GRUPO 7
Venenos e Toxinas

7.01

PHARMACOLOGICAL STUDY OF THE LOW MOLECULAR WEIGHT COMPONENTS OF *Phoneutria nigriventer* SPIDER VENOM ON NEUROTRANSMITTER RELEASE FROM BRAIN STRIATAL TISSUE.

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Aims: The venom from spiders and other invertebrates contain several different low molecular weight compounds like polyamines known as glutamate receptor blockers and small peptides with a variety of different effects. Polyamine from *Phoneutria nigriventer* spider venom have never been studied and this report presents the initial results.

Methods and Results: An initial purification step employed the ultrafiltration of crude venom through an Amicon filter with a nominal cut off of 3 kDa. The permeant compounds were further purified by C_{18}-RP-HPLC generating 14 chromatographic peaks. The desiccated fractions were then screened for pharmacological activities in the guinea pig ileum. Three fractions were capable of contracting the ileum. Further screening employed the neurotransmitter release assay where ^{3}H-dopamine was taken up and released by pulses of glutamate in a superfused striatal tissue system. This assay is sensitive to glutamate blockers and suitable for the detection of polyamines. From the 14 fractions tested only 2 modified significantly the release of labelled dopamine. The spontaneous release was increased by fractions 0 and 8. None of the fractions impaired the release stimulated by glutamate. The fractions that stimulated dopamine release also contracted the guinea pig ileum.

Conclusions: These results suggest the presence of two compounds of low molecular weight that are capable of contracting the guineas pig ileum and release dopamine from neuronal tissue. The effects of polyamines were not detected in these pharmacological assays. The use of an insect/invertebrate model of glutamate receptors may provide more reliable results. Also, an eventual oxidation of the polyamines during the purification procedure may have inactivated the polyamines. Further chemical and pharmacological analyses will be performed to answer these questions.

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Structural features of the *L. muta* thrombin-like enzyme: homology modeling, docking simulations and experimental analysis.

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Aims: The *Lachesis muta* thrombin-like enzyme (LM-TL) is a serine protease that shares not only an evident sequence homology with the serine protease domain of thrombin (38%) but also its fibrinoclotting activity. In addition, LM-TL (228 residues) is a one-chain enzyme 52% homologous to the highly nonspecific trypsin, also cleaving chromogenic substrates with similar specificity. Our aim is to construct a three-dimensional (3D) model for LM-TL based on these two homologous proteins of known 3D-structure. **Methods and results**: Spatial modeling of LM-TL revealed a serine protease with a chymotrypsin fold presenting a hydrophobic pocket on its surface, involved in substrate recognition, and an important 90 loop, related to the restriction of LM-TL catalytic site cleft. By docking analysis we predicted that LM-TL would be incapable of forming a stable complex with classical trypsin inhibitors (BPTI and ecotin), but LM-TL feasible interactions with fibrinopeptide A and mutant ecotin TSRR/R were observed. The predictions were confirmed experimentally using fibrinoclotting assay; thus LM-TL model is a reliable structure. Futhermore, comparison with the crystal structure of a plasminogen activator from *Trimerusurus stejenegeri* venom (TSV-PA) highlighted the predicted participation of the residue 193 and C-terminal region of LM-TL in the substrate and ecotin recognition. The analysis of the primary structures of LM-TL and of the seven snake venom thrombin-like enzymes family (SVTLEs) reveals a subgroup formed by LM-TL, crotalase, and bilineobin, both closely related to thrombin. **Conclusion**: LM-TL provides an initial point to compare SVTLEs with their counterparts, e.g. the mammalian serine proteases, and a basis for the localization of important residues within the little known SVTLEs family.

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