Three-finger fold peptides, a natural scaffold supporting pleiotropic functional properties

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Strikingly, despite their huge diversity, peptide toxins present in the venom of venomous animals belong to a quite limited number of structural superfamilies from which the three-finger fold is probably the most common in snake venoms.

Three-finger toxins (3FTx) have a molecular mass within the range of 6000-8000 Da, they contain four or five disulphide bridges, of which four are conserved in all members and located in the small globular core from where three β-strands emerge. Members of 3FTx family show a wide array of pharmacological effects by targeting different receptors, enzymes and ion channels with often high specificity and affinity. They interfere more particularly with the cholinergic transmission by targeting the nAChRs, the AChE or the mAChRs.

We have focused our studies on 3FTx from mambas that display the unique property to interact with various class A GPCRs. Indeed, in addition, to the well-known muscarinic toxins, we have recently identified several toxins active on α-adrenoceptors as well as on dopamine D3 subtype, highlighting the multipotent interacting property of 3FTx for aminergic GPCRs. These toxins may display either absolute selectivity for one receptor subtype or a polypharmacological property for various aminergic receptors. Moreover, to study the mode of action of some of these toxins (MT7 and ρ-Da1a) on their respective targets (muscarinic M1 and $\alpha 1_A$-adrenoceptor), binding experiments as well as functional assays were performed using wild-type and modified toxins as well as receptor mutants. Our results highlight that both toxins interact in a complete different way with GPCRs. Based on these results, toxin's engineering using a loop permutation strategy was used in order to design new three-finger toxins with original pharmacological profiles. Finally, phylogenic analyzes of these 3FTx show that muscarinic, adrenergic and dopaminergic toxins may be pooled in one family called aminergic toxins, this family coming probably from a specific radiation of ligands present in mamba venoms. We apply the ancestral protein resurrection strategy to aminergic toxins in order to pinpoint important functional mutations which have probably occurred during their evolution and analyze them to modulate their binding properties on various GPCRs.

Kessler, P., Marchot, P., Silva, M., and Servent, D. (2017) The three-finger toxin fold: a multifunctional structural scaffold able to modulate cholinergic functions. *J. Neurochem.* **142**, 7-18

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