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PURIFICATION AND AMINO ACID SEQUENCES OF PEPTIDES FROM *Phyllomedusa* SPECIES

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Amphibian skin represent an enormous source of bioactive peptide. Adult specimens of four different species of frog tree (PHYLLOMEDUSINAE) were captured in three different regions of the Brazilian flora. The skin secretion was obtained by manual compression of the parotoid glands and dissolved in distilled water. After liophylization, samples were submitted to reverse-phase HPLC on a C₁₈ column and individual peaks were collected manually. Purified peptides were sequenced using Edman-Chang double coupling method and automatic Edman degradation. Peptide lithic activity against phosphatidilcholine bilayers (large unilamellar vesicles) were investigated by measuring calcein release from the phospholipid liposomes using a Hitachi F-2000 spectrofluorimeter.

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LOW MOLECULAR WEIGHT PEPTIDIC FRACTIONS OF AFRICANIZED BEES VENOM *Apis mellifera* L. 1758 (HYM.:APIDAE).J.C. Bonfanti-Almeida¹, M.S. Palm² and N. Gobbi³¹Depto de Ciências Biológicas - FIRP - 15025-400 São José do Rio Preto, ²Depto de Biologia, ³Depto de Ecologia - I. B. UNESP - 13506-900 Rio Claro.

Protein and peptidic compounds of the honeybee venom are associated with their biological activities, and their biochemistry is well known. Venom compounds may vary in amount and quality according to caste, age, collection technique and storing. Their composition may also vary according to the *Apis mellifera* subspecies; European, African or Africanized. A pool of venoms collected from different africanized bee strains was investigated with the aim at determining the number of low molecular weights (< 5000 Da) through the method of exclusion molecular chromatography. The venom was collected with an electric extractor from several africanized bee colonies and later chromatographed in resin column Sephadex G-15. The profile of the protein and fractions of the venom was accomplished by measuring ultraviolet light absorption at 220 nm. Peak I of the molecular weight compounds was eliminated over 5000 Da, that is exclude in the V₀ of the column, which represents fraction I. The venom pool molecular weight of the six fraction found was determined: 3800 Da (fraction II), 3500 Da (fraction III), 3250 Da (fraction IV), 2850 Da (fraction V), 2550 Da (fraction VI) and 2100 Da (fraction VII).

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INFLUENCE OF GANGLIOSIDES ON RATTLESNAKE VENOM TOXICITY.

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Venoms in general are weakly immunogenic yet fairly toxic. This causes problems because serotherapy is the treatment of choice in snake-bite accidents, and horse antisera availability is dependent upon venom immunogenicity. To improve antisera production and extend the useful life of immunized horses much effort has been devoted to decreasing chronic venom toxicity.

On the other hand gangliosides, present in the ganglion cells of the central nervous system, have been showed to be able to bind cholera toxin and block its physiologic effects.

Based on these informations, this work consisted on check if those gangliosides could interact with rattlesnake venom blocking its toxicity.

Thus, different concentrations of GM1, main constituent gangliosides, were incubated with 2, 5, 10 e 20 LD₅₀ of crude venom of *Crotalus durissus terrificus* at room temperature for 30 minutes. After that groups of 4 mice each were injected with the mixture by using intraperitoneal route. Animal mortality was observed 24 and 48 hr after injection. Our results showed that 10 or 20 mg of GM1 protected 50% of the animals injected with 5 or 10 LD₅₀ respectively.

So, gangliosides, although having an unclear interaction with the venom constituents seem to be a tool to diminish rattlesnake venom toxicity and could be used to immunize horses or even be employed as a vaccine if immunological properties were conserved.

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PURIFICATION AND AMINO ACID SEQUENCE OF PEPTIDES FROM *Crotalus durissus colillineatus*A.V. Delmondes¹, A. Sebben², L. Mohry¹ and C. Bloch Jr.^{1,3}¹Laboratório de Bioquímica e Química de Proteínas/CEI/IB; ²Laboratório de Fisiologia Animal/FIS/IB and ³Departamento de Química/IB - Universidade de Brasília, Brasília-DF CEP 70910-900.

It's known that snake venoms are complex mixture of many different substances constituted mainly by proteins, peptides and ions. Crude venom (40 mg) of *Crotalus durissus colillineatus* was dissolved in phosphate buffer 0.1 mol/L pH 7.0 and submitted to gel filtration chromatography (Sephadex G-25), equilibrated with the same buffer. Eluted fraction of 12 mL were dialyzed against water using dialysis membrane of 3.5 kDa cutoff, liophylized and submitted to FPLC gel filtration chromatography using Superose 12 prepacked column. FPLC peaks were collected manually and analyzed by SDS-PAGE (Tris-Tricine, Gradient 8 to 25%) in the Phast System from Pharmacia. Highly purified peptide samples were obtained by RP-HPLC (Vydac C₂ column) of the individual FPLC peaks using isopropanol/acetone nitrile gradient for amino acid sequence determination.

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SNAKE VENOM PEPTIDES WITH ANALGESIC ACTIVITY

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Crotalic and bothropic venoms were fractionated by gel filtration on Sephadex G-75 column. The peptide fractions were pooled, desalted by ultrafiltration using an Amicon system and then lyophilized. These fractions, showing analgesic activity in mice by the hot plate assay, were submitted to paper high voltage electrophoresis (HVE) at pH 6.5. After elution and drying of the neutral band, which encloses free neutral amino acids, a second HVE was run, now at pH 3.5. By controlling the HVE time, most of the free amino acids, if not all, have been removed. Two final electrophoretic bands could finally be obtained, both of them positive to nymydrin and peptide nature. Based on their electrophoretic mobility relative to aspartic acid, they are neutral at pH 6.5. Due to the very low percentage of these peptides relative to the whole venom, several cycles of the above procedure should be carried out in order to gather enough material for the activity assay, purity tests and sequencing. Parallel experiments are being run by high performance liquid chromatography as an attempt to overcome this difficulty.

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MOLECULAR AND PHARMACOLOGICAL CHARACTERIZATION OF BRADYKININ-POTENTIATING PEPTIDES PRECURSOR, WHICH INCLUDES A NEW MEMBER OF THE NATRIURETIC PEPTIDE FROM BOTHROPS JARARACA VENOM.

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While trying to identify the precursor of the bradykinin-potentiating peptides (BPPs) from *B. jararaca* venom, we isolated a cDNA from *B. jararaca* venom gland library encoding one rosary type polyprotein, which contains 5 copies of the BPPs of known structures, and 2 unknown putative BPPs. The same precursor contains one copy of a new member of the natriuretic peptide family.

Besides the presence of processed BPPs, already isolated and characterized from snake venom, our study also showed evidences for the presence of the processed natriuretic