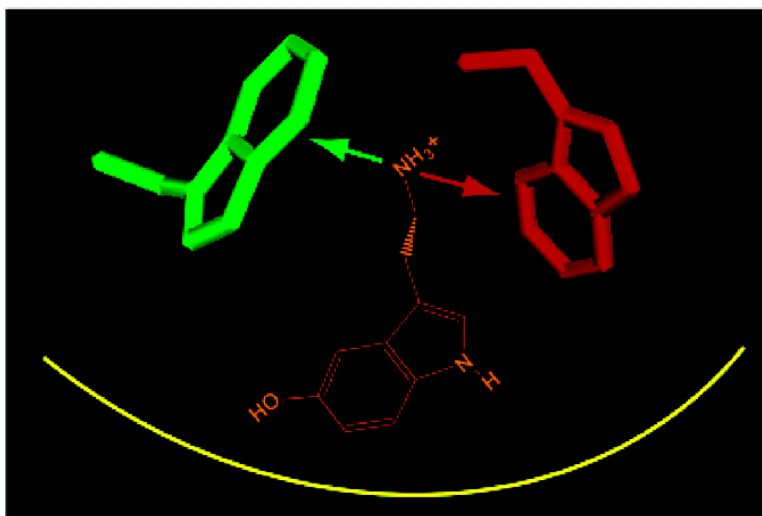## Communication

Different Binding Orientations for the Same Agonist at Homologous Receptors: A Lock and Key or a Simple Wedge?

Ting-Wei Mu, Henry A. Lester, and Dennis A. Dougherty

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 6 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Different Binding Orientations for the Same Agonist at Homologous Receptors: A Lock and Key or a Simple Wedge?

Ting-Wei Mu, Henry A. Lester, and Dennis A. Dougherty*

Division of Chemistry and Chemical Engineering and Division of Biology, California Institute of Technology, Pasadena, California 91125

Received February 21, 2003; E-mail: dad@igor.caltech.edu

The "lock and key" model for drug−receptor interactions has evolved over time. Certainly, proteins and small molecules are more flexible than their metaphorical counterparts, and some adaptation, one to the other, is common. Nevertheless, the notion that proteins craft well-defined binding sites that are custom tailored to their ligand remains central to analyses of biological recognition and drug discovery. We describe here studies of two highly homologous binding sites for the neurotransmitter serotonin (5-HT). The receptors are the 5-HT$_3$ receptor[1] (mouse) and the MOD-1 receptor[2] of *Caenorhabditis elegans*. Both are ligand-gated ion channels of the Cys-loop superfamily of receptors, which also includes the nicotinic acetylcholine (nAChR), GABA$_A$, glycine, and Glu-Cl receptors.[3] As such, 5-HT$_3$ and MOD-1 have evolved from a common ancestral gene. Both are activated by serotonin. In the region of the agonist binding site, the two are highly homologous. MOD-1 is 24% identical to and 45% similar to 5-HT$_3$. Importantly, key aromatic residues of the agonist binding site are conserved in the two (Figure 1).

Most of what we know about the agonist binding site of the Cys-loop family of receptors comes from studies of the nAChR.[4] Extensive biochemical studies implicated a large number aromatic of residues as contributing to the binding site. These are associated with five so-called "loops" (A−E), different regions of sequence space that define the agonist binding site. Here we discuss only loops B, C, and D, as these are the most conserved among the receptors considered (Figure 1). Studies of the nAChR using unnatural amino acid mutagenesis identified a key tryptophan, Trp 149 in loop B, as making a potent cation−π interaction with ACh in the binding site.[5] The key to this study was the incorporation of a series of fluorinated Trp derivatives (F$_n$-Trp, $n = 1$−4), showing that agonist potency tracked linearly with the cation−π binding abilities of the fluorinated rings (Table 1). These findings were subsequently supported by the crystal structure of the ACh-binding protein (AChBP, Figure 2), a soluble protein that is highly homologous to the agonist binding site of the nAChR.[6] This structure defines the agonist binding site as an "aromatic box", and a bound molecule of HEPES from the buffer makes a cation−π interaction with Trp 143, the analogue of the nAChR Trp 149.

More recently, we have used the same approach to establish a similar binding motif for serotonin in the 5-HT$_3$ receptor.[7] In particular, Trp 183, the analogue of nAChR Trp 149, makes a strong cation−π interaction to the ammonium of serotonin, as evidenced by the fluorinated Trp study (Table 1, Figure 3).

As shown in Figure 1, MOD-1 also contains aromatic residues at the key locations, but with a subtle difference. The residue that aligns with Trp 183 of 5-HT$_3$ is Tyr 180 of MOD-1. The key tryptophan that makes a cation−π interaction with serotonin (and ACh) is now a Tyr. Of course, Tyr (and Phe) can also participate in cation−π interactions,[8] and so this conservative change (Trp to Tyr) is not startling.

Figure 1. Sequence alignments for the key regions of the agonist binding sites. For the nAChR, loop D corresponds to the γ subunit, loops B and C to the α subunit.

Table 1. Mutations of Key Aromatic Residues (EC$_{50}$, μM)

residue	α149 nAChR[a]	183 5-HT$_3$[b]	226 MOD-1	cation−π binding[a,c]
Trp	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.3	32.6
5-F-Trp	4.7 ± 0.1	6.0 ± 0.5	12 ± 1	27.5
5,7-F$_2$-Trp	13 ± 0.1	37 ± 3	66 ± 11	23.3
5,6,7-F$_3$-Trp	34 ± 0.1	240 ± 8	240 ± 50	18.9
4,5,6,7-F$_4$-Trp	65 ± 0.3		1600 ± 210	14.4

[a] Reference 5. The receptor has a Leu9′Ser mutation in M2. [b] Reference 7. [c] Binding energy of a probe cation (Na$^+$) to the ring in kilocalories per mole; see ref 5.
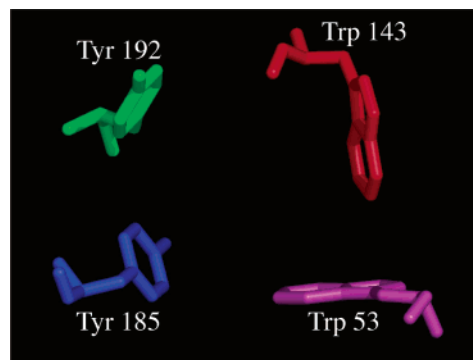

Figure 2. Arrangement of key aromatic residues as seen in AChBP. The numbering is that of AChBP (PDB 1I9B), and the color scheme matches that of Figure 1.

MOD-1 thus presented an opportunity to quantify a cation−π interaction between serotonin and a Tyr, allowing a direct comparison with the serotonin···Trp interaction in 5-HT$_3$. As such, we used the in vivo nonsense suppression methodology for unnatural amino acid incorporation[9] to substitute Tyr 180 of MOD-1 with several Tyr analogues, including fluorinated residues of the sort that were so informative in studying the tryptophan interaction. The detailed results are given in the Supporting Information; in brief, these studies led to no clear conclusions. Many substitutions had a significant effect, but the data showed no clear trend, and certainly not the remarkable trend seen when studying the Trp residues in 5-HT$_3$ or nAChR.

Given these puzzling results, we considered a second subtle difference between MOD-1 and other Cys-loop receptors. Another canonical aromatic residue of the agonist binding site is Tyr 198
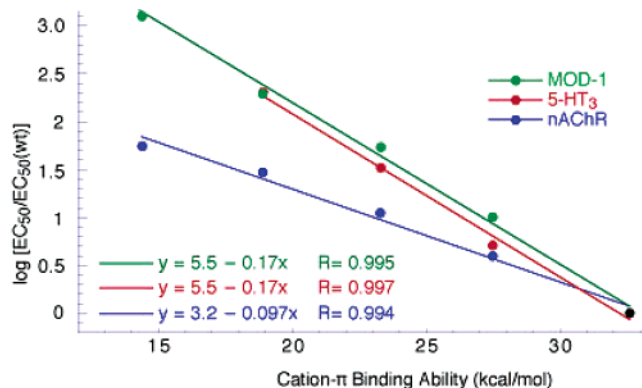
Figure 3. Plots of the data in Table 1. Note that log EC_{50} is plotted, so both axes are proportional to energy.

of the nAChR, aligning with Tyr 234 of 5-HT$_3$ and Tyr 192 of AChBP. This Tyr is conserved in essentially all members of the family, but it is *Trp* 226 in MOD-1. Given our earlier successes with studies of fluorinated Trp derivatives, we decided to apply the same protocol to Trp 226 of MOD-1.

The results of such an experiment are summarized in Table 1 and Figure 3. Stunningly, the exact same trend seen for Trp 183 of 5-HT$_3$ is seen for Trp 226 of MOD-1. Both serotonin lines have steeper slopes than the analogous ACh line (as expected on the basis of electrostatic arguments),[7] and the two serotonin lines have identical slopes. Such agreement can be interpreted only to mean that the primary ammonium of serotonin makes a strong cation−π interaction with a Trp in both systems, *but the two homologous receptors use a different tryptophan to make the cation−π interaction to serotonin!*

It thus appears that Nature has moved the critical cation−π binding interaction from the loop B site to the second loop C site on going from 5-HT$_3$ to MOD-1. We emphasize that these are highly homologous receptors. On the basis of the AChBP structure, we estimate that the two critical residues are ∼9 Å apart. Thus, while much of the serotonin molecule might occupy the same position in the two receptors, a significant rearrangement of the alkylammonium unit must occur on going from one receptor to the other. Note also that binding is not all that is happening. In both systems, serotonin induces a large conformational change in the receptor, gating the ion channel. This makes it all the more remarkable that two binding orientations are possible.

Superficially, it appears that Nature has simply swapped the Trp/Tyr pair of the agonist binding site. That is, we have Trp···Tyr in the nAChR (149···198) and the 5-HT$_3$ (183···234), but Tyr···Trp in MOD-1 (180···226). To test this notion, we prepared the MOD-1 double mutant Y180W/W226Y by conventional mutagenesis. This receptor showed an EC_{50} 1000-fold higher than that of wild type. Both single mutants were also substantially compromised. Similarly, the W183Y 5-HT$_3$ single mutant shows a 90-fold increase in EC_{50}.[10] Thus, the MOD-1 and 5-HT$_3$ receptors are precisely designed to bind serotonin, as would be expected. But the designs are different, and serotonin accommodates both. Another perspective on these

data is that they provide testimony to the strength of the RNH$_3^+$···Trp cation−π interaction. Even when a perfectly acceptable Tyr is positioned in the "classical" cation−π site (the one used in nAChR and 5-HT$_3$), a Trp is preferable, and the ligand adjusts to reach it.

The notion of a ligand held in a precise location by specific contacts to the protein has served enzymology, and drug discovery around enzymes, well. However, perhaps for receptors, molecules for which ligand binding induces a structural change that launches a signaling process, such a structured model is not necessary.[11] Perhaps all that is required is for the ligand to occupy the binding region, acting more like a wedge, to initiate the conformational change. Standard forces of molecular recognition are required to direct the ligand to the binding site, but the precise details of the binding interaction are less critical. In the present case, a cation−π interaction plays an important role in delivering serotonin to the binding site, but there is some flexibility as to the precise location of that interaction.

While the full implications of these results for studies of drug−receptor interactions remain to be established, they immediately provide a caution for efforts to model binding sites on the basis of sequence homology. It is entirely reasonable to assume that, since serotonin makes a cation−π interaction with W183 in 5-HT$_3$, it would also make a cation−π interaction with Y180 in MOD-1; any researcher would be comfortable with this modest substitution. However, our results show that, instead of settling for the tyrosine, the agonist reorients in the binding site to contact a nearby tryptophan and thus maximize the cation−π interaction.

Supporting Information Available: Electrophysiological traces, dose−reponse curves, and data for mutations at Tyr180 of MOD-1 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Maricq, A. V.; Peterson, A. S.; Brake, A. J.; Myers, R. M.; Julius, D. *Science* **1991**, *254*, 432−437.
(2) Ranganathan, R.; Cannon, S. C.; Horvitz, H. R. *Nature* **2000**, *408*, 470−475.
(3) Karlin, A.; Akabas, M. H. *Neuron* **1995**, *15*, 1231−1244. Ortells, M. O.; Lunt, G. G. *Trends Neurosci.* **1995**, *18*, 121−127.
(4) Sine, S. M. *J. Neurobiol.* **2002**, *53*, 431−446. Corringer, P.-J.; Le Novère, N.; Changeux, J.-P. *Annu. Rev. Pharmacol. Toxicol.* **2000**, *40*, 431−458.
(5) Zhong, W. G.; Gallivan, J. P.; Zhang, Y. O.; Li, L. T.; Lester, H. A.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12088−12093.
(6) Brejc, K.; van Dijk, W. J.; Klaassen, R. V.; Schuurmans, M.; van der Oost, J.; Smit, A. B.; Sixma, T. K. *Nature* **2001**, *411*, 269−276.
(7) Beene, D. L.; Brandt, G. S.; Zhong, W. G.; Zacharias, N. M.; Lester, H. A.; Dougherty, D. A. *Biochemistry* **2002**, *41*, 10262−10269.
(8) Dougherty, D. A. *Science* **1996**, *271*, 163−168.
(9) Dougherty, D. A. *Curr. Opin. Chem. Biol.* **2000**, *4*, 645−652.
(10) Spier, A. D.; Lummis, S. C. R. *J. Biol. Chem.* **2000**, *275*, 5620−5625.
(11) For other examples of structural changes in a receptor induced by ligand binding, see: Kunishima, N.; Shimada, Y.; Tsuji, Y.; Sato, T.; Yamamoto, M.; Kumasaka, T.; Nakanishi, S.; Jingami, H.; Morikawa, K. *Nature* **2000**, *407*, 971−977. Armstrong, N.; Gouaux, E. *Neuron* **2000**, *28*, 165−181.

JA0348086