Conformational entropy in drug-receptor interactions, using M-cholinolytics, μ-opioid, and D2-dopamine receptor ligands as examples

Authors: Mikhail B. Darkhovskii [1, *], Felix S. Dukhovich [2]

Affiliations:

[1] Interx Inc.

[2] Independent Scholar

***** Correspondence to: mikednet@gmail.com

Abstract

The interest to conformational entropy started from developing new drugs. We show the role of conformational component of the entropy in the complex formation between a substance and a specific neuroreceptor, using M-cholinolytics and ligands of μ-opioid receptors as examples. It is shown that conformational entropy may be used for prediction of the drug affinity to a certain receptor. Examples of directed affinity change under their conformational flexibility modification are given. The specific role of the conformational entropy in the receptor's protection from the irreversible inactivation is identified.

1 Introduction

There are known problems in computation of conformational entropy. First, conformational entropy contribution is often implicit, as varied structure of compounds is accompanied by changing many enthalpic and entropic factors in the free energy of the complex formation, besides the conformational entropy. Second, only the total entropy change of the ligand-receptor complex formation can be determined in experiments.

The entropy change during ligand-receptor complex formation is a sum of the following terms [1]:

$$\Delta S = \Delta S_{rot} + \Delta S_{tr} + \Delta S_{osc} + \Delta S_{conf} + \Delta S_{w} \qquad (1),$$

where the first four terms are respectively from rotational, translational, vibrational, and conformational entropy changes of the ligand and the receptor. Fifth term is the entropy change during water displacement (the total or partial removal of hydrate shells) from the receptor's binding site. The components of the entropy change in Eq.(1) can be estimated by semi quantitative and empirical methods.

2 Substances Selection

However, we are interested in such a model that can extract 'pure' conformational contribution to the entropy change. That is, the change in the structure of substances should minimize change in the enthalpy and other than conformational components of the entropy. As such substances, it is appropriate to choose the amino esters of substituted glycolic and acetic acids (Table 1).

These mAChR antagonists have the similar molecular weight and size, charge distribution, basicity of the amino group, and the same pharmacophore [2]. In addition, anchoring substituents in the acidic part of molecules have similar partial free energy contributions ($\Delta\Delta G$) to the mAChR binding free energy: phenyl, thienyl, and cyclohexyl give 2.80, 2.95, and 2.40 kcal/mol, respectively [2, 3]. These substances possess high selectivity to muscarinic acetylcholine receptors, not to their specific subtypes [4].

3 Methods

3.1 Determination of binding ΔG values upon M-cholinolytic – mAChR complex formation

All experimental ΔG values given in Table 1 are determined pharmacologically by suppressing contraction of rat small intestine tissue induced by acetylcholine at 37.5 ^{o}C (310 K). In experiments the equilibrium dissociation constant K_d of the 'substance – receptor' complex was determined. It is related to Gibbs free energy by:

$$\Delta G = RT \ln K_d$$

Detailed description of the method can be found in [2, 3, 5]. All investigated substances are synthesized as racemates, so ΔG values of the chiral substances are given in Table 1 for their (R)-form, for better comparison with non-chiral substances. It is known that affinities of (R)-isomers of the chiral amino esters of substituted glycolic and acetic acids greatly dominate that of (S)-isomers [2, 6, 7]. Due to possible differences in the binding mode, glycolic and acetic acid' derivatives are considered separately.

3.2 Calculation of the conformational entropy change

To calculate conformational entropy, we need to estimate the number of conformers. The value of ΔS_{conf} is derived based on the assumption that a ligand binds to its receptor at one complementary

conformation, and the rest conformations of an unbound molecule in gas phase are treated as not contributing to the overall conformational entropy change under the complex formation.

Under these assumptions, the conformational entropy change on the complex formation is expressed through Boltzmann distribution of all rotamers of the ligand [8]:

$$\Delta S_{conf} = -R \sum_{i}^{N_{conf}} p_i \ln p_i + R \ln 1 = -R \sum_{i}^{N_{conf}} p_i \ln p_i,$$

where R is the gas constant, N_{conf} is the number of conformers of an unbound ligand molecule, *i* is the ordinal number of a conformer, and p_i is the ratio of the *i*-th conformer in the set of the conformations, which is expressed at the equilibrium as:

$$p_i = \frac{e^{-\beta E_i}}{\sum e^{-\beta E_i}},$$

where E_i is the potential energy of the *i*-th conformer, β = 1 / kT (k is the Boltzmann constant).

TINKER molecular modeling program [9] is used to produce the conformations set. As for all molecules under study, the conformational analysis procedure is based on searching for the potential energy minima in main vibrational modes that correspond to changes in the certain torsion angles. To calculate the potential energy, the MM3 parametrization is used [10]. All the potential energy components used in this parameterization are taken into account. Calculations were performed for the cationic form in which molecules bind to the receptor. The entropy contribution to the binding free energy is calculated for 310.5 K (37.5 °C), at which the K_d values are determined.

In order to scan the potential energy surface, we study changes in the torsion angles along the main chain in the ester fragment of glycolates and acetates, cyclohexyl substituent at Cα atom of the acidic part, and substituents at the nitrogen atom. In these settings, the conformations with the energies difference from the minimum energy conformation < 10 kcal/mol are exploited to exclude conformations inaccessible at the standard conditions (1 atm, 298 K).

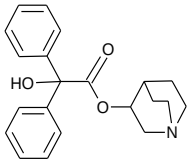
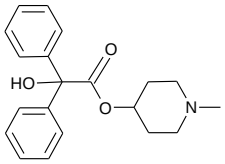
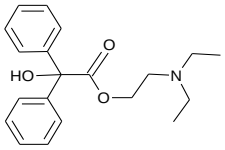
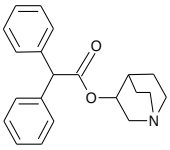
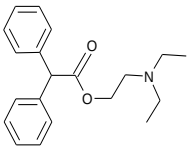
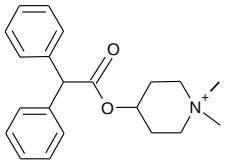
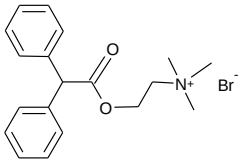
Noteworthy, ΔS_{conf} is not proportional to N, since the p_i values distribution is far from uniform. Conformational changes are not localized in one fragment in which these changes occurred but propagate to the whole molecule. Therefore, modifying one fragment can usually cause an increase or decrease in the number of conformations (N_{conf}) pertaining to the whole molecule.

4 Results and Discussion

Conformational flexibility of the substances is changed in two ways:

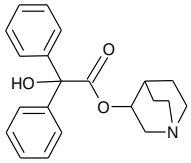
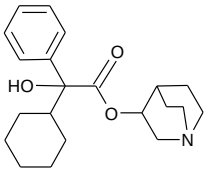
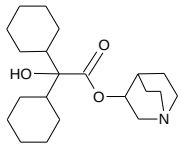
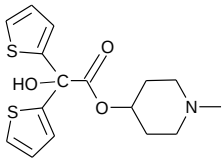
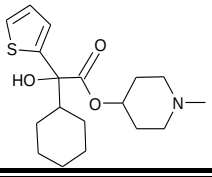
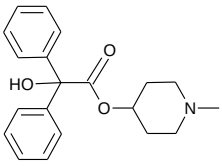
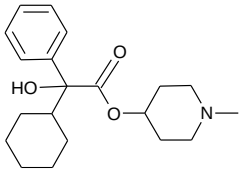
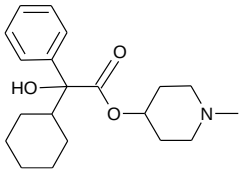
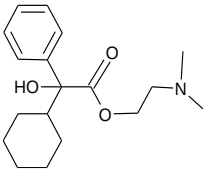
- molecular structure framework modification on substitution of more conformationally stable N-methyl-4-piperidyl and quinuclidyl groups for movable N-alkyl group;
- substitution of conformationally rigid phenyl and thienyl for cyclohexyl.

Table 1 Number of conformations and conformational entropy changes on structural modification of substances[1]

Designation	Substance	N_{conf}	$-T\Delta S_{conf}$, kcal/mol	$\Delta(T\Delta S_{conf})$, kcal/mol	$-\Delta G$, kcal/mol
1		12	0.3	1.9	14.4
2		8	0.4	1.8	14.3
3		451	2.2	-	12.1
4		5	0.3	2.0	14.2
5		415	2.3	-	10.6
6		53	0.7	1.2	12.8
7		75	1.9	-	10.4

[1] For compounds **6**, **7** K_d data is taken from [4].

Table 1 (continued)

Designation	Substance	N_{conf}	$-T\Delta S_{conf}$, kcal/mol	$\Delta(T\Delta S_{conf})$, kcal/mol	$-\Delta G$, kcal/mol
1		12	0.3	1.2	14.4
8		327	1.5	0.5	13.5
9		2329	2.0	-	12.3
10		84	0.9	0.7	14.6
11		610	1.6	-	13.5
2		8	0.4	0.3	14.3
12		103	0.7	-	13.6
12		103	0.7	1.0	13.6
13		808	1.7	-	12.4

4.1 Conformational entropy and directed affinity change of the substances

Table 1 gives the possibility to compare conformational entropy $\Delta(T\Delta S_{conf})$ and free energy ΔG changes with varied compound's structural rigidity. The following series of substances were compared: (**1**, **2**, **3**), (**4**, **5**), (**6**, **7**), (**1**, **8**, **9**), (**10**, **11**), (**12**, **13**), (**2**, **12**). An increase in the ligand's structural rigidity is accompanied by declining of the conformational entropy, and therefore the selected calculation model adequately 'reacts' to modifications in the structure of substances. In the first direction, the transition from the relatively labile compound **3** to more rigid **2**, **1** reduces the number of conformations N_{conf} from 451 to 8 and 12, and the value of $T\Delta S_{conf}$ from 2.2 kcal/mol to 0.4 and 0.3 kcal/mol, respectively. Similar structural modifications in acetic acid derivatives (compared ligands **5** and **4**) are accompanied by the reduction of N_{conf} from 415 to 5 and $T\Delta S_{conf}$ values from 2.3 kcal/mol to 0.3 kcal/mol. Analogous pattern of N_{conf} and $T\Delta S_{conf}$ changes occurs for substances with the quaternary nitrogen atom (**7** and **6**).

The second scenario of the conformational flexibility modification with the substitution of a phenyl group for cyclohexyl is followed by increasing N_{conf} from 12 to 327 and $T\Delta S_{conf}$ from 0.3 kcal/mol to 1.5 kcal/mol (compounds **1** and **8**). With the replacement of both phenyl rings for cyclohexyl, the N_{conf} sharply rises to 2329 and the $T\Delta S_{conf}$ to 2.0 kcal/ mol (compounds **1** and **9**).

So far, correspondence between increase of entropic losses and decrease of affinity is observed.

From the obtained results the subsequent conclusions follow:

1) The conformational entropy can be used to forecast drug affinity to a certain receptor.
2) The conformational entropy drives the affinity change during conformational transitions in the considered ligand molecules, together with altered steric conditions during complex formation.

Developed model takes into account conformational entropy of the ligand in gas phase. It would be useful to verify the model validity by comparison to the model taking into account conformational entropy changes in water environment.

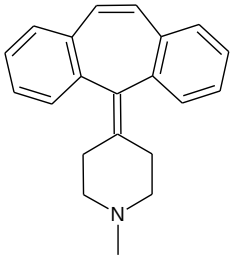
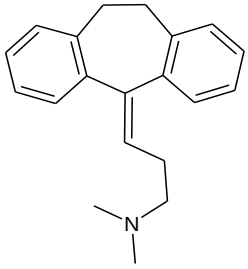
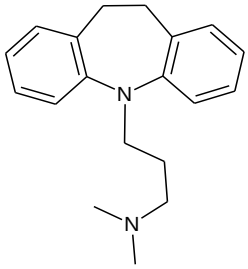
Experimental K_d, ΔG values include changes both from the ligand's side and the receptor's side in water environment and can serve as criteria for assessing the quality of models.

It is noteworthy that only two ligands from Table 1, benactyzine (**3**) and adiphenine (**5**), are used as pharmaceuticals [11]. The remaining substances have too large affinity to mAChR to be drugs. There is a relationship between complexes lifetime and K_d value, so the less K_d the larger is complex's lifetime. Substances with $K_d < 0.1$ nM almost irreversibly inactivate receptor, so they become poisons.

4.2 Antidepressants and conformational entropy

The conformational entropy dependence on structural rigidity is investigated for other classes of pharmacological agents. In Table 2 the results of N_{conf} and $T\Delta S_{conf}$ calculations (in gas phase) are given for some antidepressants.

Table 2. Conformational entropy of some antidepressants upon binding to D_2-dopmaine receptors

Designation	**Substance**	**N_{conf}**	**$T\Delta S_{conf}$, kcal/mol**	**D_2-dopamine**	
				K_d, nM	**$-\Delta G$, kcal/mol**
14	 *Cyproheptadine*	11	0.5	31 [12]	10.2
15	 *Amitriptyline*	23	0.7	210 [13]	8.9
16	 Imipramine	85	1.6	2000 [14]	8.7

Substitutions of single for double bonds and of a methylene chain for a conformationally stable N-methyl-4-piperidyl group make the substances structurally rigid (Table. 2). These structural changes certainly induce decreasing of N_{conf} and $T\Delta S_{conf}$ also, as well as the corresponding decrease of affinity to D_2-receptors.

4.3 Conformational entropy and ligands of the μ-opioid receptor

Conformational entropy may be considered as a universal factor in the preservation of receptor's

functional activity. Binding of morphine and met-enkephalin with μ-opioid receptors may serve as a visual example of this scheme.

Values of the affinity and estimation of entropy losses during binding between morphine and met-enkephalin with μ-opioid receptors mediating their analgesic effect are summarized in Table 3. Met-enkephalin is pentapeptide (Tyr-Gly-Gly-Phe-Met), acting as an endogenous μ-receptor agonist.

Table 3 $T\Delta S_{conf}$ and ΔG values for met-enkephalin and morphine binding to rat brain μ-opiate receptors

Ligand	**MW**	**N_{conf}**	**$T\Delta S_{conf}$, kcal/mol**	**K_d, nM**	**$-\Delta G$, kcal/mol**
Met-enkephalin	574	13329	3.84	3.0 [15]	11.6
Morphine	286	5	0.57	1.5 [16]	12.0

Meth-enkephalin with twice-higher molecular weight than morphine slightly differs in the affinity for μ-receptors. Similarly to morphine and other opiates, met-enkephalin is competitively displaced from μ-receptors by ^{3}H-naloxone, and this is a proof for the common binding site for these substances at the receptor. Unlike rigid morphine molecule, met-enkephalin molecule is conformationally labile, having the increased N_{conf} and $T\Delta S_{conf}$ values.

Endogenous agonists of opioid receptors are not limited to enkephalins. It is endorphins which have more sizeable molecules. Thus, α- and β-endorphins with lengths of 16 and 31 amino acids are competitively displaced from μ-receptors by low-molecular-weight opiates and have relatively comparable affinities for these receptors 11 nM [15] and 3.2 nM [17], respectively.

Even if not all conformations are frozen in large molecules at their binding, conformational entropy plays the important and likely major role in preservation of receptor's functional activity.

Little affinities of relatively large molecules are reasonably explained by steric hindrances, and this is undoubtedly may be true. However, the found relation between $\Delta(T\Delta S_{conf})$ and $\Delta\Delta G$ indicates that entropy-based control of affinity is a major factor in the protection of neuroreceptors from irreversible inactivation at binding with large ligand molecules, such as enkephalins, endorphins, hormones, peptide drugs etc.

Acknowledgments:
We would like to make a statement of author contributions here. M.B.D. designed and performed calculations on conformational analysis and subsequent entropy changes, analyzed data and prepared the manuscript; F.S.D. analyzed data and wrote the paper.

References

1. Ajay, Murcko M.A. - J. Med. Chem., 1995, v. 38, p. 4953.
2. Dukhovich F.S., Gorbatova E.N., Darkhovskii M.B., Kurochkin V.K. - Molecular Recognition: Pharmacological Aspects, New York, Nova Science Publishers, 2004.
3. Dukhovich F.S., Gorbatova E.N., Kurochkin V.K. - Pharm. Chem. J., 2001, v. 35, p. 260.
4. Shelkovnikov S.L. - Itogi nauki i tekhniki, seriya Pharmacology and chemotherapeutics (in Russian), v. 25. Moscow, VINITI, 1991, p. 24.
5. Blattner R., Classen H.G., Denhert H., Doring H.J. - Experimente an isolierten glattmuskulären Organen, Freiburg, Hugo Sachs Elektronik KG, 1978.
6. Hulme E.C., Birdsall N.J.M., Burgen A.S.V., Menta P. - Mol. Pharmacol., 1978, v. 14, p. 737.
7. McKinney M., Anderson D., Forray C., el-Fakahany E.E. - J Pharmacol Exp Ther., 1989, v. 250, No. 2, p. 565.
8. Pickett S.D., Sternberg M.J. - J. Mol. Biol. , 1993, v. 231, No. 3, p. 825.
9. Pappu R.V., Hart R.K., Ponder J.W. - J. Phys. Chem. B., 1998, v. 102, No. 48, p. 9725.
10. Allinger N.L., Yuh Y.H., Lii J.H. - J. Am. Chem. Soc., 1989, v. 111, p. 8551.
11. Mashkovskii M.D. - Lekarstvennye sredstva (in Russian), Moscow, Novaya Volna, 2000, v. 1.
12. Leysen J.E., Awouters F., Kennis L., et al. - Life Sci., 1981, v. 28, p. 1015.
13. Hall H., Ogren S.O. - Eur. J. Pharmacol., 1981, v. 70, p. 393.
14. Richelson E., Nelson A. - Eur. J. Pharmacol., 1984, v. 103, p. 197.
15. Childers S.R., Creese I., Snowman A.M., Synder S.H. - Eur. J. Pharmacol., 1979, v. 55, No. 1, p. 11.
16. Zaytsev S.V., Yarygin K.N., Varfolomeev S.D. - Narcomania. Neuropeptide morphine receptors (in Russian), Moscow, Moscow University, 1993, p. 88.
17. Goldberg I.E., Rossi G.C., Letchworth S.R., et al. - J. Pharmacol. Exp. Ther., 1998, v. 286, No. 2, p. 1007.