ACTION OF ANTIVENOM ON RENAL EFFECTS CAUSED BY *Thalassophryne nattereri* FISH VENOM.

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Thalassophryne nattereri, popularly known as Niquim is responsible for many accidents in fishermen in the Northeast of Brazil. The Niquim venom provoked pain, edema, transitory ischemia and alteration in the renal function. In this work, we have examined the action of *T. nattereri* antivenom on renal effects caused by *T. nattereri* fish venom. Isolated kidneys from Wistar rats of 240-280g weight were perfused with Krebs-Henseleit solution containing 6% of bovine serum albumin. The parameters studied included perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF), glomerular filtration rate (GFR). The Niquim venom (3mg/mL), antivenom (3mg/mL) alone or antivenom plus Niquim were added to the system 30 minutes after the beginning of each experiment. The data were analyzed by ANOVA and Bonferoni test with (*p<0.05). The antivenom reverted the increase in PP (control = 110.2 ± 3.7mmHg; niquim = 148.2 ± 6.9mmHg*; antivenom=126.8±6.7mmHg), RVR (control=5.48±0.53mmHg/mL/g/min; niquim=7.75±0.7mmHg/mL/g/min*; antivenom=6.86±0.4mmHg/mL/g/min*), UF (control = 0.16 ± 0.02mL/g/min; niquim = 0.40 ± 0.08mL/g/min*; antivenom=012±0.013mL/g/min), GFR (control=0.69±0.08mL/g/min; niquim=2.47±0.69mL/g/min*; antivenom=0.51±0.06mL/g/min) promoted by Thalassophryne nattereri fish venom. In conclusion, the antivenom was able to inhibit the effects induced by *Thalassophryne natterer* venom in the isolated kidney.

KEY WORDS: antivenom, fish venom, *T. nattereri*, renal effects.

FINANCIAL SUPPORT: CNPq.

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EXCITATORY EFFECTS OF *Thalassophryne nattereri* VENOM ON VASCULAR TISSUE

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Thalassophryne nattereri is a venomous fish found in the northeast of Brazil. The venom effect has not been investigated on vascular tissues. Our aim was to examine the functional alterations produced by the venom in vascular reactivity as well as to verify changes in the smooth muscle cell (SMC) membrane potential. Male Wistar rats (155±10g, N=6) were killed and mesenteric rings (5-6 mm) free of endothelial layer were set up in gassed (95% O2/5% CO2) Krebs-Henseileit solution, pH 7.4, at 37±1°C. Vascular reactivity were tested under a tension of 1g after 30-45min equilibration period and mechanical activity was measured isometrically. Contractile cumulative concentration-response curves were constructed with *T. nattereri* venom (1; 3; 10; 30; 100 µg protein/mL). The same procedures were taken in male C57Bl/6J mice (25-30 g, N=3) to study vascular reactivity in aorta. In a Lucite chamber with Sylgard bed, we pinned a 2-3 mm length of the mesenteric artery. Vascular SMC membrane potentials were measured using glass microelectrodes filled with 3M KCl solution, and tip resistances of about 40 MΩ. All the criteria for acceptance of recordings were observed. T. nattereri venom (10 µg protein/mL) induced a significant contractile response both mice aorta (4.3 ± 1.2 mN/mm; P<0.05) and rat mesenteric rings (0.9 ± 0.03 g; P<0.05). The EC50 value in mesenteric tissue was 6.63 µg protein/mL. The venom of *T. nattereri* when in contact with SMC from mesenteric artery caused membrane depolarization from -56 ± 0.62 mV to -28 ± 1.16 mV (N=3; P<0.05). Based on our present results we concluded that the *T. nattereri* venom has excitatory effects on smooth muscle from aortic and mesenteric artery.

KEY WORDS: *Thalassophryne nattereri*; VASCULAR REACTIVITY; MEMBRANE POTENTIAL.

FINANCIAL SUPPORT: CNPq/PROPEP/UFAL

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BIOCHEMICAL AND BIOLOGICAL VARIATION BETWEEN THE MUCUS AND THE STING VENOM FROM THE CATFISH *Cathorops spixii*

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Catfishes are known to cause the majority of the accidents in the Brazilian shoreline. These fish possess a toxic mucous-covered sting, responsible for most of the injuries, as well as a toxic epidermal secretion which may contribute to the symptoms of the accident. Our objective was to compare the biochemical and biological features presented by these two secretions (mucus and sting). 1,5 mg of each secretion were separated by RP-HPLC using a C18 analytical column and the obtained fractions were analyzed by MALDI-TOF/MS and screened by intravital microscopy, for biochemical and biological characterization. Seventeen RP-HPLC fractions for each venom were obtained and the mucus contained only four major peaks while the sting presents eight peaks, being three of them common to the mucus. The fractions showed a wide range of bioactivities in the microcirculatory system: Vasoconstriction (fractions 1, 3, 7, 16 of the mucus and 5, 6, 9, 11, 12, 14 of the sting), increase of the rolling leukocyte (fractions 3 of the mucus and 1, 5, 6, 9 of the sting), increase of the rolling leukocyte followed by a decrease (fractions 11, 12, 14 of the sting), increase the number of adhered leukocytes (fractions 1, 6, 9, 11, 12, 14 of the sting), decrease of the blood flow in the veins (fractions 1, 7, 16 of the mucus and 3, 12 of the sting), stoppage of the flow in the veins (fractions 7, 16 of the mucus and 3, 12 of the sting), vasodilatation (fraction 1 of the sting), stoppage of the capillaries (fraction 16 of the mucus), disruption of vases and hemorrhage (fraction 9 of the sting). In light of these results, we can observe that the sting contains a richer toxic-peptide pool than the mucus. Moreover, all tested fraction presented some kind biological activity in the microcirculation, thus demonstrating the presence of toxic molecules that are able to participate on innate inflammatory immune response.

KEY WORDS: fish, venom, *Cathorops spixii*, peptides.

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BCIV, A NEW PARALYZING PEPTIDE FROM THE VENOM OF THE SEA ANEMONE *Bunodosoma caissarum*. A COMPARISON WITH THE Na+ CHANNEL TOXIN BCIII

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Sea anemones produce a wide variety of biologically active compounds, such as the neurotoxins and cytolysins. Herein we report a new peptide, purified to homogeneity from the neurotoxic fraction of *B. caissarum* venom, by using gel filtration followed by rp-HPLC, naming it as BcIV. BcIV is a 41 amino acid peptide (molecular mass of 4669 Da) possessing 6 cysteines covalently linked by three disulfide bonds. This toxin has 45 and 48% of identity when compared to APETx1 and APETx2 from Anthopleura elegantissima, respectively, and 42% of identity with AmII and BDS-I and -II obtained from *Antheopsis maculata* and *Anemonia sulcata*, respectively. This neurotoxin presents only a weak paralysing action (minimal Lethal Dose close to 2000µg/kg) in swimming crabs *Callinectes danae*. This appears to be a different effect to that caused by the type 1 sea anemone toxin BcIII that is lethal to the same animals at lower doses (LD50=219µg/kg). Circular dichroism spectra of BcIII and BcIV show a high content of β-strand secondary structure in both peptides, very similar to type 1 sodium channel toxins from various sea anemones, and to APETx1 and APETx2 from *A. elegantissima*, a HERG channel modulator and an ASIC3 inhibitor, respectively. Interestingly, BcIII and BcIV have similar effects on the action potential of the crab leg nerves, suggesting the same target in this tissue. As BcIII was previously reported as a Na+ channel effector and BcIV is inactive over Na+ currents of mammalian GH3 cells, we propose a species-specific action for this new molecule. A molecular model of BcIV was constructed using the structure of the APETx1 as template and putative key residues are discussed.

KEYWORDS: *Bunodosoma caissarum*, Sea Anemone, MS/MS Spectrometry, Molecular Modelling.

FINANCIAL SUPPORT: FAPESP, CNPq

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CYTOTOXIC ACTIVITY OF PEREZONE, A QUINONE ISOLATED FROM THE CARIBBEAN GORGONIAN CORAL *Pseudopterogorgia rigida*

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Gorgonian corals are known for their ability to produce biologically active compounds. Previous studies have demonstrated antimicrobial activity in a crude extract derived from *Pseudopterogorgia rigida*. The aim of this study was to evaluate the cytotoxic potentials of a crude extract, derived fractions and a purified quinone, perezone, obtained from the Caribbean gorgonian coral *P. rigida*. The gorgonian coral was collected during an expedition cruise to the Bahamas.14 colonies were extracted in 20% MeOH in DCM, dried and fractionated over a flash silica columm eluted with a raising polarity gradient of TMP/EtOAc/MeOH. The columm yielded 13 fractions and perezone was isolated from fractions 5 and 6. The crude extract, fractions 1 through 10 and perezone were assayed for cytotoxicity against a panel of 4 human tumor cell lines - HL-60 (leukemia), MDA-MB435 (breast), HCT-8 (colon) and SF-295 (central nervous system) - and quantified colorimetrically by the MTT assay after 72 hours incubation. Perezone was also evaluated for membrane damage on mouse erythrocytes, incubated for 1, 2 and 4 hours. The crude extract showed a strong cytotoxic activity. Fractions 5 and 6 were the most powerful inhibitors of cell proliferation, showing and IC50 around 3ug/mL. Perezone showed a similar strenght in its inhibitory activity to fractions 5 and 6 (IC50 also around 3ug/mL), which suggests that it must be responsible for their cytotoxic activity. Moreover, perezone did not induce hemolysis on the mouse erythrocytes, not even at the highest assayed concentration (50ug/mL). Further studies are needed to elucidate the mode of action of perezone and its molecular targets.

KEY WORDS: *Pseudopterogorgia rigida*, perezone, cytotoxic activity.

FINANCIAL SUPPORT: CNPq, InCb, FINEP.

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EFFECT OF *Thalassophryne nattereri* FISH VENOM ON ADHESION AND VIABILITY OF NORMAL AND TUMORIGENIC CELLS

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Thalassophryne nattereri fish venom are rich sources of bioactive substances that can affect several process, e.g., inflammation by causing changes in cellular recruitment and viability, and agents that block appropriate cell-matrix interactions are know to have an important therapeutic utility in inhibiting tumor-induced neovascularization and tumor progression. The aim of the present study was to analyse the changes in adhesion and viability after *T. nattereri* venom treatment of macrophages and HeLa or NCIH292 carcinoma cells. Confluent cells at high density (1 x 106 cells/well) were cultivated for 24 hours under differential doses of venom (0.1, 0.3, 1, 10, 20, 100 mg for macrophages; 0.2, 2 or 20 mg for HeLa; 0.1, 0.3, 1, 10, 20, 100 mg for NCIH292). After culture, cells were stained with violet crystal at 0,05% for the determination of the cellular adherence and, for evaluation of the viability was used quantitative method of MTT reduction (0,5 mg/mL). In normal cells, the effect of the venom on adhesion or viability was only observed at high dose (100 mg). *T. nattereri* venom induced a significant detachment of HeLa cells at all doses used, but only with the higher dose of the venom it was observed an elevated number of death cells. The low doses of the venom (0.1 to 1 mg) induced detachment of NCIH292 cells near to 40%, and 25% of cell death. The higher doses used (10 to 100 mg) affect drasticaly the adherence, and only the doses of 20 and 100 mg induced 55.07%, and 88.13% of cell death, respectively. These results show that cells presented different pattern of susceptibility to venom, and the action of venom on cell viability is independent of its action on detachment of cells to matrix. We can suggest that the *T. nattereri* venom can block appropriate cell-matrix interactions and possesses cytotoxic activity on tumorigenic cells.

KEY WORDS: adhesion, viability, *Thalassophryne nattereri*, tumoral cell

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THE EFFECT OF THE TOXIN *Phyllorhiza punctata* IN MOUSE VAS DEFERENS

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The *Phyllorhiza punctata* species (Stomolophidae) was discovered in the Brazilian coast in middle of the decade of 1950. This species were collected during periods of low tide by free diving at different rocky shores of the São Vicente Channel on the southern coast of Sao Paulo, Brazil. The Tentacles were removed from body, imemersed in ice-cold aqueous solution of 0,1%TFA and subjected to three cycles of freezing. After that, the solution was centrifuged and the supernatant was filtered, centrifuged and submainted to desalting and final cleaning. Then this extract was conncentrated by lyophilization. In preliminary experiments this extract (PHY-N) showed mouse vas deferens (MVD) contraction effect. To investigate whether the increase of neurogenic contractions in MVD induced by PHY-N was dependent on a post-synaptic mechanism a1-adrenergic, the following protocol was used. Albinic Swiss mice had been sacrificed by cervical displacement and *vas deferens* had been removed. A segment of approximately 1 cm of length was removed of the prostate portion and chemical preparation in organic chamber that contained solution of Krebs for *vas deferens*. PHY-N was evaluated for its biological activity in the autonomic neuromuscular junction by using the eletrical stimulated MVD as a model. A cummulative concentration-response curve (0.1 to 1000 ng/mL) was perfomed for each fraction in separated protocols. The EC50 was determined by non-linear regression and the result showed the 1,172 µg/mL value. The contractions elicited after exposition to exogenous noradrenaline (NA; 10 mM; n=6), were analyzed in the absence or presence of the EC50 of the fraction. The results showed that contraction induzed by NA (72,3±19,7) was not significantly altered by PHY-N (96,0± 22,2). Suggesting that contraction induced by PHY-N was not dependent on a post-synaptic mechanism a1-adrenergic.

KEY WORDS: *Phyllorhiza punctata*, mouse *vas deferens*.

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CAISSAROLYSIN I (BCS I), A NEW HEMOLYTIC TOXIN FROM THE BRAZILIAN SEA ANEMONE *Bunodosoma caissarum*: PURIFICATION AND BIOLOGICAL CHARACTERIZATION

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Two proteins, C1 and C3, were purified from the venom of *Bunodosoma caissarum* sea anemone. The purification employed gel filtration and FPLC, being the purity and molecular weight confirmed by mass spectrometry. Protein C1, the second major peak of the hemolytic fraction, has a molecular weight of 15495 Da and an amino terminal with no similarity to all known hemolysins. When assayed for hemolysis, PLA_2 activity and acute toxicity in crabs and mice this protein had no activity in these assays and, therefore, should not be considered a toxin. The protein C3 (MW 19757), also lacks PLA_2 activity but is recognized by antiserum against Eqt II. It has a high hemolytic activity to human erythrocytes (ED50 = 0.270 µg/ml) and was inhibited by pre-incubation with sphingomyelin, being named Caissarolysin I (Bcs I). Caissarolysin I belongs to the Actinoporins and is the first hemolysin purified from a sea anemone belonging to the genus *Bunodosoma*. All this data was published in the paper Oliveira et al., 2006 (1).

KEY WORDS: Hemolysin, Sea anemone, *Bunodosoma caissarum*, sphingomyelin

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TOXICITY AND TOXIN IDENTIFICATION IN *Colomesus asellus*, AN AMAZONIAN (BRAZIL) FRESHWATER PUFFER FISH

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We evaluated the toxicity in the extracts of *Colomesus asellus*, a freshwater puffer fish from the rivers of the Amazon and identified the components responsible for its toxicity. For this, it was employed mouse bioassay, ELISA, HPLC and mass spectrometry. The T20G10 monoclonal antibody raised against TTX, and employed in an indirect competitive enzyme immunoassay, showed very low affinity for the *C. asellus* extracts, indicating that TTX and its analogues are not the main toxic components of the extracts. This antibody was efficient in detecting presence of TTX in a total extract of *Sphoeroides spengleri*, which is one of the most toxic puffer fish found in the Atlantic coast. Extracts of *C. asellus* were toxic when administered intraperitonially into mice with an average toxicity of 38.6 ± 12 MU/g, while HPLC analysis indicated a lower toxin content (7.6 ± 0 5 MU/g). No traces of TTX were evidenced by HPLC, but only the presence of PSPs (STX, GTX 2 and GTX 3). These toxins were also confirmed by electrospray ionization mass spectrometry. All these data was published in the paper Oliveira et al., 2006 (1).

KEYWORDS: *Colomesus asellus*, *Sphoeroides spengleri*, ELISA, freshwater puffer fish, saxitoxin, gonyautoxin, HPLC, mass spectrometry

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EFFECT OF SCORPIONFISH *(Scorpaena plumieri*) VENOM ON CULTURED MURINE GLIOBLASTOMAS CELLS (RT2)

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Animal venoms have been recognized as potential sources of pharmacological agents and physiological tools. The scorpionfishes (Scorpaena) are the most venomous fishes in the Atlantic Ocean. There is very little information on the venom of scorpionfishes whereas studies describing biological properties of fish venoms have focused mainly on lionfishes. These venoms often contain active components such as catecholamines, acetylcholine and some enzymatic activities such as proteolytic hyaluronidases and phosphatases. The effect of *Scorpaena plumieri* venom (SP) on tumoral cells has not been studied yet. The aim of this work was to identify and characterize the antitumoral effect of SP on cultured murine glioblastomas cells (RT2). RT2 cells were treated with varying concentrations of SP and cytotoxicity was established using MTT assay. RT2 cells were sensitive to SP in a dose-dependent way. Low concentrations of SP venom did not modified significantly the metabolism of RT2 cells, but significant reduction in metabolism could be observed at SP concentrations higher than 5mg/mL (IC50=16,7mg/mL) followed by morphological disturbs, such as rounded cell shape and reduction of the cytoplasmic volume. SP effects on cell adhesion and clonogenicity were also evaluated. Inhibition of cell adhesion and proliferation could be observed at concentrations higher than 10mg/mL. At concentration of 100mg/mL the cells were completely lysed. Metalloproteases are involved in cell-matrix interactions. Since proteolytic activity has been found in many fish venoms, we evaluated the SP venom proteolytic activity on gelatin by zymography, in order to shed some light on the mechanisms of its effects. We found that SP venom presents gelatinolytic activity that could stand, at least partially, for the anti-tumoral effect of SP.

KEY WORDS: scorpionfish, venom, glioblatoma, metabolism

FINANCIAL SUPPORT: CNPq, CDTN/CNEN

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PURIFICATION AND PARTIAL CHARACTERIZATION OF A HYALURONIDASE (SpH) FROM THE VENOM OF THE SCORPIONFISH *Scorpaena plumieri*

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Fish's envenomation involves subcutaneous or intramuscular injection of venom into the prey/human victims. The pathology of envenomation includes local effects, such as the degradation of proteins and glycosaminoglycans in the extracellular matrix, in connective tissue surrounding blood vessels and capillaries, beyond systemic effects, such as cardiovascular and neurological disorders. Agents as hialuronidases, which promote degradative process, are referred to as "spreading factors", these has been considered as an invariant factor in the venoms. In this work a new hyaluronidase (denominate SpH) from the scorpionfish venom *S. plumieri* was purified to homogeneity through a combination of three chromatographic steps: gel filtration on Sephacryl S 200 HR, anion exchange/FPLC on Mono-Q 5/5 HR and reverse phase/HPLC on a Source 15ST column. Activity was assayed by HA substrate – Hyaluronan gel zymography procedures. The molecular mass was found to be 76,955 Da by MALDI-TOF mass spectrometry and the amino terminal sequence of 19 residues was determined by automatic sequencing using a standard Edman degradation program and is APADKVAWGVKK_KLL_K_ _VMA. The carried out searches with the partial sequence shows similarily with the SFHYA1 hyaluronidase from the venom of stonefish (*Synanceja horrida*). This is the first report of the isolation and characterization of a scorpionfish venom hyaluronidase.

KEY WORDS: scorpionfish, hyaluronidase, *Scorpaena plumieri*.

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COMPARATIVE STUDY OF VENOMS AND SECRETORY CELLS OF STING APPARATUS FROM FRESHWATER (*Potamotrygon falkneri)* AND MARINE *(Dasyatis guttata*) STINGRAYS

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Stingrays are found along marine coast and in rivers in Brazil, and their venom apparatuses (bilaterally retroserrate spines covered by glandular and integument tissues) are located in their tail. Patients wounded by stingrays usually complain of pain, but only accidents by freshwater stingrays are followed by cutaneous necrosis. The aim of this work was to characterize some aspects venoms and sting apparatus of *P. falkneri* (Pf) and *D. guttata* (Dg). By SDS-PAGE, both venoms showed similar patterns above 80 kDa, but most differences were observed below this molecular mass. Major bands were located around 10 and 15 kDa. In mice, lethal, dermonecrotic and myotoxic activities were detected only in Pf venom. Edematogenic activity was similar and dose-dependent in both venoms. The presence of nociceptive activity was verified in both venoms, but Pf presented a twofold higher activity than Dg venom. No direct hemolysis and coagulant activities were observed in both venoms using human blood cells. Antigenic cross-reactivity was observed by ELISA and WB using species-specific sera produced in rabbits by ELISA and Western Blotting. By zymography, both venoms presented gelatinolytic, caseinolytic and fibrinogenolytic activities. Hyaluronidase activity was detected solely in Pf venom. Morphological differences were found between Pf and Dg venom apparatus, especially in the position and density of cells that comprise the sting tegument. Our experimental results might explain the difference in clinical picture observed in patients wounded by freshwater and marine stingrays.

KEY WORDS: stingrays, *Potamotrygon*, *Dasyatis*, venom

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SEX-LINKED VARIATION OF *Thalassophryne maculosa* FISH VENOM

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In this work we have compared some biological and biochemical properties of female and male *T. maculosa* fish venoms. Females and males were captured in deep waters of the La Restinga lagoon (Venezuela) and their venom collected from the spines. Protein content is higher in males than in females, 3.9 mg/ml vs 1.7 mg/ml, respectively. It is also noteworthy that the LC-MS profile of the venoms showed that, at least three components are only present in males, bearing molecular masses of 15135 and 15633 at RT 15,5 min and one peptide of 5066 Da at RT 13.5 min being the peaks not detected in the female venom. The visual analysis of the gels indicated in the male venom there is a group of abundant, well-stained spots of ~80 kDa and a group of weak spots of ~25 kDa which are absent in the female venom. On the other hand, the female gel showed spots of 25 kDa and 14 kDa not visible on the male gel. In vivo studies showed that mice injected with male venoms presented higher nociception when compared to those injected with female venoms, and both venoms induced migration of macrophages into the paw of mice. On the other hand, mice injected with female venoms had more paw edema and extravasation of Evans blue in peritoneal cavity than mice injected with male venoms. Finally, we observed that the female venoms had more capacity for necrosis induction when compared with male venoms (6.11 ± 0.85 mm2 vs 3.41 ± 0.42 mm2). These results suggest that there are different toxins involved in the nociception and edema induced by *T. maculosa* venom. Furthermore, the presence of exclusive toxins in the male venoms could be related with the largest capacity of nociception induction, and the severity of the lesion characterized by necrosis development can be related with the poisoning for female specimens.

KEY WORDS: *Thalassophryne maculosa*, female and male fish venom, biological and biochemical properties

FINANCIAL SUPPORT: FAPESP

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ORPOTRIN: A NOVEL VASOCONSTRICTOR PEPTIDE FROM THE VENOM OF THE BRAZILIAN STINGRAY *Potamotrygon gr. orbignyi*

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Characterization of the peptide content of venoms has a number of potential benefits for basic research, clinical diagnosis, development of new therapeutic agents, and production of antiserum. In order to analyze in detail the peptides and small proteins of crude samples, techniques such as chromatography and mass spectrometry have been employed. The present study describes the isolation, biochemical characterization, and sequence determination of a novel peptide, named Orpotrin from the venom of *Potamotrygon gr. orbignyi*. The natural peptide was shown to be effective in microcirculatory environment causing a strong vasoconstriction. The peptide was fully sequenced by de novo amino acid sequencing with mass spectrometry and identified as the novel peptide. Its amino acid sequence, HGGYKPTDK, aligns only with creatine kinase residues 97-105, but has no similarity to any bioactive peptide. Therefore, possible production of this peptide from creatine kinase by limited proteolysis is discussed. Taken together, the results indicate the usefulness of this single-step approach for low molecular mass compounds in complex samples such as venoms.

KEY WORDS: Orpotrin, *Potamotrygon* venom; Stingrays; arteriolar vasoconstriction, high performance liquid chromatography, *de novo* sequencing, natural peptides.

FINANCIAL SUPPORT: FAPESP, CNPq.

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DOSE-RESPONSE CURVE OF THE SEA ANEMONE *Bunodosoma caissarum* VENOM ON KIDNEY RENAL PERFUSION

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Sea anemones contain a variety of interesting biological active compounds including some potent toxins. The aim of this work was to study the alterations produced by *Bunodosoma caissarum* venom (BcV) in the isolated rat kidney in different doses. Isolated kidneys from Wistar rats (250-300g) were perfused with Krebs-Henseileit solution containing 6% of bovine serum albumin for 120 min, and the initial 30min was an equilibration period (internal control). BcV 1mg/mL/min (BcV1), 3mg/mL/min (BcV3) and 10mg/mL/min (BcV10), n=6 for each group, were added to system 30 min after the beginning of experiment. The external control (Ec) was perfused only with Krebs-Henseileit solution. The data were analyzed by ANOVA and Student's t-test (*p<0,05). BcV3 and BcV10 doses caused increase of perfusion pressure at 60min (EcPP =104.2±3,7; BcV3PP= 127.7±5.8*; BcV10PP= 134.7±7.4* mmHg / EcRVR = 4.9±0.16; BcV3RVR= 6.48±0.47*; BcV10RVR= 5.79±0.42*mmHg/mL/g/min), only with BcV3 at 90min (EcPP =104.7 ±4,2;BcV3PP=126.1±5.2*mmHg and EcRVR=4.47±0.2;BcV3RVR=6.36±0.37* mmHg/mL/g/min) and with BcV1 and BcV3 at 120min (EcPP =107.6 ± 3,15; BcV1PP= 122.3 ± 5.2* mmHg; BcV3PP= 140.1 ± 6.5* mmHg and EcRVR = 4.71±0.18; BcV1RVR= 5.82±0.44*; BcV3RVR= 7.04±0.39*mmHg/mL/g/min). The urinary flow was increased by 3mg/mL/min and 10mg/mL/min doses at 90 and 120min respectively (90':cFU=0.16 ±0.02; BcV3FU=0.31±0,04*;BcV10FU =0.25±0.03*mL/g/min and 120':cFU = 0.16 ± 0,02; BcV3FU=0.4 ± 0.05*; BcV10FU = 029 ± 0,03* mL/g/min). The glomerular filtration rate decrease with 1mg/mL/min and increase with 10mg/mL/min doses at 120min. BcV didn't present dose-response relation in the doses and parameters studied.

KEY WORDS: *Bunodosoma caissarum*, kidney perfusion, sea anemone

FINANCIAL SUPPORT: CNPq.

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INDOMETACIN BLOCKAGE THE *Bunodosoma caissarum* VENOM EFFECTS ON KIDNEY RENAL PERFUSION

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Sea anemones contain a variety of interesting biological active compounds including some potent toxins. For this reason many investigators have focused attention on the biological activities of the protein molecules of various species of sea anemones, with *Bunodosoma caissarum*, a sea anemone endemic in the Brazilian southern coast. The aim of this work was to study the alterations produced by block with indometacin (Idm) in the isolated rat kidney perfused with Bunodosoma caissarum venom (BcV). Isolated kidneys from Wistar rats weighing 240-300g were perfused with Krebs-Henseileit solution containing 6% of bovine serum albumin for 120 min. Indometacin was added to system 30 min before the BcV (3mg/mL;n=6). The data were analyzed by ANOVA and Student's t-test (p<0,05). Indometacin blocked the effects induced by BcV in perfusion pressure (CPP60'=104.17 ± 3.72; BcVPP60'=125.5 ± 6.1*; BcV + IndPP60'=112.4 ± 2.94mmHg), renal vascular resistance (CRVR60'=4.9 ± 0.16; BcVRVR60'=5.91 ± 0.39*; BcV + IndRVR60'=4.85 ± 0.23mmHg/mL/g/min), urinary flow (CUF90'=0.16 ± 0.02; BcVUF90'=0.241 ± 0.027*;BcV + IndUF90'=0.115 ± 0.006 mL/g/min) and glomerular filtration rate (CGFR120'=0.69±0.08; BcVGFR120'=1.046±0.127; BcV + IndGFR120'=0.489 ± 0.042* mL/g/min). The infusion of indometacin before BcV in the isolated rat kidney blocked the *Bunodosoma caissarum* effects in perfusion pressure and renal vascular resistance but this effect occur only partially in urinary flow and glomerular filtration rate. These actions suggest that inflammatory mediators could have been important substances of BcV renal effects.

KEY WORDS: *Bunodosoma caissarum*, kidney perfusion, sea anemone, Indometacin

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CLINICAL OBSERVATION IN THE ETIOLOGY AND DETERMINATION OF THE GRAVITY OF HUMAN INJURIES FOR JELLYFISH AND PORTUGUESE MAN-OF-WAR (CNIDARIA) IN BRAZIL

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Cnidarians are animals that have small organs called nematocysts, present in cnidocytes. Those structures fire up by contact or osmosis and inject polypeptides of neurotoxic and dermatonecrotic actions. The gravity of the injuries is determined by the area of the compromised skin, by the corporal area of the victim and the specie of cnidarian. Fifty and eight patients had been observed in the Ubatuba Hospital (North Coast of São Paulo State), in a period of three years (2002/2004). After to receive the first aids, they answered a questionnaire and their lesions photographed. Local (edema, erythema, blisters, necrosis and pain) and systemic manifestations (malaise, vomit, dyspnea and tachycardia) had been evaluated, as so the extension of the contact. About 80% of the patients presented only local manifestations (small, oval or circular marks and marks of small tentacles, lesser than 20 cm). Near 20% of the victims presented linear marks bigger than 20 cm, intercrossed, with frequent systemic phenomena. The pattern of short and rounded marks is caused for the hydromedusa *Olindias sambaquiensis*, being accidents of small magnitude. The long and linear marks followed of systemic phenomena and intense pain are compatible with Cubomedusa (*Tamoya haplonema* and *Chiropsalmus quadrumanus*) and Portuguese Man-of-War (*Physalia physalis*). An association between the skin marks, the probable etiology of the accidents and the gravity exists. It can be useful to standardize the attendance of the accidents, once there is no such approach in other studies around the world.

KEY WORDS: cnidarians, injuries in humans, *Chiropsalmus quadrumanus*, *Tamoya haplonema*, *Physalia physalis*, *Olindias sambaquiensis*, venomous marine animals.

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ANALGESIC EFFECTS OF A FRACTION (BcgNN) OBTAINED FROM THE SEA ANEMONE *Bunodosoma cangicum* VENOM

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A non-neurotoxic fraction (BcgNN), eluted after the fraction III (neurotoxic), was obtained from the *Bunodosoma cangicum* venom by using a Sephadex G-50 gel filtration column. It was injected via intra-plantar route in rat paws inducing a potent local analgesia. The aim of the present study is to characterize this analgesic effect and to determine the possible mechanisms involved in this analgesia. Pain threshold was assessed using the rat paw pressure test, applied before and at different times after treatments. The fraction BcgNN (10, 100 and 1000ng/mL) was administered in rat paws. Glybenclamide (80µg/paw), apamine (10µg/paw), charybdotoxin (2µg/paw), TEA (640µg/paw) and 4-aminopyridine (100µg/paw), well known K+ channels blockers, were used to evaluate the involvement of K+ channels in the antinociceptive effect of *B.cangicum* fraction. Naloxone (5µg/paw) was used to evaluate the involvement of opioid receptors in this effect. BcgNN induced dose-dependent antinociception that peaked at 30 minutes and was detected until 120 min after its administration. This antinociceptive effect was reversed by TEA, but was not altered by 4-AP, glybenclamide, apamine, charybdotoxin and naloxone. By these data we conclude that BcgNN induces analgesic effect that is mediated by voltage-gated K+ channels activation. Apparently neither ATP-dependent nor Ca2+-dependent channels appear to mediate the analgesic effects of BcgNN. Opioid receptors also are not involved in the BcgNN analgesia.

KEY WORDS: Anemone, non-neurotoxic, BcgNN, *Bunodosoma cangicum*, analgesic.

FINANCIAL SUPPORT: FAPESP, CNPq.

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