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# VASOCONSTRICTOR EFFECTS OF SARAFOTOXINS IN RABBIT AORTA: STRUCTURE-FUNCTION RELATIONSHIPS

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The sarafotoxins SRTX-a, b and c from the venom of the snake Atractaspis engaddensis are 21-amino acid peptides that affect the cardiovascular system. They are strong vasoconstrictors, the potency of which may be in correlation with their primary structure: SRTX-a, which differs from SRTX-b in a single amino acid residue (Asn instead of Tyr), shows about half of its maximal vasoconstriction, while SRTX-c, which differs in 3 additional residues is a very weak vasoconstrictor and, at high doses, shows vasodilatory effects. Sequential application of the three isotoxins result in a summated response.


The 21-amino acid residue sarafotoxins SRTX-a, b and c, isolated from snake venom, are powerful vasoconstrictors that show strong cardiotoxic effects (1, 2, 3). These toxins bind to heart and brain preparations with high affinity and activate phosphoinositide hydrolysis (4, 5). A group of recently discovered endothelium-derived peptides, the endothelins, show a high degree of homology with the sarafotoxins (Fig. 1) (4, 6) and share with them several pharmacological properties (3, 7, 8). In addition, porcine endothelin (p-Et) is as toxic as SRTX-b in mice; it induces the same disturbances in ECG and competes with the sarafotoxins for the same binding sites in heart and brain tissues (9, 10). In this study, differences in the vasoconstrictor effects of the three sarafotoxins on the rabbit aorta are presented and discussed in relation to their primary structure.

## MATERIALS AND METHODS

Sarafotoxins were separated from the whole venom of the snake Atractaspis engaddensis (Ophidia, Reptilia) as described previously (2). Synthetic p-Et (Peptide International, Louisville, KY) was kindly donated by Prof. M. Sokolovsky.

Rabbits weighing 2-3 kg were sacrificed by i.v. air injection. The thoracic aortae were removed, cleaned from extraneous connective tissue, and

Abbreviations: NE - norepinephrine; h-Et - human endothelin; p-Et - porcine endothelin; r-Et - rat endothelin; SRTX- sarafotoxin.

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SARAFOTOXINS | | | | | | | | | | | | | | | | | | | |
| SRTX-a | C | S | C | K | D | M | T | D | K | E | C | L | N | F | C | H | Q | D | V | I | W |
| SRTX-b | C | S | C | K | D | M | T | D | K | E | C | L | Y | F | C | H | Q | D | V | I | W |
| SRTX-c | C | T | C | N | D | M | T | D | E | E | C | L | N | F | C | H | Q | D | V | I | W |
| ENDOTHELINS | | | | | | | | | | | | | | | | | | | |
| r-Et | C | T | C | F | T | Y | K | D | K | E | C | V | Y | Y | C | H | L | D | I | I | W |
| h-Et; p-Et | C | S | C | S | S | L | M | D | K | E | C | V | Y | F | C | H | L | D | I | I | W |
| | 1 | | | | 5 | | | | 10 | | | | 15 | | | | 20 | | | | |

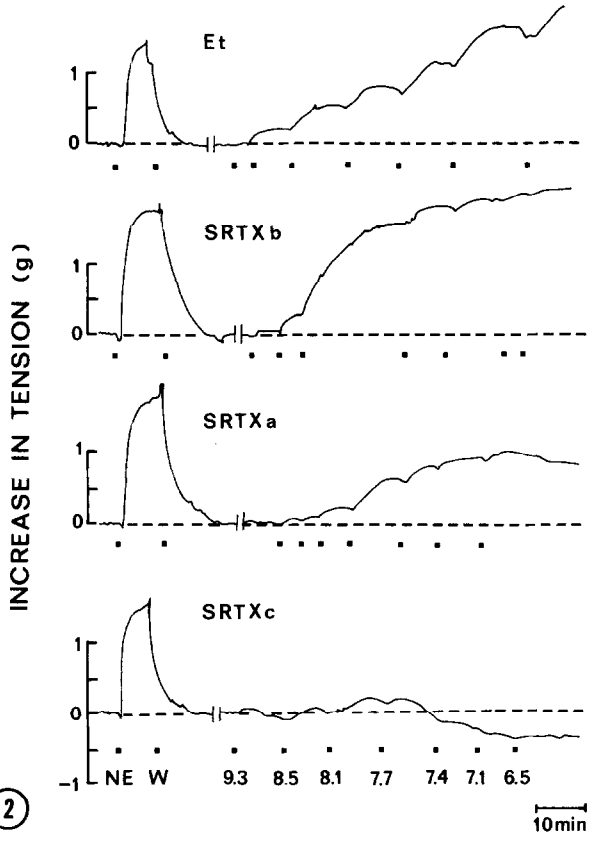
Figure 1. Amino acid sequences of sarafotoxins (2) and endothelins (7, 8). The single letter code for amino acids is used.

placed in ice cold Krebs solution composed of (mM): NaCl - 118; KCl - 4.75; $CaCl_2$ - 2.5; $MgCl_2$ - 1.19; $NaHCO_3$ - 25; $KH_2PO_4$ - 1.18; glucose - 11; the pH was adjusted to 7.4. About 2-3 mm wide rings were sliced off the ascending or descending segments of the aorta and cut open into about 10-12 mm long strips, with the endothelium kept intact. In order to perform the experiments with as similar strips as possible, no more than 2 to 4 strips from each rabbit were used. The aortic strip was suspended in an 8 ml organ bath at 31°C, containing oxygenated (95% $O_2$, 5% $CO_2$) Krebs solution. The strip was fixed by means of miniature stainless steel clips and silk threads to a glass hook at one end, and to the arm of a calibrated isometric force transducer (E&M, type A-3252) at the other end. The output of the transducer was amplified and coupled to an oscilloscope (Tektronix 502A) by an E&M Transducer Monitor Coupler. The oscilloscope was connected, via its vertical signal output, to a Gould (type 8188-2202) thermal writing recorder (filter settings: DC - 30 Hz).

The preparation was equilibrated at a passive tension of about 2 g (11), until no spontaneous drifts could be observed, and contractions elicited by supramaximal doses of norepinephrine (NE; 6 µM) were stable (usually 30-60 min). For each strip the contraction induced by this dose of NE was taken as a 100%, to which all other contractions were referred. At the end of each experiment the contraction of the aortic strip to NE was retested.

## RESULTS

Figure 2 illustrates typical cumulative constrictions of the rabbit aortic strips induced by the three SRTXs (a, b, and c), and by synthetic p-Et. Averaged cumulative dose response relationships for the three isotoxins are summarized in Figure 3. It can be seen that under these experimental conditions, p-Et induced a constriction of the rabbit aorta similar in pattern to that described previously for the porcine coronary artery (7, 12) and in other mammalian tissues (13). It can also be seen that both SRTX-a and SRTX-b induce a marked concentration dependent contraction that builds up slowly. With relatively high doses added (e.g 0.3 µM), the rise time of the contraction was usually shorter and the maximum contraction was in most cases somewhat stronger than the cumulative dose (not shown). Characteristically, a contraction was followed by a long lasting decay which could not be enhanced to any considerable degree by repetitive washing. The mean (±SD) interpolated $ED_{50}$ values (cumulative effective dose that induces half maximum response) of SRTX-a (n=5) and SRTX-b (n=5) were 3.5 ± 3.1 nM and 6.4 ± 2.4 nM,



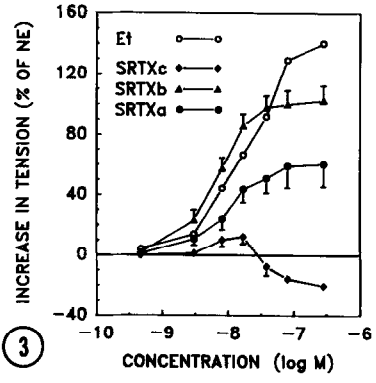




Figure 2. Representative isometric contractile responses of rabbit aortic strips to cumulatively applied SRTX-a, SRTX-b, SRTX-c and synthetic p-Et. Filled squares at the bottom of each recording represent timing of drug application or washout (W). Concentrations (expressed as negative log molar), designated by the numbers under the lower panel, refer also to the other panels. NE (6 μM) was used as reference.

Figure 3. Mean (±SEM) contractile dose response relationships of rabbit aortic strips to cumulatively applied SRTX-a, SRTX-b and SRTX-c (n=5 for all three isotoxins). The dose response to p-Et is based on one experiment. Responses are expressed as % of maximal response to NE.

respectively, and did not show a significant difference (2-tailed t-test; df=8; P= 0.2). However, the maximum increase in tension induced by SRTX-b was significantly higher (2-tailed t-test) than that produced by SRTX-a in both absolute values (1.43 ± 0.12g and 0.89 ± 0.4g, respectively; P= 0.020) and relative to maximal NE induced contraction (102.4 ± 19.5% and 60.4 ± 27.2%, respectively; P= 0.023).

Of special interest was the extremely weak vasoconstriction obtained with SRTX-c (maximal increase in tension [n=5]: 0.18 ± 0.05g, about 12% of maximal NE, at a concentration of about 18 nM, Figs 2 and 3). This contraction was followed by a spontaneous long lasting relaxation that continued even beyond the resting equilibrated tension. This demonstrates that SRTX-c also has a vasodilatory effect on the aortic smooth muscles.

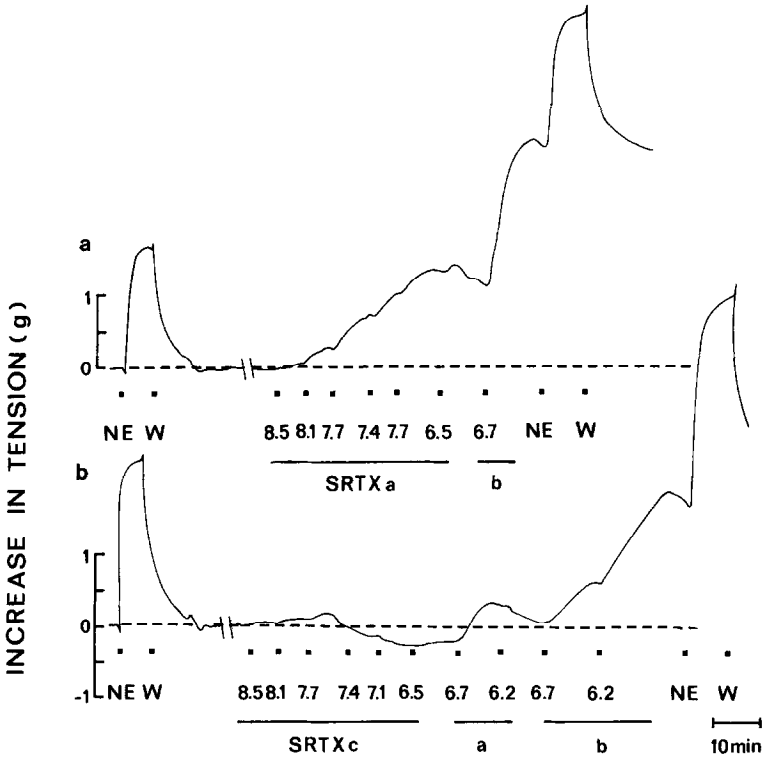



Figure 4. Cumulative combined responses of rabbit aortic strips to consecutive application of the different isotoxins. The sequence in the lower panel: SRTX-c, SRTX-a, SRTX-b, NE. In the upper panel: SRTX-a, SRTX-b, NE. All other details as in Fig. 2.

In several experiments intended to examine possible additive effects of the three sarafotoxins, a different isotoxin was added to the bath after the aortic strip reached a maximal response to one of the toxins, and ceased to respond to supplemental doses of this isotoxin. Fig 4a illustrates a case in which maximal doses of SRTX-a and SRTX-b were added sequentially on top of an SRTX-c cumulative response. SRTX-c induced the typical dual response; SRTX-a induced a somewhat weaker contraction than that usually induced by this toxin when applied separately, and the response to SRTX-b reached its regular values. The response to NE at the end of the experiment was essentially the same as the control. Fig 4b demonstrates another case in which a maximal dose of SRTX-b was added on top of cumulative doses of SRTX-a. It can be seen that the response to SRTX-b on top of a typical cumulative dose response of SRTX-a, remained unaffected.

## DISCUSSION

In this study we show that differences in the structure of the three isotoxins SRTX-a, b, and c are reflected in their vasoconstrictor potency. SRTX-a and SRTX-b have similar $ED_{50}$ values, but show a significant

difference in their efficacy when maximal responses are compared. SRTX-c is the least potent vasoconstrictor. The order of vasoconstrictor efficacy of the three isotoxins is thus b> a> c, with a mean maximal tension increase of about 1.4g, 0.9g and 0.2g respectively. It was also shown that SRTX-c has a dual effect on the aortic smooth muscles: at low doses it causes a weak vasoconstriction, whereas at higher doses it induces a long lasting relaxation even beyond the equilibrated resting length. Since all our experiments were conducted with aortic strips in which the endothelium was kept intact, it is not unlikely that the vasodilatation caused by this isotoxin is secondary, reflecting a possible triggering mechanism that causes the release of a relaxation factor(s). It should be noted that the lethality of SRTX-c, its cardiotoxic effects in mice, its competition with [$^{125}$I]SRTX-b on binding to atrial membranes, and its efficacy in inducing phosphoinositide hydrolysis, are also significantly weaker than those of the other two isotoxins (2, 4).

The differences in vasoconstriction of the three SRTXs provide information on the structure-function relationships of the Et/SRTX group. The SRTXs and the Ets all have a hydrophobic C-terminal "tail" which is essential for their activities (12). However, this alone is certainly not sufficient, as SRTX-c which has a C-terminal tail identical to the other two SRTXs is a very poor vasoconstrictor. It is thus evident that other domains are involved in the function of these peptides. SRTX-c is the most acidic peptide of this group. It has five acidic residues without any Lys (or Arg), whereas all the other peptides have at least one Lys residue in position 9 (replaced by Glu in c). The high toxicity and vasoconstrictor potency may thus be related to a cluster of charged residues which include at least one Lys residue in the main intramolecular loop (2, 6, 12). Yanagisawa et al.(8) suggested that the lower vasoconstrictor potency of the r-Et, as compared with that of p-Et, the fast decay of coronary pressor effect and the fact that r-Et could be more easily washed out, are due to the higher polarity of the amino terminal region. This suggestion seems unlikely, however, since the N-terminal halves of both SRTX-a and b are even more polar but are not easily washed out and they do have a long lasting vasoconstrictor effect.

The maximal vasoconstrictor potency of SRTX-b was found to be almost twice as high as that of SRTX-a. The only difference between the two is a change in position 13 of Tyr to Asn in SRTX-a (found also in c). The Ets also have a Tyr residue at this position which indicates its importance at the $Cys^{11}$ to $Cys^{15}$ site of the loop. The different pattern of activity of SRTX-c can thus be explained by several changes in the N-terminal half of the molecule, while the reported weak vasoconstrictor effect of the r-Et must await further studies.

The summated response of the aortic strips to a sequential application of the different isotoxins may point to the possibilty that each of these toxins activates preferentially a different receptor subtype. This notion is supported by a previous suggestion which was based on binding studies (5).

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