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Review article

The Roles of G-protein coupled receptors in health and disease conditions

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ABSTRACT

The super family of G-protein-coupled receptors (GPCRs) is the main target for the actions exerted by hormones, drugs and neurotransmitters. Each GPCR shows preferential coupling to some members of the G-protein family such as Gs, Gi and Gq which in turn activates the defined second messenger pathways. The G protein-coupled receptors (GPCRs) represent 50–60% of the current drug targets and this family of membrane proteins plays a crucial role in drug discovery, health and disease conditions. The G-protein-mediated signalling system has been used to study transmembrane signalling mechanisms in eukaryotic organisms resulting in different cellular activities and effects such as cellular growth, proliferation and differentiation. The G-protein-mediated signalling systems are made up of three main components, the receptors, the heterotrimeric G-proteins and the effectors in addition to various proteins that modulate the G-protein-mediated signalling process like the regulators of G-protein signalling (RGS) proteins. Mammalian cells express many GPCRs and several types of heterotrimeric G-proteins and their effectors. A number of drugs based on GPCRs have been developed for such different indications as cardiovascular, metabolic, neurodegenerative, psychiatric, and oncologic diseases. Most neurotransmitters of the central nervous system (CNS) act on GPCRs to mediate different cellular responses in normal and disease states. The activation of receptors that interact through Gi e.g. cannabinoid receptor types convey neuronal protection against hypoxic insult and resultant excitotoxic death.

KEYWORDS: G-proteins, G protein coupled receptors, G protein signalling, Effectors, Neurotransmitters.

INTRODUCTION

Receptors are proteins found on the cell membrane, cytoplasm or nuclear membrane which bind specific molecules such as neurotransmitters, hormones or other ligands and as such initiate the cellular response to these substances [1, 2]. Ligand-induced changes in the behaviour of receptor proteins result in physiological changes that constitute the biological actions of the ligands. There are a variety of different types of ligands. Full agonists are able to activate the receptors and produce a maximal biological response and, as such, most naturally occurring ligands are full agonists. Partial agonists are not able to maximally activate receptors resulting in a partial biological response while antagonists bind to the receptor but do not activate it and thus result in receptor inhibition of the binding of agonists. Inverse agonists are ligands which produce an effect opposite to that of an agonist, yet act at the same receptor. They promote or stabilize an inactive form of the receptor [2, 3].

Receptors are activated by the binding of the specific agonist to them, which in turn leads to the activation of the second messenger. The activation of the second messenger cascade and the final biological response is achieved only when a significant number of receptors are activated by bound ligands. Receptors are classified depending on the ligand, their function and position [1]. Some receptor proteins are peripheral membrane proteins while many hormone receptors and neurotransmitter receptors are transmembrane proteins. The transmembrane receptors are embedded in the lipid bilayer of cell membranes and allow the activation of signal transduction pathways in response to their activation by the binding molecule or ligand [2, 3]. Metabotropic receptors are coupled to G proteins and affect cells indirectly through enzymes which control ion channels, while ionotropic receptors contain a central pore which functions as a ligand-gated ion channel [1, 2]. Another major class of receptors are intracellular proteins, such as steroid hormone receptors. These receptors can often enter the cell nucleus and modulate gene expression in response to the activation by the ligand [4, 5]. The regulatory protein subunits of many ion channels and transmembrane receptors may be defined as peripheral membrane proteins [2, 4 & 5].

The transmembrane receptors are integral membrane proteins, which reside and operate in the plasma membrane of the cell and also in the membranes of some subcellular compartments and organelles [4, 6]. The binding of signalling molecule on one side of the membrane to transmembrane receptors initiate a response on the other side. In this way they play a unique role in cellular communications and signal transduction [3, 5]. Many transmembrane receptors are composed of two or more protein subunits which operate collectively and may dissociate when ligands bind or fall off. The transmembrane receptors have both extracellular domain and intracellular domain which means that within the intracellular domain enzymes are found that bring about the phosphorylation of the effectors [5, 7].

There are several ways that cells regulate the activity of a transmembrane receptor. The most important way is through phosphorylation and internalization [6, 8]. Examples of transmembrane receptors are adrenergic receptors, olfactory receptors, receptor tyrosine kinases, epidermal growth factor receptors, insulin receptors, fibroblast growth factor receptors, neurotrophin receptors, nerve growth factor (NGF) receptors and N-methyl-D-aspartic acid (NMDA) receptors [3, 9]. Neurotransmitter receptors can be classified into two broad categories based on their structural and functional characteristics, namely metabotropic and ionotropic receptors. The metabotropic receptors do not form an ion channel pore rather they are indirectly linked with ion-channels on the plasma membrane of the cell through signal transduction mechanisms [3, 6]. This class of receptor includes the metabotropic glutamate receptors, muscarinic acetylcholine receptors, γ-amino butyric acid (GABA) receptors, and most serotonin receptors, as well as receptors for norepinephrine, epinephrine, histamine, dopamine, endorphin, enkephalin and the endocannabinoids [3, 9]. All metabotropic receptors are monomeric proteins with seven transmembrane domains. While ionotropic channels have an effect only in the immediate region of the receptor, the effects of metabotropic receptors can be more widespread through the cell [3]. Although individual receptor signalling mechanisms are most frequently studied in isolation, their activity *in vivo* is likely to be highly regulated by other signal-transduction events. Receptors which primarily activate one pathway may activate a second pathway. An example is the ability of GPCRs such as α2-adrenergic receptors or muscarinic acetylcholine receptors (mAChRs), to activate the mitogen activated protein kinase (MAPK) cascade. The stimulation of GPCRs results in the activation of Adenylyl cyclase (AC) which results in the release of G protein βγ subunits. The present paper reviews the roles of G-protein coupled receptors in health and disease conditions.

G protein coupled receptors:

The GPCRs are also known as seven transmembrane (7TM) receptors and they belong to a super family of G protein receptors. Some of the examples are muscarinic acetylcholine receptors, adenosine receptors, adrenergic receptors, γ-amino butyric acid (GABA), cannabinoid receptors, dopamine receptors, glucagon receptors, metabotropic glutamate receptors, opioid receptors, calcium-sensing receptors and olfactory receptors. Hence the majority of life processes occurs through the activation of the GPCRs. Cells can regulate their receptors by either down regulating or up regulating the number of receptors to a given hormone, neurotransmitter or drug in order to alter their sensitivity to that molecule. This is called local feedback mechanism [3, 8, 9].

Several G protein-coupled receptors are capable of activating the MAPK pathway [9, 10, 11].

Research has shown that MAPK was found to induce mitogenesis in cultured fibroblasts after

stimulation of GPCRs by naturally occurring phospholipids [12]. MAPKs are localised in both the cytoplasm and nucleus and are suspected to be involved in the phosphorylation of nuclear transcription factors which regulate gene transcription [10, 12]. Activation of protein kinase C (PKC) and phospholipase C beta (PLCβ) has also been linked to MAPK activation [13]. Thus, G proteins are linked to pathways that influence not only membrane conductance but also cell proliferation and growth, implicating a possible role of G proteins in disease pathology [10, 3, 14].

The receptors for neurotransmitters can be classified into two categories: metabotropic receptors, also called GPCRs, and ionotropic receptors. GPCRs are involved in signal transduction. The binding of neurotransmitters on GPCRs may trigger series of signalling processes which may result in cellular-induced activities. The ionotropic receptors form ion channels which may be activated upon binding of specific neurotransmitters. Once activated, the influx of cations, such as Na^+, may cause the postsynaptic membrane to depolarize [7, 8]. If the depolarization reaches the threshold, an action potential can be generated on the postsynaptic neuron. The ion channel formed by neurotransmitter receptors is called the synaptic channel and each synaptic channel consists of five receptor subunits [8, 14].

All metabotropic receptors are monomeric proteins with seven transmembrane domains. The N terminus of the protein is on the extracellular side of the membrane and its C terminus is on the intracellular side [9, 11]. Metabotropic receptors have neurotransmitters as ligands, which, when bound to the receptors, initiate a cascade of events that can lead to channel-opening or other cellular effects [3, 6]. When a ligand or primary messenger, binds to the receptor, or the transducer, it activates a primary effector, which can go on to activate secondary messengers. Since the opening of channels by metabotropic receptors involves activating a number of molecules in turn, channels associated with these receptors take longer to open than ionotropic receptors do, and are thus not involved in mechanisms that require quick responses [8, 9, 11, 14]. Metabotropic receptors also remain open from seconds to minutes and have a much longer-lasting effect than ionotropic receptors, which open quickly but only remain open for a few milliseconds [7-9]. While ionotropic channels have an effect only in the immediate region of the receptor, the effects of metabotropic receptors can be more widespread through the cell [3, 9].

Metabotropic receptors can open and close channels and can make a membrane more excitable by closing K^+ channels. They can retain positive charge within the cell and thus reduce the amount of current necessary to cause an action potential [8, 10]. The metabotropic receptors on the presynaptic membrane can inhibit or, more rarely, facilitate neurotransmitter release from the presynaptic neuron [12, 15, 16]. These receptors can be further classified into receptor tyrosine kinases and G-protein-coupled receptors, known as GPCRs [8, 9, 11, 14, 16], as shown in Figure 1.

Fig 1: The effects of agonist-induced activation of receptors at the cell membrane.

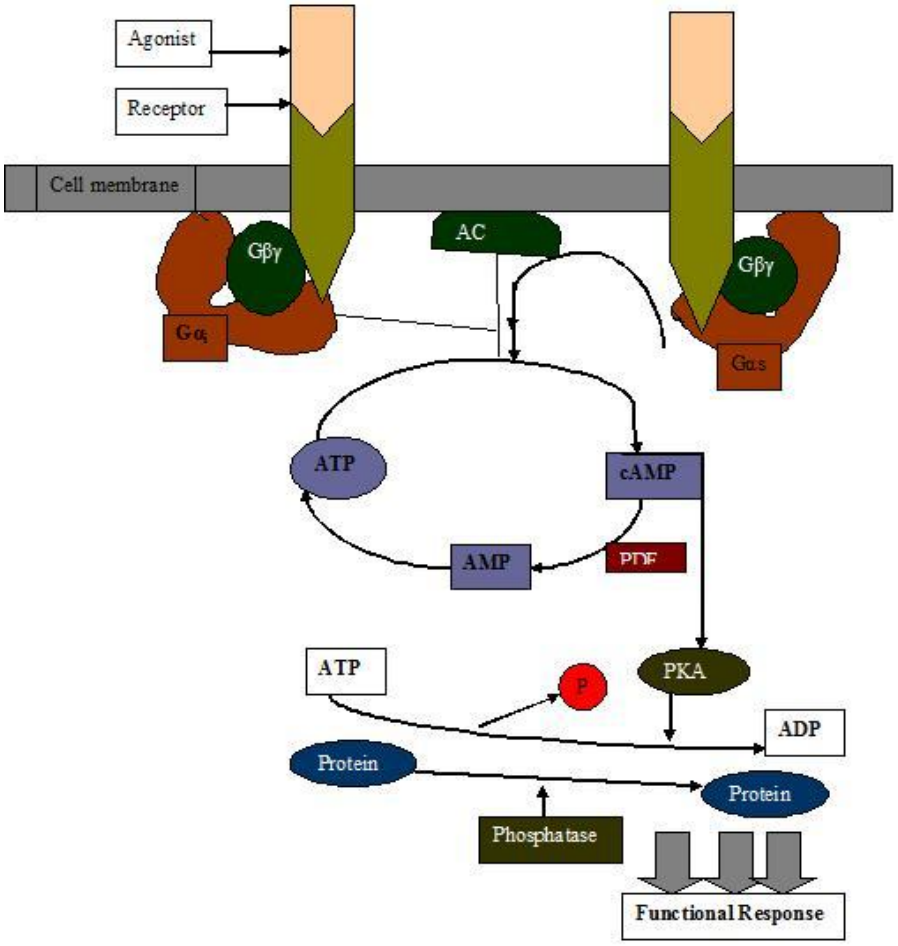

Figure1: shows the schematic representation of the effects of agonist-induced activation of G-protein coupled receptors (GPCRS) at the cell membrane. Agonists binding on the receptors result in the stimulation of the G-protein complex (Gαβγ) which then dissociate into Gα and Gβγ subunits which require the action of the adenylate cyclase (AC) enzyme. The splitting of adenosine triphosphate (ATP) for energy resulting in the formation of Cyclic Adenosine monophosphate (cAMP) which reacts with protein kinase A (PKA) enzyme resulting in protein phosphorylation which require energy from ATP breakdown for functional activity of cells.

Second Messengers

Second messengers are molecules that relay signals received at receptors on the cell surface, such as the arrival of protein hormones and growth factors to target molecules in the cytosol or nucleus. Second messengers also serve to greatly amplify the strength of the signal and the binding of a ligand to a single receptor at the cell surface. As a result they may end up causing massive changes in the biochemical and physical activities within the cell through the activities of

the second messengers which amplify the effect of the signal on the effectors [3, 7, 17]. Second messengers are intracellular molecules or ions that are regulated by extracellular signalling agents such as neurotransmitters, hormones and their ligands which are called first messengers [17]. Second messengers typically operate by activation of protein kinases that phosphorylate the target proteins, thereby altering the function of these proteins [8, 18]. Such functional effects are subsequently reversed by protein phosphatase-mediated dephosphorylation and the second messengers modulate a wide range of rapid and long-term neuronal processes [5, 18]. Examples of second messenger molecules are cAMP, Ca^{2+} and inositol triphosphate (IP_3) [16, 19].

There are three main types of secondary messenger molecules: (1) Hydrophobic molecules like diacylglycerol, inositol trisphosphate (IP_3) and phosphatidylinositols. These are membrane-associated and diffuse from the plasma membrane into the juxtamembrane space where they can reach and regulate membrane-associated effector proteins [17, 20, 18, 21]. (2) Hydrophilic molecules are water-soluble molecules, like cAMP, cGMP, and Ca^{2+}, that are located within the cytosol and (3) gases, nitric oxide (NO) and carbon monoxide (CO), that can diffuse through cytosol and across cellular membranes [6, 22]. Secondary messengers can be synthesized or released and broken down again in specific reactions by enzymes. Some, like Ca^{2+}, can be stored in special organelles and quickly released when needed and their production, release and destruction can be localized, enabling the cell to limit space and time of signal activity [19, 30, 31, 20].

Mechanisms of action of secondary messengers

Cyclic AMP is synthesized from ATP by the action of the enzyme AC and the binding of the hormone to its receptor activates a G protein which, in turn, activates AC [16, 20, 22]. The resulting rise in cAMP turns on the appropriate response in the cell by either, changing the molecular activities in the cytosol using protein kinase A (PKA) or by turning on a new pattern of gene transcription (Figure 2). This new pattern of gene transcription in cells occurs through the activity of cAMP response element-binding (CREB) proteins. The transducin family of G proteins mediate signal transduction in the visual system by regulating specific forms of phosphodiesterase (PDE) which catalyse the metabolism of cyclic nucleotides [21, 22, 32, 33]. $G\alpha_t$ activates PDE via direct binding to the enzyme [23, 28, 30, 33, 19]. The ability of neurotransmitter receptors to stimulate the phosphoinositide second-messenger pathway is mediated by the activation of phosphatidylinositol-specific phospholipase C (PI-PLC), which catalyses the hydrolysis of phosphatidylinositol bisphosphate (PIP_2) to form the second messengers inositol triphosphate (IP_3) and diacylglycerol (DAG) [22, 23, 24, 30, 34].

Fig.2. G-protein receptor activation leading to transcription of some genes.

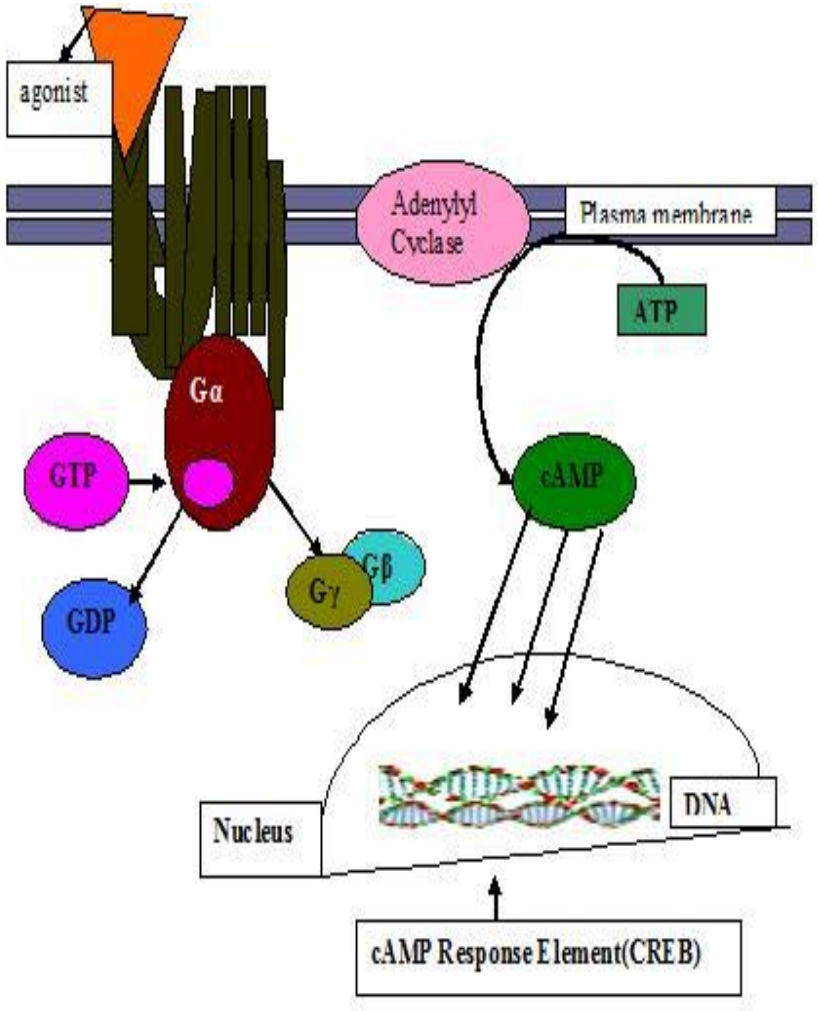

Figure2: shows representation of Guanine nucleotide binding protein (G-protein) receptor activation resulting in the splitting of G-protein complex (Gαβγ) into Gα and Gβγ subunits leading to the phosphorylation of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) which pass into the nuclei of the cells as cAMP response element-binding proteins (CREB) and function as transcription factors of some genes.

Different types of substances act as neurotransmitters. Classical neurotransmitters like acetylcholine (ACh) and norepinephrine (NE) are low molecular weight substances that have no other function but to serve as neurotransmitters [9, 25, 27, 28]. The predominant excitatory neurotransmitter in the brain, glutamate and the inhibitory neurotransmitter in the spinal cord, glycine are common and essential amino acids [26, 29, 30, 33]. They function as neurotransmitters because the membranes of secretory vesicles in glutamatergic and glycinergic nerve terminals have specific transport systems that concentrate and store these amino acids so that they can be released by exocytosis in a highly regulated manner [9, 27, 28]. Aminergic neurotransmitters, namely ACh and

GABA, also enter synaptic vesicles through specific transport proteins. Neurotransmitters released from the vesicle may diffuse through the synaptic cleft to act on their receptors in the postsynaptic neuron (Figure-3).

Fig.3: Mechanism of synaptic transmission between two nerve cell terminals.

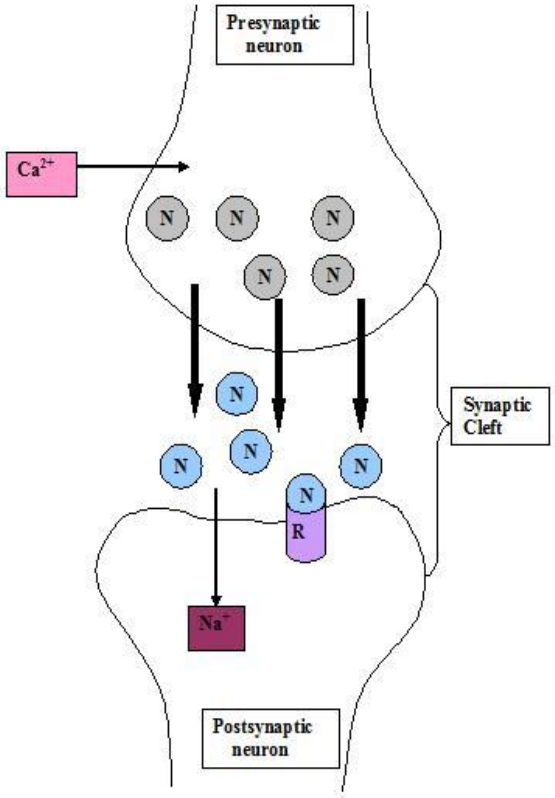

Figure 3: shows mechanism of synaptic transmission between nerve cell terminals in which neurotransmitters (N) are stored in vesicles at the presynaptic nerve terminal. The action potential at the presynaptic terminal causes the entry of Ca^{2+} ions through voltage-activated calcium channels, leading to the release of neurotransmitters (N) into the synaptic cleft which then binds to the receptors (R) with Na^{+} entry into the postsynaptic neurons through sodium channels.

Diseases Caused by Defects in the Production or Activity of G Proteins

Mutations in the heterotrimeric guanine nucleotide-binding proteins (G proteins) which relay signals initiated by photons, drugs, odorants, hormones and neurotransmitters cause many diseases. Mutations in G proteins can also lead to essential hypertension and this may be one of several common disorders caused by defects in this family of signaling molecules [34, 35, 37]. Mutations that alter G protein activation may cause disorders characterized by either insufficient or excessive transmission of signals [35, 36].

Decreased transmission of signals, loss of function, results from mutations that impair the ability of the G protein to become activated by hormone receptors. Increased transmission of signals, and gain of function result from mutations that mimic or augment the activation of receptors [34-36].

Cholera a watery diarrhoea caused by *Vibrio cholerae* is as a result of the secretion of salt and water into the intestine, stimulated by increased concentrations of cyclic AMP in mucosal cells [35, 36]. Similar toxins are responsible for the traveller's diarrhoea caused by certain strains of *Escherichia coli*. The first oncogenic Gα mutants were found in the $G\alpha_s$ genes of pituitary tumors from patients with acromegaly. The mutant $G\alpha_s$ oncogene is found in 40 percent of the somatotropic tumors in such patients [37, 38]. The protein encoded by G stimulating protein *(gsp)* is oncogenic because it mimics the intracellular signal triggered by growth hormone–releasing hormone, which normally stimulates a receptor that activates G_s and cAMP synthesis [38-40]. *Gsp* stimulates the secretion of growth hormone and increases the proliferation of somatotrophs. As with most oncogenes, *gsp* results from somatic mutations that occur in the affected tissue. Other Activating Mutations in Gα include the McCune–Albright syndrome which is characterized by polyostotic fibrous dysplasia, scattered regions of hyper pigmented skin, and autonomous hyper function of one or more endocrine glands [30, 35, 37]. This results in gonadotropin-independent precocious puberty, hyperthyroidism, Cushing's syndrome, or acromegaly. The affected tissues of patients with this syndrome contain *gsp* mutations. All manifestations of this congenital syndrome reflect a *gsp* mutation that occurred early in the development of the embryo, in the DNA of a cell whose progeny will contribute to the mosaic distribution of affected cells later in life [35, 38, 40]. In the study that used transgenic mice over-express normal or mutationally activated Gα subunits in specific tissues may prove useful in predicting Gα diseases in humans [35-39]. The over expression of $G_q\alpha$ in the heart, results in severe cardiac hypertrophy and subsequent congestive heart failure [34, 39]. Diseases are likely to result from mutations in genes encoding the regulators of G protein signaling proteins (RGS) [35, 37, 40]. Heterotrimeric G proteins attached to the cell membrane convey signals from G protein-coupled receptors in response to stimulation by a number of hormones, neurotransmitters, chemokines, and pharmacological agents to intracellular signaling cascades [41-43].

CONCLUSION

G proteins may exert other cellular functions other than acting as signaling transducers. There is evidence for their roles in different diseases including infections, inflammation, neurological diseases, cardiovascular diseases, cancer, and endocrine disorders. Mutations in G proteins are

involved in several diseases, ranging from Whooping Cough and Cholera to several endocrine disorders. G protein-related diseases are characterised by either deficient or excessive G protein signal transmission, which arises through abnormal signal initiation, defective termination, or reduced levels of G proteins. Perturbation of G-protein signalling is also central to the actions of many drugs, including those used to treat asthma, hypertension and depression. Deficient G protein signalling can arise through either reduced levels of G proteins or through decrease signal initiation. Diseases involving a decrease in the production of G proteins include Night Blindness, where mutations in Gαt protein affect the response of rod cells to light and Pseudohypoparathyroidism, where the genetic loss of Gs protein α subunits results in non-responsiveness to parathyroid hormone. Other abnormalities involve decreased signal initiation through the inability of G proteins to switch to active states. For example, the symptoms of Pertussis result from the action of a bacterial toxin which adds ADP-ribose to the receptor-binding C-terminal tail of Gαi protein, causing a reduced responsiveness of G proteins to receptor activation. Excessive G protein signalling can arise through either increased signal initiation or defective signal termination. Increased signal initiation occurs in testotoxicosis, where a mutation in the receptor for luteinizing hormone can over-stimulate Gs proteins, resulting in the excessive production of testosterone in addition and several cases of Essential Hypertension arise from mutations in Gβ protein. Diseases arising from defective signal termination result from the persistent elevated activity of downstream effectors, such as in Cholera. Two other diseases involve defective termination through mutations in Gαs protein including Adenomas, in which G proteins lose their ability to hydrolyse GTP through mutation, resulting in the excessive secretion of growth hormone and the increased proliferation of somatotrophs and McCune-Albright Syndrome with scattered regions of skin hyper-pigmentation due to the hyper-functioning of one or more endocrine glands.

REFERENCES

1) Ben-Shilomo I, Yu Hsu S, Rauch R, Kowalski HW, Hsueh AJ. Signalling Receptome: A Genomic and Evolutionary Perspective of Plasma Membrane Receptors Involved in Signal Transduction. *Sci STKE*. 2003. (187):RE9.

2) Howlett AC. Cannabinoid receptor signalling. *Handb Exp Pharmacol*. 2005.168:53-79.

3) Berridge MJ. Cell Signalling Biology. Portland Press Ltd. 2006. www.cellsignallingbiology.org.

4) Connor M and Christie MD. Opioid receptor signalling mechanisms. *Clin Exp Pharmacol Physiol. 1999.* 26(7):493-499.

5) Garzon J, Rodriguez-Munoz M, de la Torre-Madrid E, Sanchez-Blazquez P. Effector antagonism by the regulators of G

protein signalling (RGS) proteins causes desensitization of mu-opioid receptors in the CNS. *Psychopharmacol.* (Berl). 2005. 180(1):1-11.

6) Cho W and Stahelin RV. Membrane-protein interactions in cell signalling and membrane trafficking. *Ann. Rev Biophy and Biomolecular Struct*. 2005. 34: 119–151.

7) Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*. 2003. 4(7):517-529.

8) Kandel ER, Schwartz JH, Jessell TM. Principles of neural science. New York: McGraw-Hill. 2000.

9) Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia A-S, McNamara JO, Williams SM. Neuroscience: *Neural Signalling*: 2nd ed. Sinauer Associates, Inc., Sunderland, MA. USA. 2001.

10) Luttrell LM, Daaka Y, Della Rocca GJ, Lefkowitz RJ. G protein-coupled receptors mediate two functionally distinct pathways of tyrosine phosphorylation in rat 1a fibroblasts. Shc phosphorylation and receptor endocytosis correlate with activation of ERK kinases. *J Biol Chem.*1997. 272(50):31648-31656.

11) Lowes VL, Ip NY, Wong YH. Integration of signals from receptor tyrosine kinases and g protein-coupled receptors. *Neurosignals.* 2002. 11(1):5-19.

12) Howe LR and Marshall CJ. Lysophosphatidic acid stimulates mitogen-activated protein kinase activation via a G-protein-coupled pathway requiring p21ras and p74raf-1. *J Biol Chem.* 1993. 268(28):20717-20720.

13) Kolch W, Heidecker G, Kochs G, Hummel R, Vahidi H, Mischak H, Finkenzeller G, Marme D, Rapp UR. Protein kinase C alpha activates RAF-1 by direct phosphorylation. *Nature.* 1993. 364(6434):249-252.

14) Cabrera-Vera TM, Vanhauwe J, Thomas TO, Medkova M, Preininger A, Mazzoni MR, Hamm HE. Insights into G protein structure, function, and regulation. *Endocr Rev*. 2003. 24(6): 765-781.

15) Schmitz D, Mellor J, Nicoll RA. Presynaptic kainate receptor mediation of frequency facilitation at hippocampal mossy fibre synapses. *Science.* 2001. 291(5510): 1972-1976.

16) Kukkonen JP, Jansson CC, Akerman KE. Agonist trafficking of G(i/o)-mediated alpha(2A)-adrenoceptor responses in HEL 92.1.7. Cells. *British Journ. Pharmacology*. 2001. 132(7):1477-1484.

17) Nasman J, Kukkonen JP, Holmqvist T, Akerman KE. Different roles for Gi &Go proteins in modulation of adenylyl cyclase type-2 activity. *Neurochemistry*. 2002. 83(6) 1252-1261.

18) Wettschureck N, Moers A and Offermanns S. Mouse Models to study G-protein-mediated signalling. *Pharmacology and Therapeutics.* 2004. 101:75-89.

19) Heldin CH. Protein tyrosine kinase receptor signalling overview. 2003. 391–396.

20) Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem* 1997. *Sci*. 22(7):267-272.

21) Cohen P. The origins of protein phosphorylation. *Nature Cell Biology*. 2002. 4:E127-E130.

22) De Vries L; Zheng TB; Elenko FE and Farquhar MG. The Regulator of G Protein Signaling Family. *Annual Review of Pharmacology and Toxicology*. 2000. 40: 235-271.

23) Neubig RR and Siderovski DP. Regulators of G-protein signalling as new central nervous system drug targets. *Nat Rev Drug Discov*. 2002. 1(3):187-197.

24) Ross EM and Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu Rev Biochem.* 2000. 69:795-827.

25) Saugstad JA, Marino MJ, Folk JA, Helper JR & Conn PJ. RGS4 inhibits signalling by group 1 metabotropic glutamate receptors. *The Journal of Neuroscience.* 1998. 18(3) 905-913.

26) Bruno V, Copani A, Battaglia G, Raffaele R, Shinozaki H, Nicoletti F. Protective effect of the metabotropic glutamate receptor agonist, DCG-IV, against excitotoxic neuronal death. *Eur J Pharmacol*. 1994. 256(1):109-112.

27) Shen M and Thayer SA. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. *Mol Pharmacol*.1998. 54 (3):459-462.

28) Rashidian J, Iyirhiaro G, Aleyasin H, Rios M, Vincent I, Callaghan S, Bland RJ, Slack RS, During MJ, Park DS. Multiple cyclin-dependent kinases signals are critical mediators of ischemia/hypoxic neuronal death in vitro and in vivo. *PNAS. USA*.2005. 102(39):14080–14085.

29) Golla R and Seethala R. A homogeneous enzyme fragment complementation cyclic AMP screen for GPCR agonists. *J Biomol Screen.* 2002. 7(6): 515-525.

30) Siegel GJ, Agranoff BW, Albers RW, Fisher SK and Uhler MD. eds. *Basic Neurochemistry; Molecular, cellular and medical aspects*. 6th ed. Lippincott Williams & Wilkins, Philadelphia. 1999. 1023-1120.

31) Haglund K and Dikic I. Ubiquitylation and cell signaling. *EMBO J.* 2005. 24(19): 3353-3359.

32) Neer EJ. Heterotrimeric G proteins: organizers of transmembrane signals. *Cell*. 1995. 80(2):249-257.

33) Wickman KD and Clapham DE. G-protein regulation of ion channels. *Curr. Opin. Neurobiol*. 1995. 5(3):278-285.

34) Farfel Z, Bourne HR and Iiri T. The expanding Spectrum of G protein diseases. New Eng J. Med. 1999, 340:1012-1020.

35) Spiegel AM, Weinstein LS and Shanker A. Abnormalities in G-protein-Coupled signal transduction pathways in human disease. Journal of Clin Invest. 1993. 92(3) 1119-1125.

36) McDowall J. When G protein signalling is disrupted. InterPro Protein sequence analysis and classification Online accessed on 30th Nov.2010.

37) Purves G proteins in Neuroscience 4th Ed Sinauer Associates Inc 2007, pg 115

38) Melien O. Heterotrimeric G proteins and disease. Methods in Molecular Biology. 2007, 361: 119-144.

39) Lundstrom K, An Overview on GPCRs and Drug discovery; Structure-based drug design and structural biology of GPCRs. Methods in Molecular Biology 2009, 55;51-56

40) Lagerstrom MC, Schioth HB (2008). Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov*. 2008. 7: 339–357.

41) Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? *Nat Rev Drug Discov 2006.* **5**: 993–996.

42) Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol.* 2003. 63: 1256–1272.

43) Siegel GJ, Albers RW, Brady ST and Price DL. eds. *Basic Neurochemistry: Molecular, cellular and medical aspects.* 7th ed. Elsevier Academic Press, San Diego. 2006. 339-346.

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