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The Evolutionary History of the Sarafotoxin/Endothelin/ Endothelin-Like Superfamily

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Summary: The evolutionary relationships among 17 protein and nucleic acid sequences from the sarafotoxin/endothelin/endothelin-like superfamily of peptides were studied. The endothelin/endothelin-like gene family has diverged from an ancestral gene that has experienced an exon duplication event followed by two complete gene duplications. The sarafotoxin lineage diverged from the ancestral gene prior to the first gene duplication event. In several lineages, the peptides have independently accumulated identical amino acid replacements in position 2. This finding supports the hypothesis that residue 2 is crucial to biological activity. **Key Words:** Sarafotoxin—Endothelins—Ancestral gene—Residue 2.

The mammalian endothelins (ETs) and the venom sarafotoxins (SRTXs) of *Atractaspis engaddensis* are 21–amino acid–long vasoconstricting peptides that have powerful effects on the heart (1–3). SRTXs and ETs bind specifically to the brain, heart, blood vessels and other smooth muscle systems, and also activate phosphoinositide transduction (4,5). ETs are encoded by a gene family consisting of three duplicate genes, endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). The presence of these genes has been demonstrated in humans, pigs, rats, and mice (6,7). Each mature ET is produced from a prepropeptide approximately 200 amino acids long (3). In ET-1 and ET-3, where the preproendothelin sequence is known, a sequence homologous to the mature ET is found downstream of the mature ET (8). The SRTXs and the ET peptides are evolutionarily conserved. In particular, four Cys residues, i.e., Cys^{1}, Cys^{3}, Cys^{11}, and Cys^{15}, are preserved in all the SRTXs, ETs, and ET-like sequences. In a previous study we demonstrated that these sequences are homologous, i.e., they have been derived from a common ancestral gene (9). This study presents further details of the evolutionary relationships among and within the SRTX, ET, and ET-like gene families.

DATA

The analyses are based on the amino acid sequences of four sarafotoxins, SRTX-a, SRTX-b, SRTX-c, and SRTX-d, and the nucleic acid sequences of 10 ETs and 3 ET-like sequences: human ET-1, ET-2, ET-3; mouse ET-1, ET-2; dog ET-1, ET-2; pig ET-1; rat ET-3; rabbit ET-1; human ET-like-1, ET-like-3; and pig ET-like-1. The predicted amino acid sequences of the ET-1 peptide of human, mouse, dog, and pig are identical, as are those of the ET-3 peptides from human, rat and rabbit. The mouse ET-2 peptide (VIC) differs from human and dog ET-2 by a single residue.

RESULTS

The amino acid sequences of the sarafotoxins and endothelins exhibit a high degree of similarity (71–95% within the ETs, 81–95% within the SRTXs, and 52–67% between the SRTXs and ETs). Using the neighbor-joining method (10), we obtained an unrooted tree for eight peptides (Fig. 1). The SRTX and ET families are clearly distinguished from each other. We placed the root at the midpoint of the longest path, i.e., from SRTX-c to ET-3. A maximum parsimony analysis (11) on these 8 peptides resulted in 2 equally parsimonious trees (12), each requiring a total of 24 replacements. One of these

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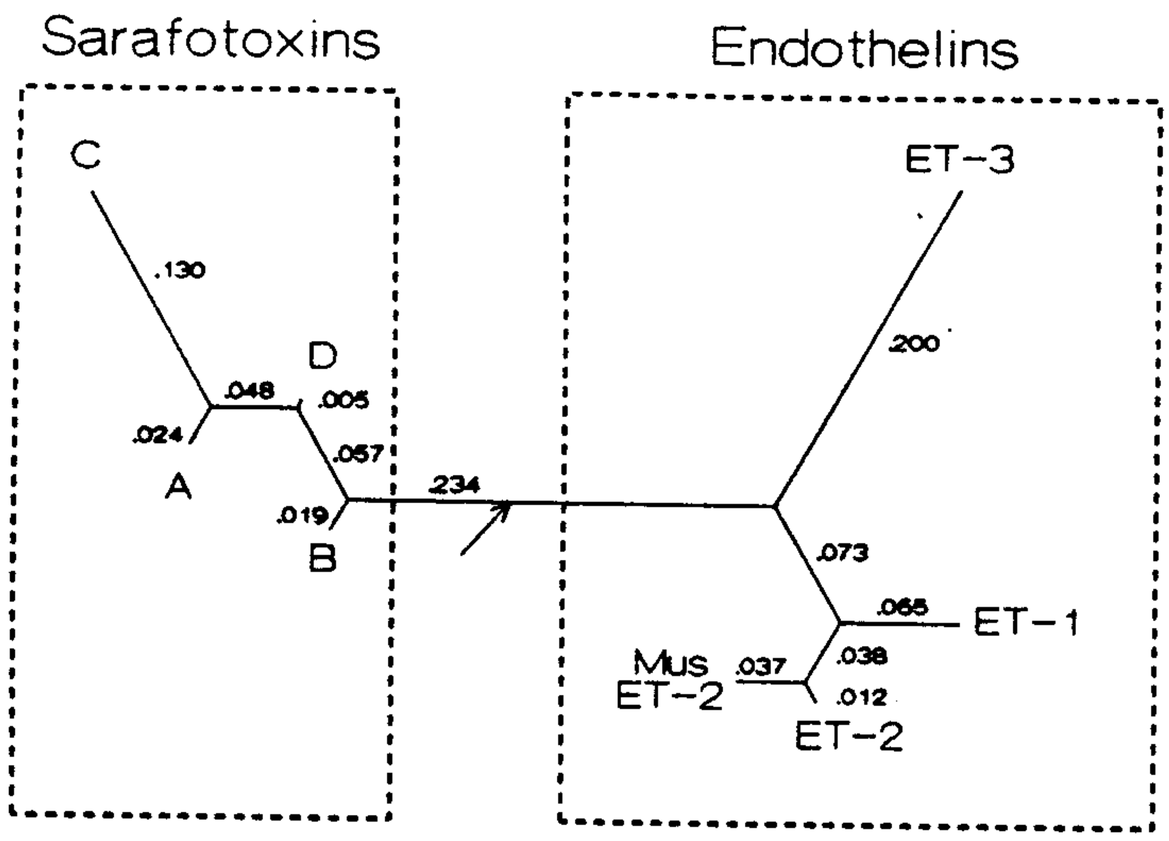

FIG. 1. Unrooted phylogenetic tree of the eight distinct sarafotoxin and endothelin peptides, obtained by the neighbor-joining method (10). The branch lengths are proportional to the number of replacements. Arrow denotes the root.

trees was identical to the neighbor-joining tree whereas the other differed in the branching order within the SRTXs. In order to reconstruct the phylogenetic relationships within the ETs and to maximize available data, we applied the maximum parsimony method to the DNA sequences of 10 ET genes (13). Three unrooted trees were obtained, each requiring a total of 35 nucleotide substitutions and differing only in the branching order of the ET-1 genes. The consensus tree is shown in Fig. 2.

DISCUSSION

An inspection of the mutational pathways of the reconstructed trees for the SRTXs and ETs reveals that the peptides experienced parallel amino acid replacements, i.e., the same type of replacement occurred independently in different lineages. Position 2 is of particular interest. Although it is not possible to determine whether the ancestral amino acid was Ser or Thr, it is certain that two or three parallel replacements must have occurred independently in three different lineages. Given that only 24 amino acid replacements occurred over the whole SRTX-ET tree and that parallel or back replacements are very rare, it is likely that these instances of parallelism represent selectively advantageous processes rather than chance events.

Several hypotheses were raised to explain the differences in activity of the SRTX and ET peptides. Kloog and Sokolovsky (5) suggested a possible effect of the net charge on activity. Inoue et al. (6) argued that the activity is correlated with the hydrophobicity of the entire peptide. Graur et al. (9) suggested that position 2 is crucial to activity.

We find the latter hypothesis to be more plausible for several reasons: (a) Classification of the peptides according to activity and according to the amino acid at the second amino acid position yields identical groupings, i.e., higher activities are associated with Ser^2 (SRTX-a, SRTX-b, ET-1, and ET-2), while lower activities are associated with Thr^2 (SRTX-c, SRTX-d, and ET-3). No other residues correlate as perfectly with activity. (b) Given the phylogenetic relationships among the peptides, position 2 has undergone two, and perhaps three, parallel replacements. As mentioned above, this can hardly be due to chance alone. (c) Cys^1 and Cys^3 create two disulfide bonds with Cys^{15} and Cys^{11}, respectively, so that the amino acid residue residing between them should influence the structure of the peptide. (d) The four SRTX peptides have the same hydrophobicity, and yet they exhibit different levels of activity. Moreover, the SRTXs are less hydro-

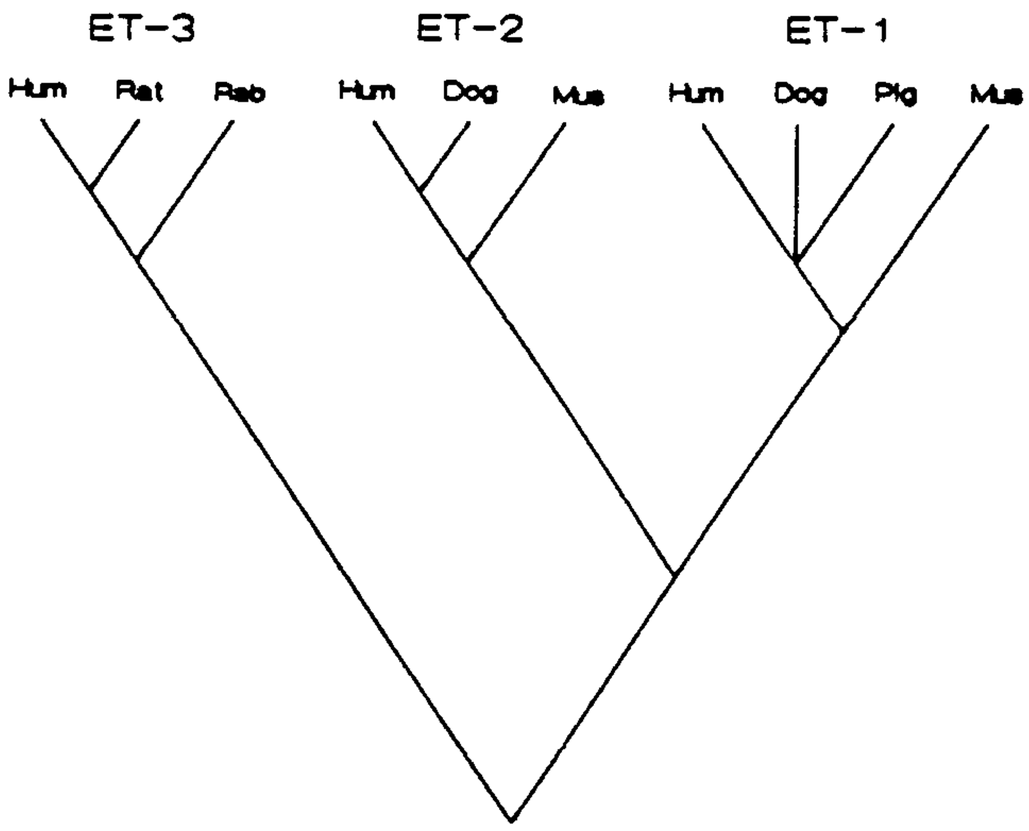

FIG. 2. Consensus tree for the nine mature endothelins (ETs), obtained by the maximum parsimony algorithm (13). The tree requires 35 nucleotide substitutions. The root was placed by taking either the sarafotoxins or the ET-like segments as outgroups.

phobic than ET-3, yet both SRTX-a and SRTX-b are as active as ET-1.

The prepropeptide structure of the ET-1 and ET-3 genes, along with the observation that the gene duplication leading to the ET-2 family took place after the ET-3 gene duplication, leads us to propose the following scenario for the evolution of the endothelins: The ET-like sequence is a product of an early exon duplication event in an ancestral ET gene (14). The duplicated gene subsequently underwent a complete gene duplication resulting in the ET-3 gene and an additional ET gene, which underwent yet another gene duplication leading to the creation of ET-1 and ET-2. All these stages took place before the main eutherian radiations, so that all mammals possess three ET genes. The four SRTX peptides, on the other hand, are the result of an independent divergence process.

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