3. Pharmacology

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3.01 Crotalphine and opioid receptor agonists hyper-activate opioid receptors underperipheral sensitization

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Introduction: Several data have shown that the peripheral efficacy of opioid drugs is enhanced in the presence of tissue injury, but the mechanisms involved in this phenomenon are not well known. Previous data of our group showed that, in rats, prostaglandin E_2 (PGE_2, intraplantar/i.pl.) and chronic constriction injury (CCI) of the sciatic nerve increase the peripheral analgesic efficacy of opioid agonists and of crotalphine (CRP), a peptide obtained from *Crotalus durissus terrificus* snake venom. CRP induces peripheral analgesia mediated by the activation of κ- opioid receptors in PGE_2-induced hyperalgesia or κ- and δ- opioid receptors in the CCI model. We have recently demonstrated that opioid receptor expression is distinctly regulated by the presence of acute or chronic injury in nerve paw (NP) and dorsal root ganglia (DRG) of rats, which may explain the increased efficacy of CRP and opioids. **Objectives:** This study aimed to further characterize some of the mechanisms involved in the increase of the analgesic efficacy of opioids caused by inflammation/tissue injury. **Methods:** For this purpose, the effect of PGE_2-induced hyperalgesia and CCI on opioid receptor activation in DRG and NP of male Wistar rats was evaluated. Activation of opioid receptors was assessed by ELISA assays in slices of NP or DRG, using antibodies to regions within the N-terminus of activated opioid receptors. This assay was performed 1 h after intraplantar injection of DAMGO (5 μg/paw), U-50488 (10 μg/paw), DPDPE (20 μg/paw), μ-, κ- and δ-opioid receptor agonists, respectively, or CRP (0.6 ng/paw) in naïve rats or in rats 3 h after i.pl. injection of PGE_2 (100 ng/paw) or 14 days after CCI. **Results and Discussion:** PGE_2 or CCI, *per se*, did not cause receptor conformational changes in opioid receptors in NP or DRG. Activation of opioid receptors was observed after treatment with CRP or opioid agonist. PGE_2 enhances the μ-opioid activation caused by DAMGO (22%) and κ opioid receptor activation induced by CRP or U50,488 (16 and 20%, respectively). In contrast, δ–opioid activation caused by DPDPE was not altered by previous sensitization. CCI enhances the κ– and δ–opiod activation caused by CRP (26 and 15%), but not by the selective agonists. These results indicate that acute and chronic sensitization increases opioid receptor activation. These alterations can contribute to the higher efficacy of CRP and opioid agonists peripherally administrated.

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3.02 Fluorescent analogues of crotalphine: antinociceptive effect and mechanism of action

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Introduction: Crotalphine (*CRP*) is a 14-mer peptide isolated from the venom of *C. durissus terrificus* that triggers long-lasting antinociception (3-5 days) mediated by κ– and/or δ-opioid receptor activation in experimental animal models of acute and chronic pain when administered *p.o.* and *i.v.* or *i.p.* injection. In spite of this, we do not know how *CRP* elicits its antinociceptive effect. Thus, the use of fluorescent analogues of *CRP* could allow us to understand its mechanism of action. Fluorescent ligands have been used for studies of processes triggered by ligand-receptor interaction due to their advantages on radioligands. However, the addition of fluorescent moieties to macromolecules can affect both their receptor selectivity as well as their biological activity. **Objectives:** The aim of this work was to synthesize *CRP* and *CRP*-fluorescent, functionally active analogues by the solid phase method and to investigate the mechanism of action of *CRP* on sensory neurons isolated from adult rat dorsal root ganglia. **Methods:** The peptides were synthesized at 60°C on a Cys(Trt)-Wang resin by Fmoc strategy. Carboxyfluorescein (CF) was introduced in the peptide-resin. Disulfide bond formation was achieved by air oxidation. The crude peptides were purified by RP-HPLC and characterized by amino acid analyses and LC/ESI-MS. Antinociceptive activity was evaluated through the paw pressure test in rats with hyperalgesia induced by prostaglandin E_2 (PGE_2, 100 ng/paw in 50 µL) treated or untreated with *CRP* or *CRP*-fluorescent analogues. The effect of *CRP*-fluorescent analogues on sensory neurons was evaluated by confocal microscopy in cell cultures pretreated with 1 µM PGE_2 and 10 µM bradykinin (BK) for 15-120 min. **Results and Discussion:** *CRP* and *CRP*-fluorescent analogues were successfully synthesized and their overall purities were higher than 96%. CF-[Glu^1]-*CRP* and CF-[Gln^1]-*CRP* did not induce an antinociceptive effect as did *CRP* and [Glu^1]-*CRP* when administered *p.o.* (0.25-5 µg/kg) in PGE_2-induced hyperalgesia. However, the administration of CF-[Glu^1]-*CRP* and CF-[Gln^1]-*CRP* by *i.p.* injection (0.5 µg/kg) induced antinociceptive effects equal to *CRP* and [Glu^1]-*CRP* in PGE_2-induced hyperalgesia. These results suggest that the large fluorescent moiety prevented the absorption of CF-[Glu^1]-*CRP* and CF-[Gln^1]-*CRP* by *p.o.*, but did not affect the pharmacophore structure require to trigger antinociception. Confocal microscopy analysis showed that CF-[Gln^1]-*CRP* internalize in sensory neurons pretreated with BK. On the other hand, sensory neurons pretreated with PGE_2 or without any pretreatment showed low fluorescence, indicating that these cells require a pretreatment or priming stimulus with BK for functional competence *in vivo*.

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3.03 A phospholipase A_2 isolated from snake venom up-regulates ADRP expression in macrophages

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Introduction: ADRP (adipocyte differentiation-related protein) is a member of the PAT protein family that is involved in the transport and storage of neutral lipids in multiple cell types. This protein is highly expressed in macrophages differentiated into foam cells from atherosclerotic lesions, where it is found on the surface of lipid bodies, which are important organelles in the inflammatory process. Recently, we showed that MT-III, a phospholipase A_2 (PLA_2) isolated from *Bothrops asper* snake venom, increased the number of ADRP-enriched lipid bodies in cultured macrophages. **Objectives:** The aims of this study were to evaluate the ADRP gene and protein expression in macrophages stimulated by MT-III, and to correlate these parameters with the number of lipid bodies. **Methods:** Thioglycolate-elicited macrophages from male Swiss mice were incubated with MT-III (0.4 μM) or culture medium (control) from 1 to 24 h, and gene and protein expression of ADRP was determined by Western blotting and real-time PCR, respectively. Lipid bodies were quantified by both the fluorescence method and staining with osmium tetroxide (1%), followed by analysis under phase contrast microscopy. **Results and Discussion:** Incubation of macrophages with MT-III significantly increased ADRP mRNA at 1 h of incubation and ADRP protein expression from 6 up to 12 h. Moreover, a significant increase in the number of LB was detected from 1 up to 24 h of stimulation with MT-III, with a maximum between 12 and 24 h of stimulation with MT-III. MT-III is able to up-regulate the ADRP gene and protein expression and lipid body formation in macrophages. Since maximal levels of LB followed ADRP protein expression, MT-III-induced ADRP expression may be relevant to the late increase in lipid body numbers induced by this venom PLA_2.

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3.04 Crotalphine reduces peripheral sensitization evoked by activation of TRPV1 receptor in mice

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Introduction: Crotalphine (CRP), a peptide first identified and isolated from *Crotalus durissus terrificus* snake venom, produces a potent and long-lasting analgesic effect mediated by activation of *kappa* and *delta* opioid receptors. Interestingly, the high effectiveness and long-lasting action of CRP is observed only in the presence of inflammation or tissue lesion, indicating that tissue sensitization is an important phenomenon for the expression of CRP effect. **Objectives:** In order to further characterize the role of previous sensitization in the action of this peptide, the aim of the present work was to evaluate the influence of peripheral sensitization induced by activation of TRPV1 receptors on the antinociception induced by CRP. **Methods:** All procedures were approved by the Institutional Animal Care Committee of the Butantan Institute (CEUAIB, protocol number 742/10). Male swiss mice (30-40 g) received an intraplantar (i.pl., 20 µl) injection of capsaicin (CPS; 0.03 or 1 nmol/paw) or prostaglandin E_2 (PGE_2; 0.01 nmol/paw) or the corresponding vehicles. In the experiments of previous sensitization, mice received the i.pl. injection of PGE_2 (0.01 nmol/paw) 3 h prior CPS administration (0.03 nmol/paw, i.pl., sub-threshold dose). Immediately after the treatments, the overt nociception [licking time (s)] was recorded for 5 min. Three hours after treatments, the development of allodynia was also evaluated, in the same animals, using the von Frey filaments (VFF). In this experiment, 0.6 g VFF was applied (10 applications with a duration of 3 s each) to the plantar surface of one of the hind paws of the mice and the % of withdrawal response frequency determined. **Results and Discussion:** Intraplantar administration of CPS induced overt nociception and mechanical allodynia as compared to controls. CRP (50-200 µg/kg), administered p.o. 1 h before CPS (1 nmol/paw), reduced, in a dose-dependent manner, the mechanical allodynia (52%, for 200 µg/kg), without interfering with overt nociception induced by the algogenic agent. The injection of PGE_2 3 h prior to CPS (sub-threshold dose) significantly increased overt nociception (CPS: 18±3 s; PGE_2: 16±4 s; CPS+PGE_2: 59±7 s) and mechanical allodynia caused by CPS. The PGE_2-induced potentiation of CPS-induced nociceptive phenomena was reduced (63% and 50%, respectively) by pre-treatment with CRP (200 µg/kg). These data confirm and extend previous findings from our group which demonstrated that the antinociceptive effect of CRP depends on prior sensitization. These results confirm the clinical importance of crotalphine as an analgesic agent, since inflammation is a component present in a great diversity of pathophysiological conditions.

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3.05 A new potential animal model of Parkinson's disease: The use of mouse strains selected for acute inflammatory response

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Introduction: One of the possible causes for the neuronal loss that leads to Parkinson's disease (PD) is a neuro-inflammatory response to chemical agents. Strains of animals disposed to produce intense or weak inflammatory responses can be used to determine the involvement of inflammation in the genesis of parkinsonian lesions. **Objectives:** We investigated the susceptibilities of two strains of inbred mice selected for high (AIRmax) or low (AIRmin) inflammatory response to stimuli such as Biogel. **Methods:** Groups of male animals (six months of age; BALBc mice) were used as controls in animal models of PD induced by treatment with 1-methyl-4-phenyl-1 ,2,3,6-tetrahydropyridine (MPTP) (5 x 20mg/kg i.p in 8 h) and rotenone (continuous infusion of 6 mg/kg/day, Alzet osmotic pump for 28 days). The animals were subjected to motor coordination assessment by Rotarod using a paradigm of rotation from 5 to 50 rpm over 5 min, measuring the time spent on the rotating bar. The lesion was quantified by immunohistochemistry for tyrosine hydroxylase in sections of the striatum and substantia nigra. **Results and Discussion:** The results showed that all strains of mice were resistant to MPTP or rotenone. The AIRmin strain showed immunohistochemistry suggestive of injury observed in substantia nigra by rotenone. Strains showed no significant motor impairment when evaluated by the Rotarod test. We suggest that the strains differ in peripheral inflammatory response, but not in neuro-inflammatory mechanisms. Still, we think the BALBc controls may not be ideal for this evaluation since the strain most often employed for Parkinson animal models is the C57BL/6. The BALBc are traditionally used as controls for AIRmax and AIRmin strains.

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3.06 Effect of gender on pain sensitivity and on the analgesic action of crotalphine, a peptide obtained from *Crotalus durissus terrificus* snake venom

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Introduction: Crotalphine (CRP), a peptide obtained from *Crotalus durissus terrificus* snake venom, induces analgesia by acting on opioid receptors. Due to its analgesic properties, pre-clinical trials with CRP are now in progress. However, the studies have always been carried out in male animals. **Objectives:** In the present study, differences in nociception and in the analgesic effect of CRP between male and female Wistar rats were evaluated. **Methods:** Differences in nociception and in the analgesic effect of CRP were evaluated using acute and chronic experimental pain models. Acute hyperalgesia was induced by intraplantar (i.pl.) injection of prostaglandin E_2 (PGE_2) into one of the hind paws. Neuropathic pain was induced by chronic constriction of sciatic nerve (CCI) and characterized by the presence of hyperalgesia and allodynia, 14 days after surgery. Mechanical hyperalgesia and allodynia were determined using the rat paw pressure test or von Frey filaments, respectively. CRP (p.o.) was administered immediately before the hyperalgesic agent, or on day 14 after surgery. To determine the influence of the estrous cycle, vaginal smears were examined. To determine whether the sex- related differences in nociception and crotalphine-induced antinociception were the result of the effects of gonadal hormones, female rats were submitted to ovariectomy. **Results and Discussion:** Female rats responded to lower hyperalgesic doses of PGE_2 than males. In PGE_2-induced hyperalgesia, females responded to lower analgesic doses of CRP (p.o.) than males. In females, the peptide, at 0.008 or 5 μg/kg, suppressed PGE_2-induced hyperalgesia for up to 3 or 6 days, respectively, whereas in males, CRP inhibited hyperalgesia for up to 3 h (0.2 μg/k) or 5 days (5 μg/kg). CRP was also more effective in inhibiting neuropathic pain in females than in males; however, in the CCI model, there were no sex differences in the duration of the analgesic action of the peptide. The antinociceptive action of CRP is mediated, in both sexes, by the activation of κ- ,and δ-opioid receptors. To determine the influence of gonadal hormones, females were ovariectomized (OVX). The nociceptive behavior of OVX rats, induced by PGE_2, was similar to that of male rats. The effect of CRP was more pronounced in intact females than in the OVX group. Hormonal replacement restored pain threshold in females. The estrous cycle phase did not interfere with pain threshold and with CRP effect. These data indicate that sex differences could be observed in relation to pain threshold. Despite displaying opioid activity, CRP is more effective in females.

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3.07 Effects of methotrexate and bee venom on plasma aminopeptidases

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Introduction: In the last years, the search for selective inhibitors for neutral (APN) and basic (APB) aminopeptidases and dipeptidyl peptidase IV (DPPIV) has increased. It is known that the inhibition of the above aminopeptidases leads to immunosuppression, due to their involvement in various immune mechanisms. Their roles include post-translational modifications of chemokines, antigenic processing, angiogenesis, molecular signaling and others. One drug commonly used for treating autoimmune diseases and cancer is methotrexate, which diminishes cell proliferation, chemotaxis and cytokine production; however, its mechanisms of action are not completely understood. In alternative therapy, bee venom is also applied for autoimmune inflammation; it contains some components to which are ascribed anti-inflammatory and antinociceptive actions. **Objectives:** The goal of the present study was to investigate the direct effects of methotrexate and bee venom on APN, APB and DPPIV activities in plasma of normal rats. **Methods:** The blood of six healthy male Wistar rats was collected with heparin and centrifuged at 200 x *g* for 10 min in order to obtain plasma. Methotrexate or bee venom at concentrations of 50, 500 and 5000 µg/mL in distilled water was incubated with plasma for 20 min at 37ºC. Subsequently, synthetic naphthylamide substrates in appropriate buffers were added to the reaction mixture. This mixture was allowed to react for 30 min at 37ºC. Aminopeptidase activities were measured fluorometrically. Samples without methotrexate or bee venom were considered controls (100%), and results were expressed as relative percentage of control±SEM. **Results and Discussion:** Bee venom caused a decrease of 60±2% of APN activity at concentration of 5000 µg/mL, did not alter APB activity, and increased DPPIV activity by 47±9% at a concentration of 50 µg/mL, 259±10% at a concentration of 500 µg/mL and 803±22% at a concentration of 5000 µg/mL. Methotrexate at a concentration of 500 µg/mL inhibited 60±9% of APN, 57±9% of APB and 55±10% of DPPIV activities, and at a concentration of 5000 µg/mL caused total inhibition of all aminopeptidases under study. The increase in DPPIV in samples incubated with bee venom corroborates data that describes DPPIV activity in this venom. Moreover, bee venom may contain a component capable of inhibiting APN activity, by which it could present its anti-inflammatory properties. APB activity is not altered by bee venom. Methotrexate may be an aminopeptidase inhibitor, which could be a novel mechanism of action of this drug.

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3.08 New cytolytic peptides from the venoms of solitary Eumenine wasps

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Introduction: In our continuing survey of biologically active substances in solitary wasp venoms, we have isolated four new cytolytic peptides from two species of Eumenine wasps, *Eumenes rubrofemoratus* (Eu) and *E. fraterculus* (Ef). Two of them, named Eu-5 (LNLKGLIKKVASLLN) and Ef-10 (LNLKGLFKKVASLLT), are quite similar to Eumenitin (LNLKGIFKKVASLLT), whereas the other two, named Eu-6 (FDIMGLIKKVAGAL-NH_2) and Ef-11 (FDVMGIIKKIASAL-NH_2), can be grouped in the Mastoparan (INLKALAALAKKIK-NH_2) class. **Objectives:** The aim of this study was to investigate the biological activity profile of these new Eumenine peptides and their structural and pore-forming activity in asolectin lipid bilayers. **Methods:** The peptides were isolated from the venom sacs by HPLC, sequenced in MALDI-TOF-TOF, and synthesized. The synthetic peptides were used in bioassays for antimicrobial, hemolytic, and mast cell degranulation activities. The pore-forming properties of the peptides were evaluated in mimetic lipid bilayers and circular dichroism experiments. **Results and Discussion:** The peptide Eu-5 was the most effective in the antimicrobial assay, showing the lowest MIC values against both gram-positive and gram-negative strains. The solitary wasp peptides displayed low (Eu-5 and Ef-10) to moderate (Eu-6 and Ef-11) hemolytic activity against mouse erythrocytes in a dose-dependent manner, and were able to induce mild mast cell degranulation with equivalent potencies and dose-dependent action. The peptides induced ion channel-like incorporation into lipid bilayers formed from GUVs of asolectin under positive and negative voltage pulses, within a 10-min incubation time, but the peptides Eu-5 and Ef-10 showed higher conductance levels. These helical peptides possess an amphipatic structure as foreseen from their helical wheel projections, and insert into the lipid membranes forming channel-like pores. Based on the results, it was shown that Eu-5 shows the highest potential as a leading compound in drug development. It was associated with an average net charge and low hydrophobicity, which resulted in improved antimicrobial activity with minimum hemolytic effect.

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3.09 Macrophage prolyl dipeptidyl aminopeptidase IV (DPPIV/CD26) and neutral aminopeptidase (APN/CD13) activities are affected by phospholipases A_2 (PLA_{2S}) isolated from *Bothrops jararacussu* and *Crotalus durissus terrificus* venom

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Introduction: Phospholipases A_2 from snake venoms are able to activate macrophages (Mϕs) to release inflammatory mediators. Mϕs are the main effector cells of the immune system. Once activated, these cells undergo morphological, biochemical and functional changes. Aminopeptidases (APs) are enzymes involved in leukocyte activation and migration. DPPIV/CD26 and APN/CD13 are expressed in monocytes/macrophages and regulate biological processes relevant to the immune response. **Objectives:** In this study, we evaluated the *in vitro* effect of both bothropstoxin II (BhTX-II), a myotoxic PLA_2 from *Bothrops jararacussu* snake venom and the crotoxin B (CB), a neurotoxic PLA_2 from *Crotalus durissus terrificus* venom, on the activities of soluble (S) and membrane-bound (M) DPPIV/CD26 and APN/CD13 in Mϕs. **Methods:** Resident Mϕs were collected from the cavities of male Swiss mice and incubated with non-toxic concentrations of either BhTX-II or CB (3.5 μg/mL) for selected periods of time (30 min, and 1 and 3 h). DPPIV/CD26 and APN/CD13 activities were quantified by a fluorimetric assay. **Results and Discussion:** Data, represented as UP/mg of protein, showed that BhTX-II increased DPPIV/CD26 activity (3416.9±27.2) after 3 h incubation and APN/CD13 activity (104.2±2.7) at 1 h in the M fraction, as compared with controls (2522.2±14.5; 51.9±1.2, respectively). CB decreased APN/CD13 activity (29.5±1.2) at 30 min in the M fraction, as compared with control (111.5±2.6), but did not affect DPPIV activity for any period of incubation tested. S fraction activity of both enzymes was not altered by either of these venom $PLAs_2$. BhTX-II and CB were able to modify the activities of membrane-bound peptidases (APN/CD13 and DPPIV/CD26). However, these PLA_2s showed distinct actions on the aminopeptidases, BhTX-II being stimulatory and CB inhibitory. Such a difference in effects may be related to the distinct roles of the two PLA_2s on inflammatory and immunological processes.

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3.10 Opioid receptors are involved in the antinociceptive effect of crotalphine in a bone cancer pain model

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Introduction: Crotalphine, a peptide first identified and isolated from the South American rattlesnake *Crotalus durissus terrificus* venom, induces analgesia mediated by the activation of δ- and κ-opioid receptors. **Objectives:** The aim of this work was to characterize the analgesic effect of crotalphine in a new rat model of bone cancer pain induced by inoculation of Walker 256 carcinoma cells ($4x10^6$) into the rat femoral cavity. **Methods:** The presence of bone metabolic alterations was determined by scintigraphy, using ^{99m}Tc-MDP, which is significantly concentrated in areas of osteogenesis. Femoral images were obtained before and 7, 14 and 21 days after tumor cell inoculation. Bone cancer pain was characterized by the presence of hyperalgesia and allodynia, determined using the rat paw pressure test or von Frey filaments, respectively. **Results and Discussion:** Incorporation ^{99m}Tc-MDP was significant 7, 14, 21 days after tumor cell injection, suggesting the development of tumor in femoral cavity. Hyperalgesia and allodynia were detected on days 1, 3, 7, 14 and 21 after cell inoculation. Interestingly, we observed that paw withdrawal threshold in the von Frey test was reduced not only in the ipsilateral hind paw inoculated with the tumor, but also in the contralateral one, demonstrating the existence of bilateral allodynia (mirror-image pain). Crotalphine (8 μg/kg, p.o) administered on day 21, blocked hyperalgesia and allodynia. The analgesic effect was detected for up to 2 days after peptide administration. This effect was mediated by *delta* and *kappa* opioid receptors. These results indicate that intrafemoral injection of Walker 256 cells causes bone cancer and pain. Crotalphine induces a potent, long-lasting and opioid-mediated antinociception in this model of cancer pain.

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3.11 Cnidaria venom as pharmacological tool for studying pain and analgesia

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Introduction: Animal toxins are directed against a wide variety of pharmacological targets, making them an invaluable source of ligands for studying the signaling pathways of pain and its control. Sea anemone (cnidaria) venoms contain many biologically active compounds such as cytolysins (18–20 kDa) and ion channel modulators (3–5 kDa). In addition, low molecular-weight compounds have been isolated and identified in these venoms; however, few studies have been carried out in order to determine the biological activity of such compounds. BDS 391 is a low molecular-weight, non-peptide compound purified from venom of the Brazilian sea anemone *Bunodosoma cangicum*. Studies on the structure of BDS 391 have demonstrated that this compound is composed of a bromoindole group connected to histidine. Our recent data have indicated that BDS 391 administered by the intraplantar route into the rat hind paw induces potent peripheral analgesia in models of acute and chronic pain. Initial results indicate that peripheral 5-HT receptors and K_V channels mediate the analgesic action of this compound. **Objectives:** The aim of the present work was to further characterize the analgesic action of BDS 391 and its mechanisms, determining the type of 5-HT receptor involved in this effect, the presence of these receptors in the inflamed tissue and the ability of BDS 391 to directly activate K_V channels. **Methods:** Male Wistar rats and Swiss mice were used. The effect of BDS 391 was evaluated in the rat paw pressure test, before and 3 h after injection of prostaglandin E_2 (PGE_2, 100 ng/paw) and against nociception induced by 1% formalin solution in mice. Spiroxatrine, ketanserin or ondansetron (6 mM/paw, antagonists of 5-HT_{1a}, 5-HT_2 and 5-HT_3 receptors, respectively), were used to characterize the type of serotonin receptors involved the analgesic effect. Expression of 5-HT receptors in the paw tissue was evaluated by immunoblotting assays. In voltage clamp studies, BDS 391, was screened in 9 cloned Kv channels. **Results and Discussion:** BDS 391 (0.15 - 1.5 μM) inhibited PGE_2-induced hyperalgesia and nociceptive response induced by formalin. Ondansetron but not spiroxatrine and ketanserin was able to totally reverse the antinociceptive effect induced by BDS 391. These pharmacological data indicated that peripheral 5-HT_3, but not 5-HT_{1a} and 5-HT_2 receptors, mediate the action of BDS 391. The immunoblotting data showed that 5-HT receptors are expressed in nerve paw and that PGE_2-induced hyperalgesia increases (15 – 20%) the expression of these receptors. BDS 391 did not modify the peak or shape of ionic potassium current. These data indicate that peripheral 5-HT_3 receptors are involved in the analgesic effect of BDS 391 and demonstrate for the first time, that inflammation induces up-regulation of 5-HT receptors. The opening of Kv channels induced by BDS 391 does not result from a direct action of the compound, but could be due to activation of 5-HT_3 receptors (a channel activated by ligands). These results also contribute to the better characterization of the role of 5-HT_3 receptors in pain control.

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