D7 SIGNAL TRANSDUCTION BY GALANIN RECEPTORS

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Galanin is a 29/30 amino acids long neuroendocrine peptide which is involved in the control of feeding behaviour, insulin secretion, acetylcholine and excitatory amino acid release in the hippocampus. It is assumed that galanin receptor antagonists may be therapeutically useful agents in treatment of feeding disorders, depression and Alzheimer's disease. The present study attempts to map the sequences of intracellular domains of galanin receptor which are responsible for coupling of the galanin receptors to specific G-proteins. Deletion and point mutations in the intracellular loops of galanin receptor have now been introduced and the influence of the mutated receptor on formation of cAMP has been measured. Most promising sequences from these loops have been synthesised and the influence of these peptides to G-protein coupled adenylate cyclase activity as well as G protein binding has been characterized. The results enable to determine the mechanism of interaction of galanin receptor with specific G-proteins.

D8 ANGIOTENSIN II RECEPTORS

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Two distinct angiotensin II receptor subtypes, AT1 and AT2, have been identified based on their differential binding of the non-peptide antagonists, DuP 753 (inverse; AT2-selective) and DuP 754 (agonist; AT1-selective). Molecular cloning has revealed that they are both members of the G-protein coupled receptor family. Many of the recognized actions of angiotensin II (Ang II) appear to be mediated by AT1 receptors, predominantly through activation of the phospholipase C signal transduction pathway. We have constructed a three dimensional model of the AT1 receptor to guide site-directed mutagenesis aimed at identifying potential residues involved in ligand binding. The consequences of substituting either Arg111, in the third transmembrane helix (TM III), or Arg221, in the seventh transmembrane helix (TM VII), on ligand binding and receptor coupling will be presented. These data suggest that a specific interaction between TM III and TM VII may be a major determinant of AT1 receptor recognition from an inactive to active conformation. In addition to receptor activation we have also observed that receptor desensitization seems to be dependent on the integrity of its C-terminus. Furthermore, we have established that AT1 receptors are phosphorylated in an agonist-dependent manner resulting in homologous desensitization. Phosphorylation of the receptor is predominantly through PKC at low concentrations of Ang II. In contrast, at high concentrations of Ang II, although PKC still contributes, receptor phosphorylation is dominated by another kinase, probably a member of the GRK family. Regulation of the AT1 receptor by two kinases, one which is second messenger-dependent, is thus in keeping with that established for other G protein-coupled receptors.

D9 STRUCTURE AND FUNCTION OF NEUROHYPOPHYSIAL HORMONE RECEPTORS

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The multifarious physiological effects of the neurohypophysial peptide hormones (arginine) vasopressin (AVP) and oxytocin (OT) are mediated by a family of related G-protein-coupled receptors (GPRs). The V1a, V1b and V2 subtypes of vasopressin receptor (VPR) and the oxytocin receptor (OTR) have now been cloned from several species. This has confirmed that although they are distinct proteins, they have related pharmacological characteristics and possess sequence homology. Identification of the domains/residues within these receptor proteins which give rise to the similarities/differences exhibited is of fundamental importance. With particular reference to the V1a receptor (V1aR) and the OTR, we have used a combination of site-directed mutagenesis, specific chemical modification and peptidic approaches to investigate receptor:ligand interaction. In addition, the role of glycosylation of the extracellular loops of the rat V1aR has been addressed. This utilised a combination of site-directed mutagenesis and metabolic labelling in an in vitro translation system.

D10 DISCOVERY AND DEVELOPMENT OF NONPEPTIDE ANTAGONISTS FOR PEPTIDE RECEPTORS

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The last 10-15 years has seen an explosion of research aimed at the identification and development of orally-active nonpeptide antagonists of receptors whose natural ligands are peptides. The success of efforts to develop antagonists of these receptors is exemplified by the large number of antagonists, particularly for the G-protein coupled class of receptors, that are now available as novel research tools and potential therapeutic agents. The general paradigm followed for these discovery efforts has involved the identification of novel lead compounds through targeted screening of a variety of sample sources in radioligand binding assays, with subsequent refinement of the structures towards high potency and selectivity and other properties suitable for clinical development. This paradigm for discovery was first established by researchers at Merck with the development of antagonists for the gut hormone, cholecystokinin (e.g. MK-329, L-365,260) which entered clinical studies for GI and CNS disorders. A similar path of discovery has been followed toward the identification of potent and selective oxytocin antagonists (e.g. L-368,899) for the potential treatment of preterm labor.

D11 GENE STRUCTURE, EXPRESSION AND REGULATION OF THE RECEPTOR FOR THE PEPTIDE HORMONE OXYTOCIN

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The oxytocin receptor conforms to the general pattern of the transmembrane-domain, G-protein-coupled surface receptors, and to date appears to be encoded by only a single gene within the mammalian genome. The gene is split by two introns common to all species, the human gene additionally has a short exon present within the second first intron, which interposes within the 5' UTR of the mRNA. Consistent with oxytocin physiology, the receptor is expressed in a number of different tissues including the male and female reproductive tracts, kidney, breast, pituitary and brain, and at lower levels in several other organs. Oxytocin receptor gene expression is greatest within the uterus, especially in the prepartum phase in both the myometrium and endometrium. In the cow, there is additionally a considerable up-regulation of the gene within the endometrium at the end of the non-pregnant cycle. Although in vivo there appears to be a correlation with sex steroid levels, there is no evidence supporting direct control by steroid receptors acting at the gene promoter. Using nuclear extracts from high, low and non-expressing tissues we are mapping the promoter regions of the human and bovine oxytocin receptor genes. So far none of the sites shown specifically to bind nuclear proteins correspond with sites identified in computer searches, nor do the genes conform to any known transcription factors. Research is supported by the Deutsche Forschungsgemeinschaft (Hu 7/1-3 and Hu 7/8-1).

D12 DOMAIN SWAPPING IN THE ACTIVATION OF G-PROTEIN COUPLED RECEPTORS

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Computer simulations were performed on models of the B2-adrenergic receptor dimer, including the 5-6 domain swapped dimer. The calculated energies of all the dimers on the domain swapping pathway are similar, suggesting that domain swapping is energetically feasible. Novel experimental support for domain swapping was obtained from the correlated mutations amongst the external residues of the known B2-adrenergic receptors. These occur mainly at the 5-6 interface at precisely the locations predicted by the simulations. Many aspects of G-protein coupled receptor activation which hitherto had no clear molecular explanation are discussed in terms of this novel domain swapping mechanism.