

Role of Tryptophan in the Structural Alterations of the Rat Ovarian LH/hCG Receptor

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The LH/hCG receptor contains 5 tryptophan residues, two of them are located in the extracellular domain [1]. Information on the importance of tryptophan residues for ligand binding to LH/hCG receptors has not been reported as yet. We therefore studied the inhibitory effect of the oxidizing agent N-bromosuccinimide (NBS) and of 2-hydroxy-5-nitrobenzyl bromide (HNB-Br), reagents with specificity toward tryptophan [2], with the aim to determine whether tryptophan residues are essential for the hCG binding to the receptor. The study analyzed also possible structure-functional relationships of the LH/hCG receptor by intrinsic fluorescence and thermal perturbation techniques [3]. Treatment of the rat ovarian membrane-bound and Triton X-100 solubilized LH/hCG receptor with the NBS and HNB-Br resulted in a dose-dependent reduction in hCG binding activity. The Stern-Volmer quenching constants (K_{sv}) determined from the Stern-Volmer plots for control and 5 mM NBS-treated membranes were found to be $7.0 M^{-1}$ and $4.4 M^{-1}$, respectively, indicating that oxidation of tryptophan residues by NBS decrease the accessibility of fluorophores for acrylamide. Preincubation of membranes with hCG and subsequent inactivation with NBS apparently increased the accessibility of tryptophan for quenching by acrylamide (K_{sv} 5.3

M^{-1}). Modification of tryptophan residues was associated with alteration in the physical state of ovarian membranes. Membrane lipid rigidity was increased after NBS and HNB-Br action. Thermal inactivation of the receptor is a rapid process. During the incubation of ovarian membranes treated with 1 mM NBS or HNB-Br at a constant temperature of $50^{\circ}C$, the damage of binding sites was apparently manifested after 5 min. Heat inactivation demonstrated that there was a significant destabilization of the LH/hCG receptor structure. Modification of the LH/hCG receptor by tryptophan-reactive reagents suggests that tryptophan residue is essential for hCG binding to the receptor.

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