Preface

The G proteins are a family of structurally homologous, plasma membrane-associated guanine-nucleotide-binding proteins. These proteins play an integral role in the transduction of extracellular signals through second messenger systems. As such, G proteins affect a wide variety of intra cellular biochemical reactions by regulating the concentration of second messengers in cells.

G proteins are heterotrimeric, consisting of α, β, and γ polypeptide chains, with G protein specificity largely determined by the α-subunit. Molecular cloning of G protein subunits has revealed 23 distinct α-subunits, encoded by 17 different genes. Based on functional measures, G proteins are generally classified into three major categories: the G_s family, which is stimulatory for adenylyl cyclase; the G_i family, which is inhibitory for adenylyl cyclase; and the G_q family, which stimulates phospholipases (Birnbaumer and Birnbaumer, 1995). Alternatively, on the basis of sequence homology, G proteins can be subdivided into four categories: G_s, G_i, G_q, and G_{12}.

Although the α-subunits are specific for a given G protein, the β- and γ-subunits share considerable homology with each other. As previously mentioned, it has generally been assumed that the diversity of the α-subunits provides the essential specificity for G protein receptor interactions. However, there is also evidence suggesting that the structural diversity of the β- and γ-subunits may also contribute to the specificity of these interactions. On the basis of molecular cloning studies, 5 β-subunits and 10 γ-subunits have been described. These two subunits can be regarded as one functional subunit, considering their tight association. The five β-subunits are highly homologous, and display a characteristic feature of repetitive tryptophan-aspartate (WD) motif,

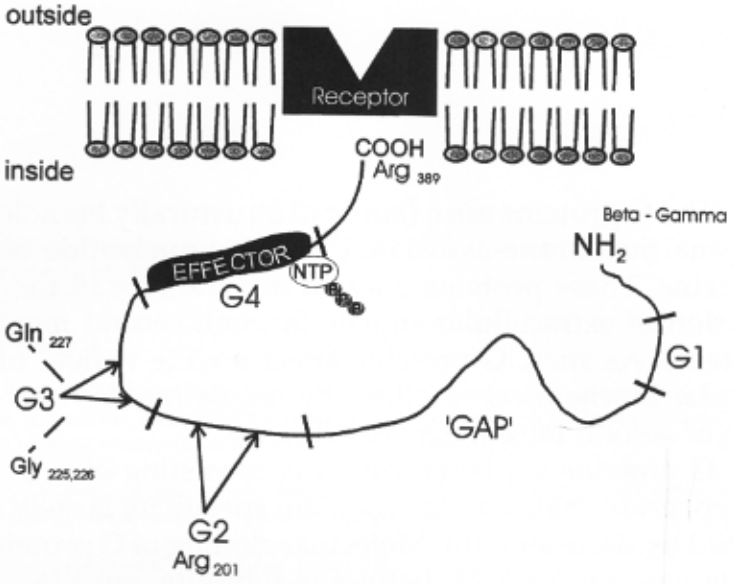

Fig. 1. A hypothetical model of G protein α-subunit (modified from Gordeladze et al., 1994). The figure displays various functions and the spatial interrelationships within the α-subunit. The C-terminus is involved in receptor binding, whereas the N-terminal and probably some other stretch of the subunit are involved in its anchoring to the β γ-subunit complex. Various other regions, G-1, G-2, G-3, and G-4, are important for the binding of GTP. The GTPase activity and the GDP/GTP exchange reside in G-2, G-3 regions. NTP stands for nucleotide triphosphate. Amino acids denoted with a number represent their corresponding position in the sequence. Mutations in these positions have led to the identification of their functional role.

whereas the γ-subunits display significantly more diverse primary structures (Simon et al., 1991; Helper and Gilman, 1992; Ray et al., 1995; Guderman et al., 1996).

G proteins cycle between a GDP-bound inactive form (which maintains the high-affinity agonist-binding state of the receptor) and a GTP-bound active form (low-affinity agonist binding state of the receptor). On agonist binding, the cell surface receptor catalyzes the GDP/GTP exchange at the α-subunit, and this active form goes on to stimulate effector pathways in transmembrane signaling. The salient features of α-subunit signaling are displayed in Fig. 1.

Table 1
G Proteins in Disease States

Disease states	Subtypes $G_{s\alpha}$	$G_{i\alpha}$	G_o	β_γ	References
Congestive heart failure	↓80%	↑	ND	ND	Horn et al., 1988
	↓	↑	—	—	Horn et al., 1995
Dilated cardiomyopathy	ND	↑37%	ND	ND	Bohm et al., 1990
Heart failure	ND	↑	ND	ND	Neumann et al., 1988
Left ventricle failure	↓50%	ND	ND	ND	Longabaugh et al., 1988
Heart—septic shock	ND	↑65%	ND	ND	Bohm et al., 1995
Schizophrenia	—	↓	↓	ND	Okada et al., 1991
Depression	↑	NC	—	—	Manji et al., 1995
	↑	↑FC	↓	—	Lesch et al., 1992; Young et al., 1994
Cocaine addiction	NC	↓NAC	↓NAC	NC, NAC	Self et al., 1994
	—	Low levels VTA, NAC, LC	Low levels VTA, NAC, LC	—	Manji, 1992
	↑30–70% (hypo)	NC	↑20% clostrum, NE	NC	Parolaro et al., 1993
Morphine tolerance	↑>50% (striatal neurons)	↓16%	—	—	Van Vliet et al., 1993

[a]Abbreviations: ND, not determined; NC, no change; FC, frontal cortex, NAC, Nucleus accumbens, VTA, ventral tegmental area; LC, locus ceruleus; NE, nucleus endopiriform.

Changes in G Protein Subunits in Diseases

In view of the critical and important role carried out by G proteins in signal transduction, it is very likely that qualitative or quantitative changes in G proteins could have significant effects on intracellular effectors. It has been recently recognized by many investigators that the simple estimation of receptor numbers in disease states may not provide a complete perspective of receptor activity (Brodde and Michel, 1989). Indeed, the modification of signal transduction pathways could provide important clues to the pathophysiology of many disease conditions. For example, it has been demonstrated that in experimentally induced diabetes, the gene expression of the $G_{i\alpha}$-subunit is significantly reduced (Gawler et al., 1987). Similarly, in patients with type IA pseudohypoparathyroidism, reduced levels of G_s protein and mRNA levels have been reported (Carter et al., 1987). In congestive heart failure, the levels of G_s in lymphocyte membranes are significantly reduced, and this reduction can be fully restored by therapeutic agents, such as angiotensin-converting enzyme inhibitor (Horn et al., 1988). A summary of alterations in G protein levels in various diseases is presented in Table 1. Although in the past decade significant advances have been made toward unraveling the role of neurotransmitter receptors in various psychiatric and neurological disorders, the role of second messenger systems in these disease states is still poorly understood. The purpose of *G Protein Methods and Protocols* is to provide novel information and techniques pertaining to the study of G proteins and their role in CNS functions.

References

Birnbaumer, L. and Birnbaumer, M. (1995) Signal transduction by G-proteins. *J. Recept. Signal Trans. Res.* **15,** 213–252.

Bohm, M., Gierschik, P., Pieske, B., Schnabel, P., Ungerer, M., and Erdmann, E. (1990) Increase of Gi alpha in human hearts with dilated but not ischemic cardiomyopathy. *Circulation* **82,** 1249–1265.

Brodde, O. E. and Michel, M. C. (1989) Disease states can modify both receptor number and signal transduction pathways. *TIPS* **10,** 383, 384.

Carter, A., Bardin, C., Collins, R., Simons, C., Bray, P., and Spiegel, A. (1987) Reduced expression of multiple forms of the alpha subunit

of the stimulatory GTP binding protein in pseudohypoparathyroidism type Ia. *Proc. Natl. Acad. Sci. USA* **84,** 7266–7269.

Gawler, D. J., Milligan, G., Spiegel, A. M., Unson, C. G., and Houslay, M. D. (1987) Abolition of the expression of inhibitory guanine nucleotide regulatory protein Gi activity in diabetes. *Nature* **327,** 229–232.

Gordeladze, J. O., Johansen, P. W., Paulson, R. H., Paulssen, E. J., and Gautvik, K. M. (1994) G-proteins: implications for pathophysiology and disease. *Eur. J. Endocrinol.* **131,** 557–574.

Guderman, T., Kalbrenner, F., and Schultz, G. (1996) Diversity and selectivity of receptor G-protein interaction. *Ann. Rev. Pharmacol. Toxicol.* **36,** 429–459.

Helper, J. R. and Gilman, A. G. (1992) G-proteins. *Trends Biochem. Sci.* **17,** 383–387.

Horn, E. M., Corwin, S. J., Steinberg, S. F., Chow, Y. K., Neuberg, G. W., Cannon, P. J., Powers, E. R., and Bilezikian, J. P. (1988) Reduced lymphocyte stimulatory guanine nucleotide regulatory protein and beta-adrenergic receptors in congestive heart failure and reversal with angiotensin converting enzyme inhibitor therapy. *Circulation* **78,** 1373–1379.

Horn, E. M., Kukin, M. L., Neuberg, G. W., Goldsmith, R. L., McCarty, M., Gratch, M., Medina, N., Yushak, M., and Packer, M. (1995) Lymphocyte G-proteins reflect response to treatment in congestive heart failure. *Am. Heart J.* **129,** 98–106.

Lesch, K. P., Hough, C. J., Aulakh, C. S., Wolozin, B. L., Tolliver, T. J., Hill, J. L., Akiyoshi, J., Chuang, D., and Murphy, D. L. (1992) Fluoxetine modulates G-protein alpha-s, alpha-q and alpha-12 subunit mRNA expression in rat brain. *Eur. J. Pharmacol. Mol. Pharmacol.* **227,** 233–237.

Longabaugh, J. P., Batner, D. E., Batner, S. F., and Homcy, C. J. (1988) Decreased stimulatory guanosine triphosphate binding protein in dogs with pressure overload left ventricular failure. *J. Clin. Invest.* **81,** 420–424.

Manji, H. K. (1992) G-proteins: implications for psychiatry. *Am. J. Psychiatry* **149,** 746–760.

Manji, H. K., Chen, G., and Shimon, H. (1995) Guanine nucleotide binding proteins in bipolar affective disorders: effect of long-term lithium treatment. *Arch. Gen. Psychiatry* **52,** 135–144.

Neumann, J., Schmitz, W., Scholz, H., von Meyerinck, L., Doring, V., and Kalmar, P. (1988) Increase in myocardial Gi-proteins in heart failure. *Lancet* **2,** 936, 937.

Okada, F., Crow, T. J., and Roberts, G. W. (1990) G proteins (Gi, Go) in the basal ganglia of control and schizophrenic brain. *J. Neural Transm. [GenSect]* **79,** 227–234.

Parolaro, D., Rubino, T., Gori, E., Massi, P., Bendotti, C., Patrini, G., Marcozzi, C., and Parenti, M. (1993) In situ hybridization reveals specific increases in G-alpha-s and G-alpha-o mRNA in discrete

brain regions of morphine-tolerant rats. *Eur. J. Pharmacol. Mol. Pharmacol.* **244,** 211–222.

Ray, K., Kunsch, C., Bonnes, L. M., and Robishaw, J. D. (1995) Isolation of cDNA clones encoding eight different human G-protein g subunits, including three novel forms designated the g4, g10 and g11 sunbunits. *J. Biol. Chem.* **270,** 21,765–21,771.

Self, D. W., Terwilliger, R. Z., Nestler, E. J., and Stein, L. (1994) Inactivation of G_i and G_o proteins in nucleus accumbens reduces both cocaine and heroin reinforcment. *J. Neurosci.* **14,** 6239–6247.

Simon, M. I., Strathmann, M. P., and Gautam, N. (1991) Diversity of G-proteins in signal transduction. *Science* **252,** 802–808.

Young, L. T., Li, P. P., Kamble, A., Siu, K. P., and Warsh, J. J. (1994) Mononuclear levels of G-proteins in depressed patients with bipolar disorder or major depressive disorders. *Am. J. Psychiatry* **151,** 594–596.