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Tandem sulfur-containing amino acids are epicritical determinants of dopamine D_2 receptor pharmacology

John A. Schetz, David R. Sibley *

Molecular Neuropharmacology Section, Experimental Therapeutics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Building 10, Room 5C-108, 9000 Rockville Pike, Bethesda, MD 20892, USA

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Abstract

The conserved aspartic acid that is required for ligand binding to the dopamine D_2 receptor is followed by three tandem sulfur-containing amino acids. While previous point mutation studies did not reveal any single one of these residues as being critical for ligand binding, we now show that simultaneously substituting all three with isovolumetric, non sulfur-containing amino acids results in large decreases in the binding affinity for dopamine, (−)-raclopride and 7-(-4(4-(2,3-dichlorophenyl)-1-piperazinyl)butyloxy)-3,4-dihydro-2(1*H*)-quinolinone (aripiprazole), but not for methylspiperone or allosteric modulators. © 2000 Elsevier Science B.V. All rights reserved.

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Previous mutation studies of catecholamine receptors have indicated that a highly conserved and negatively charged aspartic acid residue located two helical turns into the third transmembrane-spanning domain is a critical molecular determinant for the binding of agonists and antagonists (Strader et al., 1994). In the case of the dopamine D_2 receptor, the critical aspartate, which corresponds to position 114 (D114) [1], has been proposed to face the 'binding-site crevice' and to directly participate in the docking of the protonatable amine moiety of dopamine and other ligands (Mansour et al., 1992). Remarkably, point mutations of individual amino acids in the helical turns that both proceed and follow D114 have no direct effect on dopamine binding. While mutations in the helical turn that precedes D114 do moderately alter (< 30-fold) the binding affinity of antagonists with compact molecular structures, these changes appear to be due to relatively nonspecific effects on receptor conformation (Schetz et al., 1999) and they are not mimicked by point mutations in the sulfur-rich helical turn that follows D114. For instance, the point mutations, M116L, M117G, M117C (Mansour et al., 1992), have virtually no effect the binding of the agonist 2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin [^{3}H]N-0437 or the substituted benzamide antagonist [^{3}H]raclopride. Furthermore, the point mutations, M116C, M117C, C118S, C118M, C118A (Javitch et al., 1994, 1996) have virtually no effect on the binding of dopamine and only modest effects (≤ 10-fold) on the binding of the substituted benzamide antagonist (−)-sulpride. Only the rather drastic C118K mutation drastically reduces the binding affinity for dopamine concomitant with large decreases in the binding affinity for methylspiperone and substituted benzamide antagonists (Javitch et al., 1996). These results are consistent with the proposal that M116 and M117 are oriented away from and C118 is oriented at the interface of the 'binding-site crevice' (Javitch et al., 1994, 1996; Baldwin et al., 1997).

Given the overall tolerance for side chain variation for all single-point mutations at positions 116–118 (barring the most drastic modification at position 118) and their proposed orientation, it was indeed surprising that simultaneous replacement of all three tandem sulfur-containing amino acids, i.e., methionine, methionine and cysteine (MMC) at positions 116–118, with the isovolumetric amino acid substitutions isoleucine, leucine and serine (ILS) re-

* Corresponding author. Tel.: +1-301-496-9316; fax: +1-301-496-6609.

E-mail address: sibley@helix.nih.gov (D.R. Sibley).

[1] The capital letter is the one-lettered amino acid code and the corresponding number refers to the position of the amino acid in the dopamine D_2 receptor sequence.

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Table 1
Comparison of binding affinities and pseudo Hill slope values of selected drugs and modulators for *wild type* dopamine D_2 and dopamine D_2-MMC116–118ILS mutant receptors ($n = 3–4$). The D_2-MMC116–118ILS mutant receptor was constructed by DpnI-based site-directed mutagenesis as described previously (Schetz et al., 1999). Mutant and *wild type* dopamine receptors were transiently expressed in SV40-transformed African green monkey kidney cells (COS-7) and crude membranes were screened by rapid filtration radioligand binding assays with [^{3}H]methylspiperone as the primary radioligand as described previously (Schetz et al., 1999)

Drug: receptor	Equilibrium affinity constants (nM ± S.D.)[a]	Fold change in affinity (away from the *wild type* background)	Pseudo Hill slope, n_H^b
[^{3}H]methylspiperone			
D_2-WT	0.020 ± 0.004	1	
D_2-MMC116–118ILS	0.055 ± 0.013	3	
(−)Raclopride			
D_2-WT	0.56 ± 0.03	1	0.77 ± 0.01
D_2-MMC116–118ILS	150 ± 34	268	1.06 ± 0.13
Aripiprazole			
D_2-WT	0.19 ± 0.04	1	0.86 ± 0.09
D_2-MMC116–118ILS	10.5 ± 3.0	55	0.98 ± 0.09
Dopamine			
D_2-WT	442 ± 126	1	0.81 ± 0.11
D_2-MMC116–118ILS	> 78,700 ± 49,000[c]	> 178	0.77 ± 0.46
Methylisobutylamiloride			
D_2-WT	8421 ± 2526	1	1.00 ± 0.10
D_2-MMC116–118ILS	5243 ± 1274	−1.5	1.95 ± 0.80
Zinc			
D_2-WT	161 ± 38	1	0.75 ± 0.09
D_2-MMC116–118ILS	170 ± 40	1	0.51 ± 0.06

[a] For methylspiperone the affinity values (K_d) were determined directly by saturation isotherm analysis with radiolabeled [^{3}H]methylspiperone, while for dopamine, (−)-raclopride and aripiprazole the affinity values (K_i) were derived from [^{3}H]methylspiperone/drug IC_{50} values according to the competitive form of Cheng and Prusoff (1973). In the case of the allosteric modulators, zinc and methylisobutylamiloride, the IC_{50} values are listed without correction.

[b] In some cases, the competition curves were significantly better fit to a one-site model with n_H values different from unity, and therefore, the affinity values reflect an apparent $K_{0.5}$ rather than a K_i value.

[c] Since only about 50% inhibition was achieved at the highest concentration of dopamine tested (1 mM), the affinity value is only an estimate based upon extrapolation of the data.

sulted in large decreases in the binding affinity for dopamine (> 178-fold), the D_2/D_3-selective substituted benzamide antagonist (−)-raclopride (268-fold), and the dopamine D_2 receptor-selective antagonist 7-(-4(4-(2,3-dichlorophenyl)-1-piperazinyl)butyloxy)-3,4-dihydro-2(1*H*)-quinolinone(aripiprazole)[2] (55-fold) (Table 1). In contrast, the D_2-MMC116–118ILS mutant had only modest effects on the affinity for the antagonist methylspiperone and the allosteric modulators methylisobutylamiloride and zinc (< 5-fold) (Table 1). However, both allosteric modulators had significantly different pseudo Hill slope values for the D_2-MMC116–118ILS mutant as compared to the *wild type* dopamine D_2 receptor (Table 1). Consequently, the changes in zinc and methylisobutylamiloride pseudo Hill slopes coupled with the lack of an effect on their affinity suggests that the D_2-MMC116–118ILS mutation may alter the relative path of cooperative interactions between the 'primary' and allosteric sites without inducing more global and nonspecific effects on receptor conformation.[3]

A plausible explanation for the pharmacological profile of the D_2-MMC116–118ILS mutant must take into account: (1) the proposed orientation of the three sulfur-containing residues, (2) the effects of mutating these three residues individually or all at once, and (3) the differential effects on ligand-binding affinity. Since M116 and M117 are proposed to be oriented away from the water-exposed 'binding site crevice' with C118 at the interface (Javitch et al., 1994, 1996; Baldwin et al., 1997), and individually mutating each of these residues has essentially no affect on ligand binding, it seems unlikely that the sulfur-containing

[2] In some systems aripiprazole apparently has weak partial agonist activity (Lawler et al., 1999).

[3] Allosteric modulators, like methylisobutylamiloride and zinc, can serve as indicators of a receptor's conformational state, and often help to distinguish whether a particular mutation has localized and selective effects or nonspecific and global effects on receptor conformation. The reason for this is that by definition an allosteric modulator affects the 'primary' ligand site by interacting with a physically distinct 'secondary' site, and therefore, its influence on the 'primary' site must somehow be transmitted through the protein structure.

amino acids at positions 116–118 could be directly participating in ligand docking. Furthermore, the string of sulfurs is probably not indirectly altering the orientation of D114, because dopamine, (−)-raclopride, aripiprazole and methylspiperone all have protonatable amine moieties for docking to the negatively charged D114 but methylspiperone binding affinity is not substantially altered. In addition, spacial packing per se also does not appear to explain the pharmacological profile of the D_2-MMC116–118ILS mutant, because the three tandem sulfur-containing amino acids were substituted with non sulfur-containing amino acids having similar side chain volumes. Moreover, there is no distinction for effects on ligands with compact (dopamine and (−)-raclopride) and extended (aripiprazole) chemical structures. A parsimonious alternative is that the three sulfur-containing amino acids maintain the local conformation of the 'binding site crevice' by creating a relatively mild electrostatic surface which interactions with lipids or amino acids in neighboring helices or loops (e.g., sulphyl-conjugated diene interaction). The apparent indirectness of these proposed interactions in the absence of gross changes in dopamine D_2 receptor conformation suggests that the methionine, methionine, cysteine sequence should be considered an epicritical determinant for the binding of dopamine and some dopamine D_2 receptor-selective antagonists to the dopamine D_2 receptor.

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