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A new structurally atypical bradykinin-potentiating peptide isolated from *Crotalus durissus cascavella* venom (South American rattlesnake)

Denise M. Lopes [a], Norberto E.G. Junior [a], Paula P.C. Costa [a], Patrícia L. Martins [a], Cláudia F. Santos [a], Ellaine D.F. Carvalho [b, c], Maria D.F. Carvalho [b, c, d], Daniel C. Pimenta [e], Bruno A. Cardi [d], Manassés C. Fonteles [a], Nilberto R.F. Nascimento [a, 1], Krishnamurti M. Carvalho [b, d, *, 1]

[a] *Laboratório de Farmacologia Cardiovascular e Renal, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Fortaleza, Ceará, Brazil*
[b] *GENPHARMA LTDA, Fortaleza, Ceará, Brazil*
[c] *Faculdade de Medicina Christus, Fortaleza, Ceará, Brazil*
[d] *Laboratório de Toxinologia e Farmacologia Molecular, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Fortaleza, Ceará, Brazil*
[e] *Instituto Butantan, São Paulo, Brazil*

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ABSTRACT

Venom glands of some snakes synthesize bradykinin-potentiating peptides (BPP's) which increase bradykinin-induced hypotensive effect and decrease angiotensin I vasopressor effect by angiotensin-converting enzyme (ACE) inhibition. The present study shows a new BPP (BPP-Cdc) isolated from *Crotalus durissus cascavella* venom: Pro-Asn-Leu-Pro-Asn-Tyr-Leu-Gly-Ile-Pro-Pro. Although BPP-Cdc presents the classical sequence IPP in the C-terminus, it has a completely atypical N-terminal sequence, which shows very low homology with all other BPPs isolated to date. The pharmacological effects of BPP-Cdc were compared to BBP9a from *Bothrops jararaca* and captopril. BPP-Cdc (1 μM) significantly increased BK-induced contractions (BK; 1 μM) on the guinea pig ileum by 267.8% and decreased angiotensin I-induced contractions (AngI; 10 nM) by 62.4% and these effects were not significantly different from those of BPP9a (1 μM) or captopril (200 nM). Experiments with 4-week hypertensive 2K-1C rats show that the vasopressor effect of AngI (10 ng) was decreased by 50 μg BPP-Cdc (69.7%), and this result was similar to that obtained with 50 μg BPP9a (69.8%). However, the action duration of BPP-Cdc (60 min) was 2 times greater than that of BPP-9a (30 min). On the other hand, the hypotensive effect of BK (250 ng) was significantly increased by 176.6% after BPP-Cdc (50 μg) administration, value 2.5 times greater than that obtained with BPP9a administered at the same doses (71.4%). In addition, the duration of the action of BPP-Cdc (120 min) was also at least 4 times greater than that of BPP-9a (30 min). Taken together, these results suggest that BPP-Cdc presents

Abbreviations: ACE, angiotensin-converting enzyme; AngI, angiotensin I; AngII, angiotensin II; BK, bradykinin; BPPs, bradykinin-potentiating peptides; BPP-Cdc, bradykinin-potentiating peptides from *Crotalus durissus cascavella*.

* Corresponding author: Laboratório de Toxinologia e Farmacologia Molecular, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Av. Paranjana 1700, Campus do Itaperí, CEP 60.740-000, Fortaleza, Ceará, Brazil. Tel./fax: +55 85 3486 6221.
E-mail address: carvalhokris@gmail.com (K.M. Carvalho).

[1] These authors contributed equally to the paper.

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more selective action on arterial blood system than BPP9a. Besides the inhibition of ACE, it may present other mechanisms of action yet to be elucidated.

1. Introduction

The *Bothrops jararaca* venom contains enzymes that induce the release of kinins from plasma kininogen (Rocha e Silva et al., 1949). This venom was later found to contain a fraction (bradykinin-potentiating factor), which was able to potentiate the bradykinin actions on various isolated organs and hypotensive effect in the cat (Ferreira and Rocha e Silva, 1965). Thereafter, nine biologically active peptides (bradykinin-potentiating peptides, BPPs) were isolated from this venom and shown to potentiate bradykinin-induced contraction in the isolated guinea pig ileum and to increase the hypotensive effect of bradykinin, whose actions are due to inhibition of bradykinin degradation (Ferreira et al., 1970). The inhibition of bradykinin degradation by these BPBs was also associated with the inhibition of the conversion of angiotensin I to its active metabolite angiotensin II (Stewart et al., 1971). These crucial findings paved the way for the later development of angiotensin converting enzyme (ACE) inhibitors, such as captopril the prototype compound of this group, for the treatment of hypertension and heart failure (Cushman and Ondetti, 1991; Camargo et al., 2012).

Nevertheless, recent discoveries have pointed that some BPPs can distinguish between the N or C-terminal catalytic domains of ACE and these discoveries have renewed the interest in the search for more selective peptides (Perich et al., 1992; Jaspard et al., 1993; Cotton et al., 2002; Ianzer et al., 2007). Usually, the potentiation of bradykinin and the inhibition of ACE activity are related to the antihypertensive activity of these compounds, but exceptions to this rule suggest that these effects can occur independently, i.e., BPPs may act by an ACE-independent mechanism (Camargo and Ferreira, 1971; Greene et al., 1972; Mueller et al., 2005; Ianzer et al., 2007). For instance, Ianzer and coworkers (2004) demonstrated that a BPP isolated from *B. jararaca* venom inhibited both ECA and neutral endopeptidase (NEP) and strongly potentiated the hypotensive activity of bradykinin.

Although BPPs have been initially isolated from genus *Bothrops* (Ferreira et al., 1970; Ondetti et al., 1971; Cintra et al., 1990; Ferreira et al., 1992; Murayama et al., 1997; Hayashi et al., 2003; Ianzer et al., 2004; BPPs deposited in NCBI bank (AAP 83422 and AAL 09426), several others were purified from other genera of snakes, such as *Crotalus* (Politi et al., 1985; Wermelinger et al., 2005; Higuchi et al., 2006; Gomes et al., 2007; Coutinho-Neto et al., 2013), *Lachesis* (Soares et al., 2005), *Agkistrodon* (Kato and Suzuki, 1971; Kato et al., 1973; Ferreira et al., 1995; Yanoshita et al., 1999; Murayama et al., 2000), *Trimeresurus* (Higuchi et al., 1999) and *Vipera* (Komori and Sugihara, 1990).

In the present study, a new atypical BPP (BPP-Cdc) was isolated for the first time from the *Crotalus durissus cascavella (C. d. cascavella)* venom, its amino acid sequence determined, pharmacodynamics performed on the guinea pig ileum and its effects on blood pressure of Goldblatt 2R-1C hypertensive rats was examined.

2. Material and methods

2.1. The venom of C. d. cascavella

The venom of *C. d. cascavella* was a gift from the Laboratório de Animais Peçonhentos do Instituto de Ciências Biomédicas da Universidade Estadual do Ceará.

2.2. Reagents

All chemicals, salts and standards were purchased from Sigma–Aldrich (St Louis, MO, USA), unless stated otherwise.

2.3. Purification of the BPP-Cdc

A 20 mg/mL solution of crude venom of *C. d. cascavella in* 0.05% trifluoroacetic acid (TFA) (1:4; w/v) was prepared and centrifuged at 17,000*g* for 60 min. The supernatant was fractionated by reverse-phase high performance liquid chromatography (RP-HPLC-Shimadzu Co.) using a C18 column (Shim-pack PREP 25 × 250 mm). Peptides were eluted with a 0–60% gradient of acetonitrile containing 0.05% TFA, over a period of 60 min at a flow rate of 5 mL/min, detected by UV absorbance at 214 nm (Fig. 1A), lyophilized and stored at −25 °C. Aliquots of these fractions were dissolved in saline and probed in the guinea pig ileum in order to check whether they could either potentiate BK- or antagonize angiotensin I-induced contractions. In order to confirm the purity of the fraction containing the BPP-Cdc activity, an aliquot was submitted for re-chromatography, under the same conditions described above (Fig. 1B).

2.4. Animals

Albino male Guinea pigs (300–400 g) and Wistar rats (250–300 g) were obtained from the vivarium of Colégio Christus. The animals were maintained under controlled conditions (temperature: 23 ± 1 °C; relative humidity: 55 ± 1%; and light: 8:00 AM to 8:00 PM) and received a normal diet (Purina Chow®) and tap water *ad libitum*. All the protocols were approved by the Ceara State University Animal Ethics Committee under the protocol #084393384-0.

2.5. Mass spectrometry

Electrospray ionization mass spectrometry (ESI-MS) was performed on a Perkin Elmer-Sciex API-300 triple quadrupole mass spectrometer operated in positive ion mode. The samples were injected using in 50% acetonitrile