

SARAFOTOXINS, A NEW GROUP OF CARDIOVASCULAR MODULATORS FROM SNAKE VENOM

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ABSTRACT: A new group of toxins from the venom of the snake *Atractaspis*, the sarafotoxins, are highly homologous with the endothelins that originate from the endothelium of mammalian blood vessels. Both groups of compounds are 21 amino acid peptides that affect the cardiovascular system and also bind to various regions of the brain. The sarafotoxins may have originated from endogenous modulators of the cardiovascular system that evolved into toxins in the venom glands of *Atractaspis*.

KEY-WORDS: Sarafotoxins, Endothelins, *Atractaspis*, Snake Venom.

Several components of snake venoms served as tools for the elucidation of natural processes in various tissues. The best known components are the postsynaptic neurotoxins from Elapidae venoms — the alpha-bungarotoxins and alpha-cobrotoxins. These toxins bind strongly to the acetylcholine receptors of the neuromuscular junction of striated muscles and were used in order to identify, isolate and characterize these receptors (Albuquerque *et al.*¹).

During the last year (1988), several isotoxins from the venom of the Burrowing Asps genus *Atractaspis*, were shown to mimic natural products from mammalian blood vessels, the endothelins (Bdolah *et al.*,^{4,5} Wollberg *et al.*,¹⁵).

The Burrowing Asps are now considered to belong to a separate family, the Atractaspididae (Fig. 1), that differ considerably from the other veno-

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mous and non-venomous snakes in many respects (Underwood and Kochva,¹⁴). The venom of *Atractaspis* has a characteristic composition and contains both high and low molecular weight components. Some of these, hemorrhagin, protease and phospholipase A₂ are found also in the venoms of other snakes (Ovadia,¹²). However, about 1/3 of the venom consists of a new type of highly lethal low molecular weight toxins (Fig. 2; Kochva *et al.*,¹¹). These toxins were first labeled S₅ and S₆, in order of their elution from a G-50 Sephadex column; they showed one and two bands, respectively, in acrylamide gel electrophoresis. A preliminary sequence of S₅ was later identified as sarafotoxin c, while S₆ was found to contain mainly sarafotoxins a and b. These three toxins were named after the common Hebrew name of the Israel Burrowing Asp, SARAF Ein-Gedi and were shown to be highly homologous isotoxins that contain 21 amino acid residues with two disulphide bridges (Fig. 3; Takasaki *et al.*,¹³). A search of the several protein and nucleic acid sequence data banks yielded no meaningful similarities with any of the published sequences. However, the mammalian endothelins, that are also composed of 21 amino acids, are highly homologous with the venom sarafotoxins (Yanagisawa *et al.*,¹⁷, Graur *et al.*⁶).

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Fig. 1. *Atractaspis engaddensis*, Haas 1950.

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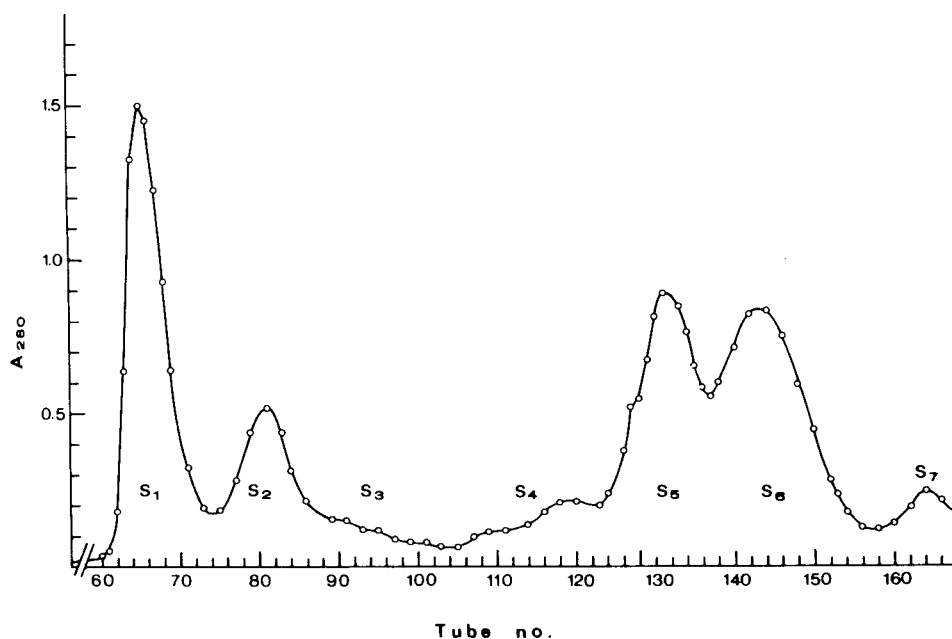


Fig. 2. Sephadex G-50 profile of *Atractaspis engaddensis* venom. Ten to 30 mg lyophilised venom were dissolved in 1 ml of 0.05 M NH_4CO_3 , applied onto a 0.9×152 cm column, and eluted with the same solution at a rate of 2ml/hr, in 1 ml fractions. S₁ contains L-amino acid oxidase and hemorrhagin; S₂ contains protease(s); S₃ contains phospholipase A₂; S₅ contains mainly SRTX-c; S₆ contains SRTX-b and SRTX-a.

SARAFOTOXIN-ENDOTHELIN SEQUENCES

Cys-Ser-Cys-Lys-Asp-Met-Thr-Asp-Lys-Glu-Cys-Leu-Asn-Phe-Cys-His-Gln-Asp-Val-Ile-Trp	SRTX-a				
Cys-Ser-Cys-Lys-Asp-Met-Thr-Asp-Lys-Glu-Cys-Leu-Tyr-Phe-Cys-His-Gln-Asp-Val-Ile-Trp	SRTX-b				
Cys-Thr-Cys-Asn-Asp-Met-Thr-Asp-Glu-Glu-Cys-Leu-Asn-Phe-Cys-His-Gln-Asp-Val-Ile-Trp	SRTX-c				
Cys-Thr-Cys-Phe-Thr-Tyr-Lys-Asp-Lys-Glu-Cys-Val-Tyr-Tyr-Cys-His-Leu-Asp-Ile-Ile-Trp	ET-3				
Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp	ET-1				
Cys-Ser-Cys-Ser-Ser-Trp-Leu-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp	ET-2				
1	5	10	15	20	

Fig. 3. Amino acid sequences of sarafotoxins and endothelins (Takasaki et. al.¹³ 1988; Yanagisawa et al.¹⁷, 1988).

To date, three endothelins were identified in the human gene, ET-1, ET-2 and ET-3 (Hiley.⁷). ET-1 was the first to be isolated from porcine endothelium and was subsequently identified in human material; ET-3 was found in the rat. The most remote members of each group, SRTX-a and c, on the one hand, and ET-3 on the other, still share between them 11 amino acids (52%).

The sarafotoxins and endothelins are also similar in their pharmacology and only a few differences were found between them (Wollberg et al.,¹⁵). Their major function, known so far, is linked with the cardiovascular system through their powerful vasoconstrictor effects on many blood vessels. In addition, both the sarafotoxins and endothelins induce a positive inotropic effect in human and animal heart muscles. When injected *in vivo*, the SRTXs and ETs show a series of disturbances in the ECG that consist of a remarkable but transient slope elevation of the S-T segment and an increase in the amplitude of the R-wave (Fig. 4). At the same time, a severe A-V block develops, starting with the prolongation of the P-R interval (first degree), followed by "dropped beats" (second degree) and by complete atrioventricular dissociation (third degree) that leads to cardiac arrest. These symptoms are characteristic of severe coronary insufficiency, which most certainly plays a crucial role in the toxicity of the SRTXs. However, experiments with isolated heart preparations show that these toxins may also directly affect the conduction system of the heart and thus contribute for their toxicity (Wollberg et al.¹⁶).

When injected *i.v.* into mice, both SRTX-b and ET-1 are highly toxic with an approximate LD₅₀ of 0.015 μ g/g (Bdolah et al.⁴).

In addition, both SRTXs and ETs bind to and compete for the same receptors and induce phosphoinositide hydrolysis in the heart and brain (Ambar et al.,²; Ambar et al.,³; Kloog et al.⁸; Kloog et al.⁹).

The structural and functional similarities between SRTXs and ETs have elicited extensive experimental work of a comparative nature, with the SRTXs being used as probes for the elucidation of the role of ETs in the regulation of blood pressure and other physiological and pharmacological phenomena of the cardiovascular system. Only time will tell whether the SRTXs will achieve the level of scientific importance of their elapid counterparts, the postsynaptic neurotoxins.

The high level of homology between the SRTXs and ETs suggests a common phylogenetic origin for the endothelin/sarafotoxin family of peptides (Fig. 5). Although endothelins are yet to be identified in non-mammalian vertebrates, they are most probably present in snakes. From the evolutionary point of view, it appears that a product already found in other tissues has evolved and adapted to a new function in the venom glands to help in the hunting for food. A similar phenomenon was recognised in other venom components, where enzymes, such as proteases and phospholipase A₂, evolved into toxic hemorrhagins and presynaptic neurotoxins, respectively (Kochva,¹⁰).

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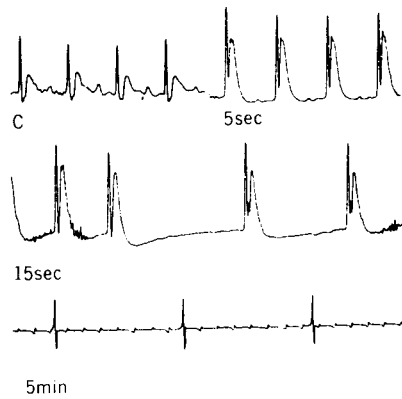


Fig. 4. Influence of sarafotoxin from *Atractaspis engaddensis* venom on ECG of mice. A lethal dose of 0.5 ug of SRTX-b was injected *i. v.* The ECG changes that resemble Prinzmetal's angina appear within less than a minute of venom injection and a complete A-V block develops gradually. Time scale: 0.5 sec for the lower line; 0.1 sec for all the others; C: control.

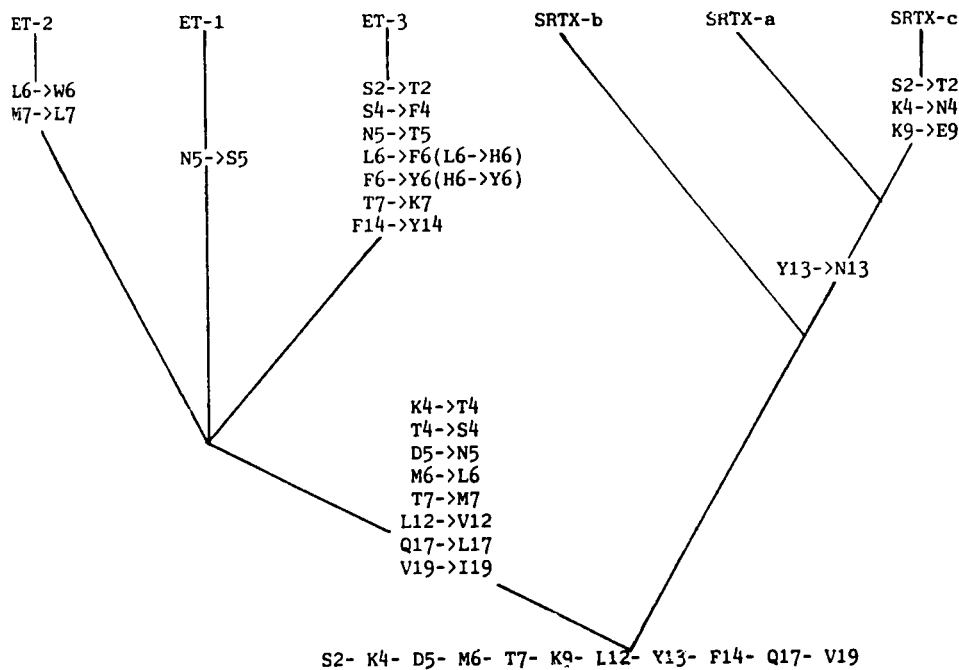


Fig. 5. Cladogram of venom sarafotoxins and mammalian endothelins. Substitutions are enumerated on the branches. Equally probable alternative substitutions are shown in parentheses.

Note added in proof: Two additional members of the endothelin/sarafotoxin family were recently described (see Bdolah, E.; Wollberg, Z.; Fleminger, G.; Kochva, E. SRTX-d, a new native peptide of the endothelin/sarafotoxin family. FEBS Lett., 256:1-3, 1989.

RESUMO: Um novo grupo de toxinas identificadas no veneno da serpente *Atractaspis*, as sarafotoxinas, tem alta homologia com as endotelinas, originárias do endotélio dos vasos sanguíneos de mamíferos. Os dois grupos de compostos são peptídeos, contendo 21 aminoácidos, que afetam o sistema cardiovascular e se ligam a várias regiões do cérebro. As sarafotoxinas podem ter-se originado de moduladores endógenos do sistema cardiovascular, que se transformaram em toxinas nas glândulas veneníferas da *Atractaspis*.

UNITERMOS: Sarafotoxinas, Endotelinas, Serpente *Atractaspis*-veneno.

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