



## Short communication

Renal effects of *Bunodosoma caissarum* crude extract: Prostaglandin and endothelin involvement

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## ABSTRACT

Sea anemones contain a variety of interesting biologically active compounds, including some potent toxins. PLA<sub>2</sub> from *Bunodosoma caissarum*, a sea anemone endemic in the Brazilian southern coast, has shown renal alterations on isolated kidney. The aim of this study was to evaluate the renal and vascular effects of *B. caissarum* crude extract (BcE) on isolated perfused kidney and arteriolar mesenteric bed, as well the involvement of prostaglandins and endothelin. BcE did not show any effect on arteriolar mesenteric bed, but increased perfusion pressure, renal vascular resistance, urinary flow, glomerular filtration rate and decreased the percentage of sodium tubular transport on isolated perfused kidney. Indomethacin blocked the renal effects induced by BcE and tezosentan only partially blocked these effects. These results demonstrate the effects of BcE on kidney *in situ*, suggesting the involvement of prostaglandins and endothelin.

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## 1. Introduction

Several types of land animals produce natural toxins that are harmful to humans. In marine/aquatic environments, people have been injured by aquatic animals in various situations (Mizuno et al., 2012). Cnidarians are the oldest extant lineage of venomous animals. Using specialized penetrating nematocysts, cnidarians inject the nematocyst content or “venom” to capture prey and as defense against predators. These venoms contain enzymes, potent pore-forming toxins, and neurotoxins, which trigger toxic and immunological reactions in the envenomated organism. In addition, certain cnidarian venoms contain or induce the release of vasodilatory biogenic amines in the host, such as serotonin, and histamine, accelerating the pathogenic effects of other venom enzymes and porins. The cnidarian attack/defense mechanism is fast and efficient, and massive envenomation of humans may result in

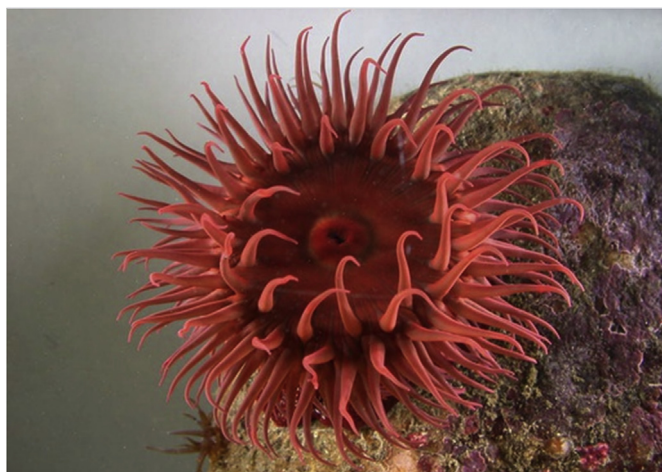
death, in some cases within a few minutes to an hour after venom inoculation (Jouiaei et al., 2015).

*Bunodosoma caissarum* (Fig. 1), a sea anemone of the Cnidaria phylum, is found only in Brazilian coastal waters. Its venom has cytolytic, hemolytic, neurotoxic and cardiotoxic actions (Martins et al., 2009). Some components of this venom such as Bcs I (hemolytic toxin) (Oliveira et al., 2006a), BcIV (sodium channel toxin) (Oliveira et al., 2006b) and PLA<sub>2</sub> (Martins et al., 2009), BcsTx3 (potassium channel blocker) (Orts et al., 2013), have been studied regarding their biological effects, but some toxic effects of *B. caissarum* crude extract are still unknown.

Some venoms or their components are highly toxic for humans and can rarely cause multiple-organ failure and lethal shock (Mizuno et al., 2012). Acute Kidney Injury (AKI) has been reported after sea anemone envenomation (Mizuno et al., 2000). In previous studies, our research group showed the renal alterations caused by PLA<sub>2</sub> from *B. caissarum*. Aiming at a better understanding of these vascular and renal effects of *B. caissarum* crude extract, we evaluated the effects of its crude extract on isolated perfused kidney and

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**Fig. 1.** *Bunodosoma caissarum* sea anemone. Alvaro E. Migotto. Anêmona-do-mar. Banco de imagens Cifonauta. Available in: <http://cifonauta.cebimar.usp.br/photo/3810/> Access in: 2017-08-01.

arteriolar mesenteric bed, as well as the involvement of prostaglandins and endothelin in its effect.

## 2. Materials and methods

### 2.1. *Bunodosoma caissarum* crude extract and drugs

Sixty specimens of *B. caissarum* (Fig. 1; weighing approximately 13 g each) were collected during periods of low tide by free diving at different rocky shores of the São Vicente Channel on the southern coast of São Paulo, Brazil. The animals were transported alive and starved in an aquarium for 72 h to eliminate any contents of the gastrovascular cavity. The animals' tentacles were removed, immediately immersed in an ice-cold aqueous solution of 0.1% trifluoroacetic acid solution (TFA) and submitted to three cycles of freezing and unfreezing. After the last cycle, the solution was centrifuged, the supernatant was recovered and filtered through a 0.45- $\mu\text{m}$  filter, followed by a second ultrafiltration through a 0.22- $\mu\text{m}$  filter. The protein from the crude extract was obtained by acid precipitation using a 10% TFA solution. The acid precipitation was performed at 4 °C and the resulting solution was centrifuged. Then, the protein pellet was dissolved in water until complete dissolution was attained and then lyophilized (Martins et al., 2009).

Indomethacin was purchased from Sigma Chemical (MO-USA) and diluted in 5% sodium bicarbonate. Phenylephrine (Phe) was purchased from Sigma (St Louis, MO) and freshly diluted in the perfusion solution on the day of experiments. Tezosentan was generously provided by Actelion Pharmaceuticals (Basel, Switzerland).

### 2.2. Isolated perfused kidney

Adult male Wistar rats (240–300) were fasted for 24 h with free access to water. The rats were anesthetized with sodium pentobarbitone (50 mg/kg, i.p) and after careful dissection of the right kidney; the right renal artery was cannulated via the mesenteric artery without interrupting the blood flow as described by Bowman (1970) and modified by Fonteles et al. (1983). The perfusate consisted of a modified Krebs-Henseleit solution (MKHS) consisting of (in mM): 114.00 NaCl, 4.96 KCl, 1.24  $\text{KH}_2\text{PO}_4$ , 0.5  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2.10  $\text{CaCl}_2$  and 24.99  $\text{NaHCO}_3$ . Bovine serum albumin (BSA 6 g%; fraction V), urea (0.075 g), inulin (0.075 g) and glucose (0.15 g) were added

to the solution, resulting in a final perfusate volume of 100 mL. The pH was adjusted to 7.4. In each experiment, 100 mL of MKHS were recirculated for 120 min. The perfusion pressure (PP) was measured at the tip of the stainless steel cannula in the renal artery. Urine and perfusate samples were collected at 10 min-intervals for analysis of sodium, potassium and chloride levels by ion-selective electrodes (RapidChem 744, Bayer Diagnostic, UK); inulin, as described by Walser et al. (1955) and modified by Fonteles et al. (1983); and osmolality, which was measured in a vapor pressure osmometer (Wescor 5100C, USA). BcE (3  $\mu\text{g}/\text{mL}$ ) was added to system 30 min after the beginning of each perfusion. Indomethacin (IND -10  $\mu\text{g}/\text{mL}$ ) and Tezosentan (TZN - 3  $\mu\text{g}/\text{mL}$ ) were added to system 30 min before the BcE (3  $\mu\text{g}/\text{mL}$ ;  $n = 6$ ). The perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF), glomerular filtration rate (GFR), the percentage of sodium (%  $\text{TNa}^+$ ), potassium (%  $\text{TK}^+$ ) and chloride (%  $\text{Cl}^-$ ) tubular transport were determined. The results were compared to the internal control group, at 30 min early in each experiment ( $n = 6$ ). This study was approved by the Ethics Committee on Animal Research (CEPA) of the Federal University of Pernambuco (Permit Number: 129/15).

### 2.3. Isolated perfused arteriolar mesenteric bed

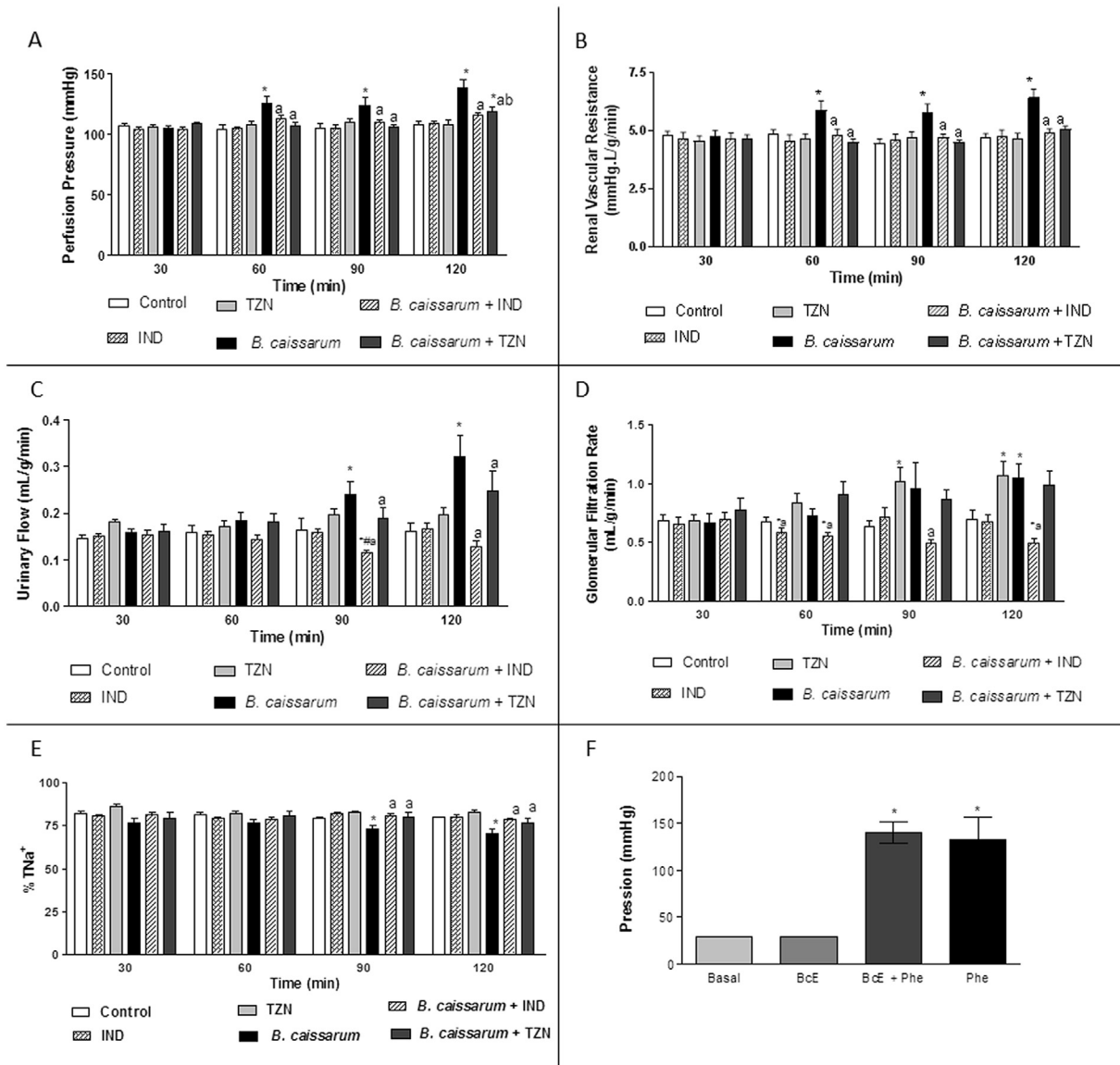
The perfusion was performed following the descriptions of McGregor (1965). Briefly, Wistar rats, weighing 280–350 g were anesthetized with sodium pentobarbitone (50 mg/kg of body weight). After opening the abdomen, the pancreatic-duodenal, ileum-colic, and colic branches of the superior mesenteric artery were tied. Then, the superior mesenteric artery was cleaned of surrounding tissue and cannulated using a polyethylene tube (PE20). The intestine was separated from the mesenteric bed by dissecting close to the intestinal border of the mesentery. The mesenteric bed was perfused with Krebs solution containing: 114.0 mM of NaCl; 4.96 mM of KCl; 1.24 mM of  $\text{KH}_2\text{PO}_4$ ; 0.5 mM of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 24.99 mM of  $\text{NaHCO}_3$ ; 2.10 mM of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; and 3.60 mM of glucose. The perfusion solution was kept warm at 37 °C and the mesenteric bed was perfused by a constant flow (4 mL/min) and variable perfusion pressure was measured at 80 min after the equilibration period. In this system, the direct vascular effects of BcE (10  $\mu\text{g}/\text{mL}/\text{min}$ ;  $n = 6$ ), infused at a constant rate (0.1 mL/min), were assessed and compared to the infusion of the vehicle alone at the same rate.

### 2.4. Statistical analysis

Results are shown as mean  $\pm$  S.E.M of six experiments for each group. Differences between groups were compared by using Student's t-test or analysis of variance (ANOVA) with significance set at 5%.

## 3. Results

*B. caissarum* crude extract caused increased perfusion pressure (PP; Fig. 2A) and renal vascular resistance (RVR; Fig. 2B) at 60 and 90 min, urinary flow (UF; Fig. 2C) at 90 and 120 min, and glomerular filtration rate (GFR; Fig. 2D) at 120 min on isolated kidney. The percentage of sodium tubular transport decreased (%  $\text{TNa}^+$ ; Fig. 2E) and no alterations were observed in the percentage of chloride (%  $\text{TCl}^-$ ) and potassium (%  $\text{TK}^+$ ) tubular transport on isolated kidney. Indomethacin caused significant decrease in glomerular filtration rate at 60 min and blocked the renal effects caused by BcE on all the evaluated parameters, when administrated 30 min before BcE. The increase in PP, RVR, UF and GFR were completely reversed (Figs. 2A and 1D). Indomethacin also blocked the effects of BcE on the percentage of sodium tubular transport (Fig. 2E).



**Fig. 2.** Effects of *B. caissarum* crude extract (BcE; 3  $\mu\text{g}/\text{mL}$ ) on perfusion pressure (A), renal vascular resistance (B), urinary flow (C), glomerular filtration rate (D), and percentage of sodium tubular transport (E). Data are expressed as mean  $\pm$  SEM from six different animals. Statistical analysis was performed by ANOVA, comparing with a control group where kidneys were perfused with only MKHS, \* $p < 0.05$  IND = Indomethacin (10  $\mu\text{g}/\text{mL}$ ) and TZN = Tezosentan (3  $\mu\text{g}/\text{mL}$ ). (a) Comparison with *B. caissarum* extract group; (b) Comparison with Tezosentan group, (#) Comparison with Indomethacin group. Effects of *B. caissarum* crude extract perfusion pressure on the mesenteric vascular bed (Phe = phenylephrine; Basal = basal measurements of pressure). Data are expressed as mean  $\pm$  SEM from six different animals. Statistical analysis was performed by ANOVA, comparing with the control group (basal), \* $p < 0.05$  (F).

BcE (3  $\mu\text{g}/\text{mL}/\text{min}$ ) did not increase the perfusion pressure on the mesenteric vascular bed. The perfusion pressure varied from control values (PP = 29.33  $\pm$  0.17 mmHg) to (29.20  $\pm$  0.26 mmHg) after BcE infusion. The mesenteric blood vessels were contracted in the presence of phenylephrine (PhePP = 140.30  $\pm$  11.86\* mmHg) (Fig. 2F).

#### 4. Discussion

Previously, our research group demonstrated the renal alterations of PLA<sub>2</sub> on isolated perfused kidney, which increased the PP, RVR, urinary flow, glomerular filtration rate, and sodium, potassium and chloride excretion. The crude extract also showed increase on PP, RVR, UF and GFR, but decreased the %TNa<sup>+</sup> and did not alter any other electrolytes transport. PLA<sub>2</sub> from *B. caissarum* also did not show any effect on the perfusion pressure on the mesenteric

vascular bed (Martins et al., 2009), as well its crude extract. This similarity on effects suggests the PLA<sub>2</sub> is partly responsible for these effects of the crude extract.

Indomethacin and tezosentan were used in order to evaluate the possible role of cyclooxygenase and endothelin on the renal changes induced by BcE. Indomethacin caused significant decrease in glomerular filtration rate at 60 min and blocked the renal effects caused by BcE on all the evaluated parameters, when administrated 30 min before BcE. This result suggests that the increase in renal PP and in RVR, previously observed in isolated perfused rat kidney, did not occur due to its direct action on renal vasculature. As an explanation, BcE might have promoted the release of substances synthesized by renal cells, such as prostaglandins, cytokines, bradykinin, complement fractions, platelet-activating factor (Barraviera et al., 1995; Facó et al., 2003) and endothelin (Chen et al., 2002). The blockage exerted by indomethacin treatment

showed that prostaglandins were important mediators of BcE renal effects.

ET1 is major renal isoform produced by and acting on the mesangial cell (MC) and numerous stimuli modulate MC ET1 gene transcription, including vasoactive substances, growth factors, cytokines, G protein-coupled receptor agonists (Sorokin and Kohan, 2003) and snake venom myotoxin (Barbosa et al., 2006). Infusion of exogenous ET1 reduces renal plasma flow and glomerular filtration rates and increases renal vascular resistance. These effects are partially or completely prevented by selective and non-selective endothelin receptor antagonists (Cheng and Harris, 2004; Sorokin and Kohan, 2003).

In the present work, we tested the effects of tezosentan, an ET-1 competitive receptor antagonist with high affinity to both ETA and ETB receptors with greater potency for the ETA subtype (Clozel et al., 1999). Tezosentan alone (3 µg/mL) caused no alterations in renal physiology. It partially blocked the renal effects caused by BcE. Our results demonstrated that endothelin is also an important mediator in renal alterations induced by BcE, which could be caused by mesangial lysis or glomerular epithelial damage.

Indomethacin and Tezosentan blocked the renal alterations promoted by BcE. Cyclooxygenase activity can be stimulated by ET-1, resulting primarily in the increase in PGE<sub>2</sub> and slight increase in thromboxane A<sub>2</sub> via ET<sub>A</sub> receptor induction of PLA<sub>2</sub> and COX-2. These effects could explain the partial blocking of tezosentan on renal alterations induced by BcE (Fukunaga et al., 1996).

Swimmers, surfers, divers, and fishermen are exposed to envenomation syndromes induced by Atlantic cnidarian. The therapy used to treat sea anemone envenomation is vinegar (5% acetic acid) and oral analgesics such as ibuprofen, acetaminophen, or acetylsalicylic acid are drugs of choice (Burnett, 2009; Hornbeak and Auerbach, 2017). In this work, the results suggest that renal alterations caused by *B. caissarum* venom could be blocked by cyclooxygenase inhibitors.

In conclusion, the effects on isolated kidney are probably determined by the release of renal prostaglandins and endothelin.

## Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.toxicon.2017.08.019>.

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