Molecular modelling of D₂-like dopamine receptors

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Three-dimensional computer models of the rat D₂, D₃ and D₄ dopamine receptor subtypes have been constructed based on the diffraction co-ordinates for bacteriorhodopsin, another membrane-bound protein containing seven transmembrane domains presumed to be arranged in a similar spatial orientation. Models were assembled by aligning the putative transmembrane domains of the dopamine receptors with those of bacteriorhodopsin using sequence similarities, and then superimposing these modelled α -helices on to the bacteriorhodopsin-derived co-ordinates. These models explore the potential hydrogen bonding, electrostatic and stacking interactions within the receptor which may be important for maintaining the conformation of these receptors, and thereby provide target sites for agonist binding. Proposed interactions between the catecholamine ligands and these receptors appear to account for the affinity, although not the specificity, of these agonist ligands for the different dopamine receptor subtypes. Such models will be useful for establishing structure–function relationships between ligands and the dopamine receptors, and may ultimately provide a template for the design of receptor-specific drugs.

INTRODUCTION

Receptors for the neurotransmitter dopamine are important targets for drug action, for example anti-parkinsonian and anti-psychotic drugs. Biochemical, pharmacological and physiological studies indicated that there were two subtypes of dopamine receptor (D₁ and D₂) with different properties and functions (Kebabian & Calne, 1979; Creese & Fraser, 1987). The application of molecular genetic techniques has shown, however, that this may be an oversimplification. At least six dopamine receptor isoforms (D_{1a}, D_{1b}, D₂, D₃, D₄ and D₅) with different properties have been recognized from gene cloning (Sokoloff *et al.*, 1990; Van Tol *et al.*, 1991; Sunahara *et al.*, 1991; Tiberi *et al.*, 1991; and references cited in Strange 1990, 1991), although these may still be grouped into D₁-like (cloned D_{1a}, D_{1b} and D₅) and D₂-like (cloned D₂, D₃ and D₄) subfamilies. There is now a need to understand the mechanism of drug binding and selectivity of drug action at the different receptor isoforms. This can be approached in a variety of ways, including chemical modification of the receptor binding site (Williamson & Strange, 1990) and site-specific mutagenesis (Neve *et al.*, 1991).

A further possible approach is to try to construct three-dimensional models of these receptors and the receptor–ligand complexes. No structural data are available for the dopamine receptors, although they do form part of the super-family of G-protein linked receptors which are thought to be formed from seven transmembrane-spanning α -helices of protein, linked by intracellular and extracellular loops. The ligand-binding site is thought to be formed by the bundling of the α -helices. It seems likely that, overall, the G-protein linked receptors will be constructed on similar principles reflecting their common mechanism of action and that this may extend to the relative positions of amino acids in space. The significant sequence similarities between different receptors, especially in the transmembrane regions that are likely to form the ligand-binding site, provide evidence supporting this point (Strader *et al.*, 1989).

Recently, a three-dimensional structural model was reported for the protein bacteriorhodopsin (Henderson *et al.*, 1990). Bacteriorhodopsin is not a G-protein-linked receptor, but it is a membrane-bound protein composed of seven transmembrane-

spanning α -helices which may be related evolutionarily to the super-family of G-protein-linked proteins. In this report we have modelled the intramembrane structures of the D₂-like receptors (D₂, D₃ and D₄) on the basis of presumed structural similarity with bacteriorhodopsin, and have shown that such models are capable of exploring potential ligand–receptor interactions at a simple level using different agonist ligands. Such studies will be important for the elucidation of the mechanism and determinants of ligand binding, which may ultimately aid in the design of receptor-specific ligands of therapeutic value.

MATERIALS AND METHODS

The cDNA sequence of the rat D₂ dopamine receptor (long form) was kindly provided by Dr. P. Vernier of the CNRS Laboratoire de Neurobiologie Cellulaire et Moléculaire, Gif-sur-Yvette, France. cDNA sequences for the rat D₃ and human D₄ dopamine receptors were retrieved from on-line databases [accession numbers P19020 (SWISSPROT) and X58497 (EMBL) respectively]. The predictions of putative dopamine receptor transmembrane α -helical domains used in the production of the models were those of Zhou *et al.* (1990) for D₂, modified in view of the paper of Van Tol *et al.* (1991) (see Table 1). Identities between the sequences of the dopamine receptors were calculated using the GAP tool (Devereux *et al.*, 1984) and helical Wheel diagrams of the putative dopamine transmembrane α -helices were generated using the HELICALWHEEL tool, both part of the University of Wisconsin Genetics Computer Group package.

The electron diffraction microscopy-derived co-ordinates of the transmembrane α -helical domains of the protein bacteriorhodopsin, used as the template for the production of the dopamine receptor models, were installed as Protein Data Bank co-ordinates into the Polygen Quanta Program (version 3.0A) on a Silicon Graphics Personal Iris computer. The sequence of amino acids in each of the seven helices was recorded from this structure. The sequences of the putative dopamine receptor transmembrane domains were converted into α -helices using the Sequence Builder and Biopolymer Builder ('Fold the Chain') tools in Quanta and were then saved separately. Identities between the predicted dopamine receptor transmembrane α -

Abbreviation used: PHNO, 4-propyl-9-hydroxynaphthoxazine.

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helix sequences and the sequences of their positional equivalents in the bacteriorhodopsin structure were calculated using Match, a program developed for us by S. S. Sturrock and W. G. Day (Biological and Computing Laboratories, The University of Kent, Canterbury, Kent, U.K.). This program provides the local similarity according to the Dayhoff scheme (Schwartz & Dayhoff, 1978) between all possible alignments of two sequences within a window defined by a maximum allowed overhang (five unmatched amino acid residues was used throughout) by effectively sliding the two search sequences past each other, calculating the similarity at each step. The Match program does not allow the insertion of gaps to improve alignments, since the insertion of gaps cannot be accommodated by the superimposition modelling method described below.

Each of the modelled α -helices of the dopamine receptor putative transmembrane domains were then individually superimposed on to their positional equivalents in the bacteriorhodopsin template structure, using the Align and Superpose sub-program of Quanta. Following superimposition, the seven putative transmembrane α -helices of each dopamine receptor were loaded into the Quanta package together as a multiple component structure using their new three-dimensional co-ordinates. These primary models were now refined by compromising the rigorous adherence to maximum identity between model and template helices in transmembrane domains TM2 and TM3, in favour of lower similarity alignments which would account for the known biochemical data concerning the role of certain conserved residues in ligand binding (see the Results section). This method represents an approach which combines the bulk structure matching method (where alignment is made blindly between helices such that their spatial positions coincide) with information which may convey the orientation of residues of similar properties in the helix being fitted with reference to residues in the template helix. The revised alignments were used to produce the secondary models of the dopamine receptors and were subjected to energy minimization using two minimization methods, the method of steepest descents and the Adopted-Basis Newton Raphson method, supported by the Quanta Charmm sub-program.

At this stage the models were transferred to Quanta (version 3.2.1). Here, models of five agonist ligands, dopamine, apomorphine, PHNO, N-0437 and N-0434, were constructed using the Quanta ChemNote sub-program. These models were minimized in the same way as the models of the receptors. The ligand models were fitted into the models of the dopamine receptors in order to show possible modes of binding. The predicted electrostatic interaction between the carboxylate side chain of the conserved Asp residue in TM3 (i.e. Asp-114 in D_2) and the cationic nitrogen group common to all of these ligands was used as an initial anchor point. Other possible interactions, such as that between the Ser side chains in TM5 (i.e. Ser-194, Ser-197 in D_2), of the receptors and the hydroxyl groups of the ligands were then sought and tested using standard hydrogen bonding parameters used within Quanta.

RESULTS AND DISCUSSION

The dopamine receptor subtypes are a highly conserved family of G-protein-linked receptors, whose protein sequences show a 40–50% identity overall and up to 70% similarity when amino acids of similar hydrophobic or ionic characteristics are compared (Table 1). When the same transmembrane domains from different receptor subtypes are compared, the identities within some of these helical domains increase to over 90%, which seems significant given that these regions are likely to contain the

important determinants for ligand binding (Strader *et al.*, 1989). For example, TM2 from D_2 and D_3 receptors are identical except for two amino acids, while TM3 from these two receptors differs in only three amino acids (Table 1). In particular, amino acids which are conserved between these receptors have been predicted to interact with catecholamine ligands. Specifically, an aspartic acid residue in TM3 (equivalent to Asp-114 in D_2) may form an electrostatic interaction with these cationic ligands, and serine residues in TM5 (equivalent to Ser-194 and Ser-197 in D_2) may form hydrogen bonds to the pair of hydroxyl groups on the catechol ring of dopamine. These ideas are based on the conservation of these sites in other G-protein-linked receptors which interact with catecholamine ligands and represent some of the primary contact points for receptor–ligand interaction based mainly on mutagenesis studies of the β_2 adrenergic receptors (Strader *et al.*, 1989). Indeed, a comparison of the protein sequences within the G-protein family of receptors may still have much to reveal about the inter-relatedness of the group and may be of evolutionary significance.

The α -helical models for the proposed transmembrane regions of the D_2 , D_3 and D_4 dopamine receptors, constructed using the QUANTA package, conformed to normal 'idealized' α -helices, with the exception of TM4 for the D_2 and D_3 receptors, and TM5 for the D_4 receptor. In each case, the existence of one or more proline residues located 2–4 turns from the presumed extracellular termini of these helices introduced a marked bend in the α -helical structure. In TM4, while the motif Cys-Pro¹⁶⁹ and Cys-Pro¹⁶⁷ in the D_2 and D_3 receptor subtypes respectively produced a definite bend in the α -helical structure, the existence of Ala-Pro¹⁶⁹ in D_4 had a much more limited effect and more closely resembled the normal α -helical pattern. It is interesting to note, however, that Pro-201 (D_2) and Pro-200 (D_3), flanked by the amino acid residues Val and Phe, do not cause a similar distortion in the TM5 helix domain, but that the existence of Pro²⁰⁴-Cys-Pro in the D_4 receptor subtype again caused a deviation of the transmembrane domain from the classical α -helical conformation. Therefore it is clear that the local environment of a residue will determine its conformation and contribution to the three-dimensional structure. Although the extent or significance of such a structure is not clear, since the torsional constraints exerted on each transmembrane helix by the lipid bilayer of the membrane have not been considered, Lefkowitz & Caron (1988) and Hulme *et al.* (1990) have proposed that these proline-induced deviations may have a role to play in conformational changes in the receptor structure which accompany the activation of receptor upon ligand binding.

Having constructed these α -helices for the D_2 , D_3 and D_4 receptor subtypes, alignments were made to the corresponding helices of the bacteriorhodopsin structure. Although the overall sequence similarity between bacteriorhodopsin and the dopamine receptor subtypes is very low, a reasonable alignment of the helices can be made using the MATCH program, in which local sequence similarity within transmembrane domains is considered using the Dayhoff scheme. By allowing a degree of wobble in the alignment around the matched position favouring maximum sequence similarity, residues of similar properties (i.e. representing conservative amino acid substitutions) could be aligned to point in similar directions in both D_2 , D_3 and D_4 receptor structures and bacteriorhodopsin. This approach combined the bulk structure matching method with information which may convey the preferred orientation of a helix within the membrane. This superimposition of these α -helices on to bacteriorhodopsin co-ordinates led to the production of the first crude structures in which there was a close conformational alignment gained in the case of all helices, with the exception of TM4 and TM5 as discussed above.

Table 1. Alignment of the protein sequence for seven selected transmembrane domains of the rat dopamine D₂, D₃ and D₄ receptors and bacteriorhodopsin (BR)

The numbering system for the D₂ receptor has been given, and conserved amino acids mentioned in the text in the dopamine receptor sequences have been underlined and are shown in bold.

Receptor		Amino acid sequence		Transmembrane domain
D ₂	-33-	HYNYYAMLLTLLIFIIVFGNVLVCMASV	-60-	1
D ₃		PHAYYALSYCALILAIIFGNGLVCAAVL		
D ₄		QGAAALVGGVLLTGAVLAGNSLVCVSVA		
BR		GRPEWIWLALGTALMGLGTLTYFLVKGMG		
D ₂	-71-	YLIVSLAVADLLVATLVMPWVVYLEVV	-97-	2
D ₃		YLVVSLAVADLLVATLVMPWVVYLEVT		
D ₄		SFIVSLAAADLLLALLVLPFLVYSEVQ		
BR		PDAKKFYAITTLVPAIAFTMYLSMLLG		
D ₂	-107-	CDIFVTLDVMMCTASILNLCAISID	-131-	3
D ₃		CDVFVTLDVMMCTASILNLCAISID		
D ₄		CDALMAMDVMLCTASIFNLCAISVD		
BR		YWARYADWLFTPLLLLDLALLVDA		
D ₂	-152-	VTVMIAIVWVLSFTISCPLLFGLN	-175-	4
D ₃		VALMITAVWVLAFAVSCPLLFGFN		
D ₄		QLLLIGATWLLSAAVAAPVLCGLN		
BR		ADQGTILALVGADGIMIGTGLVGA		
D ₂	-187-	PAFVVYSSIVSFYVPFIVTLLVYIK	-210-	5
D ₃		PDFVIYSSVVSFYVPFGVTVLVYAA		
D ₄		RDYVVYSSVCSFFLPCPLMLLLYWA		
BR		YRFVWVAISTAAMLYILYVLFPGFT		
D ₂	-374-	MLAIVLGVFIICWLPFFITHILNI	-398-	6
D ₃		MVVIVLGAFIGVWLPFFLTHVLNT		
D ₄		VLPVVVGAFLLCWTPFFVVIITQA		
BR		TFKVLNRNVTVLWSAYPVVWLIGS		
D ₂	-405-	PPVLYSAFTWLGYNVNSAVNPVIYT	-428-	7
D ₃		SPELYRATTWLGYNVNSALNPVIYT		
D ₄		PPRLVSAFTWLGYNVNSALNPVIYT		
BR		ETLLFMVLDVSAKVGFLILLRSR		

The bacteriorhodopsin structure was now subtracted to leave the first model. When the conserved residues of interest were identified by representing their side chains on the α -carbon backbone structure of the D₂ receptor transmembrane domains, the most striking feature of this first model was the immediate alignment of Ser-194 and Ser-197 (TM5) facing straight into the pocket bounded by the helices, a position ideally suited to ligand interaction. Similarly, Tyr-417 and His-394, which have been implicated in ligand binding on the basis of chemical modification (Srivastava & Mishra, 1990) and pH studies (Williamson & Strange, 1990), were also within the putative ligand-binding pocket. However, the model had to be refined by reducing the degree of 'homology matching' between the D₂ and bacteriorhodopsin transmembrane sequences to ensure that the conserved aspartic acid residues in helix 3 (Asp-114) and helix 2 (Asp-80) were located on the inside of the receptor. This refinement of the model seemed acceptable, since the sequence alignments provide only a guide to orientation. These residues are both highly conserved in catecholamine receptors and are thought to be important respectively in the interaction with agonist and antagonist ligands (Strader *et al.*, 1989) and in maintaining the receptor conformation by interaction with monovalent cations (Neve *et al.*, 1991). Since the three-dimensional models for D₃ and D₄ receptor subtypes displayed an almost identical spatial orientation of the equivalent conserved amino acid residues, the features described above for the D₂ model are equally applicable

to these other receptor subtypes, and therefore the following discussion will focus only on the D₂ receptor for clarity.

The representation of the model including the ligand dopamine (Fig. 1) shows the new spatial distribution of the Asp residues of interest within helices 2 (Asp-80) and 3 (Asp-114) with their side chains located within the central cavity. The distance between the Asp-114 carboxy groups and the hydroxy group of Tyr-417 is shown to be in the region of 0.3–0.4 nm (3–4 Å). This is interesting, since Hulme *et al.* (1990) proposed that these residues, which are conserved in and specific to the cationic amine receptors, would be in close proximity in these regions and therefore may be involved in ligand binding and receptor activation. Although this distance would appear to be too great for strong hydrogen bonds to form, the possibility of a weak interaction cannot be ruled out. Several other interactions have been shown to exist between the transmembrane domains of each of the receptor subtypes from examination of the model in the absence of the ligand. For example, there is a strong stacking interaction between Tyr-417 (TM7) and Phe-110 (TM3), and also strong hydrogen bonding interactions between Trp-387 (TM6) and Ser-194 (TM5).

These putative models now provided a template for investigating ligand–receptor interactions at a simple level using various well-defined agonist ligands. The agonists chosen for 'fitting' in the models, in addition to dopamine, were apomorphine, N-0437, N-0434 and PHNO. Each of the agonist

Fig. 1. Three dimensional model of the rat D_2 dopamine receptor interacting with dopamine

The model shows the main chain α -carbons of the transmembrane helices viewed from the extracellular side of the membrane, plus some selected amino acid side chains: Asp-80 (helix 2), Asp-114 (helix 3), Ser-194 and -197 (helix 5), His-394 (helix 6) and Tyr-417 (helix 7). (Asp-114 is visible between the hydroxyl group of Tyr-417 and the amino group of dopamine.) The primary attachment points for the dopamine are presumed to be the electrostatic interaction with the Asp-114 residue and strong hydrogen bonding interactions with the Ser-194 and -197 residues with the amino and catechol hydroxyl groups in the ligand respectively.

Fig. 2. Alternative view of the dopamine interaction with the rat D_2 receptor

This view shows the potential hydrogen bonding interactions between the ligand and the Ser-194/197 groups in helix 5 and the electrostatic interaction with Asp-114 in helix 3. The transmembrane domains have been clipped back to highlight the additional stacking interaction of the ligand with Trp-387 (helix 6).

ligands contains within its structure the nitrogen and one or two hydroxyl groups presumed to be the primary attachment points in ligand binding. Moreover, in each case, the arrangement of these 'core' (essential) atoms is conserved, with one hydroxyl group separated from the nitrogen by four carbon atoms. At physiological pH these ligands would all display a high positive charge around the nitrogen atom of the ligand due to protonation, which would be capable of strong interaction with the Asp-114 of the D_2 . (For clarity, however, the ligands have been displayed in their unprotonated forms in Figs. 1–3.) In addition, all the agonists displayed a strong hydrogen bonding interaction with Ser-194, and additionally with Ser-197 for the agonists containing two hydroxyl groups (dopamine and apomorphine). There-

fore, in agreement with structure-function analyses of other G-protein-linked receptors using site-directed mutagenesis (Strader *et al.*, 1989; Fraser *et al.*, 1989), the model appears to favour strongly the Asp residue buried in TM3 (Asp-114) and the Ser-194 (and Ser-197) residues in TM5, as the primary attachment sites for catecholamine ligands with the dopamine receptor subtypes (Figs. 2 and 3). The agonist-binding region of these receptors is therefore located about one-third of the way into the membrane. Additional interactions may occur between the side groups of these ligands and the different receptor subtypes, which may account to some degree for the specificity in ligand binding. In each case, the binding of each of these agonists produces a strong stacking interaction between the aromatic ring of dopamine (and its equivalent in the other agonists) and Trp-387 of helix 6 (Figs. 2 and 3). Also, there is a strong stacking interaction between the thiophene ring of N-0437 (or the equivalent benzene ring of N-0434) and the Tyr-417 of helix 7 (Fig. 3b), and between this Tyr residue and the Phe-110 of helix 3 of the D_2 receptor and the corresponding residues in D_3 and D_4 .

Therefore, in each case, stabilizing interactions between residues in different but spatially proximal transmembrane domains [i.e. Tyr-417 (TM7) and Phe-110 (TM3); Trp-387 (TM6) and Ser-194 (TM5); Tyr-417 (TM7) and Asp-114 (TM3)] may be responsible for maintaining the unoccupied receptor in a conformation suitable for interaction with activating ligands. The binding of agonists to the receptor then depends on similar interactions between the receptor and ligand. For example, the relatively weak interaction between Tyr-417 and Asp-114 in the unoccupied receptor is replaced by a strong electrostatic interaction between the carboxylate group of the Asp residue and the amino group of the agonist ligands. Similarly, the strong hydrogen bonding interaction between Trp-387 and Ser-194 and the stacking interaction between Tyr-417 and Phe-110 in the unoccupied receptors is replaced by stacking and hydrogen bonding interactions between these residues and the different agonists upon ligand binding (Figs. 2 and 3).

At present, the models indicate that many of the agonist ligands interact with the same residues in the dopamine receptors and that there is no obvious difference between D_2 , D_3 and D_4 subtypes which could account for the specificity of ligand binding. However, thermodynamic calculations of the free energy changes afforded by 10-fold changes in the affinity of any ligand for a particular receptor subtype represent a stabilization of the receptor-ligand complex by about $5.9 \text{ kJ} \cdot \text{mol}^{-1}$ ($1.4 \text{ kcal} \cdot \text{mol}^{-1}$). Given that the energy associated with hydrogen bonds and electrostatic interactions is significantly greater than this [about $20.9 \text{ kJ} \cdot \text{mol}^{-1}$ ($5 \text{ kcal} \cdot \text{mol}^{-1}$), Fersht, 1985], it is unlikely that the modelling approach would be able to distinguish receptor-specific interactions between different ligands, except where the affinity difference is greater than several orders of magnitude. This is the case for some agonist interactions at D_2 versus D_3 receptors. Even then, unless a change in a single amino acid is involved, it may still be difficult to distinguish receptor-specific interactions by modelling, since it is more likely that more subtle changes in several amino acids may determine specificity. In addition, one of the limitations of the modelling approach is that molecules can only be viewed statically in space, and therefore any conformational change associated with the activation of the receptor will not be evident within its structure. 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.

During the preparation of this paper, Hibert *et al.* (1991) published similar data on the three-dimensional modelling of

Fig. 3. Three-dimensional models of the rat D₂ receptor with the agonist ligands (a) apomorphine (b) N0437 and (c) PHNO

In each case, the primary attachment sites for each of the ligands are Asp-114 (helix 3) and Ser-194/197 (helix 5). In addition, stacking interactions with Trp 387 (helix 6) are shown for (a) (b) and (c). In the case of N0437, there is an additional stacking interaction between the thiophene ring of the ligand and the Tyr 417 residue in Helix 7.

other neurotransmitter G-protein-coupled receptors, including the dopamine D₁, D₂ and D₃ subtypes and their interaction with the native ligand, dopamine. These models were constructed using a similar approach to that described here, with the exception that 'standardized' α -helices were directly superimposed on to

bacteriorhodopsin coordinates, and therefore did not display the deviations from the α -helical pattern demonstrated here. In agreement with our model and with other studies (Strader *et al.*, 1989; Fraser *et al.*, 1989), they also identified the conserved Asp residue (Asp-114 in the D₂ receptor) in TM3 as an important site for electrostatic interaction with the agonist dopamine, and Ser residues (194 and 197 in the D₂ receptors) in TM5 as providing hydrogen bond interactions with the 3- and 4-hydroxyl groups of the catechol ring. Both models also postulate an interaction between a Tyr in TM7 (417 in the D₂ receptors) and the Asp in TM3 which may be important for receptor conformation and function in cationic amine receptors (Hulme *et al.*, 1991). Although this Tyr in TM7 does not seem to interact directly with dopamine, we have identified some potential additional stacking interactions between this residue and other agonist ligands, e.g. N0434, N0437. In contrast, the stacking interaction between Phe-198 (TM5) and Phe-391 (TM6) and the catechol ring of the agonist dopamine, predicted in the model of Hibert *et al.* (1991), is not shown in our model, as the distances between these groups was found to be too great for interaction.

Although these models have proven useful for identifying potential amino acids conferring ligand-binding specificity between G-protein-linked receptors (Hibert *et al.*, 1991), they do not appear to be refined enough to indicate the basis of specificity differences between the different D₂-like dopamine receptors. Nevertheless, these models now provide a good experimental tool for planning further biochemical and molecular biological studies of the dopamine receptor subtypes, specifically for structure-function analysis using site-directed mutagenesis as already successfully applied to other G-protein-coupled receptors (adrenergic and muscarinic receptors). These models remain hypothetical and will have to be refined further in light of any new biological information obtained from site-directed mutagenesis studies or three-dimensional structural studies, which ultimately may provide a template for the specificity of the interaction of ligands with the different receptor subtypes as a route to the design of new drugs.

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