Toxicon 74 (2013) 19–26

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Isolation, homology modeling and renal effects of a C-type natriuretic peptide from the venom of the Brazilian yellow scorpion (*Tityus serrulatus*)

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ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form 9 July 2013
Accepted 16 July 2013
Available online 31 July 2013

Keywords:
Tityus serrulatus
Natriuretic peptide
Kidney perfusion
Natriuretic peptide receptor
Guanylate cyclase C

ABSTRACT

Mammalian natriuretic peptides (NPs) have been extensively investigated for use as therapeutic agents in the treatment of cardiovascular diseases. Here, we describe the isolation, sequencing and tridimensional homology modeling of the first C-type natriuretic peptide isolated from scorpion venom. In addition, its effects on the renal function of rats and on the mRNA expression of natriuretic peptide receptors in the kidneys are delineated. Fractionation of *Tityus serrulatus* venom using chromatographic techniques yielded a peptide with a molecular mass of 2190.64 Da, which exhibited the pattern of disulfide bridges that is characteristic of a C-type NP (TsNP, *T. serrulatus* Natriuretic Peptide). In the isolated perfused rat kidney assay, treatment with two concentrations of TsNP (0.03 and 0.1 μg/mL) increased the perfusion pressure, glomerular filtration rate and urinary flow. After 60 min of treatment at both concentrations, the percentages of sodium, potassium and chloride transport were decreased, and the urinary cGMP concentration was elevated. Natriuretic peptide receptor-A (NPR-A) mRNA expression was down regulated in the kidneys treated with both concentrations of TsNP, whereas NPR-B, NPR-C and CG-C mRNAs were up regulated at the 0.1 μg/mL concentration. In conclusion, this work describes the isolation and modeling of the first natriuretic peptide isolated from scorpion venom. In addition, examinations of the renal actions of TsNP indicate that its effects may be related to the activation of NPR-B, NPR-C and GC-C.

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http://dx.doi.org/10.1016/j.toxicon.2013.07.016

1. Introduction

The mammalian natriuretic peptide (NP) family consists of atrial natriuretic peptide (ANP), brain natriuretic peptide (B-type; BNP) and C-type natriuretic peptide (CNP), which bind to three receptors, natriuretic peptide receptors-A (NPR-A), -B (NPR-B) and -C (NPR-C). Both ANP and BNP are abundantly expressed in the heart and are secreted mainly from the atria and ventricles, respectively. However, CNP is mainly expressed in the central nervous system, bone and vasculature (Nishikime et al., 2010; Tobias, 2011). Classically, the clearance of all NPs is carried out by NPR-C and by the neutral endopeptidase (NEP); both of these proteins are widely expressed in the kidneys, lungs and vascular walls (Chen and Burnett, 2006).

The three mammalian NPs have been extensively investigated for use as therapeutic agents in the treatment of cardiovascular diseases. Over almost 30 years of research, NPs have been found in mammals, amphibians, reptiles, fish, and in plants. Recently, they have also been found in bacteria (Vink et al., 2010).

The first natriuretic peptide isolated from animal venoms was a vasorelaxant peptide. This 38 amino acid residue peptide was isolated from green mamba venom and named dendroaspis natriuretic peptide (DNP). Many natriuretic peptides have subsequently been isolated from snake venoms, including Brazilian snakes, such as *Crotalus durissus cascavella* (Evangelista et al., 2008), *Bothrops jararaca* (Higuchi et al., 1999), *Bothrops moojeni* (Menin et al., 2008) and *Lachesis muta* (Soares et al., 2005). Many genes encoding C-type natriuretic peptides have also been described (Harvey, 2006).

Scorpion venoms are rich sources of small peptide toxins. However, no natriuretic peptides have been isolated from scorpion venom thus far. However, a new family of peptides, called hypotensins, has been isolated from the venom of the yellow scorpion, *Tityus serrulatus*. These toxins share a similar amino acid signature with the bradykinin-potentiating peptides (BPPs) found in snake venoms (Verano-Braga et al., 2008, 2010). In snakes, BPPs and CNP are encoded by the same gene (Assakura et al., 2000).

This work describes the isolation, sequencing and tridimensional homology modeling of the first C-type natriuretic peptide from scorpion venom. Its effects on the renal function of rats and the mRNA expression of the natriuretic peptide receptors in the kidneys were also evaluated.

2. Materials and methods

2.1. Materials and venom

T. serrulatus venom was acquired from the Instituto Butantan (São Paulo, Brazil). All salts and reagents were of analytical grade and were obtained from certified suppliers.

2.2. Isolation of the natriuretic peptide

Crude *T. serrulatus* venom (35 mg) was dissolved in 1.0 mL of ammonium bicarbonate buffer (1 M, pH 8.0). The solution was centrifuged at 4500 × *g* for 10 min and the supernatant was filtered with a 0.22 μm PVDF filter membrane. Then, 300 μL of the venom solution was loaded onto a Superdex® Gel Filtration Column Peptide HR10/300 GL coupled in a semi preparative Jasco HPLC system (Easton, MD, USA). This column was equilibrated with ammonium bicarbonate buffer (0.25 M, pH 7.8) for 40 min before sample application. The run conditions were adjusted to achieve an elution rate of 0.2 mL/min. The chromatography was simultaneously monitored at 280 and 214 nm, because the natriuretic peptides have a higher absorption at 214 nm. The molecular masses of each fraction were confirmed by Tricine SDS-PAGE using a 16% acrylamide gel (data not shown). Fractions with lower molecular masses were lyophilized and stored at 4 °C.

The lyophilized fractions were dissolved in TFA 0.1% buffer (buffer A) at a final concentration of 1 mg/mL and centrifuged for 3 min at 4500 × *g*. The resulting supernatant was applied onto an analytical reverse phase C18 column. The column had previously been equilibrated with buffer A for 15 min before injection of the samples. Elution was accomplished with a linear gradient of buffer B (acetonitrile 66% in buffer A). The purity of the natriuretic peptide fractions was assessed by Tricine SDS-PAGE. The fractions were lyophilized and stored at 4 °C.

2.3. N-terminal amino acid sequencing

Protein sequencing was performed as previously described by Toyama et al. (2003). In brief, 2.0 mg of purified natriuretic peptide were dissolved in 200 mL of a 6 M guanidine chloride solution (Merck, Germany) containing 0.4 M of Tris–HCl and 2 mM EDTA (pH 8.15). The surface of the protein solution was next flushed with nitrogen gas for 15 min. After this, the reducing agent DTT (6 M, 200 mL) was added to the protein solution, and the solution was then incubated under nitrogen for 90 min. Next, 80 mL of iodoacetic acid was added to the solution (50 mM of cold iodoacetic and carboxymethylated ^{14}C-iodoacetic acid), followed by a third incubation under nitrogen, after which the reaction tube was sealed. A preparative C5 reverse phase column was used to remove excess reagent and purify the peptides. The peptides were separated by a linear gradient of acetonitrile (66% in 0.1% of TFA) at a constant flow rate of 2.5 mL/min for 90 min. Buffer A was used in the first 15 min of the HPLC run to remove the salts and other reagents. The amino acid sequence of the peptide was determined using an Applied Biosystems model Procise f gas–liquid protein sequencer. The phenylthiohydantoin (PTH) derivatives of the amino acids were identified with an Applied Biosystems model 450 microgradient PTH-analyzer.

2.4. Mass spectrometry

The molecular mass of the *T. serrulatus* natriuretic peptide (TsNP) was determined using matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry on a Voyager-DE PRO MALDI-TOF mass spectrometer (Applied Biosystems®, Life Technologies™, USA). One microliter of the TsNP dissolved in 0.1% TFA was mixed with 2 μL of the matrix a-cyano-4-hydroxycinnamic

Table 1
Primer sequences used in real time RT-PCR gene expression analyses.

Gene	Primer forward (5′–3′)	Primer reverse (5′–3′)	NCBI reference
GC-A	GAACCGAAGCTTCCAAGGTG	GTGGATATCCCAGAGGCCAGT	NM_012613.1
GC-B	CATCTGCATCGTCACCGAGT	TCCACCACGCAGTTAGAGGAC	NM_053838.1
GC-C	GGCGGGATACAATCCAGAGAG	ACGGTGCCGTAGAACTTGGTC	NM_013170.1
NPR-C	CCTACAATTTCGACGAGACCAAA	TCGCTCACTGCCCTGGAT	NM_012868.1
MAPK-1	TGGTTCTACCGGCGGTTAGT	AACGAGGAGGGAGGACAACAC	NM_053842.1
TGF-β1	GGGCTACCATGCCAACTTCTG	GAGGGCAAGGACCTTGCTGTA	NM_021578.2
eNOS	CGACATTGAGATCAAAGGACTG	ACTTGTCCAAACACTCCACGC	NM_021838.2
18S rRNA	ACATCCAAGGAAGGCAGCAG	GCTGGAATTACCGCGGCTG	NR_046237.1

acid, 50% acetonitrile, and 0.1% TFA (v/v). The matrix was prepared with 30% acetonitrile and 0.1% TFA (v/v). The equipment conditions were as follows: accelerating voltage of 25 kV, laser fixed at 2890 μJ/com2, a delay of 300 ns, and using a linear analysis mode.

2.5. Homology modeling and alignment

Homology modeling of TsNP was conducted using MODELLER 9v5 (Sali and Blundell, 1993), with a standard internal setup and no additional geometrical restraints. A template search was performed through the PDB database, using the BLAST algorithm (Altschul et al., 1990) for the TsNP sequence, with the structure of lebetin 2 isoform alpha from *Macrovipera lebetina* (PDB code: 1Q01) selected. The following alignment properties with TsNP were found: E-value: 0.0181276; Score: 35.039 bits (79); Identities: 14/19 (74%); Positives: 17/19 (89%); Gaps: 0/19 (0%). The PDB 1Q01 structure was used as the template for the homology modeling. Multiple sequence alignments among the target (TsNP) and reference sequences were performed using the ClustalX program (Thompson et al., 1997) with its default parameters.

2.6. Animals

Adult male Wistar rats (weighing 260–320 g) from the Animal Facility of Universidade Federal do Ceará were used in the renal function experiments. The rats were kept in a housing room with controlled ambient humidity, room temperature maintained at 22 ± 2 °C, laminar air flux and 12 h light/dark circles. All animal studies were performed according to Brazilian laws for animal experimentation and were approved by the Ethical Committee of Animal Experimentation of Universidade Federal do Ceará under the number 107/07.

2.7. Isolated perfused kidney

The rats ($n = 6$) were fasted for 24 h with free access to water before the experiment. The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). After careful dissection of the right kidney, the right renal artery was cannulated via the mesenteric artery without interruption of blood flow, as described by Bowman (1970) and modified by Fonteles et al. (1983). A modified Krebs-Henseleit solution (MKHS, composition in mmol/L: 118.0 NaCl, 1.2 KCl, 1.18 KH_2PO_4, 1.18 $MgSO_4.7H_2O$, 2.50 $CaCl_2$ and 25.0 $NaHCO_3$) was used for the perfusion. Bovine serum albumin (BSA) fraction V (6 g) was added to 100 mL of MKHS, and this solution was dialyzed for 48 h at 4 °C against 10 volumes of MKHS. Immediately before the beginning of each perfusion protocol, 100 mg of urea, 50 mg of inulin and 50 mg of glucose were added to the dialyzed solution (100 mL), and 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. Samples of urine and perfusate were collected at 10 min intervals for the determination of sodium, chloride and potassium levels using ion-selective electrodes (Electrolyte Analyzer 9180, Roche™). Inulin levels were determined as described by Walser et al. (1955). Osmolality was measured with a vapor pressure osmometer (VAPRO® 5520,Wescor™). TsNP (0.1 μg/mL or 0.03 μg/mL) was added to the system 30 min after the beginning of each perfusion. The perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF), glomerular filtration rate (GFR), and the percentage of sodium (% TNa^+), potassium (% TK^+) and chloride (% TCl^-) tubular transport were determined (Martinez-Maldonato and Opava-Stitzer, 1978). cGMP concentrations in the urine samples were measured in triplicate

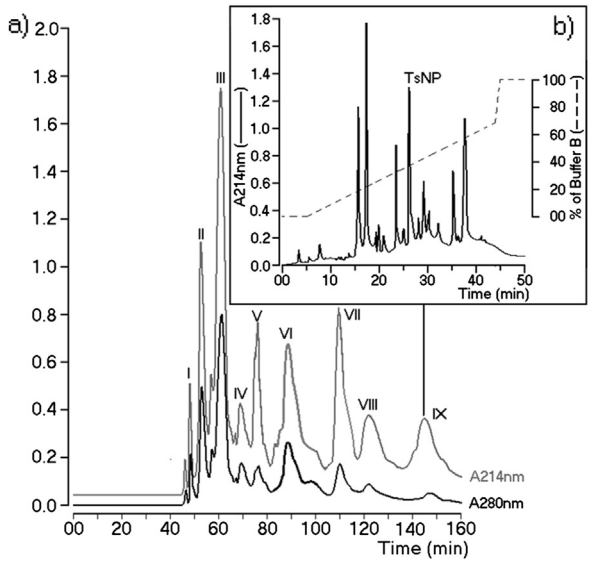

Fig. 1. (a) Chromatogram of the fractionation of *Tityus serrulatus* whole venom using a Superdex® Gel Filtration Column Peptide HR10/300 GL. The chromatography was monitored simultaneously at 280 nm and 214 nm. Only fractions VIII and IX were used for further application onto reverse phase HPLC. (b) Shows the chromatogram of the purification of the natriuretic peptide from *Tityus serrulatus* venom (TsNP).

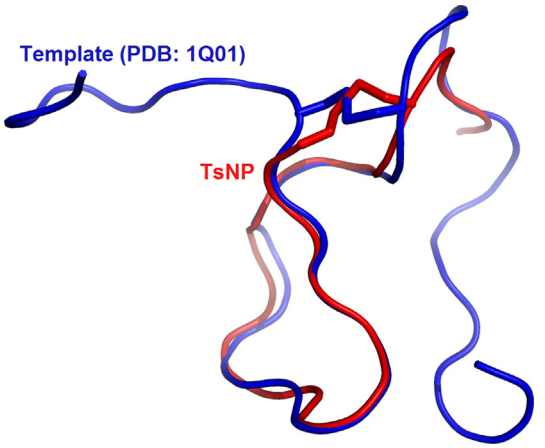

Fig. 2. Homology modeling between TsNP and the template structure of the lebentin peptide (PBD code 1Q01). The disulfide bonds between cysteine residues are detailed.

using a Direct Cyclic GMP Enzyme Immunoassay (Sigma–Aldrich, USA) according to the manufacturer's instructions.

2.8. Gene expression

Following the protocol of the RNeasy Mini Kit® (Qiagen–Valencia, CA, USA), total RNA was extracted from the kidneys of four rats per group (*Rattus norvegicus*); these animals had been perfused with two different concentrations of TsNP, as described in Section 2.7. The yield and quality of total mRNA were determined spectrophotometrically using a wavelength of 260 nm and the 260/280 nm wavelength ratio, respectively. One microgram of RNA, diluted to a final volume of 20 μL, was reverse transcribed into cDNA using the SuperScript™ III cDNA Synthesis Kit (Invitrogen Life Technologies – Carlsbad, CA, USA) with a 96-well MyCycler thermal cycler (BioRad, Hercules, CA, USA).

We investigated the relative expression of rat kidney guanylate cyclase receptors-A, -B, -C (GC-A, GC-B, and GC-C), natriuretic peptide receptor C (NPR-C), endothelial nitric oxide synthase (eNOS), mitogen-activated protein kinase-1 (MAPK-1), and transforming growth factor beta 1 (TGF-β1); 18S ribosomal RNA (18S rRNA) was used as the housekeeping gene. Real Time PCR analysis was performed using the iQ5 Multicolor Real Time PCR Detection System (Bio-Rad) and the iQ SYBR green Supermix. The specific primer sequences (5′–3′) are shown in Table 1. Thermal cycling for all genes had an initial denaturation step at 95 °C for 3 min followed by 30 cycles for 18S rRNA and 40 cycles for all the other genes. The temperature cycles were as follows: a denaturing step at 95 °C for 30 s for all the genes; an annealing step at 59 °C for GC-A, GC-B, 18S rRNA and NPR-C; an annealing step at 60 °C for eNOS, MAPK-1, TGF-β1; and an annealing step at 63 °C for GC-C also for 30 s. For all the genes underwent, an extension step at 72 °C for 45 s. The final extension step, was heat the samples at 72 °C for 3 min. After each reaction, we also performed a melting curve analysis to evaluate the specificity of the PCR amplification. Each PCR reaction well contained a final volume of 25 μL and included 2 μL of cDNA and gene specific primers at 200 nM. Negative samples were run with autoclaved Milli-Q water as the template. The threshold cycle (CT), defined as the fractional PCR cycle number at which the fluorescence reaches 10 times the baseline standard deviation, was used to compare the expression of all of the tested genes. The mathematical method described by Pfaffl (2001) was performed to evaluate the relative expression based on SYBR green staining.

2.9. Statistical analysis

The data are presented as the mean ± SEM. The means were evaluated by the Student's *t*-test or ANOVA followed by the Bonferroni test, when appropriate. Values of $p < 0.05$

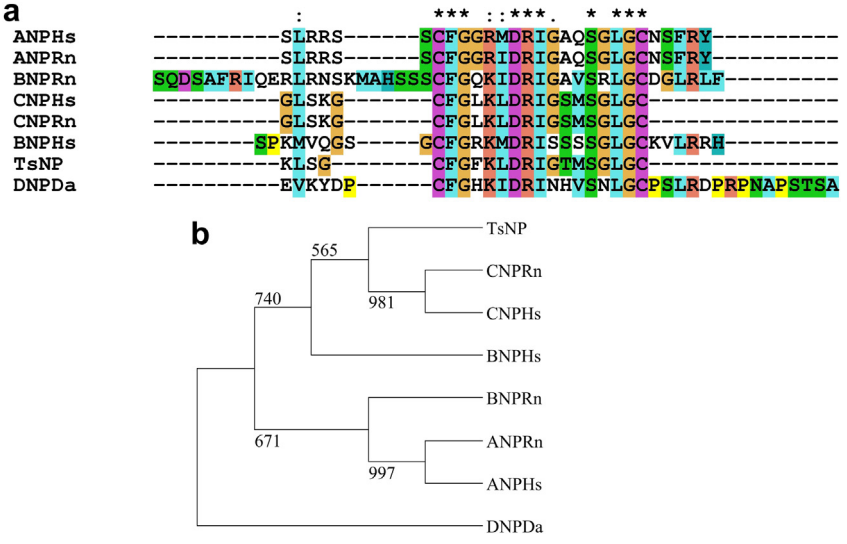

Fig. 3. **(a)** Multiple sequence alignment between TsNP and reference sequences (see details in the text). **(b)** Neighbor-joining bootstrap tree (1000 replicates).

were considered statistically significant. GraphPad Prism® 5.0 was used for all the statistical analyses.

3. Results

The fractionation of *T. serrulatus* venom showed the presence of seven major fractions when the chromatography was monitored at 280 nm; nine fractions were observed at 214 nm. The two last eluted fractions (VIII and IX) represented the lower molecular mass fractions. Fraction IX had virtually no absorption at 280 nm (Fig. 1a). Tricine SDS-PAGE analysis of fraction IX showed the presence of peptides with a molecular mass estimated to be lower than 3 kDa. Subsequently, fraction IX was lyophilized and a new fractionation was performed using reverse phase HPLC. Among the amino acid sequences of the peptides found in fraction IX, only one matched with the features of the natriuretic peptide. This peptide was designated as TsNP (*T. serrulatus* Natriuretic Peptide) (Fig. 1b). The molecular mass of TsNP was determined to be 2190.64 Da (as shown in Supplementary data). An isoelectric point of approximately 9.0 was calculated based on the N-terminal sequencing. The primary structure consisted of 21 amino acids, "KLSGCFGFKLDRIGTMSGLGC", and included the cysteine residues that allowed the formation of a 17 amino acid ring held by a disulfide bridge.

The results obtained by homology modeling of TsNP using the 1Q01 PDB structure are shown in Fig. 2. The quality of the model has been verified using PROCHECK (Laskowski et al., 1993). The overall G-factor is −0.22 and there are no residues in the disallowed regions of the Ramachandran plot.

Multiple sequence alignments among the target (TsNP peptide) and reference sequences were performed with the ClustalX program (Thompson et al., 1997) using the default parameters. The results can be found in Fig. 3. The reference structures chosen for this alignment, with their respective accession numbers, follow: 1) ANPHs (human ANP – P01160) "SLRRSSCFGGRMDRIGAQSGLGCNSFRY"; 2) BNPHs (human BNP – P16860) "SPKMVQGSGCFGRKMDRISSSSGL GCKVLRRH"; 3) CNPHs (human – P23582) "GLSKGCFGL KLDRIGSMSGLGC"; 4) ANPRn (rat ANP – P01161) "SLRRSSC FGGRIDRIGAQSGLGCNSFRY"; 5) BNPRn (rat BNP – P13205) "SQDSAFRIQERLRNSKMAHSSSCFGQKIDRIGAVSRLGCDGLR LF"; 6) CNPRn (rat CNP – P55207) "GLSKGCFGLKLDRIGSMSG LGC"; and 7) DNPDa (*Dendroaspis* DNP – P28374) "EVKYDPCFGHKIDRINHVSNLGCPSLRDPRPNAPSTSA".

In isolated perfused rat kidney assay, both concentrations of TsNP (0.03 and 0.1 μg/mL) increased the perfusion pressure and urinary flow after 90 and 120 min of exposure. The glomerular filtration rate was augmented after 120 min at both concentrations. The higher TsNP concentration (0.1 μg/mL) also increased the GFR after 90 min. These results are shown in Fig. 4a–c. Renal vascular resistance was elevated only at 120 min in the group treated with TsNP at 0.1 μg/mL (RVR 120′ Cont. 5.38 ± 0.53; $TsNP_{0.03}$ 5.82 ± 0.48; $TsNP_{0.1}$ 6.71 ± 0.52* mmHg/mL g^{-1} min^{-1}). The percentages of renal transport for sodium, potassium and chloride were decreased, as was the percentage of sodium proximal tubular transport, after treatment with TsNP 0.1 μg/mL (Table 2). Urinary cGMP concentration was elevated at both

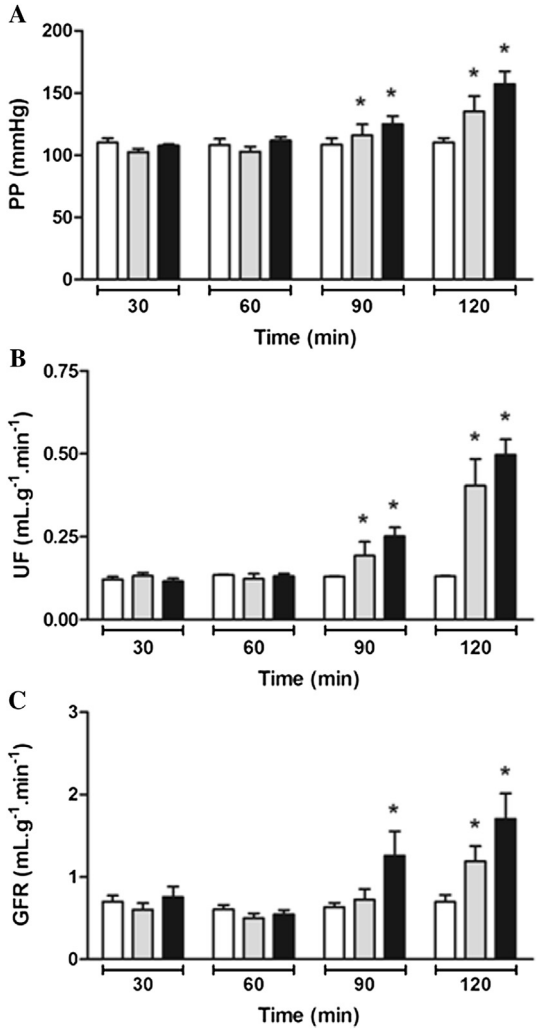

Fig. 4. Effect of TsNP on a perfused rat kidney evaluating the perfusion pressure (PP), urinary flow (UF) and glomerular filtration rate (GFR). The kidneys were perfused with 0.03 and 0.1 μg/mL solution of TsNP and were compared to those perfused with no substances. Data are expressed as the mean ± SEM and analyzed by ANOVA and the Bonferroni post test. *$p < 0.05$.

TsNP concentrations at 60 min (Cont. 8.83 ± 0.70; $TsNP_{0.03}$ 29.50 ± 5.67*; $TsNP_{0.1}$ 41.18 ± 3.25* pmol/mL) but, only for the higher concentration at 90 min (Cont. 10.81 ± 0.54; $TsNP_{0.1}$ 46.67 ± 1.60* pmol/mL) and 120 min (Cont. 9.84 ± 1.39; $TsNP_{0.1}$ 68.00 ± 7.60* pmol/mL).

mRNA expression of the natriuretic peptide receptor-A, -B, -C, and of the guanylate cyclase-C genes was analyzed in the perfused kidneys because cGMP concentrations were elevated in the urine samples. NPR-A mRNA expression was down regulated in the kidneys treated with both concentrations of TsNP (0.03 and 0.1 μg/mL). In contrast, the NPR-B, NPR-C and CG-C genes showed an up regulation following 0.1 μg/mL treatment (Fig. 5). As the expression of all of the genes was affected and because the NPR-C receptor does not act via cGMP as a second messenger, we decided to analyze other genes involved in NPR-C signal

Table 2
Electrolyte transport percentages (%T), in kidneys perfused with 0.03 and 0.1 μg/mL TsNP, were compared to those perfused with no substances. Data are expressed as the mean ± SEM and analyzed by ANOVA and the Bonferroni post test. *$p < 0.05$. % pT = proximal tubular transport percentage.

	Time (min)			
	30	60	90	120
% TNa^+				
Control	81.94 ± 1.24	81.11 ± 1.52	79.26 ± 0.90	79.76 ± 0.56
TsNP 0.03 μg/mL	79.52 ± 1.76	77.50 ± 1.86	76.59 ± 2.68	68.34 ± 4.37
TsNP 0.1 μg/mL	80.77 ± 1.42	70.88 ± 2.22*	70.72 ± 2.04*	64.84 ± 2.90*
% TK^+				
Control	69.13 ± 4.14	69.04 ± 5.68	71.84 ± 4.21	69.94 ± 6.86
TsNP 0.03 μg/mL	69.16 ± 3.68	65.41 ± 4.39	67.32 ± 4.90	65.54 ± 3.91
TsNP 0.1 μg/mL	70.04 ± 3.27	49.68 ± 7.24*	47.60 ± 9.37*	50.81 ± 6.46*
% TCl^-				
Control	79.90 ± 1.03	81.25 ± 2.44	77.32 ± 2.22	78.53 ± 2.33
TsNP 0.03 μg/mL	78.42 ± 1.78	75.95 ± 2.51	76.77 ± 1.24	71.50 ± 1.23*
TsNP 0.1 μg/mL	81.90 ± 3.68	76.15 ± 3.42	72.89 ± 3.86	69.02 ± 3.64*
% $pTNa^+$				
Control	77.69 ± 1.68	75.59 ± 0.90	77.33 ± 1.33	78.18 ± 1.97
TsNP 0.03 μg/mL	76.77 ± 1.07	73.36 ± 2.82	75.04 ± 1.93	69.02 ± 2.84*
TsNP 0.1 μg/mL	80.64 ± 1.18	71.49 ± 5.02	70.36 ± 0.98*	59.50 ± 1.75*

transduction. Therefore, we analyzed the mRNA levels of eNOS, MAPK-1 and TGFβ-1. A down regulation of eNOS mRNA was observed for both TsNP concentrations (Control 1.046 ± 0.082; $TsNP_{0.03}$ 0.156 ± 0.046*; $TsNP_{0.1}$ 0.276 ± 0.083* relative expression rate). However, an up regulation of TGFβ-1 mRNA was found for 0.1 μg/mL TsNP concentration (Control 1.124 ± 0.345; TsNP0.03 2.751 ± 0.969*; TsNP0.1 4.459 ± 1.020* relative expression rate). MAPK-1 gene expression was not affected by the TsNP treatment.

4. Discussion

Natriuretic peptides have been extensively investigated due to their potential for the treatment of cardiovascular diseases such as congestive heart failure. Despite all the advances that have been made in this field, only two NP-based drugs have been produced. Unfortunately, both of these drugs possess poor pharmacokinetic properties and have adverse effects that limit their use (Vink et al., 2010). Since the discovery of venom-related natriuretic peptides,

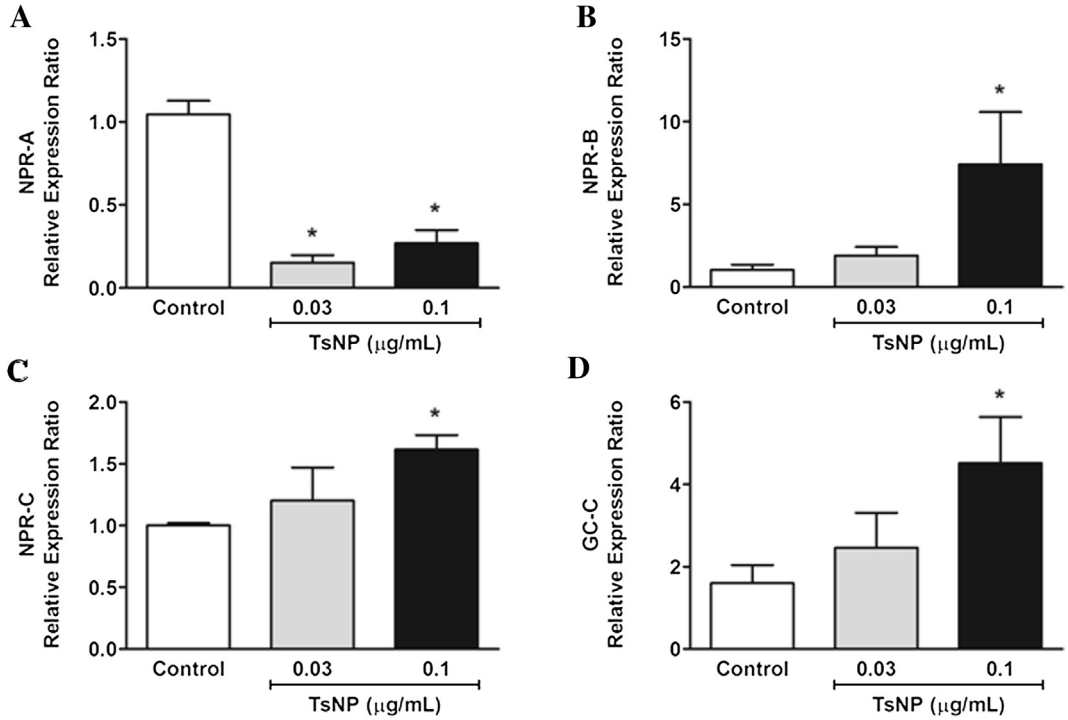

Fig. 5. Relative mRNA expression of natriuretic peptide receptors-A, -B and -C (NPR-A, NPR-B and NPR-C) and guanylate cyclase C (GC-C) in kidneys perfused with 0.03 and 0.1 μg/mL of TsNP and those of control group. The expression of 18S ribosomal RNA was used as a housekeeping gene. Data are reported as the mean ± SEM for 6 animals. *$p < 0,05$ compared to control group using ANOVA and the Bonferroni post test.

first in snakes, then in platypus, a number of chimeric peptides have been produced. These have resulted in peptides with greater stabilities and new pharmacological properties. These peptides have shown advantages over their mammalian counterparts for therapeutic use (Lisy et al., 2008; Vink et al., 2010).

In platypus and snake venoms, natriuretic peptides are encoded in the same gene regions of bradykinin-potentiating peptides (BPP) or metalloprotease-inhibiting peptides and are posttranslationally liberated (Fry et al., 2009). This is the first report of the isolation of a natriuretic peptide from scorpion venom. Sequence alignment of TsNP shows a structural similarity to C-type natriuretic peptides. Recently, a family of peptides was isolated from *T. serrulatus* venom. These peptides shared a sequence signature with the bradykinin-potentiating peptides (BPP) found in snake venom (Verano-Braga et al., 2008). Higuchi et al. (1999) showed that BPP and C-type natriuretic peptide are encoded by the same genes in species of the Crotalinae subfamily. Herein a question arises: are TsNP and the hypotensins also encoded by the same gene sequence?

Sequence alignment of TsNP with human and rat ANP, BNP and CNP (Fig. 3) showed a higher homology of TsNP for both, human and rat CNP. Based on this result, a higher affinity of TsNP for NPR-B and NPR-C is expected. NPR-B has approximately a 50-fold higher affinity for CNP than ANP. CNP essentially does not bind to NPR-A but, it binds to NPR-B and NPR-C with similar affinities (Schulz, 2005).

The effects of TsNP on the isolated perfused rat kidney were similar to those reported by Evangelista et al. (2008) for a natriuretic peptide from *C. durissus cascavella* venom. In the same perfusion system, ANP also showed an increase in GFR and urine flow, with no effect in the perfusion pressure. The urinary excretion of sodium, potassium and chloride were increased by the TsNP treatment, as has been shown for ANP, guanylin and urodilatin (Santos-Neto et al., 2006). The concentration of cGMP in the urine was elevated as would be expected for a guanylate cyclase activating drug. CD-NP, a chimeric natriuretic peptide, has been shown to enhance GFR, producing diuresis and natriuresis (Lisy et al., 2008).

Fonteles et al. (2009) showed a reduction in NPR-A expression accompanied by an increase in GC-C expression in animals receiving a high-salt diet and treated with uroguanylin (a GC-C agonist). This observation corroborates with our data, which showed that GC-C expression was elevated while NPR-A expression was down regulated following treatment with TsNP. These effects point to a possible activation of GC-C by TsNP. However, Anand-Srivastava (2005) demonstrated another possible relationship, which could explain NPR-A down regulation and link the up regulation of TGFβ-1 and NPR-C, with the down regulation of NPR-A. These results were also observed in our studies.

The increased concentration of cGMP in the urine points to the activation of guanylate cyclase, as occurs after NPR-B and GC-C activation. NPR-C could play a role in the increased perfusion pressure caused by TsNP. NPR-C has three distinct signaling pathways, which involve the activation of eNOS, MAPK-1 and phospholipase C (PLC) (Anand-Srivastava, 2005). For this reason, the gene expression of eNOS and MAPK-1 following TsNP treatment were also tested. TsNP treatment resulted in down regulation of eNOS expression, whereas MAPK-1 expression was not changed. This result possibly excludes the involvement of these two NPR-C signaling pathways in the biological actions of TsNP. Thus, activation of PLC downstream might explain the augmentation of the perfusion pressure through direct vascular smooth muscle contraction (Anand-Srivastava, 2005).

In conclusion, this work describes the isolation and modeling of the first natriuretic peptide isolated from scorpion venom. In addition, the renal actions and the effects on NPRs mRNA expression in the kidneys are delineated. Our data showed that TsNP might act promiscuously with NPR-B, NPR-C and GC-C. Future binding analyses should elucidate the relative affinities between TsNP and various receptors.

Acknowledgments

The authors wish to thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and FUNCAP (Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico) for financial support and scholarships.

Conflicts of interest

There are no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.toxicon.2013.07.016.

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