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Functional assessment of angiotensin II and bradykinin analogues containing the paramagnetic amino acid TOAC

Edson L. Santos, Kely de Picoli Souza, Regiane A. Sabatini, Renan P. Martin, Liliam Fernandes, Daniela T. Nardi, Luciana Malavolta, Suma I. Shimuta, Clóvis R. Nakaie*, João B. Pesquero*

Department of Biophysics, Federal University of São Paulo, 04023-062 São Paulo, SP, Brazil

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Abstract

This study characterized pharmacologically the functional responses to agonists angiotensin II (AngII) and bradykinin (BK) derivatives containing the TOAC (2,2,6,6-tetramethylpiperidine-N-oxyl-4-amino-4-carboxylic acid) spin label at the N-terminal ($TOAC^1$-AngII and $TOAC^0$-BK) and internal ($TOAC^3$-AngII and $TOAC^3$-BK) positions of these vasoactive peptides. Affinity constants of the ligands for AT_1 and B_2 receptors were evaluated *in vitro* by binding assays and biological effects by extracellular acidification rates and *in vivo* by blood pressure responses. In contrast to internally labeled analogues ($TOAC^3$-AngII or $TOAC^3$-BK), the $TOAC^1$-AngII and $TOAC^0$-BK derivatives dose-dependently increased the extracellular acidification rate in adherent cultured Chinese hamster ovary (CHO) cells expressing AT_1 or B_2 receptors, respectively. In addition, $TOAC^1$-AngII induced an increase in blood pressure when injected intravenously in awaken rats although with a potency four times smaller when compared to native AngII. Similarly to BK, $TOAC^0$-BK dose-dependently decreased blood pressure when injected intra-arterially in rats with a lower potency when compared to the native peptide. On the contrary, $TOAC^3$-AngII or $TOAC^3$-BK did not provoke any alteration in blood pressure levels. In summary, our results confirmed that the insertion of TOAC-probe in the N-terminal region of peptides does not significantly modify the affinity or biological activity *in vitro* and *in vivo* conditions and could be an important tool to evaluate peptide–receptor interaction mechanism. Conversely, possibly due to the unique bend-inducing property of the cyclic TOAC probe, its insertion at position 3 in both AngII and BK structures seems to restrict the interaction and the activation of the AT_1 and B_2 receptors.

* Corresponding authors. Department of Biophysics, Escola Paulista de Medicina, Federal University of São Paulo, 04023-062 São Paulo, SP, Brazil.
E-mail addresses: clovis@biofis.epm.br (C.R. Nakaie), jbpesq@biofis.epm.br (J.B. Pesquero).

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1. Introduction

Angiotensin II (AngII), a potent vasoconstrictor octapeptide, is the main product of the renin–angiotensin system and bradykinin (BK) is one important effective member of the kallikrein–kinin system. Both peptides have important roles in cardiovascular function. The main physiological action of AngII occurs through its interaction with AT_1 and AT_2 receptors [1,2] and of BK, with B_2 receptor [3,4]. These receptors are coupled to G-protein, with a seven-transmembrane-helix structure, an extracellular N-terminal tail, an intracellular C-terminal domain and three extracellular and three intracellular loops.

Spin labeling electron paramagnetic resonance (EPR) is a spectroscopic technique [5] used for the investigation, through a paramagnetic probe, of the conformation and dynamics of many types of macromolecules and systems, including peptides. In the latter case, this method enjoyed notable expansion in two-stage process when TOAC, the paramagnetic amino acid 2,2,6,6-tetramethyl-piperidine-1-oxyl-4-amino-4-carboxylic acid [6] was for the first time, derived to allow its incorporation into the peptide backbone *via* a peptide bond [7,8]. However, due to a chemical stability issue, the TOAC attachment to the peptide structure was restricted at that time to the N-terminal position. Later, a complementary chemical strategy was finally developed which permitted its insertion at any position of the sequence [9].

From this point onwards, the literature has witnessed many types of application of this piperidine-based cyclic paramagnetic probe ranging from those that investigated in depth the characteristics of this type of C^{α}-tetrasubstituted α-amino acid [10,11] until those related to some biological relevant peptides [12–15]. Taking into account the unique advantage of this conformationally constrained marker, *i.e.*, of being rigidly bound to the molecule or system and therefore, of possessing greater sensitivity in monitoring dynamics of the attaching-site [16], other applications have been designed for its use, including membrane-related investigations [17–19], solvation process of peptide-polymers and polymers [20,21] and more recently, EPR-monitoring of an enzymatic hydrolysis of TOAC-bearing substrates [22].

Following an earlier study [23] comprising the synthesis aspects of TOAC-containing AngII and BK analogues and their pharmacological properties towards isolated rabbit aorta, rat uterus and guinea-pig ileum, the present work aimed to continue the quest to deeper evaluate the effect of the TOAC-moiety in the biological properties of these important vasoactive peptides. Thus, *in vitro* and *in vivo* experiments were designed to verify further biological activities of these special TOAC-attaching analogues. Competition binding and functional assays in CHO cells transfected with AT_1 or B_2 receptors and biological effects on blood pressure in conscious Wistar rats were carried out with these paramagnetic peptides.

2. Materials and methods

2.1. Peptides synthesis

TOAC-containing peptides were synthesized manually according to the standard Boc/Bzl and Fmoc/*t*-butyl solid phase strategies, following the previously reported approach for TOAC incorporation [9,23]. After the cleavage reaction with anhydrous HF, the crude spin-labeled peptides were submitted to alkaline treatment (pH 10, 1 h, 50 °C) for complete reversion (monitored by analytical HPLC) of the N–O protonation that occurs during the HF reaction. The peptides were purified by preparative HPLC (C_{18}-column) using aqueous 0.02 M ammonium acetate (pH 5) and 60% acetonitrile solutions as solvents A and B, respectively (linear gradient of 30–70% B for 2 h, flow rate of 10 mL/min). The peptide homogeneity was checked by analytical HPLC, amino acid analysis, and LC/MS mass spectrometry (electrospray). The following peptides were synthesized: AngII and analogues where TOAC replaced Asp^1 ($TOAC^1$-AngII) or Val^3 ($TOAC^3$-AngII); BK analogues where TOAC was inserted before Arg^1 ($TOAC^0$-BK) or replacing Pro^3 ($TOAC^3$-BK). The radioactive ^{125}I-AngII and ^{125}I-Tyr^0-BK were acquired from GE Healthcare Life Sciences.

2.2. Cell culture and cell transfection

Chinese hamster ovary (CHO) cells were maintained in Dulbecco's modified Eagle's medium — DMEM (GIBICO-BRL) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (all from Life technologies) at 37 °C in a humidified 5% CO_2 atmosphere. Expression plasmids containing wild type AT_1 or B_2 receptors were permanently transfected into CHO cells by lipofectin method as described by the manufacturer (GIBCO-BRL) and 5 μg of plasmid DNA for each sample. For the establishment of stable cell lines, transfected cultures were maintained in culture medium supplemented with 500 μg of G 418 (Geneticin; GIBCO-BRL).

2.3. Binding assays

CHO cells transfected with AT_1 or B_2 receptors expression plasmids were transferred to 6-well culture plates (5×10^5 cells/well) 24 h before the beginning of the experiment. Immediately before the experiment the cells were washed briefly in 25 mM Tris–HCl buffer, pH 7.4, containing 140 mM NaCl, 5 mM $MgCl_2$ and 0.1% BSA. Binding experiments were performed at 4 °C and initiated by the addition of 50 pM ^{125}I-AngII or ^{125}I-Tyr^0-BK in the presence of varying amounts of unlabeled AngII, BK and analogs as competitors in a 1.0 mL volume assay. The binding buffer consisted of 25 mM Tris–HCl, pH 7.4, including 5 mM $MgCl_2$, 0.1% BSA, and 100 μg/mL bacitracin (Sigma). All measurements were done in duplicate. The competition binding profiles were analyzed by nonlinear regression analysis using GraphPad software (Graph-Pad). IC_{50} values were obtained from the competition binding curves and represent concentrations required to get 50% displacement of the labeled ligand bound to the specific receptors.

2.4. Microphysiometer functional assays

The cytosensor microphysiometer is a powerful technique to evaluate the responsiveness of different cell lines to a variety of bioactive ligands measuring small changes in the concentration of H^+ ions in the sensor chamber [24]. This method has been used extensively to measure the effects of signal transduction in cell surface receptor ligand agonist and analogues for many families of G protein-coupled receptors [25–28].

Cells expressing the receptors were plated onto membrane polycarbonate capsule cups at a density of 5×10^5 cells/cup in a DMEM medium, approximately 12–16 h prior the experiment. The capsule cup/insert assembly was placed into the sensor chamber on the Cytosensor system which contained the pH-sensitive system sensor. The DMEM medium not supplemented with fetal bovine serum was pumped through the chambers bathing the cells at a rate of 100 μl/min. This running medium was devoid of sodium

Table 1 Binding (IC_{50}) and functional analysis (EC_{50} and % maximum potency) of AngII, $TOAC^1$-AngII, $TOAC^3$-AngII, BK, $TOAC^0$-BK and $TOAC^3$-BK in permanently transfected CHO cells expressing wild type AT_1 or B_2 receptors

	IC_{50} (nM)	(*n*)	F_{ratio}	EC_{50} (nM)	(*n*)	F_{ratio}	% max. potency
AT_1 receptor							
AngII	19±1	3	–	37±8	9	–	100
$TOAC^1$-AngII	54±12	3	2.8	42±8	10	1.1	115±2
$TOAC^3$-AngII	ND	3	ND	ND	5	ND	ND
B_2 receptor							
BK	13±3	3	–	17±3	6	–	100
$TOAC^0$-BK	15±5	3	1.1	19±5	6	1.1	97±8
$TOAC^3$-BK	ND	3	ND	ND	6	ND	ND

Values are means±SEM, and the number of independent experiments done in duplicate is shown in parentheses. F_{ratio} is the ratio of the IC_{50} or EC_{50} values of the analogues in relation to those of native AngII or BK (and analogs). ND, not detectable (affinity or activation profile).

bicarbonate and had a buffering capacity of 1 mM supplied by phosphate. Physiological osmolarity was obtained by the addition of NaCl. The assembled cup was then transferred to sensor chambers containing 1 ml of low buffered (1 mM sodium phosphate) DMEM with 0.1% BSA and without bicarbonate but containing 2 mM glutamine and additional 44.4 mM NaCl to replace bicarbonate and adjust osmolarity. The sensor chambers were placed on the Cytosensor (Molecular Devices Corporation) microphysiometer [24,26–28] and allowed to equilibrate for more than 40–60 min before the beginning of the experiment. The medium was run through the chambers at a

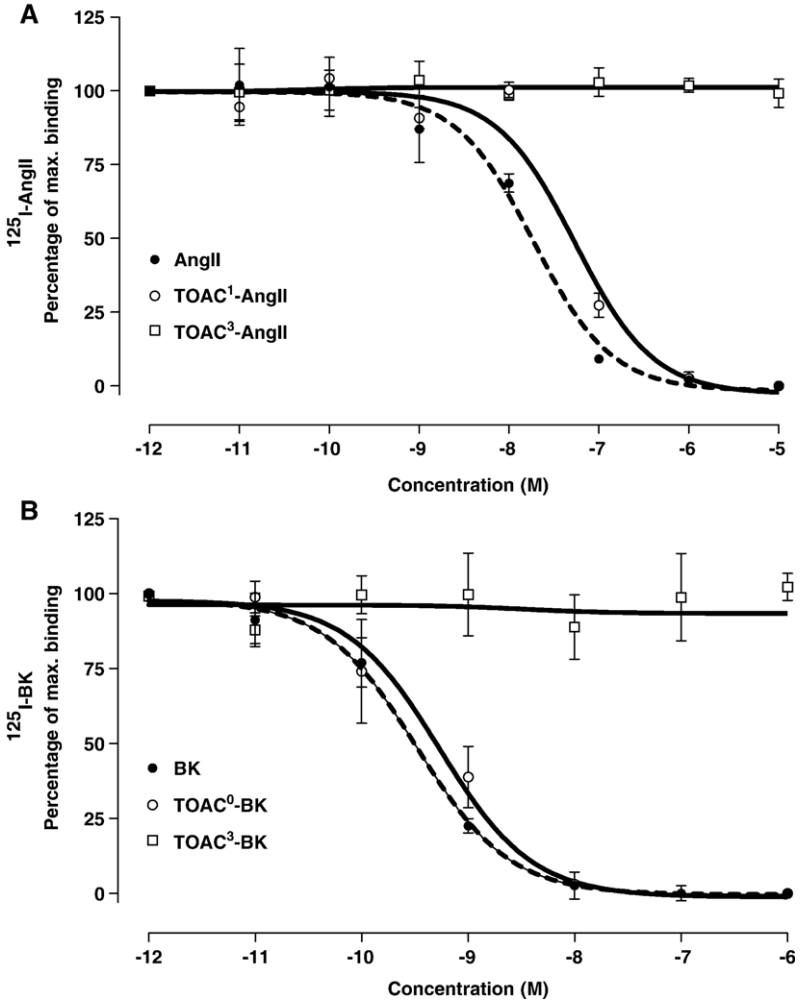

Figure 1 Competition binding profile for AngII, $TOAC^1$-AngII, $TOAC^3$-AngII (A), and for BK, $TOAC^0$-BK, $TOAC^3$-BK (B) in permanently transfected CHO cells expressing wild type AT_1 or B_2 receptors. Data are expressed as the percentage of the maximal specific binding of the radioligand ^{125}I-AngII or ^{125}I-Tyr^0-BK (mean±SEM, $n=3$).

rate of 100 μl/min and pump cycle was 90 s on, 20 s off at 37 °C. To assess shifts in the extracellular acidification rate, cells were stimulated (over a period of 30 s) with peptides in concentrations varying from 10^{-11} M to 10^{-5} M. All measurements were done in quadruplicate and the data were analyzed by nonlinear regression analysis using Graph-Pad Software (Graph-Pad, San Diego, CA). EC_{50} values were calculated from the concentration-effect curves and represent concentrations of the agonist required to elicit 50% of the maximal effect.

2.5. Blood pressure measurements

Experiments were performed in male Wistar rats (290–300 g, $n=4$ for each group). One day before the experiment, rats were anesthetized (chloral hydrate, 450 mg/kg, *ip*), and a polyethylene catheter (PE-10 connected to PE-50) was inserted into the abdominal aorta through the femoral artery for blood pressure measurements. For intravenous injections (AngII and $TOAC^1$-AngII, 50–500 pmol) a polyethylene cannula was implanted into the femoral vein, and for intra-arterial injections (BK and $TOAC^0$-BK, 50–200 pmol) another catheter was inserted into the ascending aorta through the left carotid artery. The polyethylene cannulas, closed by metallic pins and filled with isotonic saline, were driven subcutaneously to the interscapular region of the backs of the animals. After recovery from anesthesia, rats were treated with benzyl–penicillin (24,000 U, *im*) and kept in individual cages with free access to water and chow until the end of the experiment. Changes in arterial pressure were monitored by a data-acquisition system (Power Lab 8/S, ADInstruments Pty Ltd, Castle Hill, Australia).

2.6. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Statistical significance was evaluated with the Student's *t*-test for unpaired samples. Differences were considered to be significant when $P<0.05$.

3. Results

3.1. Binding assays

Binding results using AngII, $TOAC^1$-AngII, $TOAC^3$-AngII, BK, $TOAC^0$-BK, $TOAC^3$-BK as ligands and ^{125}I-AngII, ^{125}I-Tyr^0-BK as the radioligands

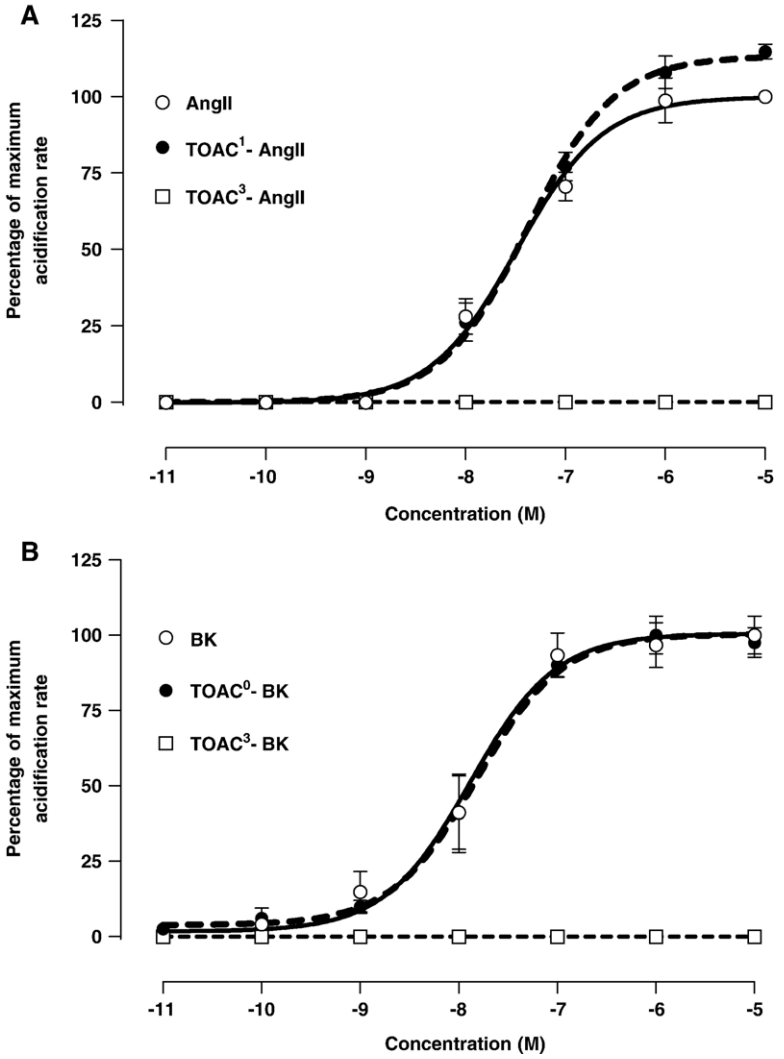

Figure 2 Effect of AngII, $TOAC^1$-AngII and $TOAC^3$-AngII in (A), and BK, $TOAC^0$-BK and $TOAC^3$-BK in (B) on concentration-response curve for extracellular acidification rate in permanently transfected CHO cells expressing wild type AT_1 or B_2 receptors. The activation is expressed as the percentage of the maximal values obtained for the wild type receptor (mean ± SEM, $n=5$–10).

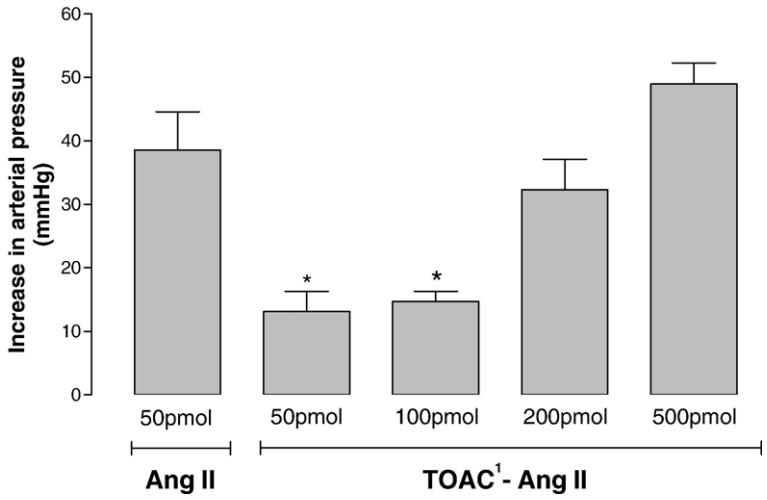

Figure 3 Dose-dependent effect of intravenous administration of AngII and TOAC^1-AngII on blood pressure in awaken Wistar rats. Results are expressed as mean ± SEM, $n=4$ for each dose. Responses statistically different when compared to basal AngII value is labeled ($^*P<.01$).

are shown in Table 1 and Fig. 1. In comparison to the native peptide hormones AngII and BK, their N-terminally TOAC derivatives (TOAC^1-AngII and TOAC^0-BK) showed similar affinity (IC_{50}). Conversely the internally labelled TOAC analogues had no affinity to their respective receptors and no binding ability was detected with the transfected cells.

3.2. Microphysiometer functional assays

The same trend of activity was also observed when dose-response curves were obtained in the cytosensor microphysiometer for the same set of peptides (Fig. 2). The TOAC^1-AngII and TOAC^0-BK derivatives presented similar EC_{50} values ($F_{ratio}=1.1$) when compared to the natural ligands AngII and BK whereas both internally marked analogues (TOAC^3-AngII and TOAC^3-BK) were completely inactive.

3.3. Blood pressure measurements

Aiming at examining the *in vivo* functionality of the TOAC-labeled AngII and BK analogs, they were next administered intravenously or intraarterially, respectively, in awaken rats and the blood pressure was evaluated. The results displayed in Fig. 3 showed that the injection of TOAC^1-AngII (from 50 to 500 pmol) induced a consistent increase in blood pressure which was 4- to 10-fold less potent when compared to the natural agonist AngII. In the case of BK and derivatives, effects of intra-arterial injection of TOAC^0-BK on the blood pressure levels are presented in Fig. 4 where a reduction in this parameter is observed. However, this effect was significantly lower when compared to the hypotension degree induced by the native BK. Finally, in agreement with the previous findings detected with *in vitro* experiments, the intra-arterial administration of

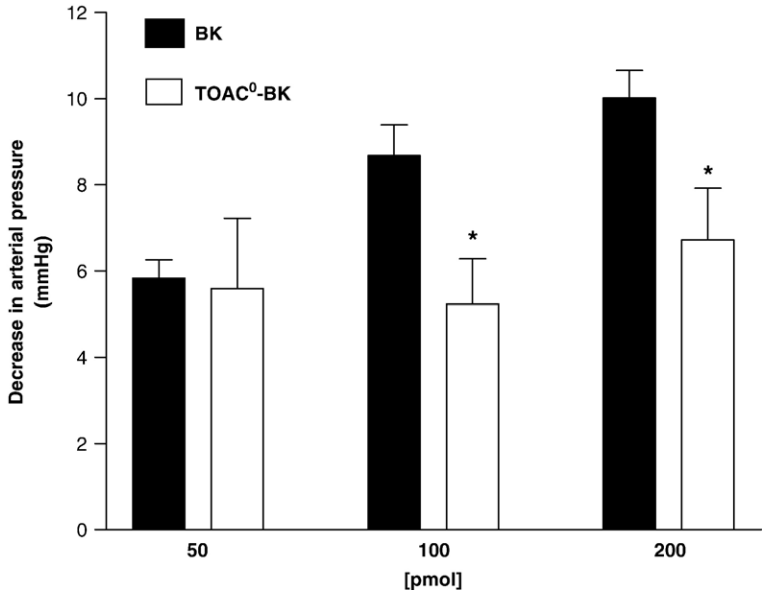

Figure 4 Dose-dependent effect of intra-arterial administration of BK and TOAC^0-BK on blood pressure in awaken Wistar rats. Results are expressed as mean ± SEM, $n=4$ for each dose. Responses statistically different when compared to basal BK value is labeled ($^*P<.05$).

TOAC^3-AngII and TOAC^3-BK had no effect on blood pressure (data not shown).

4. Discussion

In order to follow up on earlier investigation of TOAC-containing AngII or BK [23], the same set of derivatives (TOAC^1-AngII, TOAC^3-AngII, TOAC^0-BK and TOAC^3-BK) were synthesized and examined as to their activity in different *in vitro* and *in vivo* experiments. The paramagnetic amino acid is therefore replacing the Asp^1 and Val^3 residues in AngII and Arg^1 additionally at the N-terminal side or replacing Pro^3 in BK sequences. The pharmacological profiles of these peptides were evaluated *in vitro* through binding assays whereas their functional activities were measured by alteration in extracellular acidification rate in the cytosensor microphysiometer using CHO cells transfected permanently with wild type AT_1 or B_2 receptors and *in vivo* blood pressure evaluation in awaken Wistar rats. These results suggest that, in contrast to internally labeled derivatives, these N-terminal modification at AngII and BK sequences do not promote a significant alteration in the peptide conformations and these peptide-receptors interaction result in binding and functional activation of the receptors expressed in transfected CHO cells. These findings are in close accordance with previous work [23], which showed similar biological activities for these analogues in smooth muscle preparations.

Indeed, the integral or partial maintenance of different biological functions, mainly occurring when the N-terminal region of these two peptides is modified has already been verified, either in the case of AngII [29,30] or BK [31]. The explanation for these results seems to be initially related to the greater peptide chain freedom in the extremity of the sequence, where the insertion of any unnatural residue might impose lesser alteration in the overall conformation of native peptides. Moreover, one can also conclude from these findings that the Asp residue at position 1 of AngII is not essential for its biological activity whereas in the case of TOAC^0-BK, one can hypothesize that this analogue is still active as a consequence of the maintenance of the well known essential presence of Arg residue at position 1 for the BK activity [31]. Otherwise, for the case of the lack of activity of both internally labeled peptides (TOAC^3-AngII and TOAC^3-BK), the search in the literature revealed reports showing previously that Val^3 and Pro^3 are both essential for the maintenance of the AngII [32] and BK [33] activities, respectively.

Despite these observations and specifically addressing the present results originated from the TOAC insertion in AngII and BK structures, one has necessarily to take into account the special conformational effect that might be imposed by this piperidine-type cyclic probe. This compound is known as a typical peptide structure-breaking moiety, favoring the appearance of more folded conformations including bends or helices [16]. This might possibly be the main reason for the lack of biological activity of the internally TOAC-labeled AngII or BK analogues as the molecular flexibility requirement for an appropriate interaction with the receptor molecule should be lost for these samples. In contrast, the location of the TOAC probe at the N-terminal portion of the peptides seems to induce lesser variation in their conformations, allowing them to maintain at least partially, their biological potencies. A more elaborated discussion regarding the correlation degree existing between structural features and corresponding biological activities of these labelled derivatives was previously reported based on conformational data acquired from EPR, circular dichroism and fluorescence techniques [34].

In conclusion, the results presented in this work comprising further *in vitro* and *in vivo* approaches for investigating the TOAC-substituted AngII and BK analogues seem to be of relevance as they amplify significantly the knowledge existing to date, with regards to the structure-biological function relationship of these type of compounds and open new perspectives for their more fruitful application in a broad range of biological studies.

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References

[1] Oliveira L, Costa-Neto CM, Nakaie CR, Schreier S, Shimuta SI, Paiva ACM. The angiotensin II AT_1 receptor structure-activity correlations in the light of rhodopsin structure. Physiol Rev 2007;87:565–92.
[2] Catt KJ, Sandberg K, Balla T. In: Raizada MK, Phillips MI, Sumners C, editors. Cellular and molecular biology of the renin–angiotensin system. Boca Raton: CRC Press; 1993.
[3] Bhoola KD, Figueroa CD, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol Rev 1992;44:1–80.
[4] Sharma JN. Role of tissue kallikrein–kininogen–kinin pathways in the cardiovascular system. Arch Med Res 2006;37:299–306.
[5] Berliner LJ, Reuben J, editors. Biological magnetic resonance. New York: Plenum Press; 1989.
[6] Rassat A, Rey P. Nitroxydes. 23. Préparation dàminoacides radicalaires et de leurs sels complexes. Bull Soc Chim Fr 1967;3:815–7.
[7] Nakaie CR, Goissis G, Schreier S, Paiva AC. pH dependence of EPR spectra of nitroxides containing ionizable groups. Braz J Med Biol Res 1981;14:173–80.
[8] Nakaie CR, Schreier S, Paiva AC. Synthesis and properties of spin-labeled angiotensin derivatives. Biochim Biophys Acta 1983;742:63–71.
[9] Marchetto R, Schreier S, Nakaie CR. A novel spin-labeled amino-acid derivative for use in peptide-synthesis-(9-fluorenylmethyloxycarbonyl)-2,2,6,6-tetramethylpyrrolidine-N-oxyl-4-amino-4-carboxylic acid. J Am Chem Soc 1993;115:11042–3.
[10] Toniolo C, Valente E, Formaggio F, Crisma M, Pilloni G, Corvaja C, et al. Synthesis and conformational studies of peptides containing TOAC, a spin-labeled $C^{\alpha,\alpha}$-disubstituted glycine. J Pept Sci 1995;1:45–57.
[11] McNulty JC, Silapie JL, Carnevali M, Farrar CT, Griffin RG, Formaggio F, et al. Electron spin resonance of TOAC labeled peptides: folding transitions and high frequency spectroscopy. Biopolymers 2000;55:479–85.
[12] Barbosa SR, Cilli EM, Lamy-Freund MT, Castrucci AM, Nakaie CR. First synthesis of a fully active spin-labeled peptide hormone. FEBS Lett 1999;446:45–8.
[13] Bettio A, Gutewort V, Poppl A, Dinger MC, Zschornig O, Klaus A, et al. Electron paramagnetic resonance backbone dynamics studies on spin-labelled neuropeptide Y analogues. J Pept Sci 2002;8:671–82.

[14] Karim CB, Kirby TL, Zhang Z, Nesmelov Y, Thomas DD. Phospholamban structural dynamics in lipid bilayers probed by a spin label rigidly coupled to the peptide backbone. Proc Natl Acad Sci USA 2004;101:14437–42.
[15] Moraes LG, Fazio MA, Vieira RF, Nakaie CR, Miranda MT, Schreier S, et al. Conformational and functional studies of gomesin analogues by CD, EPR and fluorescence spectroscopies. Biochim Biophys Acta 2007;1768:52–8.
[16] Toniolo C, Crisma M, Formaggio F. TOAC, a nitroxide spin-labeled, achiral C^{α}–C-tetrasubstituted α-amino acid, is an excellent tool in material science and biochemistry. Biopolymers 1998;47:153–8.
[17] Pertinhez TA, Nakaie CR, Paiva AC, Schreier S. Spin-labeled extracellular loop from a seven-transmembrane helix receptor: studies in solution and interaction with model membranes. Biopolymers 1997;42:821–9.
[18] Victor KG, Cafiso DS. Location and dynamics of basic peptides at the membrane interface electron paramagnetic resonance spectroscopy of tetramethyl-piperidine-1-oxyl-4-amino-4-carboxylic acid. Biophys J 2001;81:2241–50.
[19] Inbaraj JJ, Cardon TB, Laryukhin M, Grosser SM, Lorigan GA. Determining the topology of integral membrane peptides using EPR spectroscopy. J Am Chem Soc 2006;128:9549–54.
[20] Cilli EM, Marchetto R, Schreier S, Nakaie CR. Use of spin label EPR spectra to monitor peptide chain aggregation inside resin beads. Tetrahedron Lett 1997;38:517–20.
[21] Marchetto R, Cilli EM, Jubilut GN, Schreier S, Nakaie CR. Determination of site-site distance and site concentration within polymer beads: a combined swelling-electron paramagnetic resonance study. J Org Chem 2005;70:4561–8.
[22] Teixeira LGD, Bersanetti PA, Schreier S, Carmona AK, Nakaie CR. Analogues containing the paramagnetic amino acid TOAC as substrates for angiotensin I-converting enzyme. FEBS Lett 2007;581:2411–5.
[23] Nakaie CR, Silva EG, Cilli EM, Marchetto R, Schreier S, Paiva TB, et al. Synthesis and pharmacological properties of TOAC-labeled angiotensin and bradykinin analogues. Peptides 2002; 23:65–70.
[24] McConnell HM, Owicki JC, Parce JW, Miller DL, Baxter GT, Wada HG, et al. The cytosensor microphysiometer: biological applications of silicon technology. Science 1992;257:1906–12.
[25] Bironaite D, Gera L, Stewart JM. Characterization of the B2 receptor and activity of bradykinin analogues in SHP-77 cell line by cytosensor microphysiometer. Chem Biol Interact 2004;150: 283–93.
[26] Santos EL, Pesquero JB, Oliveira L, Paiva AC, Costa-Neto CM. Mutagenesis of the AT1 receptor reveals different binding modes of angiotensin II and [Sar1]-angiotensin II. Regul Pept 2004;119:183–8.
[27] Reis RI, Santos EL, Pesquero JB, Oliveira L, Schanstra JP, Bascands JL, et al. Participation of transmembrane proline 82 in angiotensin II AT1 receptor signal transduction. Regul Pept 2007;140:32–6.
[28] Santos EL, Reis RI, Silva RG, Shimuta SI, Pecher C, Bascands JL, et al. Functional rescue of a defective angiotensin II AT(1) receptor mutant by the Mas protooncogene. Regul Pept 2007; 141:159–67.
[29] Khairallah PA, Toth A, Bumpus FM. Analogs of angiotensin II. Mechanism of receptor interaction. J Med Chem 1970;13: 181–6.
[30] Paiva TB, Goissis G, Juliano L, Miyamoto ME, Paiva ACM. Angiotensin-like and antagonistic activity of N-terminal modified [8-Leucine] angiotensin II peptides. J Med Chem 1974;17: 238–41.
[31] Rhaleb NE, Télémaque S, Rouisse N, Dion S, Jukic D, Drapeau G, et al. Structure-activity studies of bradykinin and related peptides-B_2-receptor antagonists. Hypertension 1991;17: 107–15.
[32] Nikiforovich GV, Marshal GR. 3-dimensional recognition requirements for angiotensin agonists. A novel solution for an old problem. Biochem Biophys Res Commun 1993;195:222–8.
[33] Juvvadi P, Dooley DJ, Humblet CC, Lu GH, Lunney EA, Panek RL, et al. Bradykinin and angiotensin II analogs containing a conformationally constrained praline analog. Int J Pept Prot Res 1992;40:163–70.
[34] Schreier S, Barbosa SR, Casallanovo F, Vieira Rde F, Cilli EM, Paiva AC, et al. Conformational basis for the biological activity of TOAC-labeled angiotensin II and bradykinin: electron paramagnetic resonance, circular dichroism, and fluorescence studies. Biopolymers 2004;74:389–02.