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Snake alpha-neurotoxins: Clinically relevant or irrelevant?

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Objective: Snake α-neurotoxins inhibit the nicotinic acetylcholine receptors (nAChRs) at the motor end-plate. However, their role in human paralysis remains unclear. The aims of this study were to (1) compare the ability of snake short-chain (SαNTx) and long-chain (LαNTx) α-neurotoxins to inhibit human and rat adult muscle-type nAChR and (2) to test the ability of commercially available antivenoms to reverse SαNTx- and LαNTx-mediated inhibition of nAChR.

Methods: Adult muscle-type, human and rat nAChR were expressed on *Xenopus* oocytes. The inhibition of 3 µM acetylcholine-induced membrane currents of human and rat nAChR-expressed oocytes by two SαNTx (i.e. α-elapitoxin Ppr-1 from *Pseudechis porphyriacus* and α-scutoxin-1 from *Oxyuranus scutellatus*) and two LαNTx (i.e. α-bungarotoxin from *Bungarus multicinctus* and α-elapitoxin Nk2a from *Naja kaouthia*), was measured using Two Electrode Voltage Clamping. Chick-biventer and rat-hemidiaphragm preparations were used to test the ability of commercially available antivenoms to reverse the α-neurotoxin mediated neuromuscular block.

Results: All four toxins potently inhibited both human and rat nAChR in a concentration-dependent manner with IC_{50}s below 100 nM. The two SαNTxs exhibited marked species dependence on inhibition with 5- (α-elapitoxin Ppr-1) and 17-(α-scutoxin-1) fold higher IC_{50}s at the human channel compared to that of nAChR. Inhibition by both SαNTx was rapidly reversible at the human nAChR compared to rat indicating the relative resistance of human nAChR to SαNTxs, compared to rats. The combinations of two amino acid substitutions, W187S and F189T, at the ligand binding site of the α-subunit made the rat receptor resistant to SαNTxs, similar to the human nAChR. In contrast, LαNTx showed no species differences in pharmacology. A review of venomic and clinical data of envenoming by major neurotoxic snake groups such as Cobras (*Naja*), Kraits (*Bungarus*), taipans (*Oxyuranus*) and tiger snakes (*Notechis*) indicated that paralysis in humans following snakebite is likely to be due to LαNTx,but not SαNTx. SαNTx- and LαNTx-mediated neuromuscular block in rat and chick *in vitro* nerve-muscle preparations was reversed to differing extents by commercially available antivenoms supporting the benefit of antivenom reversing post-synaptic mediated paralysis in humans.

Conclusion: Humans are resistant to snake SαNTx compared to rats hence SαNTx are unlikely to be clinically relevant. Antivenom appears to be effective in reversing post-synapitc neurotoxicity in humans due to LαNTx. The differential susceptibility of rat and human nAChR towards SαNTx challenges the usefulness of rodent lethality prevention assays in assessing antivenom efficacy.