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Original Research

Molecular modeling of interactions between tetrahydroprotoberberines and dopamine receptors[1]

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KEY WORDS molecular models; dopamine receptors; tetrahydroprotoberberines; *l*-stepholidine; ligands

AIM: To build up the structure models of dopamine receptors, then combined with the receptor models, to investigate the action mechanism of tetrahydroprotoberberines (THPB) on dopamine receptors at the molecular level. **METHODS**: Using the three-dimensional structure of bacteriorhodopsin as a template, we have constructed dopamine D_1 and D_2 receptor models on computer. *l*-Stepholidine was selected as the leading compound of THPB and docked into D_1 and D_2 receptor active sites. **RESULTS**: After manual adjustment and energy minimization, the ligand-receptor interaction models were achieved. Based on these models, the possible action mechanism of THPB on dopamine receptors was suggested that the protonated N atom of THPB form electrostatic interaction and hydrogen-bonding interaction with residue Asp in TM3 of the receptor, the two substituents in D ring of THPB form hydrogen-bonding interactions with two Ser residues in TM5 of the receptor, and the aryl groups form $\pi - \pi$ interactions with some aryl residues of the receptor around ligand. **CONCLUSION**: Our ligand-receptor interaction models should be helpful for rational design of more potent drugs.

Structure-based drug design has advanced very quickly[1]. A detailed structure information of the target macromolecule, namely receptor, is essential to it. However, receptors known three-dimensional (3D) structures are very limited and a large number of receptors remain uncertain. If the primary sequence of receptor is elucidated, we could predict its 3D structure from the amino acid sequence by molecular modeling approach, which may be helpful for rational drug design.

Dopamine receptors are the primary targets in the medical treatment of Parkinson's disease and schizophrenia. At least 5 dopamine receptor subtypes have been recognized from gene cloning, and these may be grouped into D_1-like (cloned D_1 and D_5) and D_2-like (cloned D_2, D_3 and D_4) subfamilies[2]. They belong to the superfamily of G-protein coupled receptors (GPCR) formed from seven transmembrane (TM) α-helices, linked by intracellular and extracellular loops. The ligand-binding sites are thought to be located within the 7 α-helices[3]. Tetrahydroprotoberberines (THPB, Fig 1), isolated first from the Chinese herbs and then synthesized, are novel dopamine receptor antagonists found in our Institute[4].

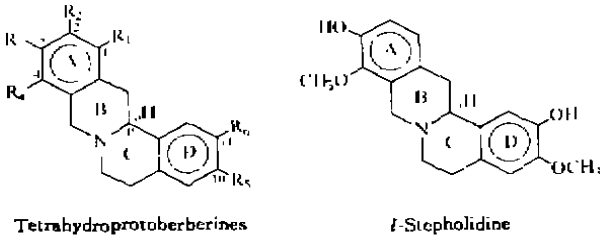

Fig 1. (a) Common structure of THPB. (b) *l*-SPD.

They have affinities toward both D_1 and D_2 receptors in brain, but preferentially toward D_1 receptors. Among THPB, *l*-stepholidine (*l*-SPD), isolated from *Stephania intermedia*, was demonstrated to be a potent antagonist, but with some agonist properties to dopamine receptor[5]. Thus *l*-SPD was selected as the leading compound of THPB in investigating the interactions between THPB and dopamine receptors.

This paper is to construct the D_1 and D_2 receptor models using the diffractional structure of bacteriorhodopsin (BR)[6] as a template and

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investigate the action mechanism of THPB on dopamine receptors at the molecular level.

METHODS

This work was performed on Silicon Graphics IRIS Indigo XZ4000 workstation using SYBYL V6.1 molecular modeling software package[7]. The structure data of BR were read from the Protein Data Bank[8]. The amino acid sequences of human D_1 and D_2 receptors were inputted manually according to reports[4,10]. D_1 receptor consists of 446 residues, and D_2 consists of 414 residues. The initial structure of *l*-SPD was built according to the crystal structure and then minimized using SYBYL/Base module. The structure of dopamine was also built and minimized in SYBYL/Base module. At first, we built up the conservative regions, ie, 7 TM α-helices of dopamine receptors. Thus, analysis of the hydropathicity and sequence alignment were necessary to define the beginning and end of each TM segment. Then, the 7 α-helices were constructed with normal φ and ψ values separately in SYBYL/Biopolymer module. Using the structure of BR as a template, each of the 7 α-helices were superimposed on to the corresponding position in the template structure. Automatic adjustments were made to remove the unfavorable steric interactions and to make the model consistent with the mutational data. The primary models were now optimized for 200 steps with the steepest descent minimizer and subsequently for 2000 steps with the conjugate gradient minimizer, using the Kollmann all-atom force field in SYBYL V6.1. A cutoff of 0.8 nm was used, and dielectric constant depended on the distance.

The natural ligand dopamine was docked into the cavity of 7 α-helices to examine the validity of these models. Then, we generated all the loop segments using LOOP SEARCH method in SYBYL/Biopolymer module, and formed intramolecular disulfide bridges between Cys96 and Cys186 in D_1, Cys107 and Cys182 in D_2. After removing the unfavorable steric interactions, the whole receptor structures were optimized by energy minimization in the same procedure as before-mentioned. Based on the dopamine receptor models, the ligand *l*-SPD and protonated *l*-SPD were docked into D_1 and D_2 receptor active sites, respectively. Then, the ligand-receptor complexes were optimized by energy minimization, with Kollmann all-atom charge for the receptor and Gasteiger-Huckel charge for the ligand in Tripos force field.

RESULTS AND DISCUSSION

Modeling of the receptors Many articles about molecular modeling of GPCR have been reported[11]. Most of them based on the high-resolution, electron cryo-microscopy structure of BR[6]. Because of the low abundance, difficult solubility and difficult purification, no GPCR have been crystallized and elucidated 3D structures to date. The only available structure is that of BR. Although there is low primary sequence homology between BR and GPCR, BR has the same seven TM α-helices as GPCR, meanwhile it has the same ligand as rhodopsin, a member of GPCR family. Moreover, a 0.9 nm resolution electron diffraction structure was obtained for bovine rhodopsin[12]. The projection footprint of bovine rhodopsin indicated the presence of 7 TM α-helices in this receptor (and probably in all GPCR). Therefore, we propose that their tertiary structures are likely to be similar, and using the structure of BR as the template of GPCR is practicable. Then, in the absence of 3D structures of dopamine receptors, we built up the structure models of D_1 and D_2 receptor by the 3D structure of BR. These models would contribute to a better understanding of the structure and function of dopamine receptors, as well as the ligand-receptor interaction.

The beginning and end of each TM segment were difficult to define accurately. What we had done mainly relied on the analysis of hydropathicity and sequence alignment, meanwhile referred other related papers (Fig 2).

Under the present situation, this definition is effective enough to analyze the ligand-receptor interaction. To examine the validity of TM regions, we docked the natural ligand dopamine into the cavity of 7 α-helices in D_1 and D_2 receptor, respectively. To D_1 receptor, after manual adjustment and energy minimization, we got the stable ligand-receptor complex conformation with low energy. Within the complex, the carboxylate group of the residue Asp103 formed a strong electrostatic interaction and hydrogen-bonding interaction with the amino group of dopamine. The 2 hydroxyl groups of dopamine formed hydrogen-bonding interactions with residue Ser199 and Ser202, respectively. Some hydrophobic residues around phenyl ring of dopamine, such as Phe156 and Phe203, could form $\pi-\pi$ interactions with the ligand (Fig 3).

The same case occurred in D_2 receptor. From these results, we found that our models were consistent with the previous sitemutagenesis studies[13]. For this reason, our

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TM1 BR    8 PEWIWLALGTALMGLGTLYFLVKGM  32
    D1   25 LTACFLSLLILSTLLGNTLVCAAVI  49
    D2   36 YYATLLTLLIAVIVFGNVLVCMAVS  60

TM2 BR   38 DAKKFYAITTLVPAIAFTMYLSMLLG 63
    D1   62 FVISLAVSDLLVAVLVMPWKAVAEIA 87
    D2   72 LIVSLAVADLLVATLVMPWVVYLEVV 97

TM3 BR   79 YWARYADWLFTTPLLLLDLALLV   101
    D1   96 CNIWVAFDIMCSTASILNLCVIS  118
    D2  107 CDIFVTLDVMMCTASILNLCAIS  129

TM4 BR  106 GTILALVGADGIMIGTGLVGAL    127
    D1  143 LISVAWTLSVLISFIPVQLSWH    164
    D2  155 MISIVWVLSFTISCPLLFGLNN    176

TM5 BR  138 WAISTAAMLYILYVLFFGFTS      158
    D1  196 ISSSVISFYIPVAIMIVTYTR     216
    D2  191 VYSSIVSFYVPFIVTLLVYIK     211

TM6 BR  170 TFKVLRNVTVVLWSAYPVVWLI     191
    D1  273 TLSVIMGVFVCCWLPFFILNCI    294
    D2  345 MLAIVLGVFIICWLPFFITHIL    366

TM7 BR  202 NIETLLFMVLDVSAKVGFGLILLRS 226
    D1  307 CIDSNTFDVFVWFGWANSSLNPIIY 331
    D2  373 NIPPVLYSAFTWLGYVNSAVNPIIY 397
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Fig 2. Sequence alignments of the TM regions between BR, D_1, and D_2 receptors.

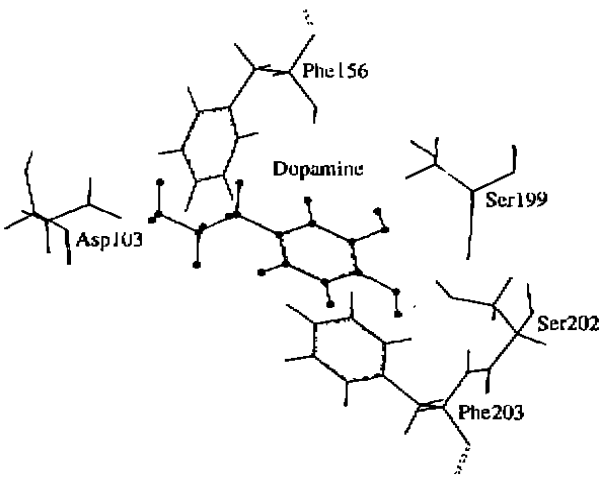

Fig 3. Dopamine interaction with the active sites of human D_1 receptor.

models may be rational, and we could investigate the interaction of THPB with dopamine receptors by these models.

To a whole dopamine receptor, loop regions are more variable than TM regions. No template could be used to build up the loop regions. Hence, the creation of loop segments is difficult in the entire model building process. However, we have also built up the loops by searching the library of the Protein Data Bank[18] for protein fragments with LOOP SEARCH program in SYBYL/Biopolymer module. According to the fitting root mean square (RMS) and van der Waals contact values, the conformation with least RMS and contact values was selected as the best loop region bridging one helix and the next. For the 2 highly conserved Cys residues in the 2nd and 3rd extracellular loops, we generated an intramolecular disulfide bridge between them. That was consistent with the biochemical analysis[13,14]. The invariance of these Cys residues in most GPCR suggests that this intramolecular disulfide bridge is a structural feature common to the entire family of receptors. After optimization, the whole receptor structure model was attained. Although the reliability of loop regions remains to be confirmed, the construction of loop regions could make us recognize the receptor model as a whole.

Modeling of the ligand-receptor interaction

In our previous work, we had carried out semiempirical quantum chemical calculations on THPB (unpublished data). The results indicated that D ring was important for THPB to bind with the receptors. So, we docked *l*-SPD into the active sites of receptors and adjusted D ring in the suitable position similar to the phenyl ring of dopamine. After energy minimization, the *l*-SPD-receptor complex models were attained. In these models, the action of the 3 basic residues was in accordance with that in dopamine-receptor complex. The docking energy of *l*-SPD with the receptor was listed in Tab 1.

Because at physiological pH these ligands

Tab 1. Docking energy of ligand-receptor interaction (kcal · mol^{-1}).

Energy	*l*-SPD	protonated *l*-SPD
D_1 receptor		
Total	−121.509	−153.575
Steric	−119.984	−117.292
Electrostatic	−1.525	−36.283
D_2 receptor		
Total	−89.540	−122.176
Steric	−86.262	−93.086
Electrostatic	−3.278	−29.893

would all display a high positive charge around the N atom of the ligand due to protonation, the protonated *l*-SPD was docked into the binding sites of the receptor (Fig 4), too.

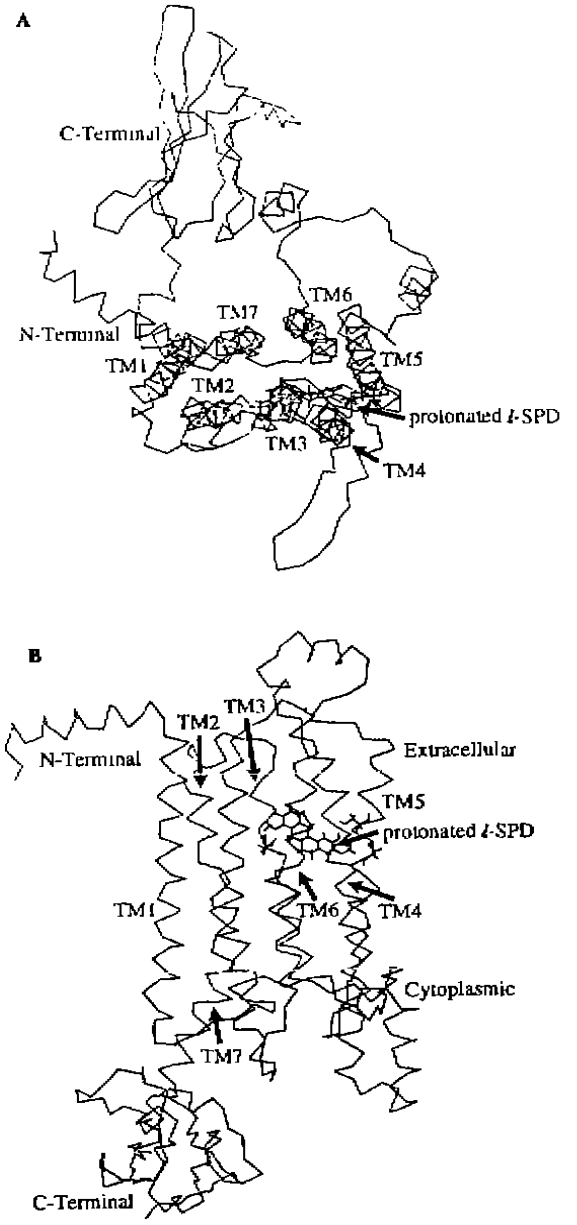

Fig 4. Complex between protonated *l*-SPD and the human D_1 receptor, shown in α-carbon atom only, except for residues Asp103, Ser199, and Ser202. (a) View from the outside of the cell, in a direction parallel to the axes of the α-helices and perpendicular to the membrane of the cell. (b) Side view from a direction perpendicular to the main axes of the 7 α-helices.

After energy minimization, the interactive energies were also shown in Tab 1. From Tab 1, we could see that the electrostatic interaction was enhanced significantly when the N atom of the ligand was protonated, whereas the steric interaction almost remained constant. It suggested that the ligand interacted with the receptor through protonation. Furthermore, the total interaction energy of *l*-SPD with D_1 receptor was more excellent than that with D_2 receptor, which might be the reason of *l*-SPD binding preferentially toward D_1 receptor. On the total interaction of D_1 and D_2 receptors, the main difference lay in the steric interaction. According to these complex models, we could explain the possible action mechanism of *l*-SPD, as well as other THPB, on dopamine receptors. As shown in Fig 4, the ligand binding site is located in a narrow dihedral cleft, near the extracellular side surrounded by α-helices 3, 4, 5, and 6. That was similar to the activation site of BR. After *l*-SPD entered into the receptor, at first it makes molecular recognition and stabilization due to the electrostatic orientation. Then, the protonated N atom of *l*-SPD could form electrostatic interaction with the carboxy group of residue Asp103 in D_1 receptor (Asp114 in D_2) as well as hydrogen-bonding interaction. The hydroxyl and methoxy in D ring of *l*-SPD formed hydrogen-bonding interactions with the hydroxyl groups of residues Ser199 and Ser202 in D_1 receptor (Ser194 and Ser197 in D_2), respectively. The aryl groups formed π — π interactions with the residues Trp99, Phe156, Trp163, and Phe203 in D_1 receptor (Phe164, Phe198, Tyr379 in D_2) (Fig 5).

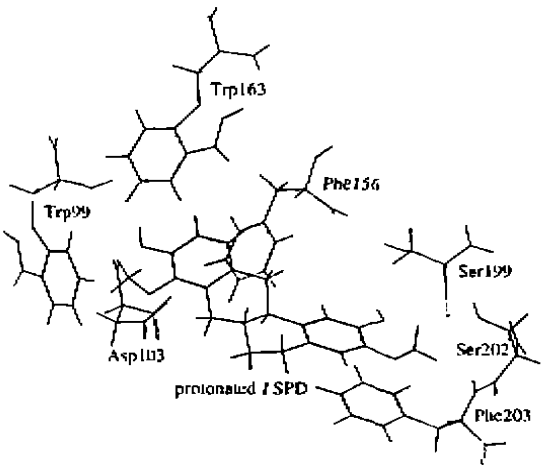

Fig 5. The detailed view of protonated *l*-SPD interaction with the binding sites of human D_1 receptor.

All these electrostatic, hydrogen-bonding and π—π interactions render *l*-SPD high binding

affinity to dopamine receptors, thus possess potent activity. The primary difference between D_1 and D_2 receptor should be attributed to the aryl residues around the ligand, which made more $\pi-\pi$ interactions in D_1 than D_2 receptor.

Molecular modeling could help us visualize and analyse the ligand-receptor interaction. One of the limitations of the modeling approach is that molecules can only be viewed statically in space, but interaction between the ligand and receptor may alter the conformation of the receptor, which results in the coupling to G-proteins and triggers the appropriate signal transduction system response. Hence, to study these models further, it is necessary to apply some other tools and methods, such as quantum mechanics and molecular dynamics simulation.

In summary, we presented here some preliminary *l*-SPD dopamine receptor interaction models. With these models, we had investigated the possible mechanism of THPB action on dopamine receptors.

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四氢原小檗碱类与多巴胺受体相互作用的分子模拟

R966

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关键词 分子模型；多巴胺受体；四氢原小檗碱类；左旋千金藤立定；配体

目的：建立多巴胺受体三维结构模型，结合受体模型从分子水平上研究四氢原小檗碱类(THPB)对多巴胺受体的作用机制。**方法**：以细菌视紫红质的三维结构为模板，在计算机上建立多巴胺 D_1 和 D_2 受体的三维结构模型，选择左旋千金藤立定作为 THPB 的先导化合物，将它分别对接到 D_1 和 D_2 受体的结合位点。**结果**：得到了配体-受体相互作用模型，提出了 THPB 对多巴胺受体可能的作用机制，即配体上质子化 N 原子与受体第三个跨膜结构的 Asp 残基形成静电和氢键作用，配体 D 环上两个取代基与受体第五个跨膜结构的两个 Ser 残基分别形成氢键作用，配体上芳香环与围绕配体的芳香残基形成 $\pi-\pi$ 相互作用。**结论**：该配体-受体相互作用模型具有一定程度的合理性，将有助于设计作用更强的药物。