

P009 The agonist binding site of the V_{1b} receptor

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The vasopressin V_{1b} receptor ($V_{1b}R$) is a subtype of a neurohypophysial receptor which belong to Family A G-protein-coupled receptors (GPCRs). Its natural ligand vasopressin is a nonapeptide comprised of a hexapeptide ring with a tripeptide tail. In humans, [arginine⁸]vasopressin (AVP) binds with a high affinity to the vasopressin receptor subtypes V_{1a} , V_{1b} , V_2 , and also to the closely related oxytocin receptor (OTR) albeit with a lower affinity. The conserved residues among these receptors in the vicinity of the putative binding site were the focus of the study. Several amino acid residues which could be involved in agonist binding to the $V_{1b}R$ were selected. These targeted amino acid residues were substituted by alanine using site-directed mutagenesis. Mutant constructs were expressed in HEK293T cell-line, and the pharmacological properties of the mutant constructs were investigated using radioligand binding assays. The cell-surface expression and the agonist-induced internalisation of the mutant constructs were compared to the wild-type receptor using an ELISA protocol based on HA epitope tag engineered in the N-terminus of the receptor.