Neurotoxicity Research
https://doi.org/10.1007/s12640-021-00351-z

ORIGINAL ARTICLE

Not Goanna Get Me: Mutations in the Savannah Monitor Lizard (*Varanus exanthematicus*) Nicotinic Acetylcholine Receptor Confer Reduced Susceptibility to Sympatric Cobra Venoms

Lee Jones[1] · Richard J. Harris[1] · Bryan G. Fry[1]

Received: 21 January 2021 / Revised: 3 March 2021 / Accepted: 8 March 2021

Abstract

Antagonistic coevolutionary relationships provide intense selection pressure which drive changes in the genotype. Predator-prey interactions have caused some venomous snakes and their predators/prey to evolve α-neurotoxin resistance through changes at the orthosteric site of nicotinic acetylcholine receptors. The presence of negatively charged amino acids at orthosteric site positions 191 and 195 is the ancestral state. These negatively charged amino acids have exerted a selection pressure for snake venom α-neurotoxins to evolve with strong positive charges on their molecular surface, with the opposite-charge attraction facilitating the binding by the neurotoxins. We aimed to test the effects of a series of mutations whereby one or both negatively charged amino acids are replaced by uncharged residues to ascertain if this was a novel form of reduced venom susceptibility in the varanid species. Using a biolayer interferometry assay, we tested the relative binding of α-neurotoxin-rich snake venoms against the orthosteric sites of *V. giganteus* (Perentie) and *V. komodoensis* (Komodo dragon), which both possess the negatively charged aspartic acid at position 191; *V. mertensi* (Merten's water monitor), which also has aspartic acid at position 195; and *Varanus exanthematicus* (savannah monitor), which lacks negatively charged amino acids at both positions 191 and 195. The orthosteric sites of these species are otherwise identical. In order to complete the structure-function relationship examination, we also tested a mutant version with the negatively charged aspartic acid at both positions 191 and 195. It was demonstrated that the presence of a negatively charged amino acid at either position 191 or 195 is crucial for the successful binding of snake venom α-neurotoxins, with *V. giganteus*, *V. komodoensis* and *V. mertensi* all strongly bound. The mutant version containing a negatively charged amino acid at both positions was bound equipotently to the native forms of *V. giganteus*, *V. komodoensis* and *V. mertensi*. Thus, the presence of a negatively charged amino acid at both positions does not increase binding affinity. In contrast, *Varanus exanthematicus*, lacking a negatively charged amino acid at either position, displayed dramatically less sensitivity to neurotoxins compared with the other species. *V. exanthematicus* is distinguished from the other species examined in this study by being a small, terrestrial, slow-moving species living sympatrically with a high density of large cobra species that have neurotoxin-rich venoms. Thus, this vulnerable prey item seems to have evolved a novel form of reduced susceptibility to snake venom neurotoxins under a strong selection pressures from these neurotoxic predators. These results therefore contribute to the body of knowledge of predator/prey chemical arm races while providing novel insights into the structure-activity relationships of the orthosteric site of the nicotinic acetylcholine receptor alpha-subunit.

Keywords Neurotoxicity · Venom · Varanid · Resistance · Evolution · Nicotinic acetylcholine receptor

Introduction

Predator-prey relationships are a main driver in the coevolution of genotypic adaptations through positive selection pressures (Dawkins and Krebs 1979). Such notable reciprocal relationships are that of venomous organisms and their prey/predators which, through antagonistic

✉ Bryan G. Fry
bgfry@uq.edu.au

[1] Venom Evolution Lab, School of Biological Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia

Published online: 20 March 2021

coevolution, can lead to the evolution of a myriad of resistance mechanisms (Arbuckle et al. 2017).

A prominent form of resistance toward venom that has convergently evolved across the animal kingdom (Khan et al. 2020) is seen at the nicotinic acetylcholine receptor (nAChR) orthosteric site (acetylcholine binding region) of some animals by substitutions of specific amino acids which reduce the relative sensitivity to α-neurotoxins (such as three-finger toxins; 3FTx). These 3FTx are positively charged and have been shown to have varying degrees of affinity towards specific amino acids at key positions along the orthosteric site (Kachalsky et al. 1995) located between positions 187-200 of the α-1 nAChR subunit (Bracci et al. 2001). Positions 187, 189, 190, 191, 194 and 195 have been shown to be integral for 3FTx binding (Bracci et al. 2001; Dellisanti et al. 2007a, 2007b; Tzartos and Remoundos 1990). Of particular importance are the negatively charged amino acids at positions 191 and 195 of the orthosteric site which guide the binding of the positively charged α-neurotoxins (Harris and Fry 2020).

One common form of nAChR orthosteric resistance is the introduction of N-glycosylation of asparagine residues, which can sterically hinder α-neurotoxin binding as the bulky glycan structure prevents the docking by the large snake venom neurotoxins but still allow the binding by the much smaller endogenous neurotransmitter acetylcholine (Khan et al. 2020; Takacs et al. 2001, 2004). This mutation has been shown to occur in position 187, such as in the mongoose (*Herpestes ichneumon*) (Barchan et al. 1992, 1995; Kachalsky et al. 1995), a predator of α-neurotoxic snakes such as cobras, or at position 189, which has also been shown in cobras as a form of auto-resistance to their own venom (Khan et al. 2020; Takacs et al. 2001). This form of resistance is widespread throughout the animal kingdom, having convergently evolved on at least 10 independent occasions at either position 187 or 189, with 6 of these evolutions being just within snakes (Khan et al. 2020).

Another form of resistance is the mutation to encode for the bulky, positively charged amino acid arginine at position 187, such as in honey badger (*Mellivora capensis*), a predator of cobras (Drabeck et al. 2015), and in wild pigs (*Sus scrofa*) which have been known to predate on venomous snakes (Tanaka and Mori 2000). In these cases, positively charged arginine replaces the ancestral aromatic residue, which significantly reduces binding affinity of *α*-bungarotoxin (Drabeck et al. 2015). This reduced α-neurotoxin susceptibility was originally thought to be because of electrostatic repulsion of the positively charged 3FTx (Dellisanti et al. 2007a; Drabeck et al. 2015); however, the precise mechanism in action is still unclear, as it has more recently been hypothesised to be steric hindrance (Rahman et al. 2020). Regardless of mechanism, it has been confirmed experimentally that an arginine at position 187 confers strong resistance to neurotoxins (Harris and Fry 2020).

An alternative form of resistance has been shown to occur through charge reversal substitutions from an ancestral negatively charged aspartic acid (D) or glutamic acid (E) at position 191 or 195 to positive amino acids that are thought to induce resistance via electrostatic repulsion (Harris and Fry 2020). A positively charged amino acid lysine (K) has been shown to have convergently evolved on 10 separate occasions in snakes (Khan et al. 2020), replacing the negative amino acids. Negatively charged orthosteric sites provide a selection pressure for a positively charged surface to be a general characteristic of neurotoxins, as opposite charges facilitate binding. Therefore, the evolved positively charged residue would cause electrostatic repulsion of positively charged α-neurotoxin. This has been observed across some snake species, where K substitutions have occurred at position 191 or 195, conferring neurotoxic resistance (Harris and Fry 2020).

Examining all sequences available from published literature (Khan et al. 2020) and public sequence databases (Genbank gnl|SRA|SRR6144715.1034956.2) showed all known varanid lizard orthosteric sites differed only at positions 191 and 195 (Fig. 1). The Australian evolved varanids *Varanus giganteus*, *Varanus komodoensis* and *Varanus mertensi* possess the negatively charged amino acid aspartic acid at either position 191 (*V. giganteus* and *V. komodoensis*) or 195 (*V. mertensi*) (Fig. 1). The presence of a negatively charged amino acid at this key bioactive site suggests high susceptibility to snake venom α-neurotoxins, particularly because these positions have been identified to be essential for the binding of α-neurotoxins (Dellisanti et al. 2007b; Tzartos and Remoundos 1990), due to opposite-charge attraction (Harris and Fry 2020). All these species are large (*V. mertensi*) to giant (*V. giganteus* and *V. komodoensis*), agile and in the case of *V. mertensi* semi-aquatic. All occupy geographical ranges where the snakes with potent α-neurotoxic venoms (e.g., *Acanthophis*) are too small to take even hatchling specimens. In contrast, *V. exanthematicus*, which possesses an uncharged residue at both positions (Fig. 1), is a small, terrestrial, slow-moving species that is

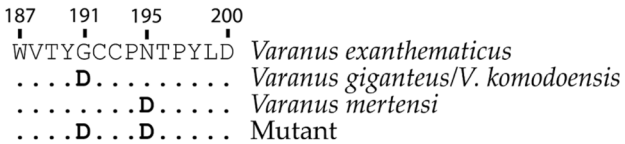

Fig. 1 Interspecies comparison of amino acid sequences of residues 187–200. A dot (.) indicates the same amino acid. Amino acid substitutions of aspartic acid (D) are in bold. *Varanus exanthematicus*, *V. giganteus*, *V. komodoensis* and *V. mertensi* sequences were taken from Khan et al. (2020). *V. exanthematicus* sequence was from Genbank gnl|SRA|SRR6144715.1034956.2

sympatric with a high density of large neurotoxic cobras able to feed upon prey of this size. This sequence analysis combined with natural history data allowed us to generate a testable hypothesis that due to the lack of a negatively charged amino acids at positions 191 and 195, *V. exanthematicus* will display reduced susceptibility to binding by α-neurotoxic venoms, while the other species will display sensitivity to such venoms.

This hypothesis was tested using a biolayer interferometry (BLI) assay platform that has been previously validated to assess α-neurotoxin binding to nAChR orthosteric mimotopes (Harris et al. 2020a, 2020b, 2020c; Zdenek et al. 2019a). To do such, we tested a wide range of α-neurotoxic snakes that were sympatric with *Varanus* species in this study (*V. exanthematicus*: *Hemachatus haemachatus*, *Naja mossambica* and *N. nubiae*) and allopatric snakes (*V. giganteus*, *V. komodoensis* (prior to going extinct in Australia) and *V. mertensi: Acanthophis antarcticus*). To ascertain if susceptibility, or reduced susceptibility, were general to α-neurotoxic venoms, we included the following species that were allopatric to all the *Varanus* species included in this study: *Bungarus flaviceps*, *N. sputatrix* and *Tropidolaemus wagleri*. In addition to testing the native versions that had a negatively charged amino acid at position 191 (*V. giganteus* and *V. komodoensis*) or position 195 (*V. mertensi*), we tested a mutant with a negatively charged amino acid at both positions to ascertain if this resulted in stronger binding than would be the case with only a single negatively charged amino acid present.

Materials and Methods

Venom Stock Preparation

All venom work was undertaken under University of Queensland Biosafety Approval #IBC134BSBS2015. Venoms were sourced from the long-term cryogenic collection of the Toxin Evolution Lab. Lyophilised venoms were reconstituted in deionised water. Concentrations were determined using nanodrop at 280 nm wavelength. They were then centrifuged (4 °C, 10 min, 14,000 RCF). A 'working stock' of 1 mg/mL was made from the supernatant with 50% glycerol to prevent freezing at −20 °C (where they were stored until use).

Mimotope Preparation

Following methods from a previously developed assay (Zdenek et al. 2019a), a 14 amino acid mimotope of the vertebrate α-1 nAChR orthosteric site was developed by GenicBio Ltd. (Shanghai, China) designed upon specification. *V. exanthematicus*, *V. giganteus*, *V. komodoensis* and *V. mertensi* sequences were from Khan et al. (2020). *V. exanthematicus* sequence was from Genbank gnl|SRA|SRR6144715.1034956.2. The C–C of the native mimotope is replaced during peptide synthesis with S–S to avoid uncontrolled postsynthetic thiol oxidation. The C–C bond in the nAChR binding region does not participate directly in analyte-ligand binding (McLane et al. 1994, 1991; Tzartos and Remoundos 1990); thus, replacement to S–S is not expected to have any effect on the analyte-ligand complex formation. However, the presence of the C–C bridge is key in the conformation of the interaction site of whole receptors (Testai et al. 2000). As such, we suggest direct comparisons of kinetics data, such as Ka or KD, between nAChR mimotopes and whole receptor testing should be avoided, or at least approached with caution. Mimotopes were further connected to a biotin linker bound to two aminohexanoic acid (Ahx) spacers, forming a 30 Å linker. Powdered stocks of synthesised mimotopes were solubilised in 100% dimethyl sulfoxide (DMSO) and then diluted 1:10 in deionised water to make a final working stock concentration of 50 µg/mL and stored at −80 °C until use.

Biolayer Interferometry

BLI is a label-free, microfluidics-free, optical technique that precisely measures the thickness of biomolecules accumulating on the interaction surface of an optical-fibre-coated biosensor. The binding of molecules to the biosensor causes a measurable spectral shift in the wavelength of light being reflected through the fibre-optic biosensor, which yields quantitative, kinetic interaction information. Full details of the developed assay, including all methodology and data analysis, can be found in the validated protocol (Zdenek et al. 2019a). In brief, the BLI assay was performed on the Octet HTX system (ForteBio, Fremont, CA, USA). Venom samples were diluted 1:20, making a final concentration of 50 µg/mL per well. Mimotope aliquots were diluted 1:50, with a final concentration of 1 µg/mL per well. The assay running buffer was Dulbecco's phosphate-buffered saline (DPBS) with 0.1% BSA and 0.05% Tween-20. Preceding experimentation, Streptavidin biosensors were hydrated in the running buffer for 30–60 min, whilst on a shaker at 2.0 revolutions per minute (RPM). The dissociation of analytes occurred using a standard acidic glycine buffer solution (10 mM glycine (pH 1.5–1.7) in ddH2O). Raw data are provided in Supporting information 1–4.

Data Analysis

All raw data is available in Supplementary Dataset 1. All data obtained from BLI on Octet HTX system (ForteBio) were processed in exact accordance to the validation of

this assay (Zdenek et al. 2019a). The association step data (a recording of the wavelength shift (nm) at 0.2 s intervals over a 120-s period) were obtained and imported into Prism8.0 software (GraphPad Software Inc., La Jolla, CA, USA) where area under the curve (AUC) and one-way ANOVA with a Dunnett's multiple comparison analyses were conducted and graphs produced.

Results and Discussion

A clear pattern emerged whereby the presence of the negatively charged aspartic acid at either position 191 (*V. giganteus* and *V. komodoensis*) or 195 (*V. mertensi*) (Fig. 1) conferred sensitivity to the α-neurotoxic venoms (Fig. 2). Conspicuously, the presence of aspartic

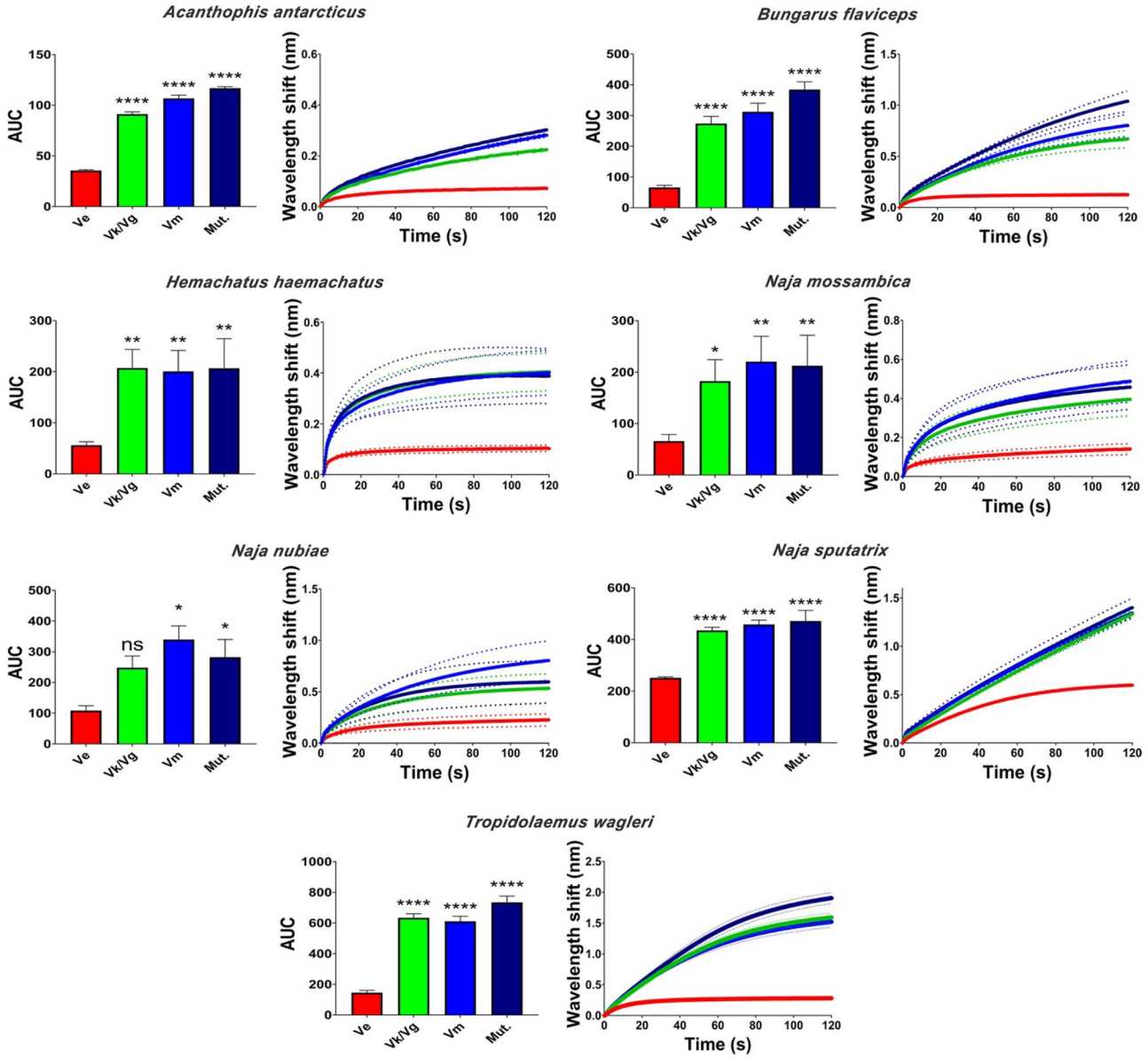

Fig. 2 Effects of African and non-African snake venom against *Varanus exanthematicus* (Ve), *V. komodoensis/V. giganteus* (Vk/Vg) and *V. mertensi* (Vm) and a synthetic mutant (Mut.) orthosteric sites and the structure-activity mutant sequence. Venom from *Acanthophis antarcticus*, *Bungarus flaviceps*, *Hemachatus haemachatus*, *Naja mossambica*, *N. nubiae*, *N. sputatrix* and *Tropidolaemus wagleri*. Bar graphs represent the area under curve (AUC) of the adjacent line graph. Line graphs show the mean wavelength shift in light (nm) of venoms binding over a 120 s period. Each venom was tested in triplicate ($N=3$). Error bars on all graphs represent SEM. AUC values were statistically analysed using a one-way ANOVA with a Dunnett's multiple comparisons post hoc test comparing with the *V. exanthematicus* mimotope. Statistical significance from the native mimotope is annotated by $*p<0.05$, $**p<0.01$ or $****p<0.0001$ above the corresponding bar

acid at both positions in the mutant version (Fig. 1) did not increase sensitivity to the α-neurotoxic venoms (Fig. 2). This indicates that a single negatively charged amino acid is sufficient to guide the binding by the positively charged α-neurotoxins. Conversely, *V. exanthematicus* was bound only very weakly by the α-neurotoxic venoms (Fig. 2) which is consistent with it lacking a negatively charged amino acid at both positions 191 and 195 (Fig. 1).

The introduction of positively charged amino acids in replacement of the ancestral negatively charged amino acids seen in other species (Harris and Fry 2020) provided a much stronger level of resistance compared with that observed in this study where the negatively charged amino acids were replaced by non-charged amino acids. This for the case of the reduced α-neurotoxin susceptibility seen in this study of *V. exanthematicus*, the neutrally charged amino acid replacements are not as effective at preventing binding as the positive charge replacements seen across some snakes, where the positive-positive same-charge repulsion is a powerful force (Harris and Fry 2020). Therefore, this reduced α-neurotoxin susceptibility may provide protection but not absolute resistance. Although full abolishment of binding was not observed in this study, the reduced α-neurotoxin susceptibility documented may lessen lethal paralytic effects and facilitate prey escape from the cobra predator. Thus, in conjunction with the thick scales that these species have as a mechanical defence, it may provide sufficient protection against predation. Further, although previously documented (Dellisanti et al. 2007a, 2007b; Tzartos and Remoundos 1990), these data also further underscore the importance of both 191 and 195 positions in the binding of snake venom α-neurotoxins. Other similar mechanisms of toxin resistance have also been found throughout nature, such as the resistance to cardiac glycosides by where neutral residues were replaced by charged residues (either positive or negative) (Ujvari et al. 2015). Thus, given these other similar mechanisms of action, it is clear that changing the charge of a binding site is crucial to alter binding of toxins and that even going from charged to uncharged such as in the current study may significantly affect neurotoxin binding.

The differences in binding seem consistent with differences in the morphology and ecology of the species and their relative sympatry with α-neurotoxic venomous snakes. *V. exanthematicus* is a small, slow moving terrestrial/burrowing monitor lizard native to Africa that is smallest of the African monitors (Bennett 2000). The combination of small size and slow gait makes them particularly vulnerable to predation by the abundant and large sympatric cobras (*Naja* spp.). *V. exanthematicus* is known to be dietary specialists, only taking a narrow range of invertebrate prey (Bennett 2000). Therefore, unlike that of the mongoose, the selection pressure to develop resistance is likely to come not from *V. exanthematicus* being predators of α-neurotoxic venomous snakes, but from this species being highly susceptible prey to such snakes. Small specimens of the closely related *V. albigularis* has been recorded in the stomach contents of *Naja annulifera* and *N. nigricincta* (Shine et al. 2007). This provides support that smaller varanids within the *albigularis/exanthematicus* clade are in a predator-prey relationship with cobras and that reduced α-neurotoxin susceptibility observed in this study is a less than that observed for other mechanisms of resistance (Harris and Fry 2020; Khan et al. 2020). However, more natural history observations are needed to confirm to what extent cobras provide a predatory selection pressure toward *V. exanthematicus* and to what degree the resistance protects them.

The other three species included in this study (*V. giganteus*, *V. komodoensis* and *V. mertensi*) are much less vulnerable to predation by α-neurotoxic snakes due to a combination of morphological and ecological factors. *Varanus komodoensis* are large upon hatching and also arboreal during neonate and juvenile stages (Imansyah et al. 2008), before undergoing extensive growth into adulthood, reaching up to 3 m (making them the largest extant lizard) and thus not vulnerable to predation by venomous snakes. *V. giganteus* reaches similar lengths, but instead of being arboreal in early life stages, they are extremely fast and highly agile (Clemente et al. 2009). The sheer size of *V. giganteus* as adults also makes them unlikely to be prey to elapids. *V. mertensi* occupies a semi-aquatic environment, separate from most elapid species (Mayes et al. 2005). Large varanids are considered top predators, with only smaller individuals predated upon by larger predators including other varanids (Christian 1995; Pianka 1994). *V. giganteus*, *V. komodoensis* and *V. mertensi* all evolved in Australia (Amer and Kumazawa 2008; Jennings and Pianka 2004; Vidal et al. 2012) where the lizard eating, potently α-neurotoxic snakes are small species (Jackson et al. 2016) unable to predate upon any but the smallest of varanids (such as *V. brevicauda*). In Australia, correspondingly, large predatory venomous snakes which take reptiles as a large part of their diet are represented by *Pseudechis* species which are myotoxic and potently anticoagulant (Zdenek et al. 2020) or procoagulant such as *Notechis* species (Zdenek et al. 2019b). While *Notechis* species have powerful neurotoxins in their venom, they are pre-synaptic neurotoxins, not post-synaptic α-neurotoxins that would interact with the orthosteric site of the alpha-1 nicotinic acetylcholine receptor examined in this study. This is in contrast to the cobras that *V. exanthematicus* lives sympatrically with, which can reach up to 3 m in length (*Naja ashei* and *N. melanoleuca*) (Shine et al. 2007; Wuester and Broadley 2007) and are powerfully α-neurotoxic (Harris et al 2020c).

Some evolutionary novelties that infer a greater survival benefit do come at an evolutionary cost (Blanchard and

Moreau 2017; Brodie III and Brodie Jr 1999). It is possible that there is an evolutionary trade-off with evolving certain amino acid changes at the orthosteric site. For example, it might be that negatively charged amino acids provide a greater binding benefit of the endogenous acetylcholine (ACh) and by removing these amino acids to non-charged or even positively charged (Harris and Fry 2020) might mean that the efficacy of ACh might be decreased. This has been suggested by viperid snakes which are resistant to cobra venom via the N-glycosylated asparagine, secondarily losing this trait in lineages which radiated into Europe where there are not any predatory α-neurotoxic snakes exerting the selection pressure to maintain resistance (Khan et al. 2020). Thus, investigating potential trade-off between acetylcholine binding efficiency and neurotoxin resistance is a fascinating area for future research.

In conclusion, our findings support the hypothesis that negatively charged amino acid at positions 191 and 195 the orthosteric site has provided the selection pressure for the evolution of α-neurotoxins which have strongly positively charged molecular surfaces. Correspondingly, the loss of negatively charged amino acids in both positions results in a significantly reduced α-neurotoxin susceptibility. Mutations at these sites occur as a result of adaptive changes in response to the presence or absence of selection pressures. Function and evolution of venom should be viewed in combination with the ecology of venomous species and their predator/prey. It is clear that there is a gap in the literature in regard to the predator ecology of the species investigated in this study. Therefore, future research should include natural history observations to bridge the gap. Future research should also undertake more in-depth sequencing to ascertain if reduced α-neurotoxin susceptibility is a basal condition in varanids, with secondary reversal in species which are not vulnerable to predation by α-neurotoxic snakes, or if it is a derived condition in the slow moving *V. exanthematicus* and thus may be present in other vulnerable species like *V. brevicauda*. Of particular note for future research is *V. albigularis*, a close relative of *V. exanthematicus*. This larger species is known to predate on neurotoxic cobras. A testable future hypothesis is whether the reduced α-neurotoxin susceptibility described in this study in the sister species *V. exanthematicus* is a mechanism that has allowed *V. albigularis* to become predators of venomous snakes. Thus, this study has revealed a novel arena for predator-prey interactions of *Varanus* species and lays the groundwork for a rich harvest of future laboratory and field research into these fascinating lizards.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12640-021-00351-z.

Author Contribution Conceptualisation, B.G.F.; data curation, L.J.; formal analysis, L.J.; funding acquisition, B.G.F.; investigation, L.J.; methodology, L.J. and R.J.H.; project administration, B.G.F.; resources, R.J.H. and B.G.F.; supervision, R.J.H. and B.G.F.; writing—original draft, L.J.; writing—review and editing, L.J., R.J.H. and B.G.F

Funding R.J.H was supported by the University of Queensland International PhD scholarship fund. B.G.F was funded by Australian Research Council Discovery Project DP210102406.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

References

Amer SA, Kumazawa Y (2008) Timing of a mtDNA gene rearrangement and intercontinental dispersal of varanid lizards. Genes Genet Syst 83:275–280

Arbuckle K, de la Vega RCR, Casewell NR (2017) Coevolution takes the sting out of it: evolutionary biology and mechanisms of toxin resistance in animals. Toxicon 140:118–131

Barchan D, Kachalsky S, Neumann D, Vogel Z, Ovadia M, Kochva E, Fuchs S (1992) How the mongoose can fight the snake: the binding site of the mongoose acetylcholine receptor. Proc Natl Acad Sci 89:7717–7721

Barchan D, Ovadia M, Kochva E, Fuchs S (1995) The Binding Site of the Nicotinic Acetylcholine Receptor in Animal Species Resistant to. alpha.-Bungarotoxin. Biochemistry 34:9172–9176

Bennett D (2000) Preliminary data on the diet of juvenile Varanus exanthematicus (Sauria: Varanidae) in the coastal plain of Ghana. Herpetol J 10:75–76

Blanchard BD, Moreau CS (2017) Defensive traits exhibit an evolutionary trade-off and drive diversification in ants. Evolution 71:315–328

Bracci L, Lozzi L, Lelli B, Pini A, Neri P (2001) Mimotopes of the nicotinic receptor binding site selected by a combinatorial peptide library. Biochemistry 40:6611–6619

Brodie ED III, Brodie ED Jr (1999) Costs of exploiting poisonous prey: evolutionary trade-offs in a predator-prey arms race. Evolution 53:626–631

Christian K (1995) Varanus panoptes (NCN) and Varanus gouldii (Sand Goanna). Diet and predation Herpetological Review 3:146

Clemente CJ, Thompson G, Withers P (2009) Evolutionary relationships of sprint speed in Australian varanid lizards. J Zool 278:270–280

Dawkins R, Krebs JR (1979) Arms races between and within species Proceedings of the Royal Society of London. Series B Biological Sciences 205:489–511

Dellisanti C, Yao Y, Stroud JC, Wang Z-Z, Chen L (2007a) Structural determinants for α-neurotoxin sensitivity in muscle nAChR and their implications for the gating mechanism. Channels 1:234–237

Dellisanti CD, Yao Y, Stroud JC, Wang Z-Z, Chen L (2007b) Crystal structure of the extracellular domain of nAChR α1 bound to α-bungarotoxin at 1.94 Å resolution Nature neuroscience 10:953–962

Drabeck DH, Dean AM, Jansa SA (2015) Why the honey badger don't care: convergent evolution of venom-targeted nicotinic acetylcholine receptors in mammals that survive venomous snake bites. Toxicon 99:68–72

Harris RJ, Fry B (2020) Electrostatic resistance to alpha-neurotoxins conferred by charge-reversal mutations in nicotinic acetylcholine receptors. Proceedings of the royal society B: biological sciences

Harris RJ et al (2020) Assessing the binding of venoms from aquatic elapids to the nicotinic acetylcholine receptor orthosteric site of different prey models. Int J Mol Sci 21:7377

Harris RJ, Zdenek CN, Debono J, Harrich D, Fry BG (2020b) Evolutionary interpretations of nicotinic acetylcholine receptor targeting venom effects by a clade of Asian viperidae snakes. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 108769

Harris RJ, Zdenek CN, Harrich D, Frank N, Fry BG (2020) An appetite for destruction: detecting prey-selective binding of α-neurotoxins in the venom of Afro-Asian elapids. Toxins 12:205

Imansyah M, Jessop T, Ciofi C, Akbar Z (2008) Ontogenetic differences in the spatial ecology of immature Komodo dragons. J Zool 274:107–115

Jackson TN et al (2016) Rapid radiations and the race to redundancy: an investigation of the evolution of Australian elapid snake venoms. Toxins 8:309

Jennings W, Pianka E (2004) Tempo and timing of the Australian Varanus radiation Varanoid Lizards of the World Indiana University Press, Bloomington, Indiana:77–87

Kachalsky SG, Jensen BS, Barchan D, Fuchs S (1995) Two subsites in the binding domain of the acetylcholine receptor: an aromatic subsite and a proline subsite. Proc Natl Acad Sci 92:10801–10805

Khan MA et al (2020) Widespread evolution of molecular resistance to snake venom α-neurotoxins in vertebrates. Toxins 12:638

Mayes P, Thompson G, Withers P (2005) Diet and foraging behaviour of the semi-aquatic Varanus mertensi (Reptilia: Varanidae) Wildlife Research 32:67–74

McLane KE, Wu X, Conti-Tronconi BM (1994) An. alpha.-Bungarotoxin-Binding Sequence on the Torpedo Nicotinic Acetylcholine Receptor. alpha.-Subunit: Conservative Amino Acid Substitutions Reveal Side-Chain Specific Interactions. Biochemistry 33:2576–2585

McLane KE, Wu X, Diethelm B, Conti-Tronconi BM (1991) Structural determinants of. alpha.-bungarotoxin binding to the sequence segment 181–200 of the muscle nicotinic acetylcholine receptor. alpha. subunit: effects of cysteine/cystine modification and species-specific amino acid substitutions. Biochemistry 30:4925–4934

Pianka ER (1994) Comparative ecology of Varanus in the Great Victoria desert. Aust J Ecol 19:395–408

Rahman MM et al (2020) Structure of the native muscle-type nicotinic receptor and inhibition by snake venom toxins. Neuron

Shine R, Branch W, Webb J, Harlow PS, Shine T, Keogh JS (2007) Ecology of cobras from southern Africa. J Zool 272:183–193

Takacs Z, Wilhelmsen KC, Sorota S (2001) Snake α-neurotoxin binding site on the Egyptian cobra (Naja haje) nicotinic acetylcholine receptor is conserved. Mol Biol Evol 18:1800–1809

Takacs Z, Wilhelmsen KC, Sorota S (2004) Cobra (Naja spp.) nicotinic acetylcholine receptor exhibits resistance to erabu sea snake (Laticauda semifasciata) short-chain α-neurotoxin. J Mol Evol 58:516–526

Tanaka K, Mori A (2000) Literature survey on predators of snakes in Japan Current. Herpetology 19:97–111

Testai FD, Venera GD, Peña C, de Jiménez Bonino MJB (2000) Histidine 186 of the nicotinic acetylcholine receptor α subunit requires the presence of the 192–193 disulfide bridge to interact with α-bungarotoxin. Neurochem Int 36:27–33

Tzartos S, Remoundos MS (1990) Fine localization of the major alpha-bungarotoxin binding site to residues alpha 189–195 of the Torpedo acetylcholine receptor. Residues 189, 190, and 195 are indispensable for binding. J Biol Chem 265:21462–21467

Ujvari B et al (2015) Widespread convergence in toxin resistance by predictable molecular evolution. Proc Natl Acad Sci 112:11911–11916

Vidal N et al (2012) Molecular evidence for an Asian origin of monitor lizards followed by Tertiary dispersals to Africa and Australasia. Biol Let 8:853–855

Wuester W, Broadley DG (2007) Get an eyeful of this: a new species of giant spitting cobra from eastern and north-eastern Africa (Squamata: Serpentes: Elapidae: Naja). Zootaxa 1532:51–68

Zdenek CN et al (2019) A taxon-specific and high-throughput method for measuring ligand binding to nicotinic acetylcholine receptors. Toxins 11:600

Zdenek CN et al (2019b) Coagulotoxic effects by brown snake (Pseudonaja) and taipan (Oxyuranus) venoms, and the efficacy of a new antivenom. Toxicol in Vitro 58:97–109

Zdenek CN et al. (2020) Anticoagulant activity of black snake (Elapidae: Pseudechis) venoms: Mechanisms, potency, and antivenom efficacy. Toxicol Lett 330:176–184