Basal Histamine H_4 Receptor Activation: Ligand Mimicry by the Diphenylalanine Motif

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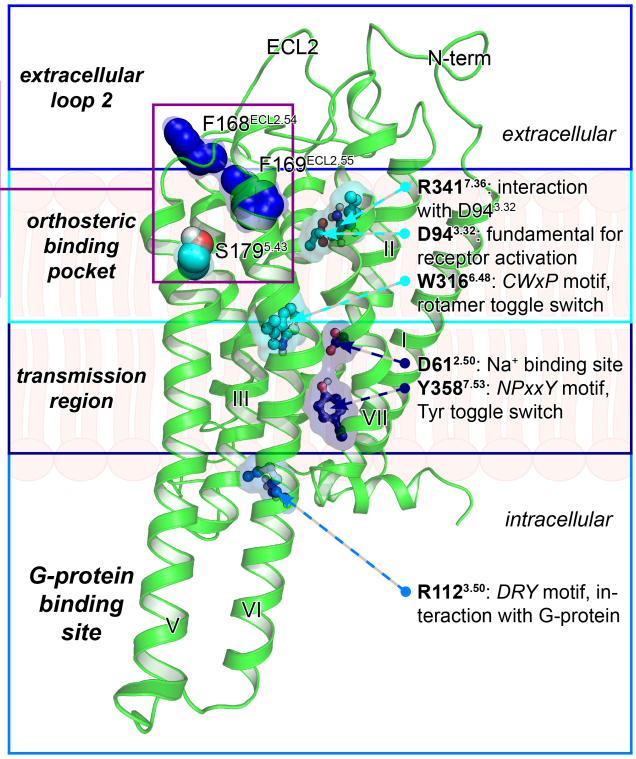
Histamine H_4 receptor (H_4R) orthologs exhibit species-dependent basal (constitutive) activity: In contrast to mouse H_4R (mH_4R), human H_4R (hH_4R) shows a high degree of basal activity. In a previous molecular-pharmacological study, the mutation F169V significantly decreased the high constitutive activity of hH_4R, and the basal activities of the hH_4R-F168A and hH_4R-F169V+S179M mutants were even comparable to that of mH_4R [1,2]. In contrast, the constitutive activity of hH_4R was maintained in the S179M mutation. Driven by such promising results, we aimed at further investigating the molecular mechanism of basal H_4R activation by performing 2 µs molecular-dynamics simulations of six H_4R variants (hH_4R, hH_4R mutants S179M, F169V, F169V+S179M, F168A, and mH_4R), each revealing a different degree of constitutive activity. Most interestingly, during the MD simulations, $F169^{ECL2.55}$ dips into the orthosteric binding pocket only in the case of hH_4R, thus adopting the role of an agonist and contributing to the stabilization of the active state. Strikingly, the overall distances between the C_α atoms of $D94^{3.32}$ and $Q347^{7.42}$, a measure of binding-pocket contraction, increased from hH_4R to the S179M, F169V, F169V+S179M, F168A mutants, and were highest for mH_4R. Remarkably, the overall C_α-C_α distances between $R112^{3.50}$ and $A298^{6.30}$, a measure of TM6 outward movement and GPCR activation, decreased in this order. Hence, these results, along with both information about additional motifs fundamental for GPCR activation and from rigidity analysis provide a molecular explanation for differential constitutive activities of H_4R variants. Moreover, this study has provided novel insights into molecular mechanisms of basal H_4R activation that are also of importance for other GPCRs.

Site-directed mutagenesis

H_4R variant	F/A$^{ECL2.54}$	F/V$^{ECL2.55}$	S/M$^{5.43}$	basal activity (%)
hH_4R	F168	F169	S179	
hH_4R-S179M	F168	F169	M179	
hH_4R-F169V	F168	V169	S179	
hH_4R-F169V+S179M	F168	V169	M179	
hH_4R-F168A	A168	F169	S179	
mH_4R	F170	V171	M181	

basal activity (%): 0 20 40 60 80 100



[1] D. Wifling, et al., *Br. J. Pharmacol.*, **2015**, 172, 785-798.

[2] D. Wifling, et al., *PLoS One*, **2015**, 10, e0117185.