50. Tetrodotoxin in North-American Newts

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Background: The red spotted newts, *Notophthalmus viridescens*, from the eastern part of North-America and newts of the genus *Taricha*, which are distributed along the west-coast of the continent, are known to be poisonous due to the presence of high concentrations of tetrodotoxin (TTX) in their skin, but also in all organs.

Methods: TTX and its analogues, 6-*epi*TTX and 11-oxoTTX were analyzed in methanolic extracts of newt specimens and of their organs by using post-column LC-fluorescent detection. TTX was also localized in tissue sections using a monoclonal antibody-based immunoenzymatic technique.

Results: TTX and its analogues were detected in varying concentrations in *Notophthalmus viridescens* newts in adults as well as in the terrestrial efts. When kept in captivity, toxin levels decreased over the years, offsprings totally lacked TTX. In a newt population from Pennsylvania more than 50 percent were found to be infected with intestinal parasites (nematodes, trematodes, cestodes) which were positively stained for TTX by immunohistochemical technique. Moreover, insect predators such as mantids (*Tenodera* spp.) were observed occasionally feeding on the newts. In the Californian newt, *Taricha torosa*, only TTX was identified in the skin and internal organs. The toxin was also present in the eggs and larvae, which was histochemically confirmed. However, none of the toxińs analogues could be detected.

Discussion: Variability of the toxin levels in the newts is a common phenomenon ranging from zero to extremely high concentrations. The biogenetic origin of TTX is still a matter of discussion. An exogenous source of TTX via the food chain or its synthesis by symbiotic bacteria like in marine animals may explain its high variability in certain populations. The role of TTX in protecting the newts from predation or parasitism is questionable, because parasites and some predators (insects, snakes) may sustain or adapt to the toxicity.

Conclusion: The occurrence of a toxin like TTX in amphibians (newts, frogs, toads) offers many opportunities to study evolutionary, ecological and biosynthetic aspects of a natural compound.

Keywords: tetrodotoxin, newts, toxin evolution
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51. Evolution of a Neurotoxin from a Defensin

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Review: Evolution of toxic proteins (toxins) represents key functional innovations convergently occurring in phylogenetically diverse animal lineages. However, the evolutionary scenario of such innovations remains largely unknown. Previous studies have shown high structural conservation between scorpion neurotoxins affecting voltage-gated potassium channels (VGPCs) (abbreviated as α-KTxs) and antibacterial insect defensins. Here we highlight an evolutionary role for a small deletion event taking place in an insect defensin which results in a neurotoxic α-KTx. In comparison with insect defensins, α-KTxs lack an amino-terminal loop (n-loop) but possess two conserved residues (LysCys$_4$XaaAsn, underlined here) involved in a direct interaction with the pore region of VGPCs. We found that five insect defensins from Hemiptera and Hymenoptera also contain the two functional residues at equivalent positions to α-KTxs and thus represent missing evolutionary links between these two classes of peptide families. Deletion of the n-loop of one such peptide navidefensin2-2 removes steric hindrance of peptide-channel interactions and results in a VGPC-targeted neurotoxin (herein termed navitoxin) that selectively blocks three different mammalian VGPC isoforms with nanomolar to micromolar affinities. Mutations of the two crucial residues diminished or significantly decreased the affinity of navitoxin on these channels, demonstrating that this defensin-derived neurotoxin binds to VGPCs in the same manner with α-KTxs. Taken together, these results indicate that evolution of toxicity can be achieved by one small deletion event. Our finding might also be important in considering toxicity of antibacterial defensins as drugs.

Keywords: defensin, voltage-gated potassium channel, neurotoxin
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52. Divergence in the Biological Activity and Composition of Venom from Mackay (QLD) and Barossa/Adelaide (SA) Populations of Australian *Pseudonaja textilis* (Serpentes: Elapidae): An Important Role of Procoagulants in Rodent Prey Incapacitation

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Background and Aims: The eastern Brown snake *Pseudonaja textilis* has evolved extremely toxic venom to subdue prey, which consists mainly of mice, rats, lizards and frogs. A study on museum specimens found that individuals from Queensland grow larger in body size than those in South Australia, and that the proportion of endothermic prey consumed increases with snake body size (Shine, 1989). A more potent venom would confer advantage in preventing escape of a large rodent prey or an injury to the snake. It was thus interesting to compare the biological activities of the Queensland *P. textilis* venoms to those of South Australia in rats to see if the venom from Queensland would affect the physiological functions of a rat more severely and more rapidly than the venom from South Australia.