ANALYSIS OF THE DOCKING RESULTS OF SOME SELECTIVE MOR LIGANDS

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ABSTRACT

Endogenous opioids produce the same effects as the chemicals known as classic alkaloid opiates, which include morphine and heroin. Endogenous opioid peptides function both as hormones and as neuromodulators. The aim of the present study was to analyze the results of docking of ligands with MOR to identify the key elements required for selectivity. Many of the ligands have been synthesized and biologically tested by our colleagues. The other part is compounds known in the literature. The analysis of the obtained ligand-receptor complexes makes it possible to determine the key structural elements associated with the manifestation of specificity with respect to the receptor. These results will assist in the design of new compounds with potential MOR agonistic or antagonistic effects.

In order to be active and effective, a ligand must have certain properties. First, it must be stable in a biological environment so that it can reach the place where it will manifest its action. Second, be of a suitable structure to allow it to reach and interact with the receptor's binding site. Third, upon binding, the resulting ligand-receptor complex should be stable, i.e. its energy to be small. Fourth, the ligand induces the appropriate conformations in the receptor molecule upon interaction, i.e. to bind to precisely defined amino acid residues. Therefore, the present study aims to analyze the docking results of dalargine derivatives with MOR and determine the necessary conditions for the manifestation of the biological effect.

Keywords: computer modelling, molecular docking, μ-opioid receptor, μ-opioid ligands.

INTRODUCTION

Opioid receptors are part of the G protein-coupled receptors (GPCR), with three main subtypes - mu (MOR), delta (DOR) and kappa (KOR) [1]. The development of new opioid-based drugs that provide analgesia without leading to addiction is important for the treatment of pain [2]. Ligands that are opioid agonists lead to an analgesic effect primarily by acting on the mu-opioid receptor (MOR) [3] sites because they are potent analgesics and are used to treat pain [4]. But frequent use of opioid agonists can lead to dependence and limit their therapeutic efficacy [5]. It is crucial that scientists understand the molecular mechanisms controlling opioid analgesia and dependence in order to discover new opioid medications that provide analgesia without inducing dependency. Pharmacological tests that offer a complete understanding of the functional selectivity of opioid candidate medications are necessary to reach this goal in order to enable the selection, planning, and *in vivo* testing of lead compounds.

The main objective of the present investigation was to examine the results of docking of ligands with MOR to identify the key elements required for selectivity and determine the necessary conditions for the manifestation of the biological effect. Using the obtained ligand-receptor complexes, it is possible to identify the key structural elements associated with receptor specificity. The results of this study will aid in the design of new compounds with potential agonistic or antagonistic effects on MOR.

EXPERIMENTAL

Many of the ligands have been synthesized and biologically tested by our colleagues [6,7] and their structures were presented in Table 1. The other part is compounds known in the literature. In the present work, the following software was used in order to perform computational studies. The crystal structure of the μ-opioid receptor was obtained from RCSB (PDB id: 4dkl) [3]. Ligand preparation was done with Avogadro: an open-source molecular builder and visualization tool [8]. Docking studies were performed by using GOLD 5.2 (Genetic Optimization for Ligand Docking) from The Cambridge Crystallographic Data Centre (https://www.ccdc.cam.ac.uk/solutions/csddiscovery/Components/gold/) [9]. The software comprises four scoring algorithms: ChemPLP, GoldScore, ASP, and ChemScore.

For generation figures, Molegro Molecular Viewer was used [10]. Graph Pad Prism statistical software was used to determine the Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Docking was carried out with GOLD 5.2 software. It uses a genetic algorithm and considers full ligand conformational and partial protein flexibility. For docking studies, the crystal structure of mu-opioid receptor, published in RCSB was used [3]. It was published that the binding site for opioid receptors was defined as residues within 10Å radius of aspartic acid of the third transmembrane domain, which is involved in the most crucial interaction [11]. In the case of MOR, this is Asp^{147}. GoldScore algorithm was used and the optimization function was calculated for each ligand. GoldScore scoring function considers mainly Van der Waals interactions and hydrogen bonds [12 - 14]. All values of the scoring function are listed in Table 2.

As a result of the docking, the scoring function of the studied ligands was obtained and the total energies of the resulting ligand-receptor complexes were calculated. The results are presented in Table 2. No correlation was found between the docking results and the inhibitory effect of the compounds. This means that the small energy of the ligand-receptor complex or a stronger binding does not lead to a stronger effect. For the manifestation of a desired biological effect, the mode of interaction of the ligand and the receptor is of great importance. It is known from previous studies that effective ligands interact with the Asp^{147} residue of the receptor molecule. This interaction must be electrostatic. Of the 10 compounds tested, six interact electrostatically with the receptor. However, dalargin does not interact with Asp^{147}, but with Asp^{216} and His^{297}.

In order for the agonistic action to occur, the neighbouring Tyr^{148} residue also participates in the interaction, with which the ligand forms a hydrogen bond. These two interactions ensure appropriate conformational changes in the receptor molecule.

The compounds with the highest inhibitory concentration (Dal4 and Dal7) do not interact electrostatically with Asp^{147},

Table 1. Structures of the ligands used in the study.

Abbreviation	Amino acids sequence
Dal1	Tyr-DAla-Gly-Phe-Leu-Arg
Dal2	Tyr-DAla-Gly-N-Me-LPhe-Leu-Arg-NH_2
Dal3	Tyr-DAla-Gly-Phe-Leu-Arg-NH-CH_2-CH_3
Dal4	Tyr-DAla-Gly-DPhe-Leu-Arg-NH_2
Dal5	Tyr-Ala-Gly-Phe-Leu-Arg
Dal6	Tyr-DAla-Gly-Phe-Met-Arg
Dal7	Tyr-DAla-Gly-N-Me-DPhe-Leu-Arg-NH_2
DAMGO	H-Tyr-D-Ala-Gly-N-MePhe-Gly
Enk1	Tyr-Gly-Gly-Phe-Leu
Enk2	Tyr-Gly-Gly-Phe-Met

but bind to it with hydrogen bonds.

Asp^{147} does not interact with the NH_2 groups in Dal4 and Dal7, although they are free. The interaction of Asp^{147} with Dal7 is weak with an amide group. In DAMGO and Enk1, Asp^{147} interacts with the NH_2-group.

Fig. 1 illustrates the obtained ligand-receptor complexes for MOR and the following ligands: 1 - DAMGO, 2 - Enk1, 3 - Dal4, 4 - Dal7.

Table 3 presents the residues with which the individual ligands interact and the binding energies with

Table 2. Scoring function, total energies of the obtained ligand-receptor complexes and IC_{50} of the studied compounds.

Compound	Fitness function	Total energy of the complex with MOR, J mol^{-1}	The inhibitory effects (IC50, nM) mouse vas deferens
Dal 1	89.10	-131.108	0.18
Dal 2	97.25	-128.487	3.98
Dal 3	82.27	-153.126	0.16
Dal 4	98.92	-147.818	1.85×10^2
Dal 5	97.24	-152.008	5.60
Dal 6	118.96	-124.561	1.50×10^{-2}
Dal 7	84.00	-136.962	1.60×10^2
DAMGO	77.13	-95.996	52.10
Enk 1	81.55	-118.089	13.90
Enk 2	86.03	-100.492	19.50

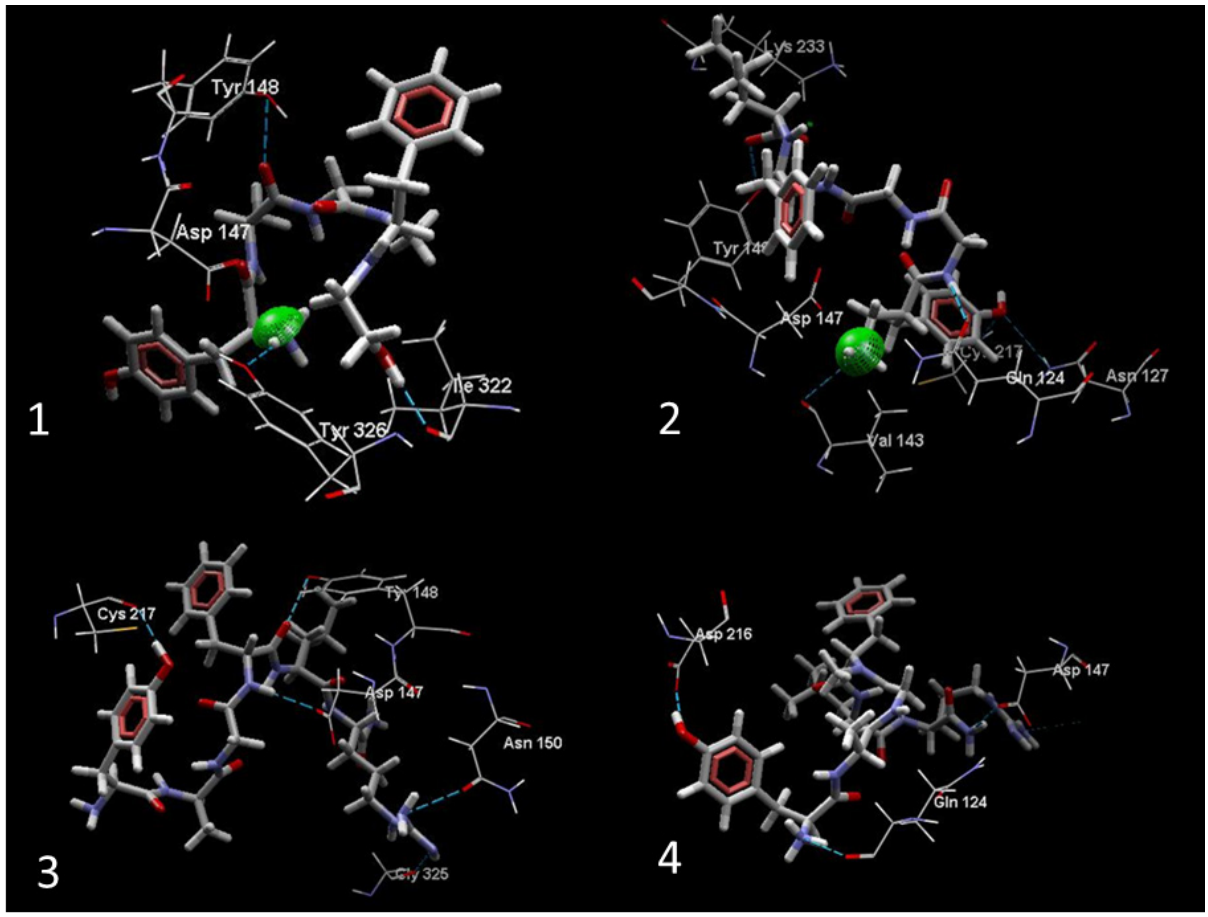

Fig. 1. Interactions of the ligands and MOR. 1 - DAMGO, 2 - Enk1, 3 - Dal4, 4 - Dal7.

Table 3. The residues with which the individual ligands interact and the binding energies with each residue.

	Electrostatic interactions, energy of the interaction, J mol^{-1}				Hbonds, energy of the interaction, J mol^{-1}												
	Asp^{147}	His^{297}	Lys^{233}	Asn^{216}	Gln^{124}	Asn^{127}	Tyr^{128}	Val^{143}	Asp^{147}	Tyr^{148}	Asn^{150}	Asn^{216}	Cys^{217}	Lys^{233}	His^{319}	Ile^{322}	Gly^{325}	Tyr^{326}
Dal 1		-7.78		-2.84	-9.4	-12.97				-23.77								
Dal 2					-15.57					-10.62	3.06				-2.54	-21.51		
Dal 3	-15.25									-9.09	-5.76							
Dal 4									-17.04	-11.41	-5.42		-9.92				-4.43	
Dal 5	-3.16						-9.52		-15.94	-10.38	-5.07							
Dal 6	-1.85				-13				-14.03		-4.54		-6.96	-16.92				
Dal 7					-19.61				-6.31			-9.05						
DAMGO	-9.22		-0.54							-4.81						-11.47		-16.8
Enk 1	-14.99				-11.94			-4.99		-10.62		-1.73	-12.89					
Enk 2					-22.71	-13.84			-12.34	-2.81						-11.28		-15.16

each specific residue.

The interaction energy of DAMGO is -42.84 J mol^{-1}, and the electrostatic interaction with Asp^{147} is -9.22 J mol^{-1}. The stronger interaction at Enk1 (from -14.99 J mol^{-1}) resulted in a significant increase in the effect, and only slightly stronger than that of Enk1 at Dal3 (from - 15.25 J mol^{-1}) increased the effect many folds.

Stronger interactions with other residues in the binding center of the receptor do not lead to an increase in effect. Such is the case with Dal7, which interacts strongly with Gln^{124} and Dal4 with Asp^{150}, Cys^{217} and Cys^{325}. These interactions likely induce different conformations that do not favour the further transmission of information to the G-protein, and this consequently significantly lowers the effect of the compound.

CONCLUSIONS

From the analysis of the obtained results, it can be concluded that it is not the energy of the resulting ligand-receptor complex that is of decisive importance for the manifestation of a biological effect, but the way of binding. The key amino acid residues Asp^{147} and Tyr^{148} must necessarily participate in the formation of the complex and as few side residues as possible interact strongly with the ligand.

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