Mapping the Binding Site Pocket of the Serotonin 5-Hydroxytryptamine$_{2A}$ Receptor

Ser$^{3.36(159)}$ PROVIDES A SECOND INTERACTION SITE FOR THE PROTONATED AMINE OF SEROTONIN BUT NOT OF LYSERGIC ACID DIETHYLAMIDE OR BUFOTENIN*

(Received for publication, April 17, 1996)

Niva Almulaat, Barbara J. Ebersole§, Daqun Zhang, Harel Weinstein¶, and Stuart C. Sealfont**

From the +Fishberg Research Center in Neurobiology, Departments of §Anesthesiology, Physiolog and Biophysics, ¶Pharmacology, and **Neurology, Mount Sinai School of Medicine, New York, New York 10029

Like other amine neurotransmitters that activate G-protein-coupled receptors, 5-hydroxytryptamine (5-HT) binds to the 5-HT$_{2A}$ receptor through the interaction of its cationic primary amino group with the conserved Asp$^{3.32(155)}$ in transmembrane helix 3. Computational experiments with a 5-HT$_{2A}$ receptor model suggest that the same functional group of 5-hydroxytryptamine also forms a hydrogen bond with the side chain of Ser$^{3.36(159)}$, which is adjacent in space to Asp$^{3.32(155)}$. However, other 5-HT$_{2A}$ receptor ligands like lysergic acid diethylamide (LSD), in which the amine nitrogen is embedded in a heterocycle, or N,N-dimethyl 5-HT, in which the side chain is a tertiary amine, are found in the computational simulations to interact with the aspartate but not with the serine, due mainly to steric hindrance. The predicted difference in the interaction of various ligands in the same receptor binding pocket was tested with site-directed mutagenesis of Ser$^{3.36(159)}$ → Ala and Ser$^{3.36(159)}$ → Cys. The alanine substitution led to an 18-fold reduction in 5-HT affinity and the cysteine substitution to an intermediate 5-fold decrease. LSD affinity, in contrast, was unaffected by either mutation. N,N-Dimethyl 5-HT affinity was unaffected by the cysteine mutation and had a comparatively small 3-fold decrease in affinity for the alanine mutant. These findings identify a mode of ligand-receptor complexation that involves two receptor side chains interacting with the same functional group of specific serotonergic ligands. This interaction serves to orient the ligands in the binding pocket and may influence the degree of receptor activation.

One goal of structure-activity studies of G-protein-coupled receptors (GPCR)$^1$ is to develop an understanding of the nature and consequences of ligand-receptor interactions at a molecular level. The serotonergic 5-HT$_{2A}$ receptor is a member of the GPCR superfamily for which such studies have identified key interactions in the ligand-receptor complexes (1–9). One notable group of ligands for this receptor are the serotonergic hallucinogens, such as LSD (lysergic acid diethylamide) and N,N-dimethyl 5-HT (bufotenin), which have high affinity for the 5-HT$_{2A}$ receptor. Studying the receptor’s binding pocket and identifying the molecular mechanisms that determine ligand affinity, specificity, and coupling efficiency may help elucidate the basis for the special biological effects of these chemicals.

The principal binding determinants of neurotransmitter GPCRs have been most fully elucidated in the adrenergic receptors (for review see Refs. 10 and 11), where they are located within the helical transmembrane domains. The 5-HT$_{2A}$ receptor, like the other receptors for biogenic amines, including the adrenergic, dopaminergic, and muscarinic receptors, has an aspartate residue at a homologous location in the putative third transmembrane helix (TMH) domain (Ref. 12; see Fig. 1). Site-directed mutagenesis studies with these receptors indicate that, for most ligands, an interaction between the basic nitrogen of the ligand and the carbonyl side chain of the TMH 3 aspartate stabilizes ligand binding (5, 13–17).

Simulations of ligand-receptor complexes of the 5-HT$_{2A}$ receptor using a three-dimensional computational model (6, 7) suggest a complex array of interactions connecting TMH 3 side chains and specific ligands. The same charged amino group of 5-hydroxytryptamine that interacts with the TMH 3 aspartate is predicted to form a hydrogen bond with the side chain of a second TMH 3 locus, Ser$^{3.36(159)}$. In the molecular model of the receptor, this residue is positioned on the same face of the helix as Asp$^{3.32(155)}$. It forms a hydrogen bond to the backbone carbonyl of the residue at position 1–4 from it, as expected (17). The simulations show a very favorable positioning of the cationic amine group of 5-HT for hydrogen bonding both the aspartate and the serine. However, some other serotonergic ligands, such as LSD and N,N-dimethyl 5-HT, are found to be unable to hydrogen bond that serine because of the steric hindrance introduced by the groups surrounding the amine in the ligand. Extensive simulations show that the ligands in which the amino group is ring-embedded or dimethyl-substituted would interact only with the aspartate side chain.$^2$ Because these predictions, if validated, provide a fine resolution mapping of this component of the binding pocket that may explain why ligands vary in their capacity to activate the receptor, this hypothesis was tested by mutating Ser$^{3.36(159)}$ → Ala and Ser$^{3.36(159)}$ → Cys. The results from ligand binding experiments with the mutant receptors were analyzed with respect to the differences from molecular dynamic simulations of the receptor-ligand complexes.

MATERIALS AND METHODS

Chemicals—LSD and N,N-dimethyl 5-HT were obtained from NIDA, National Institutes of Health. All other unlabeled ligands were from Sigma or Research Biochemicals International (Natick, MA).

Receptor Numbering Scheme—Residues are numbered according to a

*This work was supported by National Institutes of Health Grants RO1 DA09088, RO1 DA09083, T32 DA07135, and K05 DA00060. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed: Fishberg Center for Neurobiology Research, Box 1065, Mount Sinai School of Medicine, One Gustave Levy Pl., New York, NY 10029. Tel.: 212-241-7075; Fax: 212-996-9785.

1 The abbreviations used are: GPCR, G-protein-coupled receptor; LSD, lysergic acid diethylamide; 5-HT, 5-hydroxytryptamine; TMH, transmembrane helix.

2 D. Zhang and H. Weinstein, manuscript in preparation.
consensus numbering scheme described in detail elsewhere (18). The TMH 3 residues are numbered in reference to the most conserved residue in this helix, which is the arginine at the bottom of the helix. This most conserved locus is designated 3.50; adjacent residues are 3.49 and 3.51. The residue number in parentheses indicates the amino acid identity using standard amino-terminal based numbering. Thus the TMH 3 aspartate conserved among neurotransmitters is designated Asp3.32(155) in the 5-HT2A receptor, and the serine studied here is Ser3.36(159).

DNA Constructs and Transfection—The cDNA clone encoding the human 5-HT2A receptor was graciously provided by Dr. Alan Saltzman (19). Mutations were introduced as described previously (9). The expression vectors, pcDNA-Amp (binding assays) or pcDNA-3 (functional assays; Invitrogen, San Diego, CA) were used to transfect COS-1 cells (ATCC, Rockville, MD) using Lipofectamine (Life Technologies, Inc.).

Coupling and Ligand Binding Assays—Hydrolysis of [3H]phosphatidylinositol was assayed as described previously (9). Saturation and competition assays using [3H]ketanserin (DuPont NEN) were carried out as described previously (9). Nonspecific binding was defined with 10 μM methysergide. For competition studies, the concentration of [3H]ketanserin was 0.7–1.2 nM. Protein content was determined by the method of Lowry (20). Each binding assay tube contained 30–60 μg of membrane protein.

Data Analysis—Curve-fitting for data from saturation, competition, and phosphatidylinositol assays was carried out with the graphics software Kaleidagraph (Synergy Software, Reading, PA) as described previously (9).

Molecular Modeling of 5-HT2A Receptor Mutants and Computational Simulations of Interactions with Ligands—The model of the transmembrane helix bundle of the 5-HT2A receptor and its development have been reported previously (7, 22). A primary objective in the early development of this model was to reflect the pharmacological data on the structure-activity relations of 5-HT2A receptor ligands. The close agreement between the results from computational simulations of the effects of ligand binding with this receptor model and quantitative pharmacological data (6–9) supports its use to explore the role of Ser3.36(159) in the interaction with specific ligands.

The molecular models were energy-optimized, and molecular dynamics runs were carried out with the CHARMM program (23), using the same protocol as described previously (7). 5-HT, N,N-dimethyl 5-HT, and LSD were positioned in the binding site in the same manner as described, to preserve the equivalence of their initial positioning in the binding pocket (7). The same parameters for molecular dynamic simulations were used as reported previously (7). Simulation of the ligand/

RESULTS AND DISCUSSION

Affinities of Wild-type and Mutant Receptors—The hypothesis that ligands differ in their interaction with Ser3.36(159) was
tested by mutating this residue to cysteine and to alanine and by characterizing the wild-type and mutated receptors when expressed in COS-1 cells. The receptor mutants had comparable affinities for ketanserin, as determined by saturation binding (Table I).

The effects of the mutations studied on agonist affinity correlated fully with the expectations from the computational modeling of the wild-type and the alanine mutant complexes with the various ligands. The affinity of 5-HT was greatly affected by the alanine substitution (18-fold decrease), and the presence of cysteine at this position caused an intermediate 5-fold decrease in affinity (Fig. 2A). In contrast, the affinity of the N,N-dimethyl congener of 5-HT, bufotenin, showed no significant change with the cysteine mutation and a comparatively small 4-fold decrease in affinity with the alanine substitution (Fig. 2B). LSD affinity was not significantly affected by either mutation (Fig. 2C).

The affinity of the ligands for the mutant constructs is consistent with the inference from the studies of many neurotransmitter receptors, including the 5-HT2A receptor (5), that the interaction with Asp3.32 provides a major component of the binding affinity. The large decrease in the affinity of 5-HT found with mutation of Ser3.36(159) is consonant with the effect of elimination of a hydrogen bond-type interaction between the amino group of serotonin and the serine side chain. Because the Ser3.36(159) → Cys mutant constructs were not subjected to a complete dynamic modeling, the exact mechanism for the intermediate affinity changes is less clear. Notably, the cysteine side chain is a less favorable hydrogen bond acceptor than is that of serine (24), so that the moderate effect on 5-HT affinity seen with the cysteine mutation may reflect a weaker interaction between the mutant side chain and the cationic amine group of 5-HT. In contrast, the cationic amine of LSD is embedded in a ring, as shown in Fig. 2C, and is not available for the secondary hydrogen bonding. The correspondence of the ring nitrogen of LSD to the alkyl nitrogen of 5-HT is consistent with known structure-activity data (25). The lack of effect on LSD affinity of either substitution for the wild-type serine indicates that there is no interaction between serine and LSD in the wild-type receptor. The affinity of N,N-dimethyl 5-HT is unaffected by the cysteine mutation, and a very small decrease in affinity is observed with the alanine substitution. In view of the large changes seen with the same mutations for 5-HT, the relatively small decrease in N,N-dimethyl 5-HT affinity seen with one of the mutations is likely to represent an indirect effect arising from altered positioning of other side chains in the binding pocket of the Ser → Ala mutant. Because of its dimethyl substitution, the basic nitrogen of this ligand could not hydrogen-bond to the serine and also interact with the aspartate. The comparison of the results obtained with 5-HT, N,N-dimethyl 5-HT, and LSD support the presence of a direct interaction with the serine side chain only for 5-HT.

Molecular Dynamic Simulations of Ligand-Receptor Complexes—The ligands studied in binding assays were also studied in molecular dynamics simulations of ligand-receptor complexes. The results of these simulations are shown in Fig. 3. In the 5-HT-complexed serotonin receptor model, the amino group is positioned equidistant from the side chains of the aspartate and the serine (Fig. 3A). The distance from the serine, 1.8 Å, is an appropriate distance for hydrogen bonding. In contrast, the corresponding amine both in N,N-dimethyl 5-HT (Fig. 3B) and in LSD (Fig. 3C) are located at nearly 5 Å from the serine side chain of the wild-type 5-HT2A receptor model. A, 5-HT-receptor complex; B, N,N-dimethyl 5-HT-receptor complex; C, LSD-receptor complex. The distance between Asp3.32(155) and the cationic amine of LSD is 2.8 Å.
The acidic side chain of the wild-type receptor; TMH 3 can determine its ability to activate the receptor. and that the precise positioning of a ligand with respect to TMH 3 provides a crucial anchor for neurotransmitter agonists or antagonists. Thus, while all three agonists interact with the aspartate side chain, only 5-HT interacts with the serine. Consequences of Interaction with Ser 3.36(159)—The presence of agonists, both structurally similar and closely related to 5-HT, can provide evidence for the presence or absence of a hydrogen bond with Ser 3.36(159). We demonstrate that the free amino group of 5-HT interacts with a hydrogen bond and can influence the intrinsic activity of this residue. The altered positioning of structurally similar ligands may provide an explanation for their differing functional effects. Notably, both N,N-dimethyl 5-HT and LSD are partial agonists for the 5-HT 3 receptor. A variety of experiments suggest that TMH 3 provides a crucial anchor for neurotransmitter agonists and that the precise positioning of a ligand with respect to TMH 3 can determine its ability to activate the receptor. Strader et al. found that altering the length of the TMH 3 acidic side chain of the β-adrenergic receptor, by mutating Asp 3.32(113) to Glu, caused some agonists to develop partial agonist activity (21). This result shows that the capacity of the ligand to activate the receptor relates to its positioning between TMH 3 and other interaction sites. In molecular dynamic simulations of ligand-receptor complexes, we have found that partial agonists cause a smaller degree of helix rearrangement than do full agonists, whether they are structurally related to 5-HT (7) or to other families of 5-HT 3 ligands (8). It is expected, therefore, that the ability of a ligand to activate a receptor depends on the geometrical arrangement of the ligand in the ligand-receptor complex. As the presence or absence of an interaction with the second TMH 3 site in the 5-HT 3 receptor, Ser 3.36(159), alters this geometry, it can determine the differing potential of closely related compounds to activate the receptor. The contribution of interaction with Ser 3.36(159) in positioning the ligand and thereby determining efficacy is supported by the concentration-response curves obtained with the wild-type and Ser 3.36(159)→Ala mutant receptors. Whereas N,N-dimethyl 5-HT is a partial agonist for the wild-type receptor (Fig. 4A), the intrinsic activities of 5-HT and N,N-dimethyl 5-HT are similar for the Ser 3.36(159) receptor mutant (Fig. 4B). The intrinsic activity of N,N-dimethyl 5-HT relative to 5-HT was 0.73 ± 0.07 for the wild-type receptor and 0.96 ± 0.13 for the mutant (n = 4, p < 0.05). We find that the free amino group of 5-HT interacts with a second side chain of TMH 3 of the serotonin 5-HT 3 receptor. To our knowledge, this is the first example of two side chains of a receptor interacting specifically with the same functional group on the ligand. The positioning of the ligands in the binding site relates to their ability to form this second hydrogen bonding interaction and can influence their capacity to activate the receptor. The results illustrate how a detailed map of the binding site pocket of the serotonin 5-HT 3 receptor obtained through combined mutagenesis and dynamic receptor modeling can lead to a fuller understanding of the molecular basis for the effects of serotonergic ligands.

Acknowledgments—We thank Dr. Saul Maayani for helpful discussions and Irina Ivanova for superb technical assistance.

REFERENCES
1. Choudhary, M. S., Sachs, N., Uluer, A., Glennon, R. A., Westkaempfer, R. B., & Roth, B. L. (1993) Mol. Pharmacol. 43, 755–761.
2. Choudhary, M. S., Sachs, N., Uluer, A., Glennon, R. A., Westkaempfer, R. B., & Roth, B. L. (1995) Mol. Pharmacol. 47, 450–457.
3. Choudhary, M. S., Sachs, N., Uluer, A., Glennon, R. A., Westkaempfer, R. B., & Roth, B. L. (1992) Mol. Pharmacol. 42, 627–633.
4. Johnson, M. P., Lohanchic, R. J., Baer, M., & Nelson, D. L. (1994) Mol. Pharmacol. 45, 277–286.
5. Wang, C.-D., Gallaher, T. K., & Shih, J. C. (1993) Mol. Pharmacol. 43, 531–540.
6. Luo, X., Zhang, D., & Weinstein, H. (1994) Protein Eng. 7, 1441–1448.
7. Zhang, D., & Weinstein, H. (1993) J. Med. Chem. 36, 934–938.
8. Zhang, D., & Weinstein, H. (1993) Med. Chem. Res. 3, 357–369.
9. Selffon, S. C., Chi, L., Ebersole, B. J., Rodic, V., Zhang, D., Ballesteros, J. A., & Weinstein, H. (1995) J. Biol. Chem. 270, 16683–16688.
10. Kobilka, B. (1992) Annu. Rev. Neurosci. 15, 87–114.
11. Strader, C. D., Fong, T. M., Tota, M. R., Underwood, D., & Dixon, R. (1994) Annu. Rev. Biochem. 63, 101–132.
12. Wang, C.-D., Buck, M. A., & Fraser, C. M. (1991) Mol. Pharmacol. 40, 168–179.
13. Strader, C. D., Sigal, I. S., Register, R. B., Candéreore, M. R., Rands, E., & Dixon, R. A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4384–4388.
14. Strader, C. D., Sigal, I. S., Candéreore, M. R., Rands, E., & Dixon, R. A. (1988) J. Biol. Chem. 263, 10267–10271.
15. Ho, B. Y., Karschini, A., Brandner, T., Davidson, N. & Lester, H. A. (1992) FEBS Lett. 312, 259–262.
16. Fraser, C. M., Wang, C. D., Robinson, D. A., Gocayne, J. D., & Venter, J. (1991) EMBO J. 10, 99–107.
17. Gray, T. M. & Matthews, B. W. (1984) J. Mol. Biol. 175, 75–81.
18. Ballesteros, J. A. & Weinstein, H. (1995) Methods Neurosci. 25, 366–428.
19. Saltzman, A. G., Morbo, B. T., Whitman, M. M., Ivasvchenko, Y., & Felder, S. (1991) Biochem. Biophys. Res. Commun. 181, 1469–1473.
20. Ewing, W. H., Rosenthal, N., J., & Fiala, K. (1991) J. Mol. Biol. 213, 387–397.
21. Strader, C. D., Candéreore, M. R., Hill, W. S., Dixon, R. A. F. & Sigal, I. S. (1989) J. Biol. Chem. 264, 16470–16477.
22. Zhang, D. & Weinstein, H. (1994) FEBS Lett. 377, 207–212.
23. Brooks, R. R., Bucuholi, E. R., Olafson, B. D., & Swaminathan, S. (1983) J. Mol. Biol. 167, 187–217.
24. Grozdev, L. M., Rader, S. D., Fletterick, R. J., & Cohen, F. E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 99–107.
25. Glennon, R. A., Wetkamp, R. B., & Bartsch, P. (1991) in Serotonin Receptor Subtypes: Basic and Clinical Aspects (Peroutka, S. J., ed) pp. 19–64, Wiley-Liss, Inc., New York.
Mapping the Binding Site Pocket of the Serotonin 5-Hydroxytryptamine\(_2\_A\) Receptor: Ser3.36(159) PROVIDES A SECOND INTERACTION SITE FOR THE PROTONATED AMINE OF SEROTONIN BUT NOT OF LYSERGIC ACID DIETHYLAMIDE OR BUFOTENIN

Niva Almula, Barbara J. Ebersole, Daqun Zhang, Harel Weinstein and Stuart C. Sealfon

\textit{J. Biol. Chem.} 1996, 271:14672-14675.
doi: 10.1074/jbc.271.25.14672

Access the most updated version of this article at http://www.jbc.org/content/271/25/14672

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 24 references, 12 of which can be accessed free at http://www.jbc.org/content/271/25/14672.full.html#ref-list-1