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HOMOLOGY BETWEEN SNAKE VENOM SARAFOTOXINS AND MAMMALIAN ENDOTHELINS

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Almost 30 years ago, the peculiar elongated venom glands of *Atractaspis engaddensis* were described in this journal (Kochva, 1959). The venom apparatus and the venom composition of *Atractaspis* showed distinctive characters differing from those of all the other venomous snakes (see Kochva, 1987 for review). The new type of toxins purified from the venom had a very high toxicity and powerful effects on the heart, including strong vasoconstriction (Kochva et al., 1982; Wollberg et al., 1988). Several isotoxins, 21-amino acid long, were subsequently isolated and sequenced from *Atractaspis engaddensis* venom. They were named **sarafotoxins**, -a, -b, and -c after the common Hebrew name of the snake — שרף עין גדי — **saraf 'En Gedi** (Takasaki et al., 1988).

Recently, novel vasoconstrictor polypeptides named **endothelins** were described from pig, human and rat endothelium (Yanagisawa et al., 1988a; 1988b; Itoh et al., 1988). Interestingly, these compounds showed a very high degree of structural similarity with the snake venom sarafotoxins, SRTXs (Kloog et al., 1988), in addition to their common vasoconstrictor activity; therefore it was important to compare the primary structure of the mammalian and ophidian peptides and to analyze the relationship between them.

The similarity among the sarafotoxins a, b, and c and their homology with the endothelins can be seen in Fig. 1. The phylogenetic relationships were reconstructed by using the maximum parsimony method (Felsenstein, 1982). The most parsimonious cladogram (Fig. 2) requires 20 substitutions, 18 of which could be determined unambiguously. The intermediary amino acid in the leu to tyr substitution that has occurred in the rat endothelin lineage (position 6) could either be a phe or a his. We prefer the leu → phe → tyr sequence of events over the leu → his → tyr alternative, since the former involves two conservative amino acid substitutions (Grantham's distances are 22 and 22 for leu → phe and phe → tyr, respectively). The alternative assumes two rather radical changes (99 and 83 for leu → his and his → tyr, respectively). The dissimilarity measures between amino acids were taken from Grantham (1974).

Fig. 2 shows that mammalian endothelins and snake venom sarafotoxins are evolutionarily homologous. Common evolutionary ancestry between the endothelins and the

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1) Cys-Ser-Cys-Lys-Asp-Met-Thr-Asp-Lys-Glu-Cys-Leu-Asn-Phe-Cys-His-Gln-Asp-Val-Ile-Trp

2) Cys-Ser-Cys-Lys-Asp-Met-Thr-Asp-Lys-Glu-Cys-Leu-Tyr-Phe-Cys-His-Gln-Asp-Val-Ile-Trp

3) Cys-Thr-Cys-Asn-Asp-Met-Thr-Asp-Glu-Glu-Cys-Leu-Asn-Phe-Cys-His-Gln-Asp-Val-Ile-Trp

4) Cys-Thr-Cys-Phe-Thr-Tyr-Lys-Asp-Lys-Glu-Cys-Val-Tyr-Tyr-Cys-His-Leu-Asp-Ile-Ile-Trp

5) Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp

1) SRTX-a

2) SRTX-b

3) SRTX-c

4) Endothelin (rat)

5) Endothelin (human, pig)

Fig. 1. Amino acid sequences of sarafotoxins SRTX-a, -b and -c (Takasaki et al., 1988) and of rat, human and pig endothelins (Yanagisawa et al., 1988b; Itoh et al., 1988; Yanagisawa et al., 1988a).

sarafotoxins could be established despite the fact that these polypeptides are quite short. Since the most distant proteins (SRTX-a, -c and rat endothelin) share in common 11 amino acids out of 21 (52%), the probability that these two lineages represent a case of parallel evolution is remote. Several other features are conspicuous in the figure. The rat endothelin, for instance, evolves much faster than its human and porcine counterparts (7 substitutions versus 1). Interestingly, one particular substitution, a change from ser to thr in the second position, has occurred independently in the two lineages, one leading to rat endothelin and the other to SRTX-c.

The next question we are interested in, is whether the observed substitutions show signs of having been selected for or against, in the evolutionary history of these molecules. To answer this question we compared the expected effects of random mutations on the amino acid substitution pattern to the observed one. For each possible amino acid substitution we calculated the mean chemical distance between the original moiety and the substitutes that are expected to result from a random point mutation. This mean value we compared to the observed chemical distance between the original amino acid and the observed substitute. The results show that the amino-end half of these molecules (positions 1–10) is subject to a more stringent purifying selection than the carboxy-end half (positions 11–21), despite the fact that the number of substitutions in the amino-end half exceeds by a margin of 3:1 that in the carboxy-end (15 versus 5). We base this claim on the following considerations. Given that a certain amino acid has been substituted, the observed substitution can be either more or less radical in its effects on the amino acid composition of the protein than what is expected following a random, non-selected process (Graur, 1985). We show that the 15 mutations in the N-terminal half of these peptides are considerably milder in their effects than expected (paired $t=2.708$, $p=0.0151$), while no difference between observed and expected effects is evident in the C-terminal

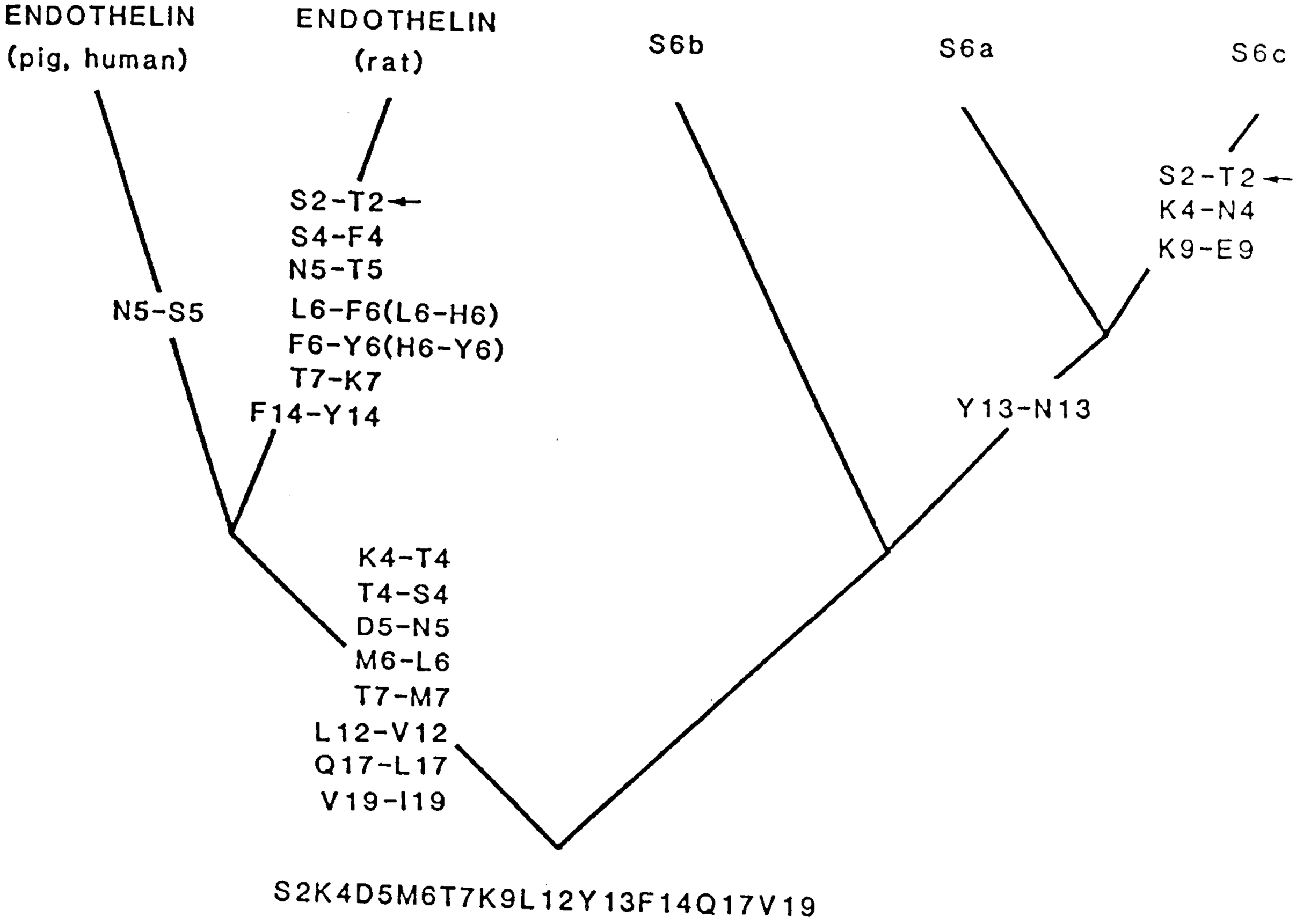

Fig. 2. Cladogram of two mammalian endothelin types and three sarafotoxins, S6-a, S6-b and S6-c. Substitutions are enumerated on the branches. Alternative substitutions are shown in parentheses.

half (paired t=0.291, p=0.4279). This means that mutations are subject to purifying selection in the N-terminal half, whereas no such selection operates on the other half.

From the functional point of view it is interesting that SRTX-c, which differs from both SRTX-a and -b, is the least toxic and has a lower affinity in binding to heart and brain membranes, and lower phosphoinositide hydrolysis and vasoconstricting activities (Takasaki et al., 1988; Kloog et al., 1988; Wollberg et al., submitted). Among mammalian endothelins, the rat's is the weakest vasoconstrictor (Yanagisawa et al., 1988b). It would be tempting to speculate that the thr residue at position 2 might be responsible for this low activity, as this position in sarafotoxin-c, itself a weak vasoconstrictor, is also occupied by a thr residue. The suggestion that the C-terminal "tail" is essential for vasoconstriction activity (Yanagisawa et al., 1988b) seems less likely, as this tail differs between the two groups (mammal versus snake), but is virtually identical within each group, despite the different degree of vasoconstriction among the members of each group. For instance, SRTX-b and pig endothelin, both strong vasoconstrictors, differ in their C-terminal "tails," while SRTX-b and -c on the one hand, and pig and rat endothelin on the other, which have similar tails, differ in their vasoconstricting powers by an order of magnitude (Yanagisawa et al., 1988b; Wollberg et al., submitted). Moreover, the syn-

thetic fragments 1–15 and 3–11 were found to be active (Takasaki, personal communication).

Regarding lethality, both pig endothelin (synthetic) and SRTX-a and -b are highly lethal, whereas SRTX-c is less so (Bdolah et al., submitted). However, since the lethality of rat endothelin is unknown at the moment, the exact lethal site is not clear. It might reside with the lys residue in position 9 of the endothelins and of SRTX-a and -b, which is replaced by glu in SRTX-c (Takasaki et al., 1988).

The origin of the endothelin/sarafotoxin protein family also remains to be elucidated. A detailed search of the PIR protein sequence data bank yielded no meaningful similarity with published amino acid sequences. Numerous statistically significant similarities have been found; however, most of them could be attributed to spurious appearances of four highly conserved cysteins in many polypeptides. The homology claimed for endothelin and scorpion alpha-toxins (Yanagisawa et al., 1988a) falls into this spurious category. Thus, the implied mechanism also remains to be re-evaluated. Not until the structure of additional peptides, such as endothelins from reptiles and other groups, becomes known, will it be possible to carry out a more detailed analysis.

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