Journal of Cardiovascular Pharmacology
13(Suppl. 5):S8–S12 © 1989 Raven Press, Ltd., New York

Synthesis of Endothelin-1 Analogues, Endothelin-3, and Sarafotoxin S6b: Structure–Activity Relationships

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Summary: Two disulfide analogues (types A and B) of endothelin-3 (ET-3; formerly, rat ET), sarafotoxin S6b, and apamin, were synthesized to determine their disulfide structures as in the case of endothelin-1 (ET-1; formerly human and porcine ET). The disulfide structures of ET-3 and sarafotoxin S6b were found to be identical with that of ET-1 (type A) but distinct from that of apamin (type B). The vasoconstricting activities of ET-3 and sarafotoxin S6b were about one-60th and one-third that of ET-1, respectively. Such different biological potencies between endothelins and sarafotoxin S6b could be largely attributed to the sequence heterogeneity at the N-terminal portion. ET-1 analogues were also synthesized to clarify the structure–activity relationships. The opening of any disulfide bond in the ET-1 molecule extremely decreased the activity, while oxidation of the Met residue did not alter it. Amidation of the terminal COOH group and extension of the Lys–Arg sequence to the N-terminus led to 16- and 540-fold decreases in activity, respectively. Removal of the C-terminal Trp residue resulted in complete loss of the activity. The other disulfide analogues (type B and C) of ET-1 showed markedly lower activity than the parent molecule (type A). These results indicated the importance of the whole molecule with the proper double cyclic structure for determining its active conformation. **Key Words:** Endothelin-1—Sarafotoxin S6b—Apamin—Vasoconstricting activity—Disulfide structure—Structure–activity relationships.

Endothelin-1 (ET-1), a 21-amino acid peptide having four Cys residues at position 1, 3, 11, and 15, shows potent and long-lasting vasoconstricting activity (1). It can be isolated from the culture medium of porcine aortic endothelial cells. The structures of human and rat ETs (ET-1 and ET-3, respectively) have also been deduced from cDNA sequences. Human ET is identical with porcine ET (2), but distinct from ET-3 (3). Recently, similar vasoconstrictor peptides, sarafotoxins, isolated from the venom of the snake *Atractapis engaddensis*, have been shown to have primary structures displaying a high degree of homogeneity with that of ETs (4), suggesting that they share a common binding site (5). Four Cys residues in these peptides are located in identical positions, which also coincide with the distribution found in apamin (6), a bee venom peptide, having two disulfide bonds between positions 1 to 11 and 3 to 15, as shown in Fig. 1.

We have previously reported the synthesis of ET-1 and the elucidation of its disulfide structure by synthesizing three possible disulfide analogues (type A, B, and C), as in the case of α-conotoxin. The peptide having the type A structure was found to be identical with natural ET-1 (7), whereas its disulfide structure was completely different from that of apamin (type B). These results as well as the high degree of similarity between the ET-1 and sarafotoxin molecules prompted us to investigate their structure–activity relationship. In the present study, we synthesized two disulfide analogues (type A and B) of ET-3 and sarafotoxin S6b, the most potent and highest homologous peptide with ETs, and compared the tendencies for disulfide bond formation with that of apamin. The vasoconstricting activities of ET-3, sarafotoxin S6b, and ET-1 analogues were measured to clarify the structure–activity relationship.

METHODS

Synthesis of peptides

To determine the disulfide structures of ET-3, sarafotoxin S6b, and apamin, two disulfide analogues (type A and

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