Cell. Mol. Life Sci. 62 (2005) 2828–2839
1420-682X/05/232828-12
DOI 10.1007/s00018-005-5286-x

Cellular and Molecular Life Sciences

Review

Endothelin-like peptides

F. Ducancel

Département d'ingénierie et d'études des protéines (DIEP), Bâtiment 152, CEA de Saclay, 91191 Gif sur Yvette Cedex (France), Fax: +33 169089071, e-mail: frederic.ducancel@cea.fr

Received 27 June 2005; received after revision 29 July 2005; accepted 2 September 2005
Online First 2 November 2005

Abstract. Mammalian endothelins (ETs) and snake venom sarafotoxins (SRTXs) comprise structurally and functionally related potent vasoconstrictor isopeptides that act on the vascular system via identical receptors. This similarity is remarkable, since SRTXs are highly toxic components isolated from the venoms of snakes of the genus *Atractaspis* of the *Atractaspididae* family, while ETs are endogenous hormones of the mammalian vascular system. Since the first functional and structural description of SRTXs in 1988, the full extent of their natural diversity has become increasingly apparent, and this has led to the characterization of new families of endothelin-like peptides. Based on a combination of conventional biochemical approaches and the latest molecular biology and mass spectrometry techniques, this review describes the more recent panel of SRTX isopeptides isolated from various snake species within the *Atractaspididae* family, but also the similarities and differences that exist between sarafotoxins and endothelins in terms of their metabolism, genetic origin, structure and functional sites.

Key words. Sarafotoxins; endothelins; *Atractaspis;* precursors; vasoactive peptides.

Introduction

Mammalian endothelins (ETs) and snake venom sarafotoxins (SRTXs) form a structurally and functionally related family of potent vasoconstrictor compounds. Snake sarafotoxins are isopeptides solely found in the venoms of the genus *Atractaspis* within the family *Atractaspididae*. The name of sarafotoxins is derived from the Hebrew spelling of the species *Atractaspis engaddensis* – *Saraf Ein Gedi* – from which these peptides were first isolated and characterized [1, 2]. These oviparous burrowing snakes (fig. 1A) live underground and are distributed throughout sub-Saharan Africa with a limited penetration into Israel and the south-western part of the Arabian peninsula. About 15 species of very similar appearance of these burrowing asps are known [3]. They are relatively small, averaging 30–70 cm in length, and mainly feed on small reptiles, amphibians and small rodents, which curiously they catch with their mouth closed, which is unique among reptiles (fig. 1B) [4]. Within the genus *Atractaspis*, venom toxicity varies greatly from one species to another. The major clinical manifestations of *Atractaspis* bites are nausea, vomiting, abdominal pain, diarrhea, sweating, profuse salivation, loss of oculomotor accommodation, loss of consciousness and respiratory difficulties [5]. Among the different venoms tested, that one of *Atractaspis engaddensis* is the most potent [6]. The latter mainly targets the cardiovascular system, and especially the heart, and this without pre- or post-synaptic neurotoxic effects [7]. SRTXs from *Atractaspis engaddensis* are among the most lethal snake toxins ever described, causing death within minutes in mice and in under one hour in humans: the mean lethal dose 50 (LD_{50}) is approximately 15 μg per kg [8].

* Corresponding author.

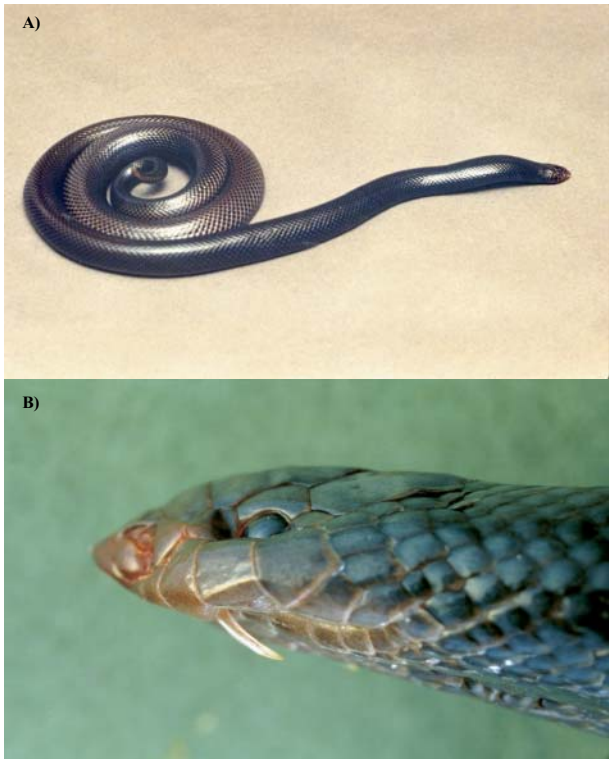

Figure 1. Views of an *A. engaddensis* specimen. (A) *A. engaddensis* in a combined defensive/offensive position. Note the arched neck and the beginning of the coiled body with exposed tip of the tail. (B) Illustration of the capacity of *Atractaspididae* snakes to strike with one fang which protrudes during the bite from the closed mouth.

In contrast to SRTXs, which are overexpressed by the venom gland cells (they may account for 30% of the venom content), endothelins in mammals are produced in very small amounts (plasma concentrations < picomole). ETs are mainly synthezised by endothelial cells but also by other cells in vertebrates, and function as autocrine or paracrine hormonal factors [9–11]. The ET system consists of four isoforms of 21-amino-acid ET peptides (fig. 2A): ET-1, ET-2 and ET-3 in human, and vasoactive intestinal contractor (VIC) in rodents [12, 13]. However, novel bioactive 31-amino-acid ETs (fig. 2A) have recently been described [14, 15]. Based on their diverse pattern of expression in mammals, the ET system components are associated with a large variety of physiological and pathophysiological roles [11, 16]. Owing to their basal vasoconstrictor activity, ET-peptides are mainly involved in regulation of the vascular system (at the level of the vessels) but also in the development of various cardiovascular diseases such as hypertension and atherosclerosis. In the heart, the ET system has cardiotropic action and also contributes to cardiac hypertrophy and remodeling in the case of heart failure. In the lungs it is involved in the development of pulmonary hypertension. In the kidneys, ETs control homeostatic functions and are associated with acute and chronic renal failure. Finally, in the brain the ET system modulates cardiorespiratory centers and the release of hormones.

Consequently, the structural and pharmacological similarities between SRTXs and ETs strongly suggest that ETs are 'endogenous toxins' in vertebrates, and on the other hand that SRTXs are 'endothelin-like' peptides in snake venoms. However, in view of the pharmacological complexity of the ET system, the study of SRTXs appears particularly attractive to further progress in its functional and pharmacological knowledge [17]. Thus, characterization of novel SRTX isopeptides from *Atractaspis* snakes studied de novo could be of particular value in identifying and/or designing clinically useful ET-receptor agonists or antagonists. Here we shall review all the data gathered since the discovery of sarafotoxins in 1982, up to the most recent findings on their structural and functional diversity, original organization, precursor maturation and the structure of their genes. These data will be analyzed and compared with those on ETs so as to establish the molecular and genetic bases of the similarities and differences between SRTXs and ETs.

Discovery and structural characteristics of SRTXs

Six years after the first reference to the existence of low-molecular-weight components in the venom of *Atractaspis engaddensis* [1], the primary sequences of three vasoconstrictor peptides named SRTX-a, b and c (fig. 2B) were published [2]. The same year Yanagisawa's group discovered ET-1 [9, 10], then ET-2 and ET-3 in 1989 [18]. A fourth member of the ET family in mammals named VIC, for vasoactive intestinal contractor, was then described in mice [19], when a new SRTX (bibrotoxin) which differs from SRTX-b solely by the presence of an alanine at position +4 instead of lysine was characterized (fig. 2B) from the venom of a specimen of *A. bibroni* [20]. SRTX-a, b and c, which display marked primary structure homology with at least 85% strict identity and an identical C-terminus, are acidic isopeptides characterized by respective isoelectric points of 5.8, 4.8 and about 3.5. SRTX-a and -b which differ by a single substitution (fig. 2B), have the same toxicity, with an LD_{50} in mice of about 0.01 $\mu g \cdot g^{-1}$. SRTX-c, which is the most abundant isoform in this venom, is also 30 times less toxic (LD_{50} = 0.3 $\mu g \cdot g^{-1}$). Comparison with mammalian 21-amino-acid ET isopeptides (fig. 2A) reveals about 60% homology with SRTXs, the most significant differences being at the N-terminus (region formed by residues +4 to +7) with several nonconservative amino acid substitutions, contrasting with the high homology of the C-terminal hexapeptides [21]. All these isopeptides contain a common core of 21 amino acids and two conserved disulfide bridges between cysteines +1/+15

	1				5					10					15							21			24	25			28		30	31	
A)	**C**	S	**C**	S	S	L	M	**D**	K	**E**	**C**	V	Y	F	**C**	H	L	D	I	I	**W**												**ET-1**
	C	S	**C**	S	S	L	M	**D**	K	**E**	**C**	V	Y	F	**C**	H	L	D	I	I	**W**	V	N	T	P	E	H	V	V	P	Y	**ET-1_{31}**
	C	S	**C**	S	S	W	L	**D**	K	**E**	**C**	V	Y	F	**C**	H	L	D	I	I	**W**												**ET-2**
	C	S	**C**	S	S	W	L	**D**	K	**E**	**C**	V	Y	F	**C**	H	L	D	I	I	**W**	V	N	T	P	E	Q	T	A	P	Y	**ET-2_{31}**
	C	T	**C**	F	T	Y	K	**D**	K	**E**	**C**	V	Y	Y	**C**	H	L	D	I	I	**W**												**ET-3**
	C	T	**C**	F	T	Y	K	**D**	K	**E**	**C**	V	Y	Y	**C**	H	L	D	I	I	**W**	I	N	T	P	E	Q	T	V	P	Y	**ET-3_{31}**
	C	S	**C**	N	S	W	L	**D**	K	**E**	**C**	V	Y	F	**C**	H	L	D	I	I	**W**												**VIC**
	C	S	**C**	A	T	F	L	**D**	K	**E**	**C**	V	Y	F	**C**	H	L	D	I	I	**W**												**ET-trout**
B)	**C**	S	**C**	K	D	M	T	**D**	K	**E**	**C**	L	N	F	**C**	H	Q	D	V	I	**W**												**SRTX-a**
	C	S	**C**	K	D	M	T	**D**	K	**E**	**C**	L	Y	F	**C**	H	Q	D	V	I	**W**												**SRTX-b**
	C	T	**C**	N	D	M	T	**D**	E	**E**	**C**	L	N	F	**C**	H	Q	D	V	I	**W**												**SRTX-c**
	C	S	**C**	A	D	M	T	**D**	K	**E**	**C**	L	Y	F	**C**	H	Q	D	V	I	**W**												**Btx**
C)	**C**	S	**C**	K	D	M	S	**D**	K	**E**	**C**	L	N	F	**C**	H	Q	D	V	I	**W**												**SRTX-a1**
	C	S	**C**	K	D	M	S	**D**	K	**E**	**C**	L	Y	F	**C**	H	Q	D	V	I	**W**												**SRTX-b1**
	C	T	**C**	K	D	M	T	**D**	K	**E**	**C**	L	Y	F	**C**	H	Q	G	I	I	**W**												**SRTX-e**
D)	**C**	S	**C**	N	D	I	N	**D**	K	**E**	**C**	M	Y	F	**C**	H																	**SRTX-m_{16}**
	C	S	**C**	N	D	I	N	**D**	K	**E**	**C**	M	Y	F	**C**	H	Q	D	V	I	**W**	D	E	P									**SRTX-m_{24}**
	C	S	**C**	N	D	M	N	**D**	K	**E**	**C**	M	Y	F	**C**	H	Q	D	V	I	**W**	D	E	P									**SRTX-$m1_{24}$**
	C	S	**C**	N	D	I	N	**D**	K	**E**	**C**																						**SRTX-$m2_{11}$**
	C	S	**C**	N	D	I	N	**D**	K	**E**	**C**	M	Y	F	**C**	H	Q	D															**SRTX-$m2_{18}$**
	C	S	**C**	N	D	I	N	**D**	K	**E**	**C**	M	Y	F	**C**	H	Q	D	I	I	**W**	D	E	P									**SRTX-$m2_{24}$**
	C	S	**C**	N	D	M	N	**D**	K	**E**	**C**	V	Y	F	**C**	H	L	D	I	I	**W**	D	E	P									**SRTX-$m3_{24}$**
	C	S	**C**	N	N	M	S	**D**	K	**E**	**C**	L	N	F	**C**	N	L	D	I	I	**W**	E	N	V									**SRTX-$m4_{24}$**
	C	S	**C**	N	D	M	N	**D**	K	**E**	**C**	V	Y	F	**C**	H	Q	D	I	I	**W**	D	E	P									**SRTX-$m5_{24}$**
E)	**C**	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**																		**SRTX-$i1_{15}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D															**SRTX-$i1_{18}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V														**SRTX-$i1_{19}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V	I													**SRTX-$i1_{20}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V	I	**W**												**SRTX-$i1_{21}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V	I	**W**	I	N										**SRTX-$i1_{23}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V	I	**W**	I	N	R	N								**SRTX-$i1_{25}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V	I	**W**	I	N	R	N	R	K	P					**SRTX-$i1_{28}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V	I	**W**	I	N	R	N	R	K	P	S	P			**SRTX-$i1_{30}$**
	C	S	**C**	A	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	R	L																**SRTX-$i2_{17}$**
	C	S	**C**	A	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	R	L	D															**SRTX-$i2_{18}$**
	C	S	**C**	A	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	R	L	D	V	M													**SRTX-$i2_{20}$**
	C	S	**C**	A	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	R	L	D	V	M	**W**	V	N										**SRTX-$i2_{23}$**
	C	S	**C**	A	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	R	L	D	V	M	**W**	V	N	R	N								**SRTX-$i2_{25}$**
	C	S	**C**	A	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	R	L	D	V	M	**W**	V	N	R	N	R	K	P	S	P			**SRTX-$i2_{30}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V	I	**W**	V	N										**SRTX-$i3_{23}$**
	C	S	**C**	T	D	M	S	**D**	L	**E**	**C**	M	N	F	**C**	H	K	D	V	I	**W**	V	N	R	N								**SRTX-$i3_{25}$**
F)	**C**	S^{20} T^{4}	**C**	N^{8} K^{5} S^{4} A^{3} T^{2} F^{2}	D^{15} S^{5} T^{3} N^{1}	M^{14} W^{3} L^{2} I^{2} F^{1} Y^{1}	S^{6} T^{5} N^{5} L^{4} M^{2} K^{2}	**D**	K^{20} L^{3} E^{1}	**E**	**C**	V^{10} L^{8} M^{6}	Y^{17} N^{7}	F^{22} Y^{2}	**C**	H^{22} N^{1} R^{1}	Q^{11} L^{11} K^{2}	D^{23} G^{1}	I^{13} V^{11}	I^{23} M^{1}	**W**	D^{5} V^{4} I^{2} E^{1}	N^{7} E^{5}	P^{5} R^{3} T^{3} V^{1}	P^{3} N^{3}	E^{3}	Q^{2} H^{1}	T^{2} V^{1}	V^{2} A^{1}	P^{3}	Y^{3}	

Figure 2. Sequence alignments of various ETs and SRTXs. The numbering is indicated in the first lane. ***A)*** Short- (21 aa) and "long-endothelins" (31 aa) from mammals, VIC (vasoactive intestinal contractor) peptide, and ET from trout. ***B)*** SRTX isoforms previously isolated from the venom of *A. engaddensis* (SRTX-a, -b, and –c), and from *A. bibroni* (Btx). ***C)*** Deduced SRTX sequences from *A. engaddensis* precursors. ***D)*** *de novo* characterized 24-amino-acid SRTX sequences from *A. microlepidota microlepidota*. Shorter SRTX-m and m2 isopeptides ranging from 11 to 18 amino acid residues are respectively gathered between dotted-lines ***E)*** *de novo* characterized SRTX sequences from *A. irregularis*. Shorter and longer SRTX-i1, i2 and i3 isopeptides ranging from 15 to 30 amino acid residues are respectively gathered between dotted-lines ***F)*** Amino acid variability. Invariant residues are in bold. The frequencies of the different amino acid residues within the twenty-four more abundant SRTX sequences are indicated as upper signs.

and +3/+11, which constitute a typical and unique Cys_1-X-Cys_3…Cys_{11}-X-X-X-Cys_{15} 'signature' among mammalian bioactive peptides. Nuclear magnetic resonance (NMR), circular dichroism (CD), fluorescence and X-ray analyses have been used to determine the three-dimensional (3D) conformation of the 21-amino-acid peptides. NMR resolution and molecular modeling of the 3D structures of ET-1 [22, 23] and SRTX-b [24] in solution have identified several structural features common to the whole family of these peptides. SRTXs and ETs adopt a cysteine-stabilized α-helical motif characterized by: (i) an extended structure of the first three or four residues, (ii) a β-turn structure between positions +5 and +8, (iii) an α-helical, or helical-like conformation of the segment Lys_9-Cys_{15} and (iv) the absence of conformation of the C-terminal domain (fig. 3). Furthermore, comparison between the NMR and X-ray structures of human ET-1 [25] revealed conformational differences in the C-terminus (helical in the crystal structure, but not in any of the NMR structures), but also in the central loop/turn region (especially residues +5, +6 and +9), that are important for defining the specificity of binding (see below).

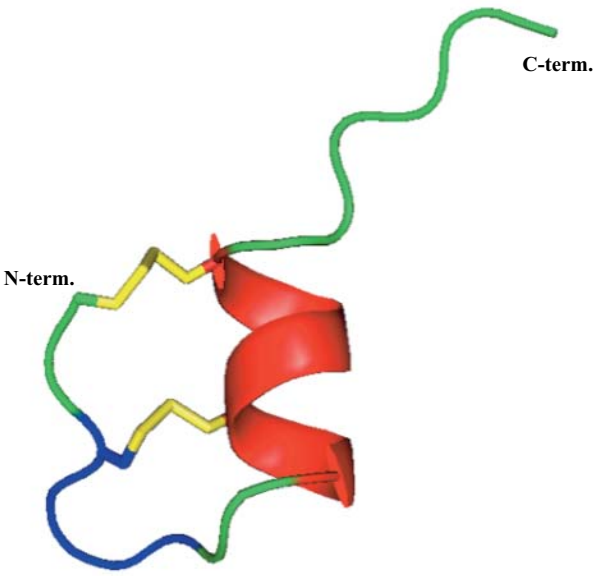

Figure 3. NMR structure of SRTX-b**.** Schematic representation of the structure adopted by the best conformer. The two disulfide bridges are indicated in yellow, extended structures are in green, the turn is in blue and the α-helical segment is in red. The Protein Data Base accession number is 1SRB.

Biosynthesis of SRTXs: precursor organization and identification of new SRTX isoforms

SRTXs are abundant in venoms, whereas ETs are present at low concentration in mammals. To study the metabolism of SRTXs by venom gland cells, their precursors have been cloned in three different species of the genus *Atractaspis*: *A. engaddenis* [26], *A. microlepidota microlepidota* [27] and *A. irregularis* [28]. Interestingly, these studies led to the identification of two differently organized precursors encoding several novel SRTX-like peptides variable in length and amino acid composition, which allowed definition of three distinct families of ET-like peptides. The structure and organization of these SRTX precursors were investigated and compared with those of ET precursors [29, 30].

The precursor of SRTXs from *A. engaddensis:* an original organization

The complete nucleotide sequence of one of the complementary DNAs (cDNAs) encoding SRTXs in *A. engad-*

```
         5′ AGACGCTCCTGTAGCGAAAGCGCTCGGCTCGGCTCGGCTCGGCTCGGCTCTCTTCGGCTGTCTTCGGCTCCGCGGTCCGCAC
         CGTCGAGGATTCTCTCGCTCTCCTTCTCGCCCACCTGCTCTGCTCAGCCCGGGAA ATG GCC CTC CTC CCC CGT
                                                                  M   A   L   L   P   R  6(163)
         CTG GCG GCC GGC GGG CTG CTG CTG CTG CTG GCC CTG GCC GCC CTC GAG GGG AAG CCG GCG
          L   A   A   G   G   L   L   L   L   L   A   L   A   A   L   E   G   K   P   A  26
         CCC TCG GCG CTG TCG CAG CTG CTG GAG AAG CGC TCC GAG GAC CAG GCG GCA GCA GGG CGC
          P   S   A   L   S   Q   L   L   E  K   R  S   E   D   Q   A   A   A   G   R  46
         ATC ATC GAC GGA GGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          I   I   D   G   G   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  66
SRTX-a1  GAG CCA CTT TGC TCC TGT AAA GAC ATG TCG GAT AAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   S   C   K   D   M   S   D   K   E   C   L   N   F   C   H   Q  86
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  106
SRTX-c   GAG CCA CTT TGC ACC TGT AAC GAC ATG ACG GAT GAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   T   C   N   D   M   T   D   E   E   C   L   N   F   C   H   Q  126
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  146
SRTX-b   GAG CCA CTT TGC TCC TGT AAA GAC ATG ACG GAT AAA GAG TGC CTC TAT TTC TGC CAT CAG
          E   P   L   C   S   C   K   D   M   T   D   K   E   C   L   Y   F   C   H   Q  166
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  186
SRTX-c   GAG CCA CTT TGC ACC TGT AAC GAC ATG ACG GAT GAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   T   C   N   D   M   T   D   E   E   C   L   N   F   C   H   Q  206
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  226
SRTX-c   GAG CCA CTT TGC ACC TGT AAC GAC ATG ACG GAT GAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   T   C   N   D   M   T   D   E   E   C   L   N   F   C   H   Q  246
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  266
SRTX-e   GAG CCA CTT TGC ACC TGT AAA GAC ATG ACG GAT AAA GAG TGC CTC TAT TTC TGC CAT CAG
          E   P   L   C   T   C   K   D   M   T   D   K   E   C   L   Y   F   C   H   Q  286
         GGC ATC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          G   I   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  306
SRTX-a1  GAG CCA CTT TGC TCC TGT AAA GAC ATG TCG GAT AAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   S   C   K   D   M   S   D   K   E   C   L   N   F   C   H   Q  326
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  346
SRTX-c   GAG CCA CTT TGC ACC TGT AAC GAC ATG ACG GAT GAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   T   C   N   D   M   T   D   E   E   C   L   N   F   C   H   Q  366
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  386
SRTX-c   GAG CCA CTT TGC ACC TGT AAC GAC ATG ACG GAT GAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   T   C   N   D   M   T   D   E   E   C   L   N   F   C   H   Q  406
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  426
SRTX-b   GAG CCA CTT TGC TCC TGT AAA GAC ATG ACG GAT AAA GAG TGC CTC TAT TTC TGC CAT CAG
          E   P   L   C   S   C   K   D   M   T   D   K   E   C   L   Y   F   C   H   Q  446
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  466
SRTX-a1  GAG CCA CTT TGC TCC TGT AAA GAC ATG TCG GAT AAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   S   C   K   D   M   S   D   K   E   C   L   N   F   C   H   Q  486
         GAC GTC ATC TGG AGA GAC ACG AAG CAG GCC GCG AGA GAC CCC TCG CCG CAG CGC AAC GTG
          D   V   I   W   R   D   T   K   Q   A   A   R   D   P   S   P   Q   R   N   V  506
SRTX-a   GAG CCA CTT TGC TCC TGT AAA GAC ATG ACG GAT AAA GAG TGC CTC AAT TTC TGC CAT CAG
          E   P   L   C   S   C   K   D   M   T   D   K   E   C   L   N   F   C   H   Q  526
         GAC GTC ATC TGG AAA AAT GCG GAC ACC AGC GCC AAT CCA GAG TTC CTA GGC TAG CTAGGAA
          D   V   I   W   K   N   A   D   T   S   A   N   P   E   F   L   G  end         543(1791)
         AGACATCCAGTCTCTGAAGGGACCCCCACCCCCCATCCATGGACATTACTGGACATCCCCTGCAATCATCCAGGGCCCCACCGG
         CGGGACCCCCAACGGTCAACACCCCTTTTCAATATGTCCCTTCAAATAAACTCACTAGACTGGAAAAAA 3'
```

Figure 4. Complete structure of the precursor of SRTXs of *A. engaddensis*. The 5' and 3' non-coding ends are shown in italics. The polyadenylation site 'AATAAA' is underlined. The nucleotide sequences and deduced amino acid sequences which constitute the ORF are numbered to the right of the figure. The amino acids are indicated in the one-letter international code. Each sequence of SRTX is underlined in the precursor, and its name indicated in bold type to the left of the figure. The doublet of basic amino acids K-R is in bold.

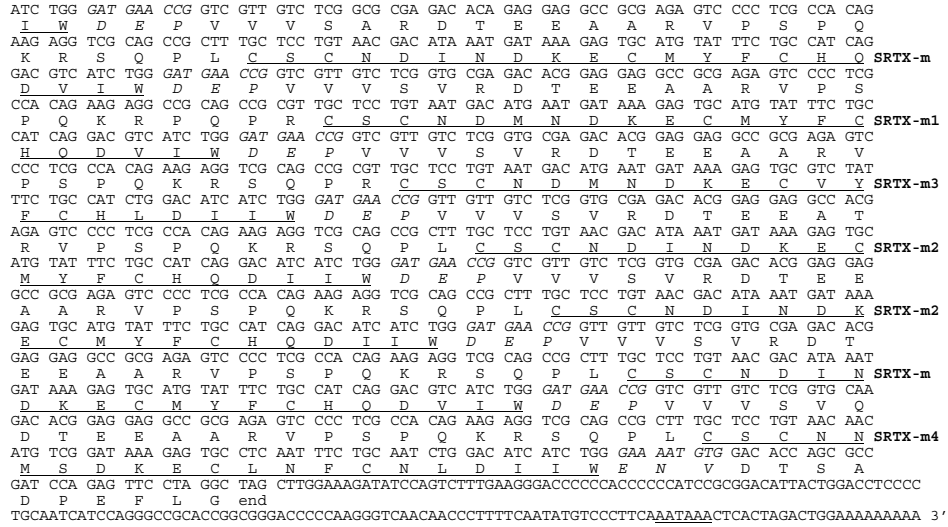

Figure 5. Nucleotide and deduced amino acid sequences of a large C-terminus cDNA fragment encoding *A. m. microlepidota* SRTXs. The sequence of each SRTX isoform is underlined, and their names are indicated on the right. The poly (A^+) addition signal is underlined within the 3' untranslated region. The three additional C-terminus amino acid residues are in italics. GenBank accession number: AY485934.

densis is presented in figure 4. It comprises 1948 base pairs (bp) including one open reading frame (ORF) of 1629 bp coding for a long pre-pro-polypeptide of 543 amino acids which starts with a methionine that initiates translation followed by a hydrophobic peptide characteristic of a signal sequence. This sequence presents no significant homology with the signal sequences of other precursors of snake venoms [31], and its cleavage site has not been identified with precision, despite predictive methods [32]. Unexpectedly, the amino acid sequence deduced for this precursor has an original repetitive structure comprising one sequence of 39 amino acids followed by 11 sequences of 40 residues (fig. 4). Each of these contains a SRTX sequence preceded by an invariant spacer peptide of 19 amino acids (18 upstream of the first sequence of sarafotoxin). The last copy of SRTX is followed by a peptide that differs from the previous one both in terms of its sequence and length (13 amino acids). In all, five different isoforms of sarafotoxin, SRTX-a, -a1, -b, -c and -e, are encoded by this precursor. SRTX-a1 is the name we gave to an isoform of SRTX-a containing a serine instead of a threonine at position +7 (fig. 2C). A sixth isoform called SRTX-b1, differing from SRTX-b in the same way as the -a1 isoform differs from -a, was identified during sequencing of an incomplete cDNA [26]. So there are at least six different isoforms of sarafotoxin in the venom of *A. engaddensis* that are produced from two precursors. This so-called rosary-type organization is unique in terms of its regularity in precursors of snake toxins with disulfide bridges. These 'three-fingered' toxins, such as neuromuscular toxins, muscarinic toxins, fasciculins and type A_2 phospholipases for which numerous isoforms have been identified, are all produced from mono-cistronic precursors [31]. Thus, this peculiar organization seems to constitute a simple, economical and effective way of amplifying the production of SRTXs in the venom of *A. engaddensis*. The SRTX-c isoform, which is the most abundant in the venom, is also the isoform with the greatest number of copies (five in all) in the precursor. However, such a peculiar organization poses the problem of the complete maturation of these precursors, which must end with the release of SRTX isoforms into the lumen of the venom gland (see below).

Identification of longer SRTX isoforms in the venom of *A. m. microlepidota*

A second poly-cistronic precursor encoding SRTXs was recently described in an exhaustive study of the venom of a specimen of *A. m. microlepidota*, another species of the genus *Atractaspis* [27]. Interestingly, combination of mass spectrometry analysis of the venom composition [33], with molecular cloning of the precursors encoding SRTXs, allowed identification of a new family of longer SRTXs containing three additional residues at their C-terminus.

Figure 5 shows the nucleotide sequences and the deduced amino acid sequences for a 1100 bp fragment of DNA, corresponding to the C-terminal end of a precursor encoding SRTXs of *A. m. microlepidota.* This DNA fragment contains seven 144-nucleotide repeats, each coding for a peptide link followed by a sarafotoxin sequence. Even if incomplete, this fragment displays a poly-cistronic organization as in the case of the precursor encoding SRTXs from *A. engaddensis.* However, it is noteworthy that there are several differences between these two rosary-type precursors (see below). Five different isoforms, named SRTX-m, -m1, -m2, -m3 and -m4, are encoded by this incomplete precursor, and all but SRTX-m4 successively present downstream of the invari-

ant tryptophan at position +21 an aspartate, a glutamate, and a proline. The C-terminal extremity of the SRTX-m4 isoform displays a different extension comprising a glutamate, an asparagine and a valine. Comparison of the primary structures of the five isoforms reveals between 54 and 96% homology (fig. 2D). Thus, SRTX-m1 and -m2 differ from SRTX-m by only one substitution, when SRTX-m4 is the most distant isoform with eight substitutions in the +1/+21 sequence, as well as a different C-terminal extension. Furthermore, a sixth isoform of long-SRTX (SRTX-m5; fig. 2D) was also identified in another incomplete precursor [unpublished data] that differs from SRTX-m2 by a ponctual substitution at position +6. SRTX-m was chemically synthesized, and its biological properties were studied, showing that despite its longer C-terminus SRTX-m (i) adopts a typical SRTX-like three-dimensional structure, (ii) induces toxicity in mice, mostly due to vasoconstriction, and (iii) has a lower toxicity and potency than the most potent SRTX described to date: SRTX-b from *A. engaddensis* [27]. Moreover, several fragments of these long SRTXs ranging from 1.24 to 2.16 kDa were also identified in the venom (fig. 2D), but their biological activity, if any, remains to be determined [33]. Such molecular diversity in SRTX content in this venom might be explained either by the existence of other SRTX precursors not yet characterized and/or by a process of RNA editing as previously described in the case of α-bungarotoxin isoforms present in the venom of the snake *Bungarus multicinctus* [34, 35]. However, only an analysis of the genomic DNA of SRTX from *A. m. microlepidota* would confirm or reject that hypothesis.

In conclusion, taken together these findings establish that *A. m. microlepidota* venom contains a new family of SRTXs isopeptides. The fact that the sequence extension of these novel SRTXs occurs at the level of the C-terminal end devoid of any particular structure strongly suggests that these new SRTXs adopt a three-dimensional structure that is globally unchanged compared with the 21-amino-acid SRTXs. Also, and even if differences do exist between the precursors encoding SRTXs from *A. engaddensis* and *A. m. microlepidota* (see below), this is the second example of a poly-cistronic organization of a precursor encoding SRTXs in two different species of *Atractaspididae*.

Atractaspis irregularis: a different SRTX-precursor organization encoding an even larger family of new SRTX isoforms

A similar and first ever molecular study on the venom of a third *Atractaspididae* species i.e. *Atractaspis irregularis* was recently reported [28]. Direct analysis by nanospray-Fourier-transform ion cyclotron resonance (FT-ICR) of the crude venom revealed about 60 distinct molecular masses in the range 0.6–15.0 kDa, half of

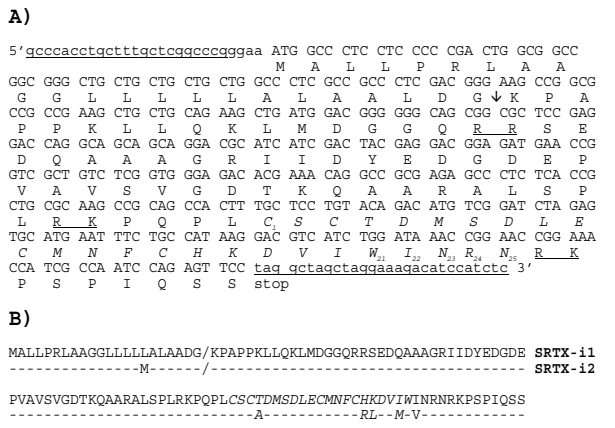

Figure 6. (A) Nucleotide and deduced amino acid sequences of the cDNA encoding *A. irregularis* SRTX isoform 1. The amino acid sequence is given in single letters. Positions of the PCR primers are underlined. The arrow indicates the probable cleavage site of the signal peptide. The mature 25-amino-acid SRTX isoform is in italics. Predicted dibasic processing sites are underlined. (B) Deduced amino acid sequences of the two cloned precursors. The names of isoforms are indicated on the right. '/' indicates the putative cleavage site of the signal peptide. The classical signature of 21-amino-acid sarafotoxins is indicated in italics. Identical amino acid residues are represented by '-'.

which were between 2.0 and 3.5 kDa, which corresponds to peptides of between 20 and 30 amino acids, a characteristic length for SRTXs. To gain more insight into the sequences of these compounds, the putative precursors of *A. irregularis* SRTXs were cloned by homology, and a de novo sequencing by mass spectrometry in the reduced crude venom was carried out. Figure 6A shows the structure of one of the two mRNAs that have been cloned. The presence of a SRTX sequence at the C-terminus of the 118-amino-acid residue precursor protein clearly establish that it corresponds to the first *A. irregularis* SRTX precursor. Surprisingly, this precursor displays a different organization compared with those encoding SRTXs from *A. engaddensis* [26] or *A. m. microlepidota* [27]. In the case of *A. irregularis* only one copy of mature SRTX emerges from each precursor when several tandem copies of mature SRTXs are encoded per molecule of the rosary-type precursors in the two previously studied *Atractaspididae* snakes.

One particularly interesting point is that the two pro-polypeptides that contain the SRTX isoforms -i1 and -i2 (fig. 6B) are not processed in a single compound but more likely in different length molecules that form two families of compounds ranging from 15 for the shorter to 30 amino acids for the longer (fig. 2E). Such molecular diversity of SRTX-like peptides has already been observed, albeit to a lesser extent, in the venom of *A. m. microlepidota* [33], and can be explained either by a different level of degradation in solution of an initial 30-amino-acid SRTX isoform or by a different state of maturation of the SRTX pro-polypeptides. The complete

sequences of the compounds of higher intensity were determined from MS/MS (tandem MS) experiments on the reduced molecular species [28]. Within these new families of compounds, the most abundant molecular species is 25 amino acids long with four additional C-terminus residues: 'V/I_{22}NRN$_{25}$' as compared with SRTXs from *A. engaddensis*. It is noteworthy that the C-terminus extension of SRTX-i1$_{25}$ is unrelated to that of long SRTX from *A. m. microlepidota*: V/I_{22}NRN$_{25}$ vs. D/E_{22}Q/N_{23}P/V_{24}.

The MS/MS analysis of the venom led also to the identification of a new isoform named SRTX-i3, which has been retrieved with at least two lengths: 23 and 25 amino acids (fig. 2E). Again, the 25-amino-acid isoform is the most abundant. The characterization of this third family of compounds relies only on mass spectrometry (MS) data since its amino acid sequence does not match any cloning results. Finally, SRTX-i1 and SRTX-i3 have very close structures that differ by only one amino acid: a valine (SRTX-i3) or an isoleucine (SRTX-i1) at position +22 following the conserved Trp$_{21}$ (fig. 2E). It is noteworthy that none of the molecular masses corresponding to the SRTX-i3 family are derived from the two precursors that were cloned. This might be explained either by a failure in cloning of the corresponding precursor or by the possibility that SRTX-i3 isopeptides could derive from an edited mRNA [28], as has been proposed in the case of α-bungarotoxin isoforms [34, 35], and suggested to explain the molecular diversity in SRTX isopeptides in the venom of *A. m. microlepidota* [27].

In conclusion, during the last 12 years a combination of cloning and MS approaches has led to the identification of an increased number of ET-like peptides in three families of SRTX isopeptides characterized by 21, 24 or 25 amino acids (fig. 2). Within the 24- and 25-amino-acid families of SRTXs, and around these major compounds, several smaller or longer related peptides do exist in the venom, but very little is known about the biological significance of such molecular diversity. Pharmacologically it was only shown that SRTX-m (one of the isopeptides of the 24-amino-acid SRTX family) and SRTX-b have similar effects upon injection in mice. However, the potency of SRTX-m is lower, and binding studies, using brain or atrial membrane preparations, failed to reveal competition between these two isopeptides [27]. Hence it is tempting to hypothesize that an ET/SRTX receptor subtype that does not bind SRTX-b [36, 37] could mediate the effects of some of these new SRTX members, as is probably the case for recently described 'long-endothelins' [38].

SRTX vs. ET precursors and gene organization

Comparison of the three cloned SRTX precursors reveals two structurally distinct organizations: mono vs. poly-cistronic (rosary-type precursors), which is unique for isopeptides that belong to the same superfamily of compounds within the same genus of venomous animals. However, and despite their distinct overall organization, these two types of SRTX precursors share several common features, such as the predicted signal peptides that display about 95% sequence identity. Also, the pro-sequence located between the predicted signal peptides and the first (in rosary-type precursors) or unique (in *A. irregularis* precursors) SRTX sequence is similar both in length, 61 vs. 69 amino acids, and its sequence since they display 72% sequence identity and/or homology. However, and even though SRTXs from *A. engaddensis* and *A. m. microlepidota* both emerge from long and repeated precursors, the length and composition of the repeats are significantly different. Repeats within the precursors encoding 24-amino-acid (*A. m. microlepidota*) or 21-amino-acid (*A. engaddensis*) SRTXs are composed of 48 or 40 amino acid residues, respectively. That difference in length results from the three additional C-terminus amino acid residues of l-SRTXs (24-amino-acid SRTXs), but also from the presence of a new stretch of amino acid residues: -Val-Val-Val-Ser-Val-, located at the N-terminus of the spacer within the l-SRTX repeats (figs. 4, 5). Furthermore, comparison of the spacers found in the precursors of SRTXs of *A. engaddensis* or of *A. m. microlepidota* indicates only 50% amino acid identity. Finally, the C-terminus peptides that follow the last (in rosary-type precursors) or the unique (in *A. irregularis* precursors) SRTX sequence are identical in length: 13 amino acids, but whereas those of the two poly-cistronic precursors are conserved (85% sequence identity), those of *A. irregularis* precursors contain only two identical amino acid residues. Together these observations strongly suggest that the genes encoding these two types of precursors have followed a different evolutionary pathway: a plausible complete duplication of a common ancestral gene in the case of *A. irregularis*, when the rosary-type precursor organization most probably reflects a combined duplication/mutation event of a SRTX-encoding exon only. This hypothesis is further supported by the structural characterization of an *A. engaddensis* exon encoding SRTX-c [39]. The latter contains the sequence of a mature 21-amino-acid SRTX preceded by a 16-amino-acid pro-peptide that appears to be conserved in both mono- and poly-cistronic types of SRTX precursors, suggesting that such a SRTX-encoding exon might be present in the corresponding genes. However, the differences observed between the two types of precursors also suggest that the genes encoding SRTXs and l-SRTXs could have evolved independently.

Surprisingly, the structure and organization of the precursors encoding ETs and VIC appear to be intermediate between those of the poly- and mono-cistronic pre-pro-polypeptides encoding SRTXs. The precursors of ETs and VIC contain a single and complete ET or

VIC sequence, followed by one ET- or VIC-like peptide of 16 amino acid residues that displays a highly divergent amino acid composition, except for the conserved cysteine residues. However, comparison with the precursors encoding SRTXs reveals a higher variability in length and sequence of the ET or VIC signal peptides, and no similarities between the pro-peptides and the linker sequences. Finally, cloning and characterization of the ET genes show that the latter are cut into five different exons, and also that the ET and ET-like sequences, which coexist in the same precursor, are encoded by two different exons [12, 18, 40, 41]. However, apart from this parallel between the existence of distinct exons coding for the different isoforms of SRTXs and those of ET and ET-like sequences, no other similarity in sequence and structure is observed. In spite of strong homologies in structure and biological activity between SRTXs and ETs, and despite cloning of a complete SRTX gene, it is likely that their genes have different organizations, suggesting distinct evolutionary paths.

SRTX vs. ET precursor maturation

The precursors of mammalian ETs [9, 12, 19, 42, 43] and those of SRTXs have different structures, suggesting distinct maturation processes. ETs are produced in the form of big-ET, an inactive proform of 39 amino acids produced by the maturation of long precursors of 178–224 amino acids, thanks to the presence of two basic doublets on either side of the big-ET [9]. Such dibasic sequences are known to be cleavage sites for trypsin-like processing enzymes such as Kex2-like serine protease, and they are often involved in the post-translation maturation processes of numerous peptide precursors [44, 45]. One biologically active molecule of endothelin is generated per precursor molecule, after the action of ECE (endothelin-converting enzyme) which is specific for the peptide bond -Trp_{21}-Val_{22}- [46, 47]. Note that two other basic doublets surround the ET-like sequence, but their role and origin are at present unknown. On the other hand, the difference in organization of SRTX precursors reflects the problem of maturation processes necessary for the release of SRTXs into the lumen of the venom glands, a process that the yeast cell seems able to mimic in the case of the poly-cistronic precursor of SRTXs from *A. engaddensis* [48]. It is likely that maturation of the SRTX precursors starts by the recognition via a trypsin-like endoprotease of the dibasic pairs of residues that are present within the pro-sequences of the precursors of SRTXs from *A. engaddensis* and *A. irregularis*, respectively. At this stage, several scenarios for the maturation of the intermediate pro-polypeptides can be envisaged, that likely require the action of uncharacterized and unlocalized endoproteases with particular specificity of cleavage, combined or not with amino and/or carboxy peptidases. It also remains to be established whether the invariant peptide link plays a role, and if so whether this role in the maturation processes is structural, or functional, or both. The only pointer available at present is the MS data on venom of a specimen of *A. engaddensis*, which shows that this venom lacks protein fractions of masses compatible with those of the peptide link, either alone or combined with an SRTX sequence [33].

Cellular targets and biological activities of SRTXs

Owing to the presence of a tyrosine residue at position +13 (fig. 2B), SRTX-b can be labeled with iodine 125 without loss of toxicity, thus enabling its use in binding studies. Iodinated SRTX-b binds specifically with high affinity to preparations of atrial membranes, in a saturable, rapid and reversible manner [2]. Scatchard analyses show that SRTX-b recognizes a homogeneous population of sites, with a maximum binding capacity of 110 fmol per mg of protein and a dissociation constant (K_D) of 3–5 nM. This binding is effectively inhibited by SRTX-a, -b and -c, at mean inhibitory concentrations (IC_{50}) of 30, 25 and 100 nM, respectively. In contrast, various ligands of ion channels and of receptors, and different bioactive peptides, are unable to block the binding of ^{125}I-SRTX-b. Other binding experiments have also shown that ^{125}I-SRTX-b recognizes sites in rat brain, particularly in the cerebellum (K_D = 3.5 nM) and cerebral cortex (K_D = 0.3 nM) [49]. It has been shown that (i) the biological activity of SRTXs is associated with a mobilization of intracellular Ca^{2+} ions, (ii) the binding of iodinated SRTX-b is unaffected by the action of blockers specific to Ca^{2+} channels, such as verapamil or ninodipine, and (iii) the binding of SRTXs induces the hydrolysis of phosphoinositides (PIs), as shown by the accumulation of inositol mono-, di- and triphosphate [2]. The concentrations of the three isotoxins that induced 50% of maximal PI hydrolysis are 100, 60, and 300 nM for SRTX-a, -b, and -c, respectively. Thus, SRTX-c is both the least toxic and potent isotoxin of *A. engaddensis*, whereas SRTX-b is also able to induce PI hydrolysis in other regions of the brain. Taken together, these results strongly suggest that SRTXs as well as ETs use the phosphoinositide signal transduction pathway via specific rhodopsin-like G-protein-coupled receptors, which seems to activate type C and D phospholipases [16, 50, 51]. This assumption was confirmed by use of iodinated ETs and the screening of cDNA libraries that revealed the existence of several functionally distinct and ubiquitous subtypes of receptors recognized by SRTXs and ETs [52-55]. These glycosylated proteins [56, 57] range in size from 30 to 70 kDa and contain seven potential transmembrane

domains likely to be regulated by phosphorylation, and share numerous structural features with other receptors coupled to G proteins. The recombinant forms of these receptors for the different isoforms of ETs and SRTXs are currently classified into two main subtypes, depending on their relative affinities: ET_A-R and ET_B-R. The isoforms ET-1 and ET-2 have similar and high affinities for ET_A-R, whereas ET-3 recognizes ET_A-R 1000 times less well. The second family of receptors, ET_B-R, appears less selective since the three isoforms of ETs and SRTXs interact with the same affinity. A new subtype of endothelin receptor called ET_{B1}-R has recently been identified [58]. It is characterized by high-affinity (picomolar) sites that do not hydrolyze phosphoinositides, and whose properties are affected differently depending on the degree of glycosylation. To determine the tissular distribution of the endothelin-binding sites, and the different biological effects demonstrated, it is probable that other receptor subtypes exist. It has been suggested that the capacity of the Egyptian mongoose to resist very high concentrations of SRTX-b or ET-1 is due to a new family of binding sites specific to SRTXs and ETs in the brain and cardiovascular tissue [59]. This assumption is further emphasized by the distinct binding specificity mentioned in the case of long-ETs and long-SRTXs [14, 15, 27, 38]. All these findings and observations underscore the incompleteness of our knowledge of the pharmacology of endothelins and toxicology of sarafotoxins, in terms of the variable, tissue-dependent concentrations of ETs and/or SRTXs, and with regard to the equally broad heterogeneity among the receptors.

Structure-function relationships of SRTXs

Numerous structure-function studies have been devoted to identifying the functional site by which SRTXs and ETs recognize their receptors and act as vasoconstrictors. It has been established that the vasoconstrictor potencies of the three SRTX isoforms on rabbit aorta preparations vary according to primary structure: SRTX-b > SRTX-a > SRTX-c [60]. STRX-b differs from SRTX-a by a single substitution: tyrosine instead of asparagine at position +13 (fig. 2B). The three ETs, and vasoactive intestinal contractor peptide, also have a tyrosine at this position (fig. 2A). SRTX-c, the least toxic isoform of *A. engaddensis* SRTX, has respectively three and four substitutions compared with the -a and -b isoforms, including a replacement of serine by threonine at position +2, which is also observed in the ET-3 isoform, the least active of the ETs (fig. 2A–C). A study has sought to establish the biological characteristics of [Thr^2]SRTX-b, a synthetic analogue of SRTX-b which, together with ET-1, is one of the two most toxic isopeptides and whose contractile activity is greater on the smooth muscles of organs such as the uterus, intestine, aorta and blood vessels [61]. The results of this study confirm that a serine at position +2 is important for high vasoconstrictor activity as confirmed by sequence alignments (fig. 2), but also show that its replacement by threonine does not alter the overall toxicity of this analogue. This suggests that other substitutions are responsible for the low pharmacological activity of SRTX-c and ET-3. Other structure-function studies have completed the identification of the amino acids respon-

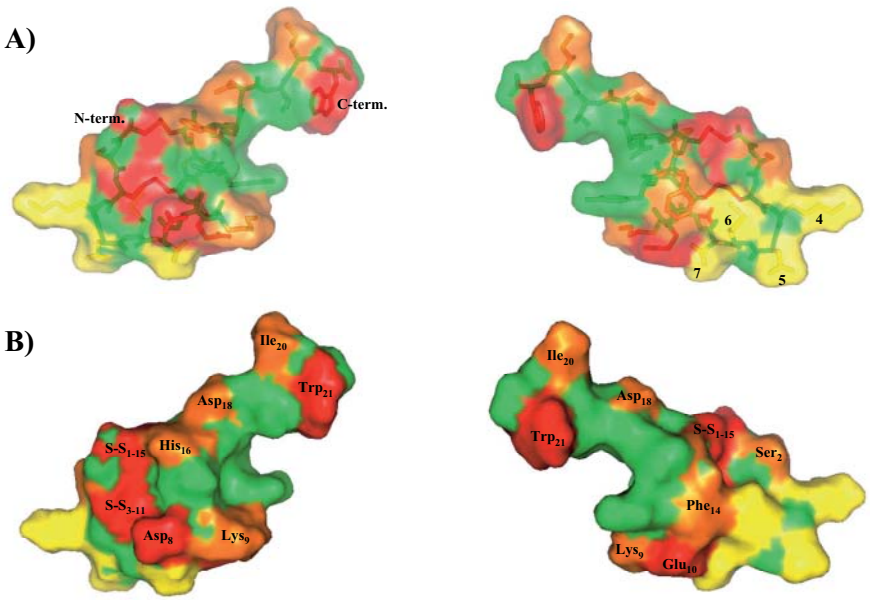

Figure 7. Surface representations of SRTX-b. (A) Faces A and B of SRTX-b are shown. The backbone is shown by transparency. N- and C-termini are indicated. Invariant and highly conserved amino acid residues are indicated in red and orange, respectively. The four positions within the turn that are the most degenerate in ETs and SRTXs in terms of amino acid composition are indicated in yellow and labeled from 4 to 7. The remainder of the molecule is shown in green. (B) Surface representations of faces A and B of SRTX-b. The residues that are labeled and numbered correspond to those found in SRTX-b. The pairing of the two disulfides is indicated as follows: $S–S_{x–y}$.

sible for the vasoconstrictor potency of ETs and SRTXs. Kimura et al. have shown that the C-terminus of ET-1, and particularly the tryptophan 21 and the two disulfide bridges, are crucial for its vasoconstrictor activity [62]. The functional importance of the amino and carboxy terminal groups, and that of the carboxylic groups of aspartic acid 8 and glutamic acid 10, has been established by modification or chemical mutagenesis of ET-1 [63]. The differences in biological activity and in toxicity which characterize these isoforms depend on (i) the nature of the amino acids constituting the loop formed between the cysteines +3 and +11 where the principal sequence variations are located, and (ii) recognized receptor subtypes. Hence, various observations suggest that ET_A-R recognizes the tertiary structures of both the amino and carboxy ends of the ETs, whereas ET_B-R is more specific to the C-terminal segment between amino acids Glu_{10} and Trp_{21} [16]. Thus we can define a 'common' and 'minimum' vasoconstrictor site at the surface of ETs and SRTXs that seems topographic and constituted by the N- and C-terminal ends, and by the COOH groups of the side chains at positions +8 and +10 (fig. 7). This is confirmed by the fact that all amino acids: $Cyst_1$, $Cyst_3$, Asp_8, Glu_{10}, $Cyst_{11}$, $Cyst_{15}$ and Trp_{21}, are invariant (fig. 2F) in ETs and SRTXs.

Even though little is presently known about the vasoconstrictor potencies of the newly identified SRTX isoforms, especially from the venoms of the snakes *Atractaspis microlepidoda microlepidota* and *irregularis,* and despite their differences in primary structure, these new isoforms display the overall structural and functional characteristics of the SRTXs of *A. engaddensis* and of ETs of mammals mentioned above. However, although these isoforms share a common structural pattern with other SRTXs, we can nevertheless notice a diversification in the amino acid composition since six new amino acids are found in positions where they were absent until now: (i) a threonine residue at position +4, (ii) an isoleucine residue at position +6, (iii) an asparagine residue at position +7, (iv) a leucine residue at position +9, (v) a methionine at position +12 and (vi) a lysine at position +17 (fig. 2). It is noteworthy that the higher sequence variability is located in the β-turn structure between positions +5 and +8 (fig. 7). Finally, the differences in length and amino acid composition of the C-termini downstream of the invariant tryptophan at position +21 seem to confer on these long-SRTXs or ETs a different receptor and tissue specificity [14, 15, 27, 38].

Conclusions

Since their discovery in 1982, all research on SRTXs, and particularly recent molecular biology and mass spectrometry data, show that the venoms of *Atractaspididae* snakes, like other venomous animals, contain a great range of isoforms of a given toxin. This diversity, which is thought to reflect a predator's need to adapt evolutionarily to the ecological diversity of its prey, goes beyond the scope of toxicology. Animal toxins are specific to precise molecular targets (receptors, ion channels, enzymes) and are involved in vital physiological machinery (central and/or peripheral nervous system, muscles, or blood circulation). For this reason, animal toxins in general and ET-like peptides from *Atractaspididae* snakes in particular, constitute unique molecular probes in identifying and studying the targets they recognize. However, the studies carried out on ET-like peptides also raise various academic questions. What are the enzymes responsible for the maturation of the precursors of SRTXs and how do they function? Do these enzymes participate in the maturation of other poly-cistronic precursors, and do they have endogenous equivalents? Another series of questions concerns the origin of and biological justification for the diversity of SRTXs. Why so many different isoforms? Does this reflect an equally great diversity among receptors, and, if so, what are they from a molecular point of view, and where are they localized? What is the biological activity and specificity of recognition of these various peptides? From a molecular evolutionary point of view, how is this diversity generated? What are the genomic structures that raise these differently organized precursors? For all these reasons, toxins in general and ET-like peptides in particular continue to fascinate researchers, clinicians and industrialists alike.

Acknowledgements. I would like to thank Avner Bdolah, Jean-Claude Boulain, Julie Bourdais-Jomaron, Julia Chamot-Rooke, Yvon Doljansky, Philippe Favreau, Andrzej Galat, Mirian Hayashi, Elazar Kochva, Hung Lamthanh, Jean-Pierre Le Caer, Caroline Ligny-Lemaire, Vilborg Matre, André Ménez, Laure Mennin, Bruno Muller, Tomohisha Ogawa, Gilles Phan, Loïc Quinton, Alexandra Savatier, Reto Stöcklin, Michaël Wéry, Zvi Wollberg and Sophie Zinn-Justin for their contributions to different aspects of current knowledge of ET-like peptides.

1 Kochva E., Viljoen C. C. and Botes D. P. (1982) A new type of toxin in the venom of snakes of the genus *Atractaspis* (*Atractaspidinae*). Toxicon **20:** 581–592
2 Kloog Y., Ambar I., Sokolovsky M., Kochva E., Wolberg Z. and Bdolah A. (1988) Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat and brain. Science **242:** 268–270
3 David P. and Ineich I. (1999) Venomous snakes in the world: systematic and repartition. Dumerilia **3:** 18–28
4 Golani I. and Kochva A. (1988) Biting behaviour of *Atractaspis*. Copeia 792–797
5 Bdolah A., Wollberg Z. and Kochva E. (1991) Sarafotoxins: a new group of cardiotoxic peptides from the venom of *Atractaspis*. In: Snake Toxins, pp. 415–423, Harvey A. L. (ed.), Pergamon Press, New York
6 Kochva E. (2002) *Atractaspis* (Serpentes, *Atractaspididae*) the burrowing asp; a multidisciplinary minireview. Bull. Nat. Hist. Mus. Lond. **68:** 91–99

7 Weiser E., Wollberg Z., Kochva E. and Lee S. Y. (1984) Cardiotoxic effects of the venom of the burrowing asp, *Atractaspis engaddensis* (*Atractaspididae*, Ophidia). Toxicon **22:** 764–774

8 Kochva E., Bdolah A. and Wollberg, Z. (1993) Sarafotoxins and endothelins: evolution, structure and function. Toxicon **31:** 541–568

9 Yanagisawa M., Kurihara H., Kimura S., Tomobe Y., Kobayashi M., Yasaki Y. et al. (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature **332:** 411–415

10 Yanagisawa M., Inoue A., Ishikawa T., Kasuya Y., Kimura S., Kumagaye S. I. et al. (1988) Primary structure, synthesis and biological activity of rat endothelin, and endothelin-derived vasoconstrictor peptide. Proc. Natl. Acad. Sci. USA **85:** 6964–6967

11 Kedzierski R. M. and Yanagisawa M. (2001) Endothelin system: the double-edged sword in health and disease. Annu. Rev. Pharmacol. Toxicol. **41:** 851–876

12 Bloch K. D., Hong C. C., Eddy T. B., Shows T. B. and Quertternous T. (1991) cDNA cloning and chromosomal assignment of the endothelin 2 gene: vasoactive intestinal contractor is rat endothelin 2. Genomics **10:** 236–242

13 Masuo Y., Ishikawa Y., Kozakai T., Uchide T., Komatsu Y. and Saida K. (2003) Vasoactive intestinal contractor/endothelin-2 gene expression in the murine central nervous sytem. Biochem. Biophys. Research Comm. **300:** 661–668

14 Nakano A., Kishi F., Minami K., Wakabayashi H., Nakaya Y. and Kido H. (1997) Selective conversion of big-endothelins to tracheal smooth muscle-contracting 31-amino acid-length endothelins by chymase from human mast cells. J. Immunol. **159:** 1987–1992

15 Kishi F., Minami K., Okishima N., Murakami M., Mori S., Yano M. et al. (1998) Novel 31-amino-acid-length endothelins cause constriction of vascular smooth muscle. Biochem. Biophys. Res. Comm. **248:** 387–390

16 Sakurai T., Yanagisawa M. and Masaki T. (1992) Molecular characterization of endothelin receptors. TIPS Rev. **13:** 103–108

17 Remuzzi G., Perico N. and Benigni A. (2002) New therapeutics that antagonize endothelin: promises and frustrations. Nat. rev. Drug discov. **1:** 986–1001

18 Inoue A., Yanagisawa M., Kimura S., Kasuya Y., Miyauchi T., Goto K. et al. (1989) The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl. Acad. Sci. USA **86:** 2863–2867

19 Saida K. and Misui Y. (1991) cDNA cloning, sequence analysis and tissue distribution of a precursor for vasoactive intestinal contractor (VIC). Biochem. Biophys. Acta **1089:** 404–406

20 Becker A., Dowdle E. B., Hechler U., Kauser K., Donner P. and Schleuning W.-D. (1993) Bibrotoxin, a novel member of the endothelin/sarafotoxin peptide family from the venom of the burrowing asp *Atractaspis bibroni*. FEBS Lett. **315:** 100–103

21 Ducancel F. (2002) The sarafotoxins. Toxicon **40:** 1541–1545

22 Tamoaki H., Kobayashi Y., Nishimura S., Ohkubo T., Kyogoku Y., Nakajima K. et al. (1991) Solution conformation of endothelin determined by means of ^{1}H-NMR spectroscopy and distance geometry calculations. Prot. Eng. **4:** 509–518

23 Andersen N. H., Chen C., Marschner T. M., Krystek S. R. and Bassolino D. A. (1992) Conformational isomerism of endothelin in acidic aqueous media: a quantitative NOESY analysis. Biochemistry **31:** 1280–1295

24 Atkins A. R., Martin R. C. and Smith R. (1995) ^{1}H-NMR studies of sarafotoxin SRTb, a nonselective endothelin receptor agonist, and IRL 1620, an ET_B receptor-specific agonist. Biochemistry **34:** 2026–2033

25 Wallace B. A., Janes R. W., Bassolino D. A. and Krystek S. R. (1995) A comparison of X-ray and NMR structures for human endothelin-1. Prot. Science **4:** 75–83

26 Ducancel F., Matre V., Dupont C., Lajeunesse E., Wollberg Z., Bdolah A. et al. (1993) Cloning and sequence analysis of cDNAs encoding precursors of sarafotoxins. J. Biol. Chem. **268:** 3052–3055

27 Hayashi M. A. F., Ligny-Lemaire C., Wollberg Z., Wery M., Galat A., Ogawa T. et al. (2004) Long-sarafotoxins: characterization of a new family of endothelin-like peptides. Peptides **25:** 1243–1251

28 Quinton L., Le Caer J.-P., Phan G., Ligny-Lemaire C., Bourdais-Jomaron J., Ducancel F. et al. (2005) Characterization of new toxins within crude venoms by combined use of Fourier Transform Mass Spectrometry and cloning. Analytical Chem., in press

29 Ducancel F., Matre V., Dupont C., Wollberg Z., Bdolah A., Kochva A. et al. (1995) Endothelins in endocrinology: new advances. Structure and function of mRNAs encoding sarafotoxin precursors. In: Frontiers in Endocrinology, vol. 15, pp. 35–45, Baldi E., Maggi M., Cameron I. T. and Dunn M. J. (eds.), Ares-Serono Symposia, Rome

30 Ducancel F., Wery M., Hayashi M. A. F., Muller B. H., Stöcklin R. and Ménez A. (1999) Les sarafotoxines de venins de serpent. Ann. Inst. Pasteur. **10(2):** 183–194

31 Ducancel F., Bouchier C., Tamiya T., Boulain J.-C. and Ménez A. (1991) Cloning and expression of toxin cDNAs. In: Snake Toxins. pp. 385–414, Harvey A. L. (ed.), Pergamon Press, New York

32 von Heijne G. A. (1986) A new method for predicting signal sequence cleavage site. Nucleic Acids Res. **14:** 4683–4690

33 Mennin L., Favreau P., Doljansky Y., Ducancel F. and Stöcklin R. (2004) The proteome of *Atractaspis microlepidota microlepidota* snake venom reveals long-sarafotoxins, a family of endothelin-like peptides. Proceedings of the 15th European Symposium on Animal, Plant and Microbial Toxins (Slovenia), p. 68

34 Liu L.-F., Chang C.-C., Liau M.-Y. and Kuo K.-W. (1998) Genetic characterization of the mRNAs encoding α-bungarotoxin: isoforms RNA editing in *Bungarus multicinctus* gland cells. Nucleic Acids Res. **26:** 5624–5629

35 Ashkenas J. (1997) Gene regulation by RNA editing. Am. J. Hum. Genet. **60:** 278–283

36 Valdenaire O., Giller T., Breu V., Ardati A., Schweizer A. and Richard J. G. (1998) A new family of orphan G protein-coupled receptors predominantly expressed in the brain. FEBS Lett. **424:** 193–196

37 Zeng Z., Su K., Kyaw H. and Li Y. (1997) A novel endothelin receptor type-B-like gene enriched in the brain. Biochem. Biophys. Res. Comm. **233:** 559–567

38 Kitamura H., Cui P., Sharmin S., Yano M. and Kido H. (2003) Binding of new bioactive 31-amino-acid endothelin-1 to an endothelin ET_B or ET_B-like receptor in porcine lung. Eur. J. of Biochem. **465:** 31–38

39 Takasaki C., Itoh, Y., Onda H. and Fujino M. (1992) Cloning and sequence analysis of a snake *Atractaspis engaddensis* gene encoding sarafotoxin S6C. Biochem. Biophys. Res. Comm. **189:** 1527–1533

40 Bloch K. D., Friedrich S. P., Lee M.-E., Eddy R. L., Shows T. B. and Quertermous T. (1989) Structural organization and chromosomal assignment of the gene encoding endothelin. J. Biol. Chem. **264:** 10851–10857

41 Onda H., Ohkubo S., Ogi K., Kosaka T., Kimura C., Matsumoto H. et al. (1990) One of the endothelin gene family, endothelin 3 gene, is expressed in the placenta. FEBS Lett. **261:** 327–330

42 Itoh Y., Yanagisawa M., Ohkubo S., Kimura C., Kosaka T., Inoue A. et al. (1988) Cloning and sequence analysis of cDNA encoding the precursor of a human endothelium-derived vasoconstrictor peptides, endothelin: identity of human and porcine endothelin. FEBS Lett. **231:** 440–444

43 Marsden P. A., Sultan P., Cybulsky M., Gimbrone M. A., Brenner B. M. and Collins T. (1992) Nucleotide sequence of endothelin-1 cDNA from rabbit endothelial cells. Biochem. Biophys. Acta **1129:** 249–250

44 Gubler U., Seeburg P., Hoffman B. J., Gage P. L. and Udenfrirnd S. (1982) Molecular cloning establishes proenkephalin

as precursor of enkephalin-containg peptides. Nature **295:** 206–208
45 Steiner D. F. (1998) The proprotein convertase. Curr. Opin. Chem. Biol. **2:** 31–39
46 Xu D., Emoto N., Giaid A., Slaughter C., Kaw S., deWit D. et al. (1994) ECE-1: a membrane-bound metalloprotease that catalyses the proteolytic activation of big endothelin-1. Cell **78:** 473–485
47 Opgenrth T. J., Wu-Wong J. R. and Shiosaki K. (1992) Endothelin-converting enzymes. FASEB J. **6:** 2653–2659
48 Borgheresi R. A., Palma M. S., Ducancel F., Camargo A. C. and Carmona E. (2001) Expression and processing of recombinant sarafotoxins precursor in *Pichia pastoris*. Toxicon **39:** 1211–1218
49 Ambar I., Kloog Y., Kochva E., Wollberg Z., Oron U. and Sokolovski M. (1988) Characterization and localization of a novel neuroreceptor for the peptide sarafotoxin. Biochem. Biophys. Res. Comm. **157:** 1104–1110
50 Kloog Y. and Sokolovski M. (1989) Similarities in mode and sites of action of sarafotoxins and endothelins. Trends Pharmacol. Sci. **10:** 212–214
51 Sokolovsky M. (1994) Endothelins and sarafotoxins: receptors heterogeneity. Int. J. Biochem. **26:** 335–340
52 Arai H., Hori S., Aramori I., Ohkubo H. and Nakanishi S. (1990) Cloning and expression of a cDNA encoding an endothelin receptor. Nature **348:** 730–732
53 Sakurai T., Yanagisawa M., Takuwa Y., Miyazaki H., Kimura S., Gota K. et al. (1990) Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature **348:** 732–735
54 Lin H. Y., Kaji E. H., Winkel G. K., Ives H. E. and Lodish H. F. (1991) Cloning and functional expression of a vascular smooth muscle endothelin 1 receptor. Proc. Natl. Acad. Sci. USA **88:** 3185–3189
55 Sokolovsky M. (1991) Endothelins and sarafotoxins: physiological regulation receptor subtypes and transmembrane signaling. Trends. Biochem. Sci. **16:** 261–264
56 Bousso-Mittler D., Galron R. and Sokolovsky M. (1991) Endothelin/sarafotoxin receptor heterogeneity: evidence for different glycosylation in receptors from different tissues. Biochem. Biophys. Res. Comm. **178:** 921–926
57 Davenport A. P. and Maguire J. J. (1995) Endothelins in endocrinology: new advances. Endothelin receptor sub-types and their function. In: Frontiers in endocrinology, vol. 15, pp. 35–45, Baldi E., Maggi M., Cameron I. T. and Dunn M. J. (eds.) Ares-Serono Symposia, Rome
58 Sokolovsky M., Ambar I. and Galron R. (1992) A novel subtype of endothelin receptor. J. Biol. Chem. **267:** 20551–20554
59 Bdolah A., Kochva E., Ovadia M., Kinamon S. and Wollberg Z. (1997) Resistance of the Egyptian mongoose to sarafotoxins. Toxicon **35:** 1251–1261
60 Wollberg Z., Bdolah A. and Kochva E. (1989) Vasoconstrictor effects of sarafotoxins in rabbit aorta: structure-function relationships. Biochem. Biophys. Res. Comm. **162:** 371–376
61 Lamthanh H., Bdolah A., Créminon C., Grassi J., Ménez A., Wollberg Z. et al. (1994) Biological activities of [Thr^2]sarafotoxin-b, a synthetic analogues of sarafotoxin-b. Toxicon **32:** 1105–1114
62 Kimura S., Kasuya Y., Sawamura T., Shinmi O., Sugita Y., Yanagisawa T. et al. (1988) Structure-activity relationships of endothelin: importance of the C-terminus moiety. Biochem. Biophys. Res. Comm. **156:** 1182–1186
63 Nakajima K., Kubo S., Kumagaye S. I., Nishio H., Tsunemi M., Inui T. et al. (1989) Structure-activity relationship: importance of charged groups. Biochem. Biophys. Res. Comm. **163:** 424–429