

P006 The role of Helix 8 residues of the V1b vasopressin receptor in receptor function

Amelia Baker & Mark Wheatley

*School of Biosciences, University of Birmingham,
Edgbaston, Birmingham, B15 2TT, UK.*

[Arginine⁸]vasopressin (AVP) exerts its physiological effects through three distinct G-protein-coupled receptors (GPCRs); V_{1a}R, V_{1b}R and V₂R. V_{1b}R regulates the secretion of ACTH and is associated with the regulation of stress and anxiety. GPCRs share a common topology with seven transmembrane domains. The crystal structure of bovine rhodopsin also revealed the presence of a short eighth helix (H8) located in the C-terminal tail. The H8 region of the V_{1b}R was predicted using sequence alignments with rhodopsin and other Family A GPCRs. The function of this H8 region of the V_{1b}R was investigated by alanine mutagenesis. Mutant receptors were characterised pharmacologically using a combination of radioligand binding assays and intracellular signalling assays. In addition, cell-surface expression and internalisation of the mutant constructs were also studied using ELISA. The V_{1a}R contains a di-cysteine motif at the C-terminus of the putative helix 8 region. This acts as a palmitoylation site anchoring the receptor into the lipid membrane. The effect of perturbing the conformation of H8 was investigated and found to be important for high affinity binding of the agonists AVP and dDAVP.