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Structural comparison of μ-opioid receptor selective peptides confirmed four parameters of bioactivity

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ABSTRACT

Structural determinants of binding to the μ-opioid receptor, an important target in analgesia, attract great scientific attention. Many natural and synthetic peptides and peptidomimetics were shown previously to bind to this receptor selectively but there is no consensus about the structure responsible for biological activity. No high resolution structure of this receptor is available and the binding site of ligands is not exactly known. However, μ-opioid ligands with similar affinity and selectivity should possess at least one common structural feature. Comparative structural analysis of such ligands, considering adequate representation of binding conditions, may reveal key features of bioactivity. In this study ten μ-opioid receptor ligands, DAMGO, Tyr-W-MIF-1, morphiceptin, endomorphin-1 and 2 and their analogues, possessing different affinity and selectivity, were examined using molecular dynamics. Conformational preference of these molecules was determined in H_2O and DMSO along with structural properties previously proposed to be important for binding. Four of such structural requirements were confirmed to be important, providing a possible structural model of receptor binding. Simultaneous fulfillment of these requirements results most likely in high affinity binding to the μ-opioid receptor.

1. Introduction

The μ-opioid receptor (MOR) is an important target in the search for novel analgesics. Thus structural determinants of binding to this receptor attract considerable scientific attention [1–6]. Endomorphin-1 (EM-1) and endomorphin-2 (EM-2) were proposed in the past decade as endogenous ligands of the MOR, characterized by their exceptionally high affinity and selectivity [7]. In addition, several modified opioid peptides were shown previously to bind to the MOR selectively, such as DAMGO [8], morphiceptin [9] and disulfide bridged cyclic analogues of somatostatin [10,11]. The members of the Tyr-MIF-1 family [12,13] were the first hypothalamic peptides which were shown to act also in the brain besides the pituitary. Investigation of this family established the field of selective endogenous opioid peptides and eventually led to the discovery of EMs. This opened a new era in the development of novel analgesics and tremendous efforts were taken to substitute morphine with novel EM-based painkillers devoid of dramatic side effects. Because of their short *in vivo* half-life [14] exogenous application of synthetic neuropeptides to suppress chronic pain is greatly limited. To increase stability against proteases while maintaining opioid activity, hundreds of synthetic analogues of EM-1 and EM-2 were prepared by inserting non-natural amino acid residues [15–25], introducing conformational constraints [26–29], modifying peptide bonds [30] and by designing stereoisomers [31] or peptidomimetics [32–34]. Many promising μ-selective analogues were found but in most cases structural modifications led to decreased selectivity toward the MOR. Nevertheless, these findings are of great importance and provided further information about possible structural requirements of binding to opioid receptors (vide infra).

Endogenous opioid peptides are flexible as short peptides are in general. According to the message-address concept [35] opioid sequences can be subdivided into two functional parts. The message part, usually the N-terminal part of the sequence, is necessary for recognition, while the address part provides selectivity. However, these sub-units vary greatly among different opioids. By general consensus the phenolic OH group of an N-terminal Tyr residue with a free cationic α-amino group (similar to the tyramine moiety of morphine) and an aromatic amino acid separated by one or two residues are the key requirements for the binding of opioid peptides [36–38] (Fig. 1). Additionally, a polar but not acidic C-terminal function was found to be essential for MOR binding of EMs [17]. A structural model of μ-opioid activity was first proposed based on ^{1}H-NMR studies of morphiceptin and its stereoisomeric analogues. The distances between the three

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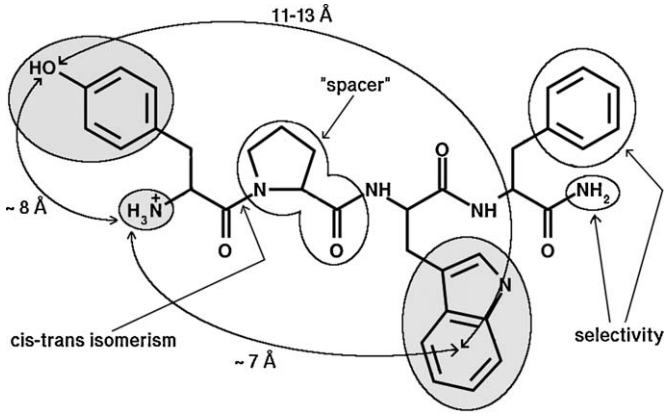

Fig. 1. General structural characteristics and pharmacophore definition of μ-opioid receptor ligands, shown on the example of endomorphin-1 (EM-2, **2**). Important chemical moieties are circled and pharmacophore groups are additionally highlighted. Pharmacophore distances proposed by Yamazaki et al. [37] are also shown.

pharmacophore groups, Tyr^1 N to Tyr^1 OH, Tyr^1 N to the center of Phe^3 aromatic ring and Tyr^1 OH to the center of Phe^3 aromatic ring were found to be ~8, ~7 and ~11–13 Å, respectively [37]. Another topographical model of MOR selective ligands was proposed based on the structural analysis of cyclic somatostatin analogues. In that model the optimal spatial arrangement of pharmacophores is furnished by bent backbone structure and *gauche+* conformation, *gauche−* conformation and increased flexibility of the first, second and third aromatic side chains, respectively [39].

The solution structure of EMs and their analogues, in relation with their bioactivity, was investigated extensively and the results were summarized in excellent reviews [29,40], but the backbone and side-chain conformation responsible for the μ-opioid activity of EM-1 and EM-2 is still debated. There is no agreement about whether peptides bind to the MOR in an extended- [41,43] or in a more compact, bent [25,26,32] backbone structure, since both conformational families are readily accessible for EMs and other short opioid peptides in solution [43,44,45]. It was shown, that the peptide bond preceding Pro^2 in the EMs is prone to *cis*/*trans* isomerization [41,42] and there is exclusive experimental evidence in support of both the *cis* [16] and the *trans* [25,26] conformer. Synthesis and biological evaluation of stereoisomeric analogues of EM-2 showed that different stereoisomers adopt different backbone structures, which results in remarkable variation of bioactivity [31]. However, a stereodiversified library of 1,5-enediol-based MOR ligands did not demonstrate such high diversity of MOR affinity [33,34]. As well as previously proposed topographical models [37,39] the solution conformation of the aromatic side chains of EMs and other MOR ligands were determined in numerous studies and several suggestions were given for their conformation in the receptor-bound structural state [17,24,38,44,46]. Generally, the flexibility of side chains, or in other words the free rotation around the χ^1 side-chain torsional angle was found to increase from the N- to the C-terminus in the EMs and the Pro^2 residue was suggested to function as a stereochemical spacer, responsible for the proper orientation of the pharmacophore groups [38]. Another important property of aromatic side chains is that they stabilize local structures [38] through various aromatic–aromatic and aromatic–proline interactions [47] which results in slightly lower backbone flexibility compared to other peptides of this size. Nevertheless, backbone and side-chain conformations of MOR ligands should not be examined and discussed separately as they contribute concurrently to the orientation of pharmacophore groups. Furthermore, a similar spatial arrangement of pharmacophores may be achieved through more than one combination of backbone- and side-chain conformations [38].

Receptor-based investigation of possible binding modes of ligands is difficult, because no high resolution experimental structure of the MOR is available. Structural models of the MOR as a member of the G-protein coupled receptor superfamily were proposed based on the X-ray crystallographic structure of bovine rhodopsin, electron cryomicroscopic studies, site-directed mutagenesis results and the analysis of variability and hydrophobicity patterns [48–54]. However, sequence similarity between the MOR and rhodopsin is approximately 20% which may lead to unrealistic assumptions about the position and chemical environment of the putative binding pocket in MOR models. Chimeric and point mutated receptors were constructed to locate regions which are responsible for ligand binding [55–59]. While several amino acid residues and loop regions were identified to be essential for ligand binding, it is still difficult to determine the exact location of a binding site. This supports the emerging concept that the receptor possesses considerable plasticity in ligand engagement [52,53] and suggests that studies of opioid activity should rather focus on the structure of ligands. Identification of possible structure–activity relationships is supported by a tremendous amount of biological data available for various EM-1 and EM-2 analogues. MOR ligands with similar affinity should possess at least one common structural feature in which they differ from other ligands of different affinity. Comparative structural analysis of such ligands may reveal key features of bioactivity. Such a ligand-based study was performed partly by our group previously and a slightly bent backbone structure was proposed for receptor-bound ligands [43]. The receptor-bound structure proposed in that study was in agreement with a rhodopsin-based receptor–ligand complex model [54].

Structural studies of biologically relevant molecules involve the adequate representation of binding conditions. The fundamental question is what solvent environment has to be employed and how does that possibly relate to the microscopic environment in which the action of the studied molecule is exerted. Since an unambiguously confirmed MOR–ligand complex model has not yet been published, different environmental conditions of receptor binding have to be taken into account. In other words, it is not known if ligands bind on the surface of the MOR or immerse into the transmembrane region. Therefore the use of solvents mimicking intersynaptic transport fluid, membrane- and protein environment has to be considered. Water is used generally as the paradigmatic biological environment but intersynaptic fluids have much higher viscosity and lower relative permittivity [60]. These two physical parameters can have a dramatic influence on the conformational equilibrium of short peptides. In high viscosity fluids conformational transitions are much slower, while lower relative permittivity modulates intra- and intermolecular electrostatic interactions significantly. Several solvents and solvent mixtures were proposed previously to mimic various biological environments [60,61]. Dimethyl-sulfoxide (DMSO) was shown to be a fairly good physical approximation to transport fluid environments as it has lower relative permittivity and higher viscosity, in the range of that of intersynaptic fluids. Moreover, being a good hydrogen bond acceptor, DMSO induces rather inter- than intramolecular interactions, which may reveal intrinsic conformational preferences and may mimic the physical circumstances of receptor–ligand interactions [61]. Many may dispute this latter statement and refer to DMSO as a denaturing agent despite the fact that it is routinely used as solvent for NMR spectroscopic studies and there is experimental evidence of folded structures in DMSO [60,61].

In this study 150 ns molecular dynamics (MD) simulations were performed in a comparative manner for ten previously proposed MOR ligands [7–9,13,25,26], listed in Table 1. This structurally