Pharmacologic and Molecular Characterization of the Vascular ET_A Receptor in the Venomous Snake *Bothrops jararaca*

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Endothelins (ETs) and sarafotoxins (SRTXs) are active isopeptides that have very similar structures and functions. All isoforms interact with two specific G-protein–coupled receptors, ET_A and ET_B. To characterize functional vascular ET receptors in the poisonous snake, *Bothrops jararaca*, cumulative concentration-response curves to ETs and SRTXs were performed in isolated aortic rings, in the absence and presence of selective ET receptor antagonists. Vascular expression of ET receptor messenger RNA (mRNA) was evaluated by reverse transcriptase (RT) polymerase chain reaction (PCR) analysis, and a fragment of the ET_A receptor was cloned and sequenced. *In vivo*, ET-1 induced a dose-dependent biphasic response on anesthetized *B. jararaca* snakes. *In vitro*, ET-1, SRTX-b, ET-3, SRTX-c, and IRL-1620 induced concentration-dependent vasoconstriction, with a potency order suggesting the presence of typical ET_A receptors. BQ-123, a selective ET_A antagonist, inhibited contractions induced by ET-1 and SRTX-b with expected negative log of the dissociation constant, K_B, (pK_B) values for mixed ET_A/ET_B receptor populations. The nonselective ET_A/ET_B receptors antagonist, PD-142893, produced similar inhibition. The ET_B antagonist, IRL-1038, potentiated contractile responses to SRTX-c. ET-1 and SRTX-c responses were also potentiated when aortic rings were pretreated with N_ω-nitro-L-arginine methyl ester (L-NAME) plus indomethacin. Processing of the *B. jararaca* aortic first-strand complementary DNA, by RT-PCR with primers designed from the *Gallus gallus* ET_A receptor sequence, enabled isolation, purification, cloning, and sequencing of a single band. The partial sequence of the *B. jararaca* ET_A receptor showed a very high sequence similarity with ET_A receptor sequences from chicken, rat, human, and Xenopus. In conclusion, vascular responses to SRTXs/ETs in the *B. jararaca* aorta are mediated predominantly, but not exclusively, by typical ET_A receptors. Exp Biol Med 231:729–735, 2006

Key words: reptile; endothelin; sarafotoxin; endothelin receptors; vascular reactivity; cloning

Introduction

Endothelins (ETs) are a family of vasoactive peptides comprised of four endogenous 21-amino acid isoforms (ET-1, ET-2, ET-3, and ET4 or vasoactive intestinal contractor), as well as the 31-amino acid ET isoforms (ET-1, ET-2, and ET-3[1–31]; Refs. 1–3). Peptides with a high degree of sequence similarity to the ETs are the sarafotoxins (SRTXs), S6a, S6b, S6c, and S6d, a group of venom toxins first isolated from the gland of the snake *Atractaspis engadensis* (4–6). Recently, a new family of SRTXs was isolated from the venom of *A. microlepidota microlepidota*. This new family displays three additional amino acid residues at the C-terminus and were, therefore, named long-SRTXs (l-SRTXs; Ref. 7).

ET-1, the predominant isoform of the ET peptide family, and the other family members exert their effects through two specific G-protein–coupled receptors, called ET_A and ET_B (8–11). ET receptors are widely distributed in tissues from all vertebrates and in some invertebrates (12). In the vascular system of mammals, ET_A receptors are detected mainly on vascular smooth-muscle cells and mediate contraction (10), whereas ET_B receptors can be found both on vascular endothelial and smooth-muscle cells (13–15). Little evidence exists on the presence of ET receptors in tissues from reptiles.

The class Reptilia, including arboreal and terrestrial snakes (order Squamata), are the first vertebrates adapted to live in a terrestrial habitat (16). Because of their elongated body shape with long fluid columns, they are highly susceptible to hydrostatic pressure disturbances by the influence of gravity (17). In an earlier study, by using SRTX peptides, we found evidence of the presence of highly sensitive ET receptors in the aorta of the Brazilian

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poisonous snake *Bothrops jararaca* (18). Because one of the main physiologic functions of the ET system is to modulate the vascular tonus, we thought it would be interesting to characterize the ET receptors in the vascular system of this terrestrial snake. Therefore, in the present study we evaluated the blood pressure effects of ET-1, and functionally and molecularly characterized the vascular ET receptors in the *B. jararaca* snake.

Materials and Methods

Animals. Experimental protocols followed standards and policies of the Committee for Animal Care and Use of the Butantan Institute. Briefly, adult *B. jararaca* snakes of both sexes, weighing up to 150 g, were maintained under controlled conditions of temperature and humidity, as previously described (19). The snakes were anesthetized with sodium pentobarbital, 30 mg/kg ip, before blood pressure measurements or decapitation, as previously described (20, 21).

Vascular Reactivity Studies. A segment of approximately 4 cm of the aorta was excised from the area near the aortic arch, and 0.50-cm rings were cut and suspended in 10-ml organ baths, as described previously (18, 22). Contractile force was recorded with a Biopac MP100WS system, using the AcqKnowledge BioPac software (BioPac Systems Inc., Santa Barbara, CA).

Cumulative concentration-response curves (CCRCs) to ET-1, SRTX-b, ET-3, and SRTX-c (10^{-11}–10^{-6} *M*) were obtained in the presence and absence of the antagonists BQ-123, IRL-1038, and PD-142893. The antagonists were added to the bath 30 mins before beginning of the agonist CCRCs. Vascular reactivity to ET-1 and SRTX-c in the presence of 10^{-4} *M* N_{ω}-nitro-L-arginine methyl ester, hydrochloride (L-NAME; a nitric oxide synthase inhibitor) plus 3×10^{-6} *M* indomethacin (Indo; a cyclooxygenase inhibitor) as well as to the selective ET_B agonist, IRL-1620 (10^{-8}–3×10^{-6} *M*), was also evaluated. A single CCRC was performed in each aortic ring to avoid the possibility of time-dependent changes in vascular responsiveness and tachyphylaxis phenomena.

Cloning and Sequencing of Partial Complementary DNA (cDNA) of Snake ETA-Receptor. Total cellular RNA was isolated from the thoracic aortae using TRizol reagent. First-strand cDNA was synthesized using 2 μg total RNA, Moloney murine leukemia virus (M-MLV)–reverse transcriptase (RT), RNAase inhibitor (RNAsin), and oligo(dT) primer, at 42°C for 50 mins. cDNA was amplified by polymerase chain reaction (PCR), using the set of primers (5′–3′): TAC GAG AAC AAG TGT ATG AGG (forward) and AAG ACA TGA CTG AAA ACA ATT (reverse), which were designed from the *Gallus gallus* ET_A receptor sequence (Genebank AF472618); and Taq Platinum polymerase; with denaturing cycles at 94°C for 30 secs and annealing at 62°C. PCR products were resolved in 1% agarose gel electrophoresis, stained with ethidium bromide, and band intensities were measured using Kodak Digital Science software (Eastman Kodak Co., Rochester, NY). A unique band (with the expected size of ~836 bp) was excised, purified, and cloned using the pGEM-T vector system. The vectors were sequenced using a Sequencing MegaBACE kit with a capillary sequencer MegaBACE 1000, and the sequence obtained was aligned (Clustal W, http://www.ebi.ac.uk/clustalw/) and compared with other ET_A sequences.

Solutions and Drugs. Bovine serum albumin, norepinephrine, acetylcholine hydrochloride, dimethyl sulfoxide, L-NAME, and indo were obtained from Sigma Chemical Company (St. Louis, MO); ET-1, ET-3, SRTX-b, SRTX-c, BQ-123, IRL-1038, IRL-1620, and PD-142893 were from American Peptide Co. Inc. (Sunnyvale, CA); TRizol, M-MLV-RT, Taq Platinum polymerase, oligo(dT), and primers were from Invitrogen Corp. (Carlsbad, CA); DNase I, RNAase inhibitor, PCR purification kit, and pGEM-T vector system were from Promega Bioscience Inc. (Granada, CA); *Nco*I and *Pst*I were from MBI Fermentas (Hanover, MD); Sequencing MegaBACE kit and sequencer MegaBACE 1000 were from Amersham Bioscience Corp. (Piscataway, NJ). Pentobarbital sodium was kindly donated by the Cristália laboratory (Campinas, São Paulo, Brazil).

Data Analysis and Statistics. Data are expressed as mean ± SEM; *n* indicates the number of animals. The concentration of the agonist producing a half-maximal response (EC_{50}) was determined after logarithmic transformation of the normalized concentration-response curves, and is reported as the negative logarithm (–log EC_{50} = rank order of potency [pD_2] values) of the mean of individual values for each tissue. The antagonist potencies were expressed as pK_B values, that is, the negative log of the dissociation constant, K_B, which is equal to the molar concentration of the antagonist divided by the concentration ratio minus one (23). The groups were compared by one-way analysis of variance (ANOVA) followed by the Bonferroni test for multiple comparisons, or by the two-tailed Student's *t* test. $P < 0.05$ was considered statistically significant.

Results

***In Vivo* Effects of ET-1 on *B. jararaca* Blood Pressure.** Intravenous infusion of 1.2–40 pmol/kg ET-1 on anesthetized *B. jararaca* snakes induced a dose-dependent biphasic response: a small hypotension followed by a long-lasting hypertensive response (Fig. 1). Higher doses of ET-1 (~40 nmol/kg) initially produced greater changes in blood pressure, which were followed by irreversible cardiac arrest (data not shown). Similar results were observed with infusion of SRTX-b.

Pharmacologic Characterization of ET Receptors. As shown in Figure 2, the agonists ET-1, SRTX-b, ET-3, SRTX-c, and IRL-1620 each contracted *B. jararaca* aortic rings in a concentration-dependent manner. The rank

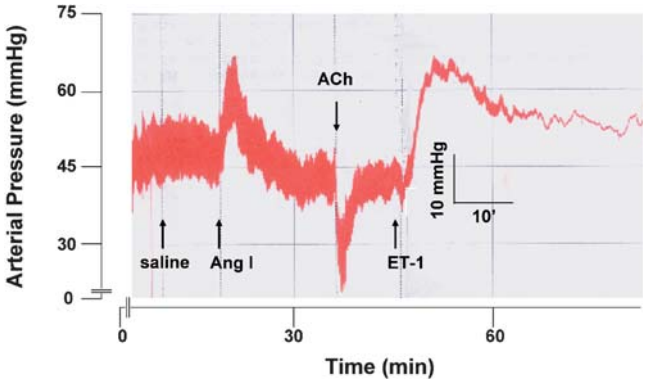

Figure 1. Representative trace of the effects of ET-1 on blood pressure of a 225 g female *B. jararaca* snake under 30 mg/kg pentobarbital anesthesia. ET-1 (1.2 pmol/kg) produced a biphasic response, a small hypotension followed by a long-lasting hypertensive response. The return to baseline occurred after approximately 1 hr. The effects of intravenous injections of a 0.6% saline solution, 97 nmol/kg angiotensin I (Ang I), and 34 nmol/kg acetylcholine (ACh) are also shown. (Color figure available in the on-line version.)

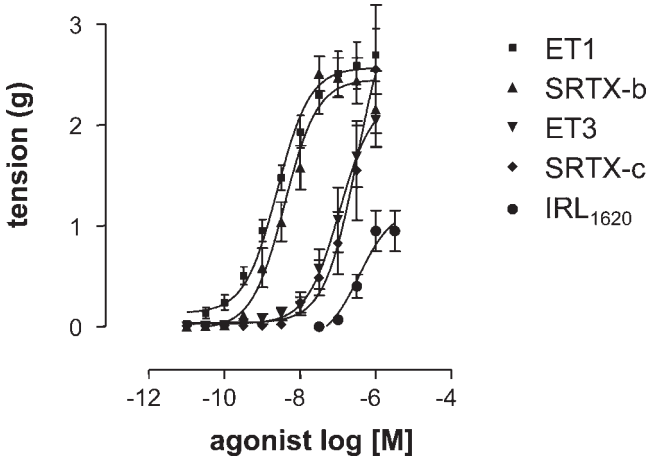

Figure 2. CCRCs to ET receptor agonists in *B. jararaca* aortae. ■, ET-1 ($n = 35$); ▲, SRTX-b ($n = 18$); ▼, ET-3 ($n = 13$); ♦, SRTX-c ($n = 15$); and ●, IRL-1620 ($n = 3$). Each point represents the means ± SEM for agonist-induced contractile responses (in grams).

order of potency (pD_2) of these peptides was ET-1 > SRTX-b >>> ET-3 > SRTX-c >> IRL-1620. Responses to ET-3 and SRTX-c were observed only at concentrations higher than 10^{-8} *M*, and maximal responses were not observed at the highest concentration tested (10^{-6} *M*). The selective ET_B agonist, IRL-1620, only induced contraction at concentrations greater than 10^{-7} *M*, and at the concentration of 3×10^{-6} *M*, the maximum effect was not achieved. The pharmacologic parameters are summarized in Table 1.

BQ-123, a selective ET_A antagonist, right-shifted the CCRCs to ET-1 and SRTX-b in *B. jararaca* aortae in a concentration-dependent manner, with pK_B values of 5.57 ± 0.16 ($n = 10$) and 5.60 ± 0.26 ($n = 11$), respectively (Fig. 3A and B). The nonselective ET_A/ET_B receptors antagonist, PD-142893, similarly inhibited the contractile response to ET-1 (Fig. 3C), with a pK_B value of 5.45 ± 0.26. The effects of both BQ-123 and PD-142893 on the CCRCs to ET-3 and SRTX-c were not clearly demonstrated.

In the presence of IRL-1038, a selective ET_B antagonist, the CCRC to SRTX-c, but not to ET-1, was left-shifted (Fig. 4A). Responses to ET-1 and SRTX-c were potentiated when *B. jararaca* aortae were pretreated with 10^{-4} *M* L-NAME plus 3×10^{-6} *M* Indo (Fig. 4B).

Molecular Characterization of ET Receptors. We used various sets of primers from different species (human, rat, chicken, and Xenopus), in attempt to identify the presence of ET_A and ET_B receptors in the *B. jararaca* aortae. The analysis of PCR products showed a single band (with the expected size, ~ 836 bp) for the set of primers designed from the *Gallus gallus* ET_A receptor sequence (GenBank AF472618). No bands were identified for the ET_B receptor with the sets of primers we have used so far. Purification, cloning and sequencing of the cDNA fragment was performed, and oligonucleotides as well as the predicted amino acid sequences are shown in Figure 5. The *B. jararaca* ET_A sequence has been deposited in the GenBank Data Library under accession number AY849868. The alignment made by CLUSTAL W showed an identity of 74% with human (24), 73% with rat (25), 77% with chicken (26), and 71% with Xenopus (27) ET_A receptors. The respective accession numbers are L06622 (hET_A), NM012550 (rET_A), AF472618.1 (cET_A), and U06633 (xET_A). A very high sequence similarity with the chicken embryo ET_A receptor was observed (BLAST identities = 62/

Table 1. Comparison of Potencies and Maximum Contractile Responses for ET Receptors Agonists in Aortae from *B. jararaca*[a]

	ET-1	SRTX-b	ET-3	SRTX-c	IRL_{1620}
pD_2	8.87 ± 0.09	8.14 ± 0.08	6.90 ± 0.25[b]	<6.0 ± 0.47[b]	<6.0 ± 0.5[b]
EC_{50} (*M*)	1.39×10^{-9}	6.83×10^{-9}	1.10×10^{-7}[b]	>4.85 × 10^{-7}[b]	>4.9 × 10^{-7}[b]
E_{max}	2.49 ± 0.24	2.61 ± 0.29	2.43 ± 0.41[b]	nd	nd
n	35	18	13	15	3

[a] pD_2 negative lagarithm of EC_{50} values; EC_{50}, concentration (*M*) of the agonist producing a half-maximal response; E_{max}, maximum contractile response obtained; nd, not determined; *n*, number of animals.
[b] Estimated values from CCRC without maximal saturation at the highest concentrations of agonist.

74 [83%]; 91/107 [85%]; 155/185 [83%]; and 172/197 [87%]).

Discussion

The sarafotoxin and ET peptide families have a common evolutionary ancestry, despite their evolution in very diverse systems (28). *In vivo* and *in vitro* experiments have shown that SRTXs and ETs resemble each other in their pharmacologic activity and binding properties in different mammalian tissues as well as in tissues from lower vertebrates, such as tilapia fish, torpedo, toads, and lizards (29). Similar to mammals, SRTXs/ETs also modulate cardiovascular responses in lower vertebrates.

To pharmacologically characterize vascular ET receptors in the *B. jararaca*, we evaluated functional responses to SRTXs and ETs in isolated aortic rings. Vascular responses to ET-1 were very similar to those produced by SRTX-b, whereas responses to ET-3 were similar to SRTX-c, in agreement with previous reports showing a clear relation-

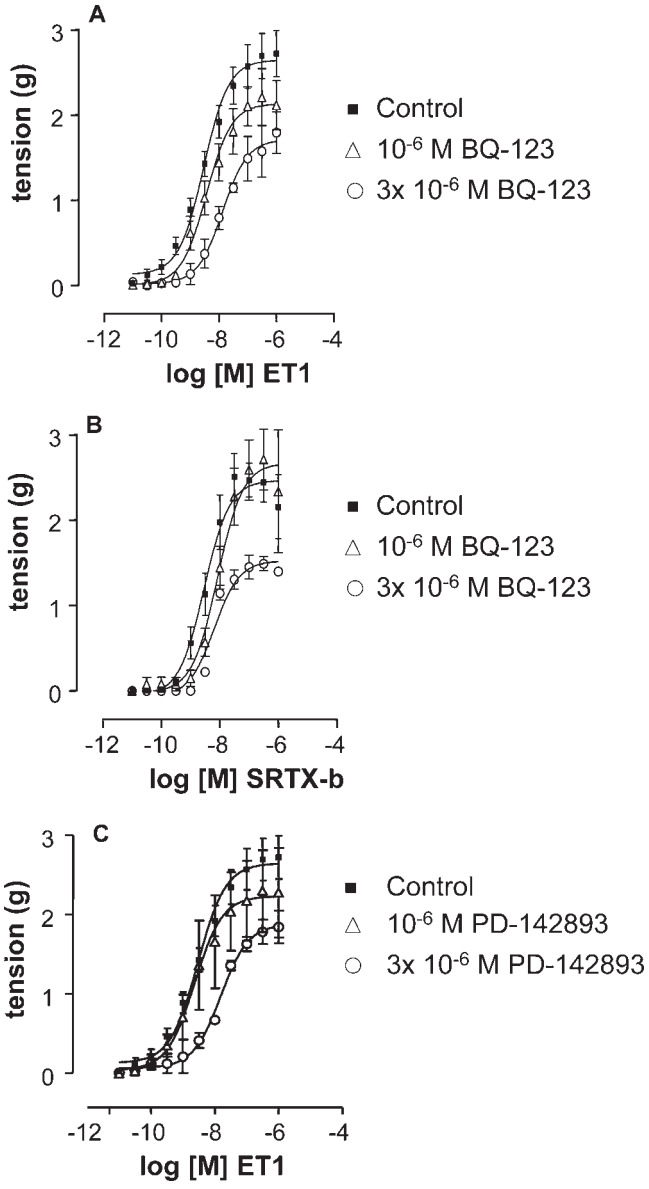

Figure 3. Effects of BQ-123 and PD-142893 in the CCRCs to ET-1 and SRTX-b. Each agonist was tested in control conditions, i.e., in the absence of the antagonist (■) or in the presence of BQ-123 or PD-142893 at 10^{-6} *M* (△) and 3×10^{-6} *M* (○). ET-1 ($n = 33$, 10, 8 for control, 10^{-6} *M*, and 3×10^{-6} *M* BQ-123; and $n = 35$, 4, and 3 for control, 10^{-6}, and 3×10^{-6} *M* PD-142893, respectively); SRTX-b ($n =$ 18, 11, 10). Each point represents the mean ± SEM of agonist-induced contractile responses (in grams) for *n* experiments.

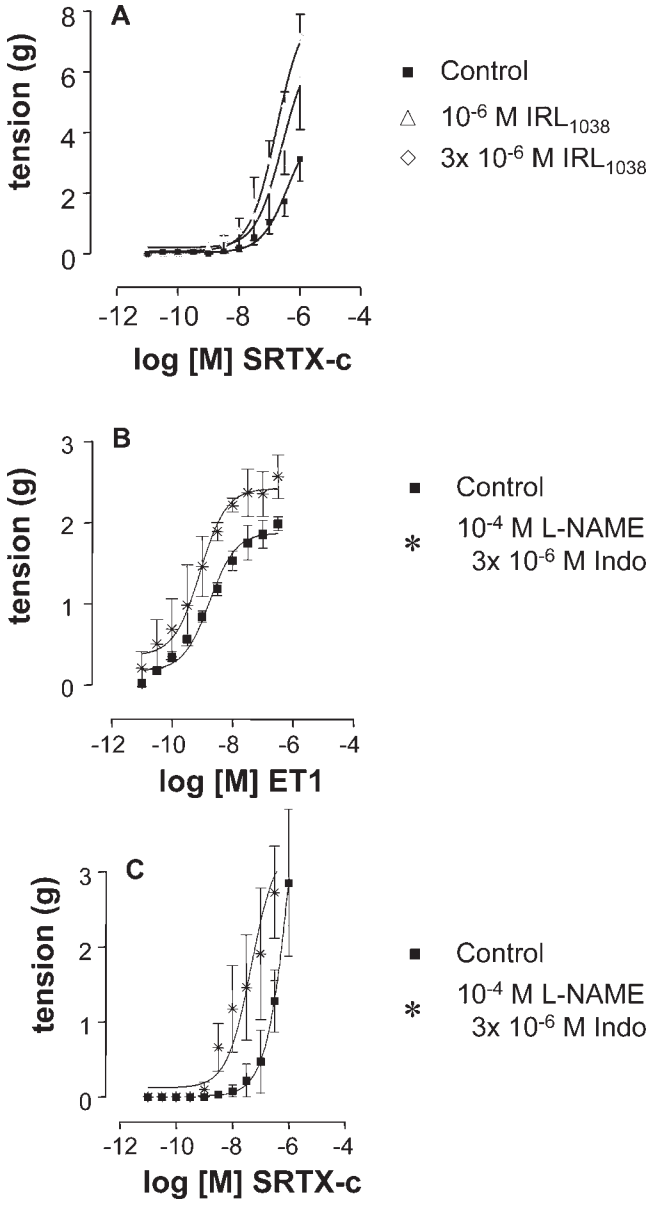

Figure 4. Effects of IRL-1038 on the CCRCs to SRTX-c (A) and of L-NAME plus indo on the contractile responses to ET-1 (B) and SRTX-c (C) . Each agonist was tested in control conditions, i.e., in the absence of the antagonist (■) or in the presence of 10^{-6} *M* IRL_{1038} (△), 3×10^{-6} *M* IRL_{1038} (◇), or 10^{-4} μ*M* L-NAME plus 3×10^{-6} *M* indomethacin (*). SRTX-c ($n = 6$, 5, and 4 for control, 10^{-6} *M*, and 3×10^{-6} *M* IRL_{1038}, respectively). In the experiments with L-NAME plus indomethacin, ET-1, and SRTX-c, *n* is equal to 5. Each point represents the mean ± SEM of agonist-induced contractile responses (in grams) for *n* experiments.

A

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Gallus      ATGCAACTCTGCTGAGGATCATTTACCAGAACAAGTGTATGAGGAATGGCCCGAATGCAC  524
Bj          ------------------------------------------- AATGGACCCAATGCAC   16
                                                        ***** ** *******

Gallus      TGATAGCCAGTCTGGCACTAGGAGACCTTATCTATATTGTCATTGATATTCCTATCATTG  584
Bj          TGATAGCTAGTCTGGCCTTGGGAGATCTCATCTACATCATCATAGATATCCCTATCCATG   76
            ******* ********  * ***** ** ***** **  **** ***** ******  **

Gallus      TGTACAAGCTCCTGGCTCAGAAGTGGCCTTTTGGAGATTCTGAATTTGGGCAGTTTCTTT  644
Bj          TGTATAAGCTTCATTTTCCAAAGCGGCCTTTGGGAGATACNGATTTTGAACAATTTCTTT  136
            **** ***** *    **  *** ******* ****** * ** ****  ** *******

Gallus      GCAAATTCCTTCCCTTTATACAGAAGGCATCAGTGGGAATCACAGTCCTTAATCTCTGTG  704
Bj          GCCGTTTTTTTCCTTTTATTCAGAAGGCATCTGTTGGGGTCACTGTTCTTAATCTCTGTG  196
            **   **  **** ***** ********** ** **  **** ** **************

Gallus      CACTTAGTGTGGACAGGTATAGAGCAGTTGCCTCCTGGAGCCGTGTTCAGGGAATCGGAA  764
Bj          CTCTAAGTGTGGACAGATACCGAGCAGTTGCTTCCTGGAGTCGTGTTCAGGGAATTGGTG  256
            * ** ********** **  ********** ******** ************** **

Gallus      TCCCTATGATCACTGCTATTGAAATTTTCTCCATTTGGCTTCTGTCTTTTATACTGGCTA  824
Bj          TCCCTTTGACTACAGCTATTGAAATTTTTTGTATTTGGATTCTTTCCTTCATCCTGGCTA  316
            ***** ***  **  ************** *  ****** **** ** ** ** *******

Gallus      TTCCAGAAGCCATTGGTTTTGCCGTGGTACCTTTCAGATACAAGGATGAAAGTTATGTTA  884
Bj          TTCCAGAAGCAATTGGTTTCACCAGTGTTGATTTTGTTTATAAGAAAAAACGGCTCAAAA  376
            ********** ********  **   **   ***    ** *** *  ** *       *

Gallus      CCTGCATGCTCAACCCTACAAATAAATTTATGTTGTTTTACAAAGATGCAAAGGATTGGT  944
Bj          CCTGTTTGCTAGTAGCAAGCAACAAGTTCTTGGAATTTTACATTAAAGTAAAAGAGTGGT  436
            ****  ****     * *  ** ** **  **   *******   * * *** ** ****

Gallus      GGCTGTTCGGTTTCTATTTCTGCATGCCACTGGCGTGTACTGCCATCTTCTATACACTAA 1004
Bj          GGCTCTTTGGTTTCTATTTCTGCATGCCACTTGCTTGCACAGCATTCTTTTATACCCTGA  496
            **** ** ************************ ** ** ** **  **** ***** ** *

Gallus      TGACTTGCGAAATGTTAAACAGAAGGAACAGCAACTTGAGAATTGCTCTCAGTGAACACC 1064
Bj          TGACCTGTGAAATGTTAAACAGGAGGAAGAGTAATTTGAGAATTGCTCTCAGTGAACATC  556
            **** ** ************** ***** ** ** ************************ *

Gallus      TTAAGCAGCGTCGAGAAGTTGCAAAAACAGTTTTCTGCTTAGTAGTGATATTTGCTCTTT 1124
Bj          TTAAGCAGCGCCGAGAAGTTGCTAAAACAGTTTTCTGTTTAGTTGTGATTTTTGCTCTCT  616
            ********** *********** ************** ***** ***** ******** *

Gallus      GCTGGTTCCCTCTTCATTTGAGCCGAATTTTGAAGAAAATGGTATATAATGAAAGAGATC 1184
Bj          GCTGGTTTCCTCTGCATCTAAGCCAGATACTGAGATCAATGCTGTATAACGACAAAGACC  676
            ******* ***** *** * ****  **  ***    **** * ***** ** * *** *

Gallus      CCGGCAGATGCGAACTGCTCAGTTTCTTGCTGCCATTGGATTATATCAGCATCAACCTGG 1244
Bj          CCAATAGATGTGAATTCCTCAGTTTTTTGTTGACTTTGGATTATATCAGCATAATCCTAG  736
            **   ***** *** * ******** *** ** * ***************** * *** *

Gallus      CAACTATGAACTCTTGTATAAACCCAATAGCACTCTATTTTGTGAGCAAGAAGTTTAAAA 1304
Bj          CAACCATCAACTCCTGCATAAATCCAATAGC----------------------------  767
            **** ** ***** ** ***** ********
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B

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N G P N A L I A S L A L G D L I Y I I I D I P I H V Y K L H
F P K R P L G D T D F E Q F L C R F F P F I Q K A S V G V T
V L N L C A L S V D R Y R A V A S W S R V Q G I G V P L T T
A I E I F C I W I L S F I L A I P E A I G F T S V D F V Y K
K K R L K T C L L V A S N K F L E F Y I K V K E W W L F G F
Y F C M P L A C T A F F Y T L M T C E M L N R R K S N L R I
A L S E H L K Q R R E V A K T V F C L V V I F A L C W F P L
H L S Q I L R S M L Y N D K D P N R C E F L S F L L T L D Y
I S I I L A T I N S C I N P I
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Figure 5. (A) Partial nucleotide sequence of the vascular ET_A receptor from the serpent *B. jararaca.* *, Identical bases in the sequence of ET_A receptors from *B. jararaca* with ET_A receptors from chicken (*Gallus gallus*). (B) Predicted amino acid sequence of the vascular ET_A receptor from the serpent *B. jararaca*. Amino acids that differ between the *B. jararaca* and *Gallus gallus* sequences are underlined.

ship between the sequences of the peptides and their biologic activity (6). The presence of typical ET_A receptors in the *B. jararaca* aorta was indicated by the rank order of potency for the agonists, with ET-1 being significantly more potent than ET-3. The potencies of the agonists observed in the present study are similar to those described in mammalian veins and arteries (13, 30) and in the guinea pig ileum (9). However, although pharmacologic data provides indirect evidence for the possible presence of ET_B-receptors, conclusive proof will require additional studies.

Few studies have addressed the cardiovascular actions of ETs/SRTXs in lower vertebrates, but it seems that the biologic activities of these peptides are well conserved across evolutionary lines. ET-1 induces potent constriction in arteries and vein of the frog *Rana pipiens* (31) and in various vascular tissues of trout (32). In the *Alligator mississipiensis*, ET-1 produces hemodynamic responses similar to those in mammals, but with a hypotensive phase of greater magnitude and duration (33). Interestingly, although nitric oxide production seems to be important in tonic regulation of the *A. mississipiensis* vasculature, it is not involved in ET-1–induced hypotension in this reptile (33).

The high degree of sequence similarity between the ET_A receptors expressed by the chicken and by the reptile used in this study may be related to the fact that the *B. jararaca* snake holds a terrestrial habitat. Interestingly, only negligible cardiovascular effects are observed in the aquatic snake *Natrix tessellata* in response to SRTX-b and ET-1 stimulation. Furthermore, very few binding sites for ET-1 were found in hearts from this animal and they were nonspecific (29). One may suggest that differences in response to SRTXs/ETs between aquatic and terrestrial snakes may be related to cardiovascular physiologic adaptations undergone by the terrestrial animals. It is well documented that terrestrial snakes are characterized by a number of attributes that are deficient or lacking in species that do not experience the hydrostatic stress of gravity (17).

In summary, our results suggest that both ET_A and ET_B receptors mediate vascular responses to SRTXs/ETs in *B. jararaca*. Characterization of ET receptors in lower vertebrates may contribute to a better understanding of the evolution as well as mechanisms involved in the functioning of the cardiovascular system.

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1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411–415, 1988.
2. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci U S A 86:2863–2867, 1989.
3. Kishi F, Minami K, Okishima N, Murakami M, Mori S, Yano M, Niwa Y, Nakaya Y, Kido H. Novel 31-amino acid-length endothelins cause constriction of vascular smooth muscle. Biochem Biophys Res Commun 248:387–390, 1998.
4. Kochva E, Viljoen CC, Botes DP. A new type of toxin in the venom of snakes of the genus *Atractaspis* (Atractaspidinae). Toxicon 20:581–592, 1982.
5. Takasaki C, Tamiya N, Bdolah A, Wollberg Z, Kochva E. Sarafotoxinas S6: several isotoxins from *Atractaspis engaddensis* (Burrowing Asp) venom that affect the heart. Toxicon 26:543–548, 1988.
6. Lamthanh H, Bdolah A, Creminon C, Grassi J, Menez A, Wollberg Z, Kochva E. Biological activities of [THR2] sarafotoxin-b, a synthetic analogue of sarafotoxin-b. Toxicon 32:1105–1114, 1994.
7. Hayashi MA, Ligny-Lemaire C, Wollberg Z, Wery M, Galat A, Ogawa T, Muller BH, Lamthanh H, Doljansky Y, Bdolah A, Stocklin R, Ducancel F. Long-sarafotoxins: characterization of a new family of endothelin-like peptides. Peptides 25:1243–1251, 2004.
8. Ambar I, Kloog Y, Schvartz I, Hazum E, Sokolovsky M. Competitive interaction between endothelin and sarafotoxin: Binding and phosphoinositides hydrolysis in rat atria and brain. Biochem Biophys Res Commum 158:195–201, 1989.
9. Wollberg Z, Bdolah A, Galron R, Sokolovsky M, Kochva E. Contractile effects and binding properties of endothelins/sarafotoxins in the guinea pig ileum. Eur J Pharmacol 198:31–36, 1991.
10. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding and endothelin receptor. Nature 348: 732–735, 1990.
11. Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K, Masaki T. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 348:732–735, 1990.
12. Zhang J, Leontovich A, Sarras MP Jr. Molecular and functional evidence for early divergence of an endothelin-like system during metazoan evolution: analysis of the Cnidarian, hydra. Development 128:1607–1615, 2001.
13. Sumner MJ, Cannon TR, Mundin JW, White DG, Watts IS. Endothelin ETA and ET_B receptors mediate vascular smooth muscle contraction. Br J Pharmacol 107:858–860, 1992.
14. Haynes WG, Strachan FE, Webb DJ. Endothelin ET_A and ET_B receptors cause vasoconstriction of human resistance and capacitance vessels in vivo. Circulation 92:357–363, 1995.
15. D'orleans-Juste P, Labonte J, Bkaily G, Choufani S, Plante M, Honore JC. Function of the endothelin (B) receptor in cardiovascular physiology and pathophysiology. Pharmacol Ther 95:221–238, 2002.
16. Romer AS. Aquatic adaptation in reptiles—primary or secondary. Ann South African Mus 64:221–230, 1974.
17. Lillywhite HB. Circulatory adaptations of snakes to gravity. Amer Zool 27:81–95, 1987.
18. Borgheresi RAMB, Palma MS, Ducancel F, Camargo ACM, Carmona E. Expression and processing of recombinant sarafotoxins precursor in *Picchia pastoris*. Toxicon 39:1211–1218, 2001.
19. Breno MC, Yamanouve N, Prezoto BC, Lazari MFM, Toffoletto O, Picarelli ZP. Maintenance of the snake *Bothrops jararaca* (WIED, 1824) in captivity. Snake 22:126–130, 1990.
20. Abdalla FMF, Hiraichi E, Picarelli ZP, Prezoto BC. Kallikrein-Kinin system in the plasma of the snake *Bothrops jararaca*. Br J Pharmacol 98:252–258, 1989.
21. Borgheresi RAM, Dalle Lucca J, Carmona E, Picarelli ZP. Isolation and identification of angiotensin-like peptides from the plasma of the snake *Bothrops jararaca*. Comp Biochem Physiol B Biochem Mol Biol 113: 467–473, 1996.
22. Yamanouye N, Salomão LC, Picarelli ZP. Effects of catecholamines on

the isolated aorta of the snake *Bothrops jararaca*. Comp Biochem Physiol 101C:197–201, 1992.

23. Besse JC, Furchgott RT. Dissociation constants and relative efficacies of agonists acting on alpha-adrenergic receptors in rabbit aorta. J Pharmacol Exp Ther 197:66–78, 1976.
24. Elshourbagy NA, Korman DR, Wu HL, Sylvester DR, Lee JA, Nuthalaganti P, Bergsma DJ, Kumar CS, Nambi P. Molecular characterization and regulation of the human endothelin receptors. J Bio Chem 268:3873–3879, 1993.
25. Lin HY, Kaji EH, Winkel GK, Ives HE, Lodish HF. Cloning and functional expression of a vascular smooth muscle endothelin-1 receptor. Proc Natl Acad Sci U S A 88:3185–3189, 1991.
26. Kempf H, Linares C, Corvol P, Gasc J-M. Pharmacological inactivation of the endothelin type A receptor in the early chick embryo: a model of mispatterning of the branchial arch derivatives. Development 125: 4931–4941, 1998.
27. Kumar C, Mwangi V, Nuthulaganti P, Wu HL, Pullen M, Brun K, Aiyar H, Morris RA, Naughton R, Nambi P. Cloning and characterization of a novel endothelin receptor from *Xenopus* heart. J Biol Chem 269:13414–13420, 1994.
28. Landan G, Bdolah A, Wollberg Z, Kochva E, Graur D. The evolutionary history of the sarafotoxin/endothelin/endothelin-like superfamily. J Cardiovasc Pharmacol 17(Suppl 7):S517–S519, 1991.
29. Zigdon-Arad T, Bdolah A, Kochva E, Wollberg Z. Activity of sarafotoxin/endothelin peptides in the heart and brain of lower vertebrates. Toxicon 30:439–448, 1992.
30. Warner TD, Allcock GH, Corder R, Vane JR. Use of the endothelin antagonists BQ-123 and PD-142893 to reveal three endothelin receptors mediating smooth muscle contraction and the release of EDRF. Br J Pharmacol 110:777–782, 1993.
31. Poder TC, Silberberg SD, Rampe D. Contraction of reptile, amphibian, and fish blood vessels by endothelin-1. Can J Physiol Pharmacol 69: 215–217, 1991.
32. Hoagland TM, Weaver L Jr, Conlon JM, Wang Y, Olson KR. Effects of endothelin-1 and homologous trout endothelin on cardiovascular function in rainbow trout. Am J Physiol 278:R460–R468, 2000.
33. Platzack B, Wang Y, Crossley D, Lance V, Hicks JW, Conlon JM. Characterization and cardiovascular actions of endothelin-1 and endothelin-3 from the American alligator. Am J Physiol 282:R594–R602, 2002.