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MEETING ABSTRACT

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Deuteration changes the binding of some histaminergic agonists to the histamine H_2 receptor in astrocytes

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Background: A crucial step in the binding of histaminergic ligands, *e.g.* histamine to the H_2 receptor, is the formation of three hydrogen bonds between amino acid residues (Asp^{98}, Asp^{186} and Thr^{190}) present in the third and the fifth transmembrane α-helices and three nitrogen atoms of the histamine molecule. In order to estimate the relevance of hydrogen bonds in the process of binding of ligands to the H_2 receptor we compared the binding properties of [^{3}H]tiotidine to histamine H_2 receptor binding sites in cultured neonatal rat astrocytes in control and deuterated medium.

Methods: To test this hypothesis we performed saturation and inhibition binding studies using [^{3}H]tiotidine as a biomarker in cultured glial cells. We modeled changed binding affinity upon deuteration of histamine in conjunction with quantum chemical calculations and quantization of nuclear motion of the protons involved in hydrogen bonding.

Results: [^{3}H]tiotidine binds in a reversible and saturable manner to a single population of binding sites with maximal binding-site density (B_{max}) of 22.0 ± 3.2 fmol/mg protein and equilibrium dissociation constant (K_d) of 6.3 ± 1.9 nM. Histamine, 2-methylhistamine and 4-methylhistamine displaced the radioligand with pIC_{50} values of 7.6 ± 0.14, 8.5 ± 0.16, and 7.4 ± 0.25, respectively. Binding characteristics changed upon deuteration: the B_{max} dropped nonsignificantly to 17.4 ± 5.2 fmol/mg protein; the K_d of [^{3}H]tiotidine changed to 8.6 ± 5.0 nM ($p > 0.05$; determined by Student's *t*-test; $n = 6$); the pIC_{50} values for histamine, 2-methylhistamine and 4-methylhistamine in deuterated conditions were 8.0 ± 0.15, 6.8 ± 0.16 ($p < 0.05$; Student's *t*-test; $n = 6$) and 7.7 ± 0.13, respectively ($n = 6$). The experimental data show that deuteration significantly attenuated binding free energy of 2-methylhistamine (2.15 kcal/mol), but decreased binding free energy for 4-methylhistamine (−0.51 kcal/mol) and histamine (−0.78 kcal/mol). *Ab initio* calculations of the isotope effect were performed for the endogenous ligand histamine for transfer of monoprotonated histamine ion from the aqueous environment to the receptor binding site. Implicitly quantized NH and OH motion revealed that the changes can be rationalized by attenuated strength of hydrogen bonding upon deuteration which is known as Ubbelohde effect.

Discussion: Replacing hydrogen atoms involved in binding of histamine ligands to H_2 receptor binding sites with deuterium atoms results in different length of intermolecular and intramolecular distances. This leads to a structural change of ligand and receptor binding sites which significantly affects the binding affinities of methylhistamines The effects of deuteration on the affinity is the difference between the interaction free energy receptor–ligand and water–ligand giving rise to increased or decreased values. Our study offers a simple and practical approach how to treat nuclear quantum effects in drug–receptor binding and will hopefully help reaching a distant goal that is *in silico* discrimination between agonists and antagonists.

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