THE CCK_A RECEPTOR IN PANCREAS AND GALLBLADDER ARE IDENTICAL: MOLECULAR CLONING AND FUNCTIONAL STUDIES

de Weerth A, Pisegna JR, Wank SA Digestive Diseases Branch, NIDDK, National Institutes of Health, Bethesda, MD, USA

Cholecystokinin (CCK) receptors mediate pancreatic acinar secretion and gallbladder contraction. Pharmacological and functional studies in pancreas and gallbladder demonstrate a CCK_A receptor class in both tissues. However, some pharmacological studies and affinity crosslinking studies of CCK receptors on pancreatic acini and gallbladder which result in different molecular weights in each tissue, suggest that these tissues possess two different subtypes of the CCK_A receptor. To determine whether the CCK_A receptor on pancreatic acini and gallbladder smooth muscle are the same, we cloned these receptors in guinea pig using a cDNA clone of the CCK_A receptor from rat pancreas. The guinea pig pancreas CCK_A receptor cDNA was cloned via the polymerase chain reaction (PCR) using primers corresponding to the rat pancreas CCK_A receptor 5' and 3' non-coding regions.The guinea pig gallbladder CCK_A receptor was cloned by hybridization screening of a gallbladder cDNA library using a ^{32}P random primed labelled cDNA probe from the rat CCK_A receptor coding region. CCK_A receptor clones from guinea pig pancreas and gallbladder had identical nucleotide sequences, which were 95% homologous to the rat CCK_A receptor cDNA sequence. The deduced amino acid sequence from guinea pig CCK_A receptors was 98% homologous to the rat CCK_A receptor sequence. Northern blot hybridization studies identified a 4.4 Kb hybridizing mRNA for guinea pig pancreas and gallbladder versus a 2.7 Kb mRNA from rat pancreas. Guinea pig CCK_A receptor cDNA clones were transfected in COS-7 cells using DEAE/dextran. Dose inhibition binding studies of transiently expressed receptors by CCK agonists and antagonists exhibited a CCK_A receptor pharmacologically similar to the rat CCK_A receptor. These studies indicate that CCK_A receptors in guinea pig pancreas and gallbladder have the same nucleotide sequence and do not support previous proposals they may represent different receptor subtypes.

CONSTRUCTION OF A NOVEL BIFUNCTIONAL RECEPTOR VIA MUTAGENESIS OF THE FIFTH TRANSMEMBRANE DOMAIN OF THE HISTAMINE H2 RECEPTOR.

J.DelValle, L.Wang, I.Gantz, Y. Guo, T.Yamada.
Depts.of Internal Medicine and Surgery, University of Michigan Medical School, Ann Arbor, MI.

Structural analysis of the histamine H2 and β-adrenergic receptors (βAR) indicate that both have an aspartic acid residue in the third transmembrane domain (TMD) that is essential for ligand recognition and biological activity. In addition, an aspartic acid residue (Asp^{186}) in the fifth TMD of the H2 receptor defines ligand selectivity while two serine residues (Ser^{204},Ser^{207}) in this same region of the βAR are thought to link to the catechol ring through hydrogen bond. In view of the marked similarities of the two receptors at this critical site, we explored the possibility that the histamine H2 receptor might be converted into one which also functions as a βAR if a serine residue were incorporated into the appropriate site in the fifth TMD. We mutated Asp^{186} to Ala^{186} and Gly^{187} to Ser^{187}, subcloned it into the eukaryotic expression vector CMVNEO and transfected both wild type and mutant H2 receptor into HEPA cells. Clones expressing comparable levels of receptor mRNA for the wild type and the mutated histamine H2 receptor were selected by Northern blot analysis and used for characterizing ligand mediated cAMP generation. Non transfected HEPA cells expressed an endogenous βAR (EC50 for epinephrine $5x10^{-8}M$, Max response = 278 ± 44% above control). The response to low dose epinephrine (10^{-11} - $10^{-9}M$)was markedly enhanced in the Ala^{186} Ser^{187} mutant (Max response= 280±15% above control) and this response was inhibited completely with both cimetidine and propranolol. High dose epinephrine response remained sensitive to propranolol but not to cimetidine. Thus the histamine H2 receptor can be converted into a bifunctional histamine H2/βAR via two simple mutations in the critical fifth TMD. Our data indicate that these distinct sites in seven TMD G-protein linked receptors may be sufficient to define ligand selectivity.