Volume 310, number 1, 83–87 FEBS 11573 September 1992

Molecular modelling of the structures of endothelin antagonists

Identification of a possible structural determinant for ET-A receptor binding

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Received 4 August 1992

Computer-aided molecular modelling of the endothelin (ET-A) receptor antagonists, BQ-123 and BE-18257B, shows that they have very similar 3D structures. Parts of their 3D structures are also shown to match closely with that reported for residues 6–8 in endothelin-1. On the basis of these similarities (and with supporting evidence from literature data on endothelin structure–activity relationships) a structural determinant is proposed for ET-A receptor binding, and novel designs of peptide are suggested for providing more potent and selective ET-A receptor antagonists.

Endothelin; Endothelin antagonist; Endothelin receptor; Computer modelling; Peptide structure; Cyclic peptide; Drug design

1. INTRODUCTION

The endothelins are a novel family of vasoconstrictor peptides, each consisting of 21 amino acid residues with two intra-chain disulphide linkages (see Fig. 1). The first member of the family identified, endothelin-1 (ET-1), was isolated from the cultured supernatant of porcine aortic endothelial cells [1]. Subsequent screening of a genomic DNA library (using a synthetic oligonucleotide probe encoding a portion of the ET-1 sequence) led to the discovery of the other two members of the family, endothelin-2 (ET-2, also known as vasoactive intestinal contractor) and endothelin-3 (ET-3) [2]. ET-1 was first shown to have potent vasoconstrictor activity [1] but was later also shown to have contractile activity on various non-vascular smooth muscles and some activity in the CNS [3].

Binding studies of labelled ET-1 have shown a wide distribution of ET-specific binding sites, not only in the vascular system but also in the kidneys, lungs, adrenal glands and neurons [4]. The sequences for five different ET receptors have so far been determined and these are divided into sub-types A and B. The ET-A receptors are distinguished by a higher affinity for ET-1 and ET-2 than ET-3 [5–7], and the ET-B receptors have an equal affinity for all three endothelins [8–10]. A further receptor with a higher affinity for ET-3 than ET-1 has been found in the anterior pituitary cells but its sequence has not yet been determined [11].

It has been suggested that ET and ET receptors have vital functions associated with haemodynamic regulation and/or modulation of the cardiovascular system, but the details of their physiological and pathological roles are still unclear (see [12] for a recent review). In order to gain further understanding of these phenomena, it is thus essential to identify specific ET-receptor antagonists.

Although various ET-1 analogues have been shown to have some antagonistic activity [13–14] none of the compounds so far considered have sufficiently high receptor specificity and/or affinity. However, Ihara et al. have recently reported the discovery of a natural antagonist, BE-18257B [15], and its chemical modification to a more potent antagonist, BQ-123 [16]. Both of these antagonists are specific to ET-A receptors and are cyclic pentapeptides with alternating D- and L-amino acids (see Fig. 1).

In the following study, the 3D structure of these two antagonists are predicted, and the resulting models are then compared against the various solution structures for ET-1 that have been determined by means of 2D NMR and distance geometry calculations [17–19]. On the basis of these analyses a possible structural determinant of ET-A receptor binding is identified, and novel peptide designs are proposed for consideration as synthetic ET-A receptor antagonists.

2. MATERIALS AND METHODS

2.1. *Modelling of the ET antagonists*

Ranges of possible conformers for the two ET antagonists, BE-18257B and BQ-123, were generated using a combination of conformational search, cluster analysis and energy minimization. The various stages involved in the model building were as follows:

(i) 100 conformers were first generated for the cyclic main chains of the two antagonists using the program RAMBLE [20]. The main chain torsion angles were constrained in the ranges:

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Φ = −180° to −30°	ψ = −90° to 180° (for L-amino acids except Pro)
Φ = −80° to −40°	ψ = −90° to 180° (for L-pro)
Φ = 30° to 180°	ψ = −180° to 90° (for D-amino acids)

All peptide bonds were fixed as trans planar, and bond lengths and bond angles were taken from Creighton [21]. The cyclization of the linearly generated structures was accomplished by constraining the distance between the N and the C′ atoms of Trp[1] and Leu[5] in the range 1–3 Å, with the peptide bond torsion angles and bond angles formed on ring closure constrained to their ideal values ±20°.

(ii) Cluster analyses of the resulting main chains were then performed to eliminate redundant structures [20]. This gave 10 unique main chain conformers for BE-18257B, and 17 for BQ-123.

(iii) After addition of hydrogen atoms, each of these cyclo-pentaalanyl peptides was then energy minimized (using the COSMIC force field [22], but with neglect of Coulombic interactions) essentially just to regularize the geometry at the ring closure point.

(iv) Next, the appropriate amino acid side chains (and a corrected set of hydrogen atoms) were added to each of the antagonist main chain structures, using the program GLUE [20]. For each residue a range of possible side chain conformations was considered. The preferred conformations for the side chains of the L-amino acids were taken from Perkins [23], and those of the D-amino acids were constructed according to the rule: preferred χ angle for D-amino acid = −preferred χ angle for L-amino acid [24]. In total there were 2,250 full atom models considered for BE-18257B, and 3,060 for BQ-123.

(v) Partial atomic charges for these two sets of structures were determined using CNDO/2, and their potential energies calculated using COSMIC [22].

(vi) For each antagonist the 200 conformers of lowest initial potential energy were subjected to 100 cycles of Newton Raphson/Simplex minimization using the full COSMIC force field. The two sets of 50 conformers of lowest final potential energy were then employed in the subsequent analyses.

2.2 *Modelling of ET-1 residues 3–11*

In view of the similarities between the ET-1/ET-2 sequences Leu[6]-Met[7]-Asp[8]/Trp[6]-Leu[7]-Asp[8] and the sequences Leu-D-Trp-D-Glu in BE-18257B and Leu-D-Trp-D-Asp in BQ-123, it was decided to construct models of the ET-1 loop encompassing residues 3–11, in order to investigate its possible structural similarity with the antagonists. The models of the loop were built using RAMBLE [20], employing the Φ/ψ angles given by Endo et al. [17] and Krystek et al. [18]; the χ_1 and χ_2 angles for Cys[3] and Cys[11] were kept variable, and the separation of their Sγ atoms was constrained in the range of 1–3 Å in order to allow disulphide formation. Regularization of the geometry of the structures was carried out as described in (iii) above.

3. RESULTS

For each of the 50 low energy structures for BQ-123, the main chain torsion angles were compared against those determined from NMR studies of this peptide in aqueous acetonitrile [25]. In all cases the torsion angles compare very favourably, with mean deviations typically of the order of ±30°. The match with the NMR structure also extends to the side chain conformations, and many of the low energy conformers have very similar χ_1 angles for Trp[1], (g^-), Asp[2] (t) and Val[4] (g^-). (These similarities give encouragement that the modelling procedures adopted provide an adequate description for the peptide's conformation in solution, but are not entirely unexpected: the peptide cycle is highly constrained since it involves only 5 amino acid residues, and is further restricted by the alternating pattern of L- and D-amino acids.)

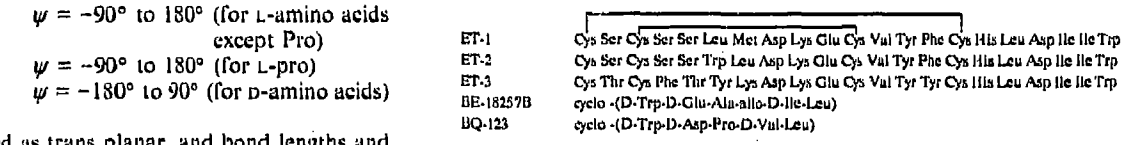

Fig. 1. Amino acid sequences of peptides from the endothelin family [12] and the two ET-A receptor antagonists, BE-18257B [15] and BQ-123 [16].

In virtually all of the low energy conformers for BQ-123 (see for example Fig. 2a), the peptide cycle is approximately planar, with a γ turn stabilized by hydrogen bonding between the Val[4] NH and Asp[2] CO groups. In contrast with the structures derived from preliminary NMR data [25], but wholly consistent with more recent findings [26], there is no type II β turn observed over residues Val[4]-Leu[5]-Trp[1]-Asp[2]; instead the CO groups of Leu[5] and Trp[1] are oriented away from the peptide cycle, directed out into solvent.

For BE-18257B, the low energy structures match closely with those obtained for BQ-123 (see Fig. 2b); there is a root-mean-square difference (rmsd) of the order of 0.5–2.5 Å for least squares fits over all the main chain and Cβ atoms, and very similar preferences for the χ_1 angles of all residues except Pro[3]/Ala[3]. More significantly, however, the two sets of structures display a particularly close match over the 10 atoms: Leu[5] (Cβ, Cα, C′, O), Trp[1] (N, Cα, C′, O) and Glu[2]/Asp[2] (N, Cα), with the majority of the pairwise comparisons giving an rmsd <0.5 Å.

Comparisons of these parts of the two antagonists against the corresponding 10 atoms in residues 6–8 in ET-1 showed encouraging similarities (see Fig. 2c). The lowest rmsd's obtained for the least squares fits to the models from Endo et al. [17] were 0.27 Å for BQ-123 and 0.28 Å for BE-18257B. The corresponding rmsd's for fits to the models from Krystek et al. [18] were 1.0 Å and 0.83 Å. Since there is a better fit between the two antagonists and the solution structure of ET in DMSO, it is tentatively suggested that this conformation of ET more closely resembles that required for ET-receptor activity. Note also therefore, that it is this model that appears most consistent with the data from fluorescence energy transfer experiments [27].

4. DISCUSSION AND CONCLUSIONS

From the molecular modelling studies performed here it is apparent that the main chain conformations and general side chain dispositions for the residues Leu[5]→Asp[2] in BQ-123 and Leu[5]→Glu[2] in BE-18257B are very similar to those reported for residues Leu[6] → Asp[8] in ET-1. Given the similarity in the sequences of these parts of the two antagonists and ET-1, we therefore propose that the loop residues 6–8 in ET-1 are

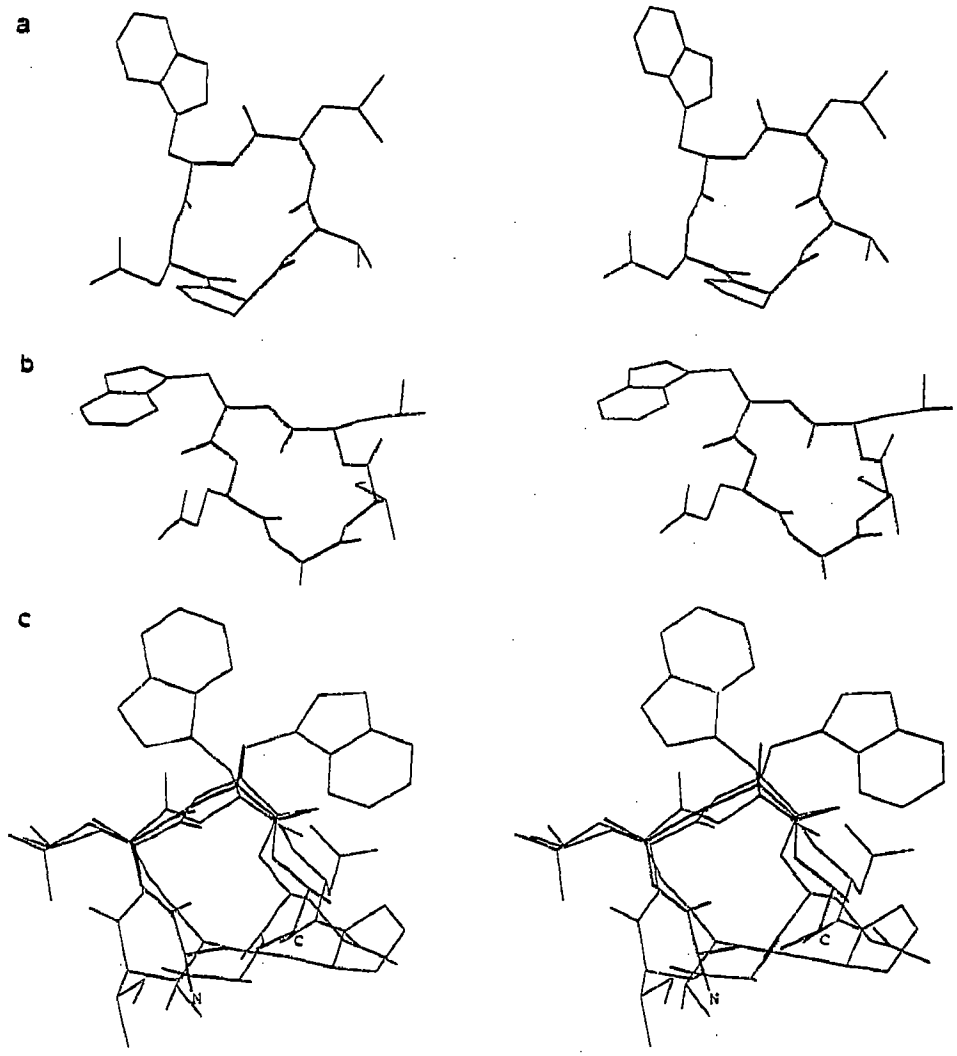

Fig. 2. Stereo-views showing (a) a representative model of the 3D structure of BQ-123, (b) a representative model of the 3D structure of BE-18257B, and (c) the superposition of residues Leu^5-D-Trp^1-D-Asp^2 in BQ-123, residues Leu^6-Met^7-Asp^8 in ET-1, and residues Leu^5-D-Trp^1-D-Glu^2 in BE-18257B. In (a) and (b) the antagonists structures are viewed with the N → C-terminal sense running anti-clockwise. In (c) the ET-loop is presented in bold, with the N- and C-termini labelled and only the main-chain and Cβ atoms shown.

involved in the binding of this peptide to the ET-A receptor, and that BQ-123 and BE-18257B achieve their antagonist activities by mimicking the structure of this loop.

In view of the sequence similarity between the two antagonists and the ET C-terminus, it is also possible that the antagonists might work by mimicking the ET residues Leu^{17} → Trp^{21}. It has been shown by Maggi et al. [28], however, that although ET (16–21) exhibits full agonist activity at the ET-B receptor, it is completely devoid of both agonist and antagonist activity at the ET-A receptor. Since BQ-123 and BE-18257B are specific for the ET-A receptor, it seems improbable, therefore, that they mimick this part of the peptide. Indeed, several groups of workers [29–32] have now provided evidence to show that it is the tertiary structure of ET (in particular, the loop dictated by the two disulphide bridges) that represents the most critical factor in its recognition at the ET-A receptor. Specifically, it is noted that the disulphide-reduced ET [29], fully Cys-protected ET [30] and [Ala^1, Ala^3, Ala^{11}, Ala^{15}] ET [31], as well as the ET derivative formed by cleaving at Lys^9 with lysyl endopeptidase [29], all have greatly reduced potency by comparison with native ET-1. (It is surprising to observe, however, that there is no apparent loss in receptor binding for ET cleaved at Met^7 by cyanogen bromide treatment [33], but the full effects of this modification remain to be determined.)

From the least squares superpositions of the two antagonist structures and the ET-1 loop residues, it is postulated that the key features on the ET-A receptor surface involved in agonist/antagonist recognition will

include: two hydrogen bond donor sites, two fairly non-specific hydrophobic binding sites, and a third binding site bearing a positive charge. The first hydrophobic binding site must be large enough to accommodate Trp (as in ET-2), but will also readily bind the smaller Leu side chain (as in the antagonists and ET-1). The second hydrophobic binding site will also accommodate Trp (as in the two antagonists), but equally well accommodates Nle [34], Met (as in ET-1) or Leu (as in ET-2). Since BQ-123 exhibits a significantly higher affinity for the receptor by comparison with either BE-18257B or [Glu^2]-BQ-123 [16], it is apparent that the cationic binding site on the receptor preferentially binds the shorter side chain, Asp, rather than the longer side chain, Glu. The two key hydrogen bond donor groups on the receptor will hydrogen bond to the CO groups of residues 6 and 7 in ET-1 and ET-2, or those of Leu^5 and Trp^1 in the antagonists.

Once again these hypotheses are consistent with experimental observations. An L-Ala scan of the ET-1 sequence [35] shows that: (i) the Ala^6 analogue binds just as well as ET-1 to h-vsm cells, and shows much the same contractile activity in rabbit vena cava; (ii) the Ala^7 analogue shows no diminution in contractile activity, but has a 3.5× higher receptor affinity; (iii) the Ala^8 analogue shows a 100-fold reduction in contractile activity, but binds more or less as well as ET-1. In addition, it is noted that the substitutions Leu^6 → Gly^6 and Met^7 → Met(O)^7 lead to ET derivatives with 70–75% activity, whereas the substitution Asp^8 → Asn^8 gives a peptide with <1% activity [31]. These results would thus appear to support the view that the binding sites on the ET-A receptor for the ET residues, 6 and 7, are relatively non-specific and hydrophobic, whereas the site for accommodating residue 8 requires a negatively charged carboxylate group.

From a scan of the ET-1 sequence using D-amino acids [36], it is noted that the D-Leu^6 and D-Met^7 analogues have similar affinities and activities to ET-1, and that the D-Asp^8 analogue exhibits a 33-fold reduction in binding and a 100-fold reduction in activity. Hence, the orientations (and by implication, therefore, the sizes) of the side chains at positions 6 and 7 in ET-1 would not seem to be stringently dictated by their ET-A receptor binding sites. In contrast, however, the conformation of residue 8 appears to be critical, not only for receptor binding, but also for biological activity.

In conclusion, we note that novel designs of ET-1 peptide antagonist with improved ET-A receptor affinity and selectivity may be produced using a minimum of 3 residues: the first of these will ideally be Leu (or possibly Nle, cf. [34]), the second Ala, and the third, Asp. Also, since the conformation of the molecule must be arranged so that the two peptide CO groups are available for intermolecular hydrogen bonding, there must be some constraints employed to ensure that the structure forms a loop. It is instructive therefore to see that Fujisawa have recently proceeded to in vivo testing of an ET-1 antagonist which is a pseudo-tripeptide with Leu and methyl-Trp side chains, a C-terminal carboxyl, and N- and C-terminal hydrophobic groups to stabilize an intramolecular turn [37].

Acknowledgements: T.S. and D.B. gratefully acknowledge the financial support of Eisai Co. Tokyo, Japan.

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