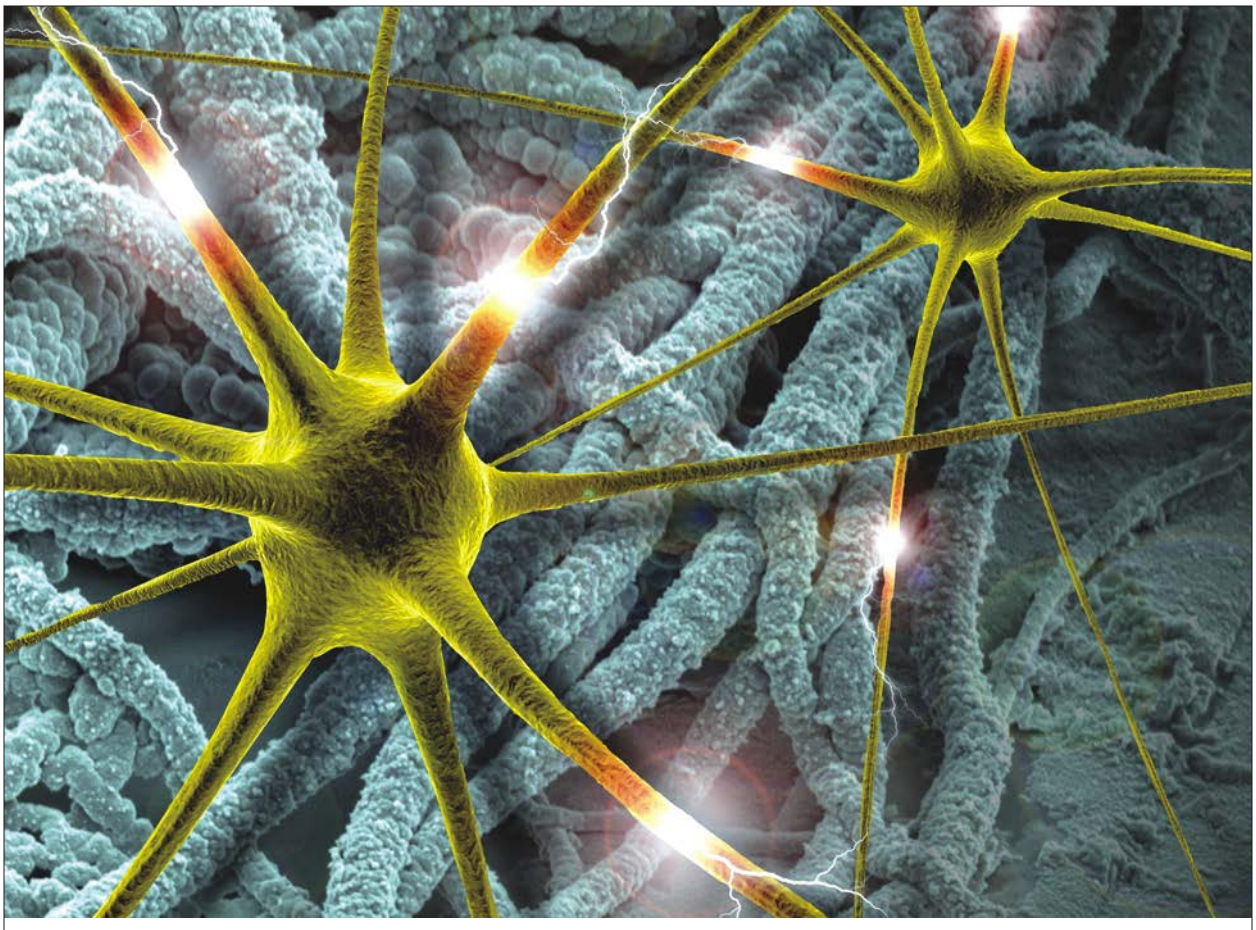

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Applications of G Protein-coupled Receptors in Clinical Medicine

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ABSTRACT

Progress in areas of research from the field of G Protein Coupled Receptor (GPCR) signalling, now shows that GPCRs are involved in a broad range of cellular regulatory activities. By virtue of widespread distribution and important roles in cell physiology and biochemistry, GPCRs play multiple important roles in clinical medicine. The understanding of how the GPCR interact (G proteins and effectors, as well as other regulatory proteins) thus has enormous implications for clinical medicine. The rapid progress in determining three-dimensional structures of GPCRs and more recently their regulators and effectors, has illuminated the search for mechanisms of activation and regulation and has allowed structure-based mutagenesis to test these ideas. The structural and mechanistic studies will in the future also hopefully provide opportunities to alter those interactions in pathological situations. A compilation of the most relevant research topics about the implication of heterotrimeric G proteins in the etiology of genetics and neurobiology of mood will provide a broad perspective of this potential therapeutic target field. In this review, we tried to show how far GPCR research involved in recent medicine.

Key words: Gproteins, GPCR mutations, signal transduction, 7TM structure

INTRODUCTION

G-protein-coupled receptors (GPCRs) mediate most of our physiological responses to hormones, neurotransmitters and environmental stimulants and so have great potential as therapeutic targets for a broad spectrum of diseases. They are also fascinating molecules from the perspective of membrane-protein structure and biology. Great progress has been made over the past three decades in understanding diverse GPCRs, from pharmacology to functional characterization *in vivo*. More than a thousand such receptors are known and more are being discovered all the time. Heterotrimeric G proteins transduce ligand binding to these receptors into intracellular responses, which underlie physiological responses of tissues and organisms. GPCR, also known as seven-transmembrane domain receptors, 7TM receptors, heptahelical receptors, serpentine receptor (Hamm, 1998). G protein-linked receptors (GPLR), comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways and ultimately, cellular responses. GPCRs are found only in eukaryotes, including yeast, choanoflagellates (King *et al*., 2003) and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones and

neurotransmitters and vary in size from small molecules to peptides to large proteins. GPCRs are involved in many diseases and are also the target of approximately 30% of all modern medicinal drugs (Overington *et al*., 2006).

G-protein-coupled receptors (GPCR) have well-recognized roles in clinical medicine. Their expression on the plasma membrane makes GPCR readily accessible, especially by hydrophilic hormones and drugs, including both agonists and antagonists and their non-uniformity of expression in different tissues and cell types provides selectivity (in some cases, specificity) in the targeting of these receptors for the activation or blockade of physiological events. Studies in recent years have provided a number of new insights, many of them gleaned from application of the tools of the genetic revolution (Insel *et al*., 2007). In this review, we have covered GPCR structure and classification, signal transduction, physiological role and various disorders related to GPCR structural dysfunction and neurobiology of mood disorders. The primary focus of this review will be to summarize recent advances and applications of GPCR in clinical medicine, which will help the researchers.

STRUCTURE

GPCRs are integral membrane proteins that possess seven membrane-spanning domains or transmembrane helices. The extracellular parts of the receptor can be glycosylated. These extracellular loops also contain two highly-conserved cysteine residues that form disulfide bonds to stabilize the receptor structure. Some seven-transmembrane helix proteins (channelrhodopsin) that resemble GPCRs may contain ion channels, within their protein. In 2000, the first crystal structure of a mammalian GPCR, that of bovine rhodopsin (1F88), was solved (Palczewski *et al*., 2000). In 2007, the first structure of a human GPCR was solved (2R4R, 2R4S) (Rasmussen *et al*., 2007). This human β_2-adrenergic receptor GPCR structure, proved to be highly similar to the bovine rhodopsin in terms of the relative orientation of the seven-transmembrane helices.

SIGNAL TRANSDUCTION

There are two principal signal transduction pathways involving the G protein-coupled receptors: the cAMP signal pathway and the Phosphatidylinositol signal pathway (Gilman, 1987). When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). The GPCR can then activate an associated G-protein by exchanging its bound GDP for a GTP. The G-protein's α subunit, together with the bound GTP, can then dissociate from the β and γ subunits to further affect intracellular signalling proteins or target functional proteins directly depending on the a subunit type ($G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$).

GPCRs were classified into 6 classes based on sequence homology and functional similarity (class A to F) (Attwood and Findlay, 1994; Foord *et al*., 2005; Kolakowski, 1994). The very large rhodopsin A group has been further subdivided into 19 subgroups (A1-A19) (Joost and Methner, 2002). More recently, an alternative classification system called GRAFS (Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, Secretin) has been proposed (Bjarnadottir *et al*., 2006).

PHYSIOLOGICAL ROLES

The human genome encodes thousands of G protein-coupled receptors (Vassilatis *et al*., 2003), about 350 of which detect hormones, growth factors and other endogenous ligands. Approximately 150 of the GPCRs found in the human genome have unknown functions. They comprise the largest family of receptors in the human genome.

Table 1: Examples of rare mutants of GPCR that cause human diseases

Receptor/Gene name	Mutation	Disease
Calcium-Sensing (CaS)/CaSR	Multiple (e.g., Arg185Gln)	Autosomal Dominant Hypocalcemia (ADH) Sporadic Hypoparathyroidism Familial Hypoparathyroidism
CXCR4	Multiple (e.g., Ser338X)	WHIM syndrome
Endothelin receptor B (ET_B)/EDNRB	Multiple (e.g., Trp276Cys)	Hirschsprung's disease
Follicle-stimulating hormone (FSH)/FSHR	Multiple (e.g., Ala189Val)	Female infertility
N-formyl-peptide (FPR)/FPR1	Phe110Ser, Cys126Trp	Juvenile periodontitis
Frizzled (FZD_4)/FZD4	Multiple (e.g., Arg417Gln)	Familial exudative vitreoretinopathy (FEVR)
Goandotropin-releasing hormone (GnRH)/GNRHR	Multiple (e.g., Arg262Gln)	Hypogonadotropic hypogonadism (HH)
GPR54/GPR54	Multiple (e.g., Cys223Arg)	Hypogonadotropic hypogonadism (HH)
GPR56/GPR56	Multiple (e.g., Cys223Arg)	Bilateral frontoparietal polymicrogyria (BFPP)
vGPCR/KSHV-GPCR	(constitutively active)	Kaposi's sarcoma (KS)
Relaxin family peptide receptor 2 (RXFP2)/LGR8	Multiple (e.g., Thr222Pro)	Cryptorchidism
MASS1 (also called VLGR1, USH2C)/MASS1	Multiple (e.g., Ser2652X))	Usher syndrome Febrile seizures (FS)
Melanocortin (MC_4)/MC4R	Multiple (e.g., Pro78Leu)	Dominant and recessive obesity
Rhodopsin/RHO	Multiple (e.g., Pro23His)	Retinitis pigmentosa (RP)
Vasopressin receptor (V_2)/AVPR2	Multiple (e.g., Arg113Trp)	Nephrogenic diabetes insipidus (NDI)

One such critical action is in the visual system where rhodopsin in photoreceptor-capturing neurons, retinal rods and color (red, blue and green) opsins in retinal cones, transduce the input from photons of light into electrical impulses that then travel to the brain and are decoded. A second major class of physiologically important GPCR are those that mediate the action of hormones, especially polypeptide hormones but also including the action of such hormones, such as the calcium-sensing receptor or other chemical entities (e.g., lipids, amines, fatty acids). A third class is receptors for physiologically important neurotransmitters, such as norepinephrine (and to a lesser extent, epinephrine), acetylcholine (at muscarinic cholinergic receptors), dopamine, serotonin (at certain receptors), glutamate (at metabotropic receptors) as well as numerous peptides and lipids that function as neuromodulators. In addition, a number of hormonally responsive GPCR have been identified as pathologic entities in a variety of endocrine disorders such as Autosomal Dominant Hypocalcemia (ADH), Hirschsprung's disease, Cryptorchidism etc., (Table 1). The latter disorders include those with either activating mutations or mutations that block hormonal response. 7-TM receptors are the target of around half of all modern medicinal drugs. Their expression on the cell surface makes them readily accessible to hydrophilic drugs and their non-uniform expression provides selectivity in activating or blocking physiological events. Agonists and antagonists of 7-TM receptors are used in the treatment of disease in almost every organ system.

INHERITED DISORDERS OF GPCR

Genetic diseases and genetic variants associated with those diseases are generally quite rare, occurring in <1% of the population and often variably among subjects of different ethnicities. non-lethal mutations can occur in GPCR, especially those that are expressed in sensory and hormonal systems, where they serve as mediators of information transfer from the extracellular environment to the cell interior (Fredriksson and Schioth, 2005). To date, mutations that lead to human disease have been identified in a relatively limited number of GPCR. Typically disease can be caused by: (Insel *et al*., 2007).

- Non-functional receptors (GHRH and familial growth hormone deficiency)
- Constitutive activation (Rhodopsin and retinitis pigmentosa)
- Changes in ligand binding specificity (Thyroid-stimulating hormone receptor and hyperthyroidism of pregnancy)
- Improper receptor processing (vasopressin receptor and diabetes insipidus)
- Antibodies directed against the receptors (Thyroid stimulation hormone receptor and Graves disease)
- Constitutively active or inactive G proteins

A large number of monogenic mutations have been identified in rhodopsin, in particular in patients that have the disease retinitis pigmentosa. Rhodopsin belongs to Family A, which contains the largest group of GPCR super family members; receptors in this Family generally contain a relatively short extracellular N-terminus and highly conserved amino acids within each transmembrane domain. Family A members are activated by small ligands such as biogenic amines and nucleotides, although rhodopsin itself is stimulated by photons of light that activate a retinal bound in a transmembrane pocket, which highlights the sites that are mutated in rhodopsin, a wide variety of residues have been identified in patients with retinitis pigmentosa, a leading cause of blindness and visual disability in younger people that occurs with an overall frequency of one in 4000.

The V2 receptor belongs to Family B, whose receptors recognize large peptides. Nephrogenic Diabetes Insipidus (NDI), which results from failure of vasopressin (antidiuretic hormone, ADH) to act on the renal collecting duct to facilitate water reabsorption, is another well-studied monogenic disorder of a GPCR. Mutations in the arginine vasopressin receptor 2 (AVPR2, V2), causes congenital nephrogenic diabetes insipidus in ~90% of patients via an X-linked recessive mode of inheritance. In most cases, these mutations lead to the intracellular trapping of the V2 receptors, such that few receptors reach the plasma membrane to trigger the activation of Gs and adenylyl cyclase and thereby, the generation of cAMP.

Genetic disorders of the Calcium-Sensing Receptor (CaSR) are a third example of a monogenic disease in GPCR (Tfelt-Hansen *et al*., 2005). This receptor is found on numerous tissues involved in calcium homeostasis, including the parathyroid glands, kidney and intestine. Binding of calcium to the CaSR is the mechanism by which parathyroid cells detect changes in ionized calcium concentration and in turn, modulate parathyroid hormone secretion so as to maintain normal serum calcium levels. CaSRs in renal tubules modulate calcium reabsorption in response to alterations in extracellular calcium concentrations. Several hypocalcemic and hypercalcemic disorders have been identified that derive from rarely occurring mutations of CaSR: loss-of-function CaSR mutations in the hypercalcemic disorders of familial benign (hypocalciuric) hypercalcaemia (FHH, FBH or FBHH) and neonatal severe primary hyperparathyroidism (NSHPT) (each of which occur <1/10,000) and gain-of-function CaSR mutations in autosomal dominant hypocalcaemia with hypercalciuria (ADHH) and Bartter's syndrome type V.

In addition to such "classical" monogenic diseases, another type of such disease can involve the generation of antibodies as monoclonal (or polyclonal) expansion of immune cells with the antibody products directed against GPCR or in some cases, their downstream targets. The most prevalent example is Graves disease, a form of hyperthyroidism with enhanced response to Thyroid-Stimulating Hormone (TSH) that is most commonly secondary to autoimmune activation of TSH receptors (Schott *et al*., 2005). Another example of activating (as well as inhibitory) antibodies

Table 2: Examples of polymorphisms of GPCR associated with human diseases

Receptor	Polymorphisms	Examples of disease associations
β_1 Adrenergic receptor	Arg389Gly	Heart failure
β_2 Adrenergic receptor	Multiple	Hypertension, Asthma
β_3 Adrenergic receptor	Trp64Arg	Obesity
CC chemokine receptor 2 (CCR2)	Val64Ile	Delayed progression of AIDS
CC chemokine receptor 5 (CCR5)	Multiple	Associated with progression of AIDS
Dopamine receptor 2 (D_2)	3'UTR52A/G	Associated with depression and anxiety
Dopamine receptor 3 (D_3)	Ser9Gly, Promoter SNPs	Haplotype associated with schizophrenia
Muscarinic receptor subtype 3 (M_3)	Promoter haplotype	Possible association with asthma and atopy
Neuropeptide S receptor	Haplotypes H1, H5	
(NPSR; also called GPR154, GPRA)	Asn107Ile, rs324981	Asthma susceptibility
$P2Y_{12}$	CA deletion at Codon 240	Associated with bleeding diathesis

directed at GPCR (e.g., β-adrenergic and muscarinic cholinergic) is Chagas' cardiomyopathy, which is triggered by infection with the protozoan *Trypanosoma cruzi* (Kierszenbaum, 2005).

In several other settings, antibodies directed at GPCR can blunt hormone action, preventing G-protein activation (Jahns *et al*., 2006; Wallukat *et al*., 2000). Of note, such disorders are almost invariably ones that occur in adults rather than children whereas monogenic disorders of the receptors themselves often manifest clinical abnormalities much earlier in life.

Genetic variants/polymorphisms identified in GPCRs can influence receptor expression, targeting, function and receptor turnover; as well as the ability of receptors to recognize and respond to pharmacologic agents. Some of important sequence variants identified in human GPCR genes that relate to human diseases such as Hypertension, Obesity, Haplotype associated with schizophrenia etc. listed in Table 2.

NEUROBIOLOGY OF MOOD DISORDERS

Suicide is a one of the major mood disorders and it is a leading cause of death (Joiner *et al*., 2005). As per a recent report from Centers for Disease Control and Prevention (CDC) in the United States in 2002, suicide was the eleventh most common cause of death (in 1998 it was the eight most common cause) (Licinio and Wong, 2005). One of the first studies showing a clear relation between suicide and psychiatric diseases reported that 45% of the suicide victims suffered mood disorders and 23% alcoholism (Robins *et al*., 1959). Thus, several researchers have demonstrated that depression, alcoholism, drugs of abuse and schizophrenia are the psychiatric pathologies more commonly related to suicide (Cheng, 1995; Isometsa and Lonnqvist, 1998; Rodriguez-Puertas *et al*., 1996). Depression is another major cause of suicide. In this regard, mood disorders seem to be present in at least 50% of all suicide victims (Balazs *et al*., 2003; Henriksson *et al*., 1993).

Several hypotheses have linked the pathogenesis of depression to alterations in the neurotransmission systems in the central nervous system (Ramakrishna *et al*., 2008). During the last four decades, alterations in the serotonergic and/or noradrenergic neurotransmission have been related to mood disorders. This monoaminergic theory of depression was based on several reports (Bunney and Davis, 1965; Coppen, 1969; Schildkraut, 1965).

GPCRs are involved in cellular responses to the majority of hormones and neurotransmitters and represent an enormously significant target for drug discovery. Alterations associated with depressive disorders of certain GPCR subtypes, such as a-adrenoceptor (Devaki *et al*., 2006; Subhash *et al*., 2003) and their subtypes (Ramakrishna *et al*., 2004; Ramakrishna and Subhash,

2010), 5-HT1A serotonin (Subhash and Ramakrishna, 2006) μ-opioid (Escriba *et al.*, 2004; Gabilondo *et al.*, 1995) and CB1 cannabinoid (Hungund *et al.*, 2004) receptor, both in terms of density and functionality, have been reported using different approaches.

Drug discovery in depression has been limited by the lack of a universally accepted animal model that can be used to screen for antidepressant effects. Although there are several animal models that reproduce some features of depression, their relevancy to the specifically human disorder major depression has been debated (Wong and Licinio, 2004).

Several works have reported that the antidepressant drugs modulate the density of different heterotrimeric G proteins (Lesch *et al.*, 1991a; Lesch and Manji, 1992), as well as their functional response (Delgado *et al.*, 1990; Menkes *et al.*, 1983; Okada *et al.*, 1986). Experimental evidences have shown that lithium attenuates the GPCR-induced modulation of the second messengers, even in the absence of changes in the density of the receptor (Devaki *et al.*, 2006). It has been consistently reported that chronic lithium affects G protein function (Avissar *et al.*, 1988; Colin *et al.*, 1991; Drummond, 1988; Lesch *et al.*, 1991b).

CONCLUSION

GPCR are physiologically important in maintaining normal homeostasis, in particular via their ability to mediate responses to circulating hormones and neurotransmitter input in the central, peripheral and autonomic nervous systems.

The cellular mechanisms of action of many antidepressants are still unknown and many depressed patients do not respond to antidepressant therapy. By understanding the cellular and molecular alterations associated with depressive disorders, the design of more efficacious therapeutic chemicals could be achievable. Several discrepancies have been reported in the literature according to the possible alterations in G proteins associated with mood disorders.

PCR will continue to be highly important in clinical medicine because of their large number, wide expression and role in physiologically important responses. We speculate that future discoveries will reveal new GPCR drugs which will help the genetic and various neurological disorders. In addition, further insights into GPCR biology may reveal novel, unexpected therapeutic targets that influence the GPCR life cycle or "ligand directed signalling".

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