Abstract

Insulin and insulin-like growth factor 1 (IGF-1) are peptide hormones that are important regulators of cellular metabolism, proliferation and apoptosis. Disruptions in signalling pathways may cause a whole range of diseases from diabetes mellitus type 1 and type 2 to cancer or neurodegenerative diseases.

The cellular response to these hormones is mediated by insulin (IR) and IGF-1 receptors (IGF-1R) with a tyrosin-kinase activity. Receptors are created as hetero-tetramers of two extracellular α-subunits and two intracellular β-subunits. Studies of receptor structures try to elucidate the basic principles of the interaction of receptors with their ligands. However, the role of some amino-acid residues in binding remains unclear. It was suggested that the arginine 704 of IGF-1R may interact with Glu58 IGF-1. In comparison with IGF-1R, the equivalent arginine 717 IR was not associated with an important role in insulin binding in previous studies.

This thesis is focused on clarifying the role of Arg704 IGF-1R and for comparison analogically on Arg717 IR isoform A (IR-A) in ligand binding to the receptors. Therefore, mutant variants of IGF-1R in positions His697 and Arg704 and variants IR-A in positions His710 and Arg717 were created. The role of histidines 697 IGF-1R and 710 IR was already elucidated so the mutants served as a control. The binding and activating properties of all full-length mutant receptors or their purified ectodomains were determined by saturation binding assays and stimulation assays. It was shown that mutations of His697 and Arg704 cause a decrease in a binding affinity to IGF-1. Thus, it documents the importance of these residues in the IGF-1 binding. Furthermore, we have shown the presence of two binding sites in IGF-1R. The mutation of His697 caused a reduction of the maximal binding to one half. The more surprising result was that both mutations of His710 and Arg717 lead to inactivation and complete disruption of the binding of insulin. It demonstrates the critical role of Arg717 IR-A in insulin binding.

Keywords: insulin, IGF-1, insulin receptor, IGF-1 receptor, site-directed mutagenesis, saturation binding assay, complex ligand-receptor