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Structural basis of cholecystokinin receptor binding and regulation ☆

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Abbreviations:
Bpa, *p*-benzoyl-L-phenylalanine
CCK, cholecystokinin
ECL, extracellular loop
G protein, guanine nucleotide-binding protein
GPCR, G protein-coupled receptor
pNO_2-Phe, *p*-nitro-phenylalanine

ABSTRACT

Two structurally-related guanine nucleotide-binding protein-coupled receptors for two related peptides, cholecystokinin (CCK) and gastrin, have evolved to exhibit substantial diversity in specificity of ligand recognition, in their molecular basis of binding these ligands, and in their mechanisms of biochemical and cellular regulation. Consistent with this, the CCK_1 and CCK_2 receptors also play unique and distinct roles in physiology and pathophysiology. The paradigms for ligand recognition and receptor regulation and function are reviewed in this article, and should be broadly applicable to many members of this remarkable receptor superfamily. This degree of specialization is instructive and provides an encouraging basis for the diversity of potential drugs targeting these receptors and their actions that can be developed.

Contents

1. Introduction

Guanine nucleotide-binding protein (G protein)-coupled receptors comprise the largest and most diverse group of receptors in the genome, representing the predominant site of action of existing approved drugs on the market. This superfamily includes three distinct families of receptors that include targets for regulatory molecules as different as photons, odorants, biogenic amines, peptides, proteins, glycoproteins, lipids, and even viral particles. This

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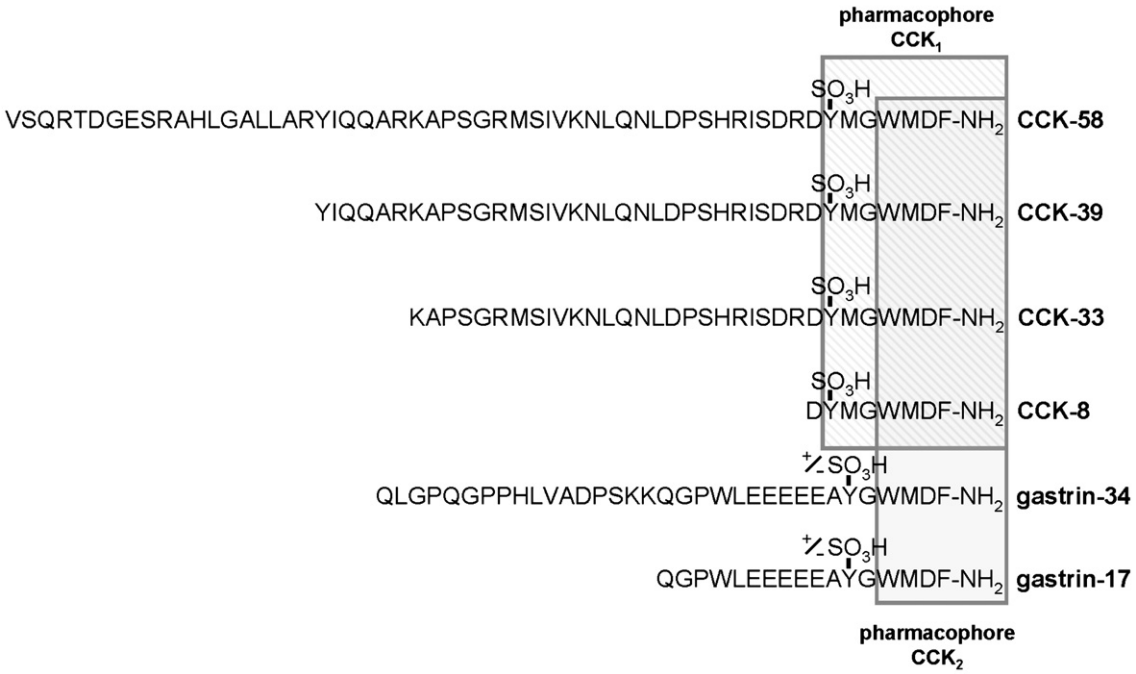

Fig. 1. Shown are the amino acid sequences of major molecular forms of human CCK and gastrin peptides. The pharmacophoric regions recognized by the CCK_1 and CCK_2 receptors are highlighted.

list includes molecules of markedly diverse structures, with sizes, charges, solubilities, and biological behaviors vastly different. Remarkably, these have been postulated to have evolved from a single evolutionary precursor (Kolakowski, 1994).

The family of G protein-coupled receptors that has been most extensively studied and that is best understood is Family A (or Class I), the rhodopsin-β adrenergic receptor family. Included in Family A are structurally highly homologous receptors for the gastrointestinal endocrine and neural polypeptide, cholecystokinin (CCK). These are classified as CCK_1 and CCK_2 based on their highly distinctive ligand selectivities (previously identified as types A and B CCK receptors, related to their prominent presence in "alimentary tract" and "brain") (Dufresne et al., 2006). While there had been the prediction of a distinct receptor for gastrin, another structurally-related polypeptide hormone produced in the gastric antrum, this turned out to be the CCK_2 receptor (Kopin et al., 1992). Despite being highly homologous and approximately 50 percent identical, particularly in the predicted transmembrane segments where these two receptors are 70 percent identical, and having clearly evolved from a common precursor, the CCK_1 and CCK_2 receptors exhibit remarkable functional differences. These differences are the focus of the current report. We believe that the ability for variation in mechanisms of ligand binding and activation of receptors that are even closely related and in mechanisms of the regulation of these receptors provide extraordinary opportunities for the introduction of highly selective therapeutic agents.

2. Naturally-occurring peptide ligands — structure-activity relationships

CCK was discovered in porcine duodenal extracts, based on its ability to stimulate gallbladder contraction and pancreatic exocrine secretion (Ivy & Oldberg, 1928). It was subsequently identified in the brain where it was found to represent one of the most abundant neuropeptides present (Miller et al., 1984). We now recognize that CCK is present as a variety of different length peptides that are produced from a single 115-residue preprohormone precursor, all sharing their carboxyl-terminal-amide sequence. These range in length from 58, 39, 33 and 8 residues, with each containing a sulfated tyrosine residue seven residues from the carboxyl terminus (Eysselein et al., 1990; Rehfeld et al., 2001). Shorter, non-sulfated CCK peptides have also been isolated from the brain (Rehfeld, 1980). The carboxyl-terminal peptapeptide-amide is also shared with gastrin polypeptides. Those peptides are produced from a distinct 101-residue preprohormone precursor, yielding dominant mature products 34 and 17 amino acid residues in length, with a carboxyl-terminal amide and a tyrosine that is sulfated approximately half of the time located six residues from their carboxyl terminus (Dockray et al., 2001). Fig. 1 illustrates the sequences of major molecular forms of CCK and gastrin peptides, highlighting the region shared between the two hormones. The CCK_1 receptor requires the carboxyl-terminal hepatapeptide-amide that includes the sulfated tyrosine found in CCK peptides for high affinity binding and biological activity (Miller, 1991). For this reason, gastrin is a low affinity ligand and weakly potent agonist at this receptor. The CCK_2 receptor requires the carboxyl-terminal tetrapeptide-amide that is shared between all CCK and gastrin peptides (Miller, 1991). Table 1 illustrates the structure-activity relationships for the action of CCK and gastrin peptides at both types of CCK receptors.

3. Receptor distribution and actions

CCK_1 receptors are responsible for a number of biological activities important for nutrient assimilation. These include stimulation of postcibal gallbladder contraction, pancreatic exocrine secretion,

Table 1
Structure–activity relationships for natural CCK and gastrin peptide action at CCK receptors

CCK_1 receptor: relative potencies and binding affinities- CCK-58 ≥ CCK-8>>> CCK-8 desulfate > gastrin-17, CCK-4 CCK-58 and CCK-8 bind with approximate K_i values of 0.6-1 nM, with desulfation of CCK-8 resulting in a 500-fold reduction in affinity, and gastrin and CCK-4 having a 1000–10,000-fold reduction in affinity.
CCK_2 receptor: relative potencies and binding affinities- CCK-8, CCK-58 ≥ gastrin-17, CCK-8 desulfate> CCK-4 CCK-8, CCK-58, gastrin, and CCK-8 desulfate all bind with approximate K_i values of 0.3–1 nM, with CCK-4 losing approximately 10-fold affinity.