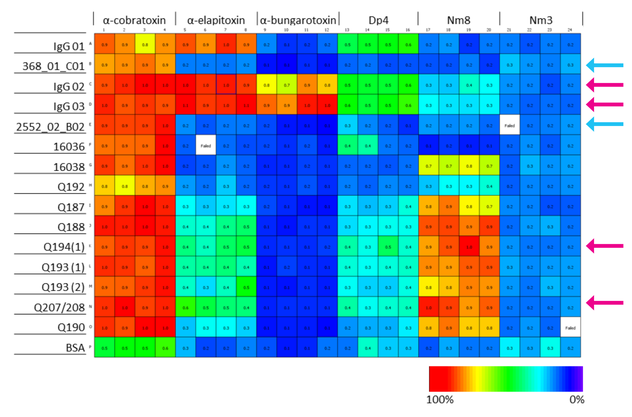


Fig. 3: **Cross-screen of IgG mAbs against α-neurotoxins.** A 384-well compound plate was created comprising six different α-neurotoxins (from four different snake species) with 15 different IgG mAbs (and a 16th test of BSA as a negative control) were tested in quadruplicate to evaluate their ability to neutralise the neurotoxin inhibition of nAChR currents (6x16x4 = 384 test conditions). The heat map represents the 384-well plate, showing the neutralization ability of the antibodies (oranges/reds are greater, greens/blues lesser neutralization), indicating whether an antibody neutralises a single toxin (e.g. IgGs indicated by blue arrows) or possesses broader neutralization capacity for multiple neurotoxins (e.g. IgGs indicated by magenta arrows).

Summary

- Engineered human IgG mAb → neutralise venom α-neurotoxins
- IgG mAb potency determined via APC
- Broader, multi-toxin (and from different snake venoms) neutralization capacity detected
- QPatch II and Qube assays → efficient characterisation of both peptide toxins and mAb function

Conclusion

The study demonstrated the potential of a range of IgGs to neutralize α-neurotoxins from several snake species. This is a critical step to enable the design of novel, broadly-neutralizing recombinant antivenoms against snakebite envenoming.

Further reading on this work:

Ledsgaard *et al.* (2021). *In vitro* discovery and optimization of a human monoclonal antibody that neutralizes neurotoxicity and lethality of cobra snake venom. *BioRxiv*, 2021.09.07.459075.

Miersch *et al.* (2022). Synthetic antibodies block receptor binding and current-inhibiting effects of α-cobratoxin from *Naja kaouthia*. *Protein Science, (accepted).*