

S014 The MC4 receptor: organisation, ligand binding and activation

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The MC4 Receptor plays an important role in the regulation of food intake. Some natural mutations are associated with obesity. This study was concerned with the structure/function properties of the human receptor. Immunopurification and Western Blotting indicated that the receptor occurs in the membrane in an oligomeric form which could be covalently crosslinked through a sulphhydryl near the cytoplasmic surface, indicating a TM2:TM2 interface. A combination of crosslinking and immunoprecipitation indicated a higher order species is also present, possibly a tetramer involving a TM4:TM4 interface. Treatment with GTP γ S, GNPpNHp and GTP γ DPATP to dissociate the G-protein has the unexpected effect of inhibiting this crosslinking and increasing ligand binding, suggestive of negative co-operativity mediated by the G-protein. Formation of the disulphide bond between monomers markedly inhibited ligand binding indicative of conformational change and cross-talk between monomers. Removal of the palmitoylation site (C318A) had little effect on ligand binding but more surprisingly abolished activation (or binding) of the G-protein. Perhaps a significant structural alteration occurred at the inner face of the receptor. Mutation of ligand-binding sites, M292C and D126C, abolished or substantially decreased activations respectively when co-expressed with the C318A mutant, however, some activation was restored. This study supports the concept of transactivation and attests to the functional importance of oligomer formation in the MC4 receptor. Finally, the incorporation of cysteine residues into both the receptor and the peptide ligand produced a series of peptide-receptor covalent crosslinks which helps to define more accurately nearest neighbour relationships at the ligand-binding site of the receptor.