Volume 45(1-4):13-21, 2001
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

REVIEW ARTICLE

Heterotrimeric G-proteins and their role in opioid receptor function

Gabriella Fábián

Department of Zoology and Cell Biology, University of Szeged, Szeged, Hungary

ABSTRACT Heterotrimeric G-proteins are signal transducers of heptahelical receptors. They consist of α and βγ subunits, both capable of interacting with several different effectors. Specific domains in their structures enable them to connect different intracellular signaling cascades, such as the adenylyl cyclase, phosphoinositol-bisphosphate or MAP kinase pathways. Their activity is synchronized by several components, one of them being a new protein family termed RGS (regulators of G-protein signaling). Members of this family inhibit the G-protein function. The intracellular localization of G-proteins indicates their role in plasma membrane-independent processes. Opioid receptors transmit their signals mainly via $G_{i/o}$ proteins. Although the heterogeneity of opioid ligands (peptides and alkaloids) and their receptors (μ, δ, κ and suggested subtypes in these classes) reveals a complicated picture, their unique characteristic of a high dependence capacity can not be explained without the analysis of the G-protein function.
Acta Biol Szeged 45(1-4):13-21 (2001)

KEY WORDS

signal transduction
G-proteins
opioid receptor
tolerance

G-proteins

General features of structure and function

The heterotrimeric guanine nucleotide binding proteins (G-proteins) have been discovered about 20 years ago, and the key nature of their participation in signal transduction led to their discoverers being honored with the Nobel Prize for medicine in 1994. They function as intermediaries in transmembrane signaling pathways that involve three proteins: receptors, G-proteins, and effectors (Gilman 1987). They belong in the superfamily of GTPases, which includes factors that take part in protein synthesis (e.g. elongation factor Tu) and small molecular weight (20-25 kDa) monomeric G-proteins, such as p21 ras and its relatives (Hall 1990; Bourne et al. 1990; Bourne et al. 1991; Kaziro et al. 1991). G-proteins consist of three subunits, designated α, β and γ. Traditionally, the type of the α subunit is used to define the G-protein oligomer. To date, 23 distinct α subunits encoded by 17 genes have been cloned with molecular masses between 39 and 46 kDa (Gudermann et al. 1997). They can be divided into four subfamilies, G_s, G_i, G_q and G_{12}, based on amino acid sequence homology. Some of them are ubiquitous, e.g. α_s, while others are more or less specialized, for example, α_o for brain tissue or α_{t1} and α_{t2} for retinal rods and cones, respectively. G-protein α subunits are enzymes with inherent GTPase activity. They are also subject to several cotranslational and posttranslational modifications. α_i, α_o and α_z are myristoylated at their N-terminus (Mumby et al. 1990); others are modified by different saturated or non-saturated 12- and 14-carbon fatty acids, facilitating the membrane attachment of α subunits and increasing their affinity for βγ dimers (Linder et al. 1991). In addition to this irreversible lipid modification, some α subunits, e.g. α_s, are reversibly palmitoylated on the cysteine residue nearest the amino terminus, which seems to have a regulatory function (Wedegaertner and Bourne 1994). While irreversible modifications are usually observed in the endoplasmic reticulum, this reversible lipidation occurs in the cytoplasm. Upon receptor activation, $G_s\alpha$ undergoes substantial depalmitoylation, which may be further increased by cholera toxin. Inactivation of the $G_s\alpha$ subunit is associated with repalmitoylation, which inhibits the interaction of this subunit with other regulatory proteins, e.g. Gα-interacting protein (GAIP). The lipid sensitivity of the G-protein function implies also that the lipid composition of the membrane microdomains can influence the signaling (Green et al. 1999). A characteristic modification of certain types of G-protein α subunits is the ADP-ribosylation by bacterial toxins. Pertussis toxin catalyzes the covalent binding of ADP-ribose to a cysteine residue located four amino acids from the C-terminus. All α_o and α_i subunits can be modified in this way, resulting in uncoupling from the receptor by inhibiting the activation of the α subunit. Cholera toxin specifically ADP-ribosylates an arginine residue in α_t, α_s and α_{olf}, leading to inhibited GTPase activity, that hence to constitutive activation of those α subunits (Hepler and Gilman 1992). There are also several possible sites for phosphorylation.

Accepted May 18, 2001
E-mail: fabiang@bio.u-szeged.hu

Five β (35-37 kDa) and 12 γ (8 kDa) subunits have been described to date (Watson et al. 1994; Ray et al. 1995; Morishita et al. 1995). They are tightly associated and form one functional unit. There is evidence that a degree of specificity governs βγ dimer assembly, and not all possible combinations are formed (reviewed in Gudermann et al. 1997). Gamma subunits are either farnesylated or geranylgeranylated, which furnishes the anchorage to the plasma membrane. It is generally considered that the β subunit interacts with the α subunit, while the γ subunit determines the effector specificity in the action of the dimer.

Role of G-proteins in signal transduction

Receptor-G-protein interaction

G-proteins serve as membrane-bound transducers of chemically and physically coded information. This extracellular information is received by receptor (R) molecules that are integrated plasma membrane proteins. Certain classes of such receptors (e.g. ligand-gated ion channels or tyrosine kinase receptors) themselves have effector domains, whereas others, characterized by 7 transmembrane α helical domains (7TM receptors or G-protein-coupled receptors, GPCRs), first activate G-proteins, which in turn activate the effector molecules. The steps in this cycle are presented in Fig. 1.

It is usually the third intracellular domain and the C-terminal intracellular tail of the receptor molecule that determine the R-G-protein interaction. For the activation of G-proteins, Mg^{2+} and GTP are essential. Little is known about the regulation of the GTPase cycle, since it proceeds 10 to 100 times faster in vivo than in vitro. However, several proteins with GTPase-activating properties (GAPs) for Gα subunits were recently described. They are termed regulators for G-protein signaling (RGS; Watson et al. 1996). At least 20 different mammalian proteins have been reported to have an RGS core, a common 120 amino acid domain. Although the number of different Gα subunits is close to this, there is not a one to one correspondence between them, and no RGS specific for $G_s\alpha$ and $G_{12}\alpha$ has so far been identified. The GTPase-activating domain acts catalytically: a single molecule of RGS can accelerate the GTPase activity of 4-6 Gα subunits. They not only provide enhancement of the enzymatic activity for most of the Gα subunits, but may also function as effector antagonists and integrators of different signaling pathways, in consequence of their C- and N-terminal protein binding motifs (Burchett 2000). One of them is the GGL (G-protein gamma subunit-like) domain, which, e.g. in human RGS11, has been shown to form a complex with Gβ5 (Snow et al. 1998). The RGS11/Gβ5 complex is a selective regulator of $G_o\alpha$.

G-proteins are also signal amplifiers. This can be achieved at different levels. First, a single receptor can activate several G-proteins in turn; second, the dissociation of α and βγ subunits leads to bifurcation of the signal; and on the third level, G-protein subunits can activate several effector molecules before reassociation (Milligan 1996).

G-protein-effector interaction

Recent results show that, upon activation of a G-protein, both α and βγ subunits are able to interact with different effectors (Birnbaumer 1992) to induce further changes in the state of the cell, leading to a response to the extracellular stimulus, or, in a broader sense, to adaptation. The effectors and their activator G-protein subunits are listed in Tables 1 and 2.

Influence of G-proteins on the gene expression

One main pathway for the regulation of gene expression by extracellular signals transduced by GPCRs proceeds via the activation of adenylyl cyclase and the subsequent production of cyclic AMP (cAMP). cAMP regulates the transcription of a variety of genes through a distinct DNA sequence termed the cAMP response element (CRE), present in their promoter regions. This element is recognized by the CRE-binding protein (CREB), a transcription factor of 43 kDa. Activation of CREB is achieved by cAMP-dependent protein kinase

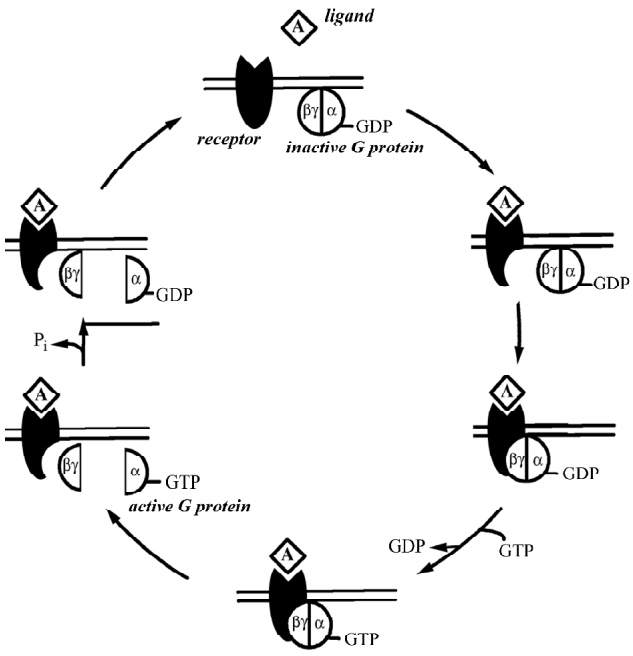

Figure 1. Ligand-activated GTPase cycle of G-proteins. In the resting state, heterotrimeric G-proteins bind GDP. The ligand-bound receptor can activate the G-protein resulting in the exchange of GDP by GTP and subsequent dissociation of α-GTP and the βγ dimer, each of them capable of activating effectors. The effect is terminated by the inherent GTPase activity of the α subunit and the reassociation of α-GDP with βγ. R: receptor, A: agonist ligand.

(PKA; Goodman 1990; Montminy et al. 1990; Collins et al. 1992; Zazopoulos et al. 1997)

The other pathway by which G-proteins can exert an influence is the signaling route of the receptor tyrosine kinases, such as epidermal growth factor, leading to cell differentiation, proliferation and cytoskeletal effects through the mitogen-activated protein kinase (MAPK) cascade. There are several convergence points between the two signal transduction pathways (for reviews, see Selbie and Hill 1998; Seasholtz et al. 1999; Pierce et al. 2001).

Role of intracellular G-proteins

Heterotrimeric G-proteins are found not only in the plasma membrane fractions, but also inside the cell, in the cytoplasm or connected to the endomembrane systems such as the Golgi and the endoplasmic reticulum. They can be detected in the non-nervous tissues, such as the liver (Lanoix et al. 1989; Toki et al. 1989), in the muscle (Carrasco et al. 1994) and also in the brain (Bem et al. 1991; Holz and Tutner 1998).

These intracellular G-proteins can be newly synthesized molecules, which are transported to the cell surface, probably in a fully functional state, able to interact with receptors and also with effectors (Zarbin et al. 1990; Vogel et al. 1991). Intracellular G-proteins may also be conveyed from the cell surface as part of the signal transduction process (Zarbin et al. 1983; Laduron 1992; Szűcs and Coscia 1992). Several plasma membrane receptors have a nuclear localization signal in their cytoplasmic tail; accordingly, they, or part of them, can enter the nucleus either alone or with other proteins recruited during the signaling process (Laduron 1994). However, recent results have revealed that G-proteins are not only transported as passive molecules, but they also have important intracellular functions. They have been suggested to regulate various membrane trafficking processes, including several steps of secretion. Coat assembly and the sorting of newly synthesized proteins secreted constitutively in polarized cells appear to be controlled by heterotrimer G-proteins (Ktistakis et al. 1992; Robinson and Kreis 1992; Pimplikar and Simons 1993). The processes of exocytotic and endocytotic membrane fusion are also under the stimulatory control of G_i and the inhibitory control of G_o (Bomsel and Mostov 1992; Ahnert-Hilger et al. 1994; Colombo et al. 1994; Helms 1995). A role of G-proteins in the maintenance of the highly specialized structure of the blood-brain barrier has also been suggested (Brett et al. 1989; Hoyer et al. 1991; Raub 1996; F bi n et al. 1998).

The opioid receptors

Opioid receptor types and function

Opioid receptors also belong in the family of GPCRs, and are characterized by 7 hydrophobic transmembrane segments and the ability to interact with different G-proteins (McKenzie and Milligan 1990; Offermanns et al. 1991; Laugwitz et al. 1993). Opioid receptors have been identified in pharmacological studies through the use of peptide and alkaloid ligands, and have been classified into three main classes, μ, δ and κ (Martin et al. 1976). Cloning of the receptors has verified this model (Kieffer et al. 1992; Evans et al. 1992; Chen et al. 1993; Yasuda et al. 1993), but failed to prove the

Table 1. Mammalian G-protein a subunits and effectors interacting with them

Subtype	Expression	Effectors
α_{s5} (2 forms)*	Ubiquitous	Adenylyl cyclase ↑ (all types)
α_{sL} (2 forms)*	Ubiquitous	Ca^{2+} channel ↑ (L-type)
α_{olf}	Olfactory epithelium	Adenylyl cyclase ↑ (type V)
α_{gust}	Taste buds, gut	?
$\alpha_{t\text{-}r}$	Retinal rods	cGMP phosphodiesterase ↑
$\alpha_{t\text{-}c}$	Retinal cones	
α_{i1}	Widely	Adenylyl cyclase ↓
α_{i2}	Ubiquitous	(types I, III, V, VI)
α_{i3}	Nearly ubiquitous	K^+ channel ↑
α_{o1}*	Neuronal and neuroendocrine	Ca^{2+} channels ↓
α_{o2}*	Neuronal and neuroendocrine	(Types L and N)
α_z	Neuronal, platelets	Adenylyl cyclase ↓ ?
α_q	Ubiquitous	Phospholipase-Cβ ↑
α_{11}	Ubiquitous	(β4 ≥ β1 ≥ β3 > β2)
α_{14}	Kidney, lung, spleen	
α_{15} (mouse)	Hematopoetic cells	
α_{16} (human)		
α_{12}	Ubiquitous	?
α_{13}	Ubiquitous	?

G-protein a subunits form 4 families based on sequence homology. * Splice variants. The data was taken from Weiland et al. (1997).

existence of the opioid receptor subtypes proposed for all three classes on the basis of pharmacological studies. This suggests that the pharmacological subtypes may result from posttranslational splicing modifications or differential protein-protein interactions between receptors or with associated proteins. The gene structures of all three opioid receptors afford the possibility of alternative splicing, and different mRNA products of single opioid receptor gene have indeed been detected (Gaveriaux-Ruff et al. 1997; Schulz et al. 1998). It has also been demonstrated that proteins encoded by the mRNA isoforms of the μ opioid receptor are desensitized at different rates (Koch et al. 1998). Extensive evidence of pharmacological and functional interactions between opioid receptor types has accumulated (reviewed by Jordan et al. 2000). These studies show that heterodimers, such as μ/δ, exhibit distinct ligand binding and signaling characteristics. Additional signaling features and regulation occur when δ or κ opioid receptors form heterodimers with β_2-adrenergic receptors (Jordan et al. 2001). This heterooligomerization does not alter the ligand binding or coupling properties of the receptors (although they couple to different classes of G-proteins, i.e. $G_{i/o}$ and G_s), but it affects their trafficking. When coexpressed with β_2 receptors, δ opioid receptors undergo isoproterenol-mediated endocytosis. Conversely, the β_2 receptors in these cells undergo etorphine-mediated endocytosis. However, the coexpression of κ opioid receptors with β_2 receptors blocks opioid- and isoproterenol-mediated endocytosis. Ligand-specific regulation of the endocytosis has been detected for homooligomers of the δ receptor. Homodimer formation is reduced by increasing concentrations of agonists, such as DADLE, DPDPE or etorphine, while morphine is ineffective. Depolimerization of the dimers correlated with the internalization of the receptor (Cvejic and Devi 1997).

Pharmacological effects of the opioid receptors are listed in Table 3.

Ligand binding to the opioid receptor

Radioligand binding studies combined with site-directed mutagenesis of the receptor molecules have provided a great deal of information on the interactions of opioid ligands with their receptors (for a review, see Raynor et al. 1996). It is thought that only agonist binding leads to activation of the receptor, followed by conformational changes and information transfer. In contrast, antagonist binding does not elicit a biological response. Certain charged amino acids in the transmembrane regions TM II (Asp114), III (Asp147) and VI (His297) have been shown to be important for ligand binding and subsequent activation of effectors (Surrat et al. 1994). Further, opioid peptides and alkaloids, and also agonists and antagonists bind to different parts of the receptor molecule (Zastrov et al. 1993; Surrat et al. 1994). In the case of δ receptors, the third extracellular loop is likewise important for ligand selectivity (Quock et al. 1999). Identification of the specific residues in the κ receptor involved in agonist and antagonist binding may facilitate the further development of therapeutically useful opioids since κ agonists have minimal abuse potential and do not cause respiratory depression, two major side-effects of the use of μ receptor-selective agonists. Nonetheless, κ agonists are effective analgesics and useful diuretic agents. Previous results have revealed that frog (Rana esculenta) brain membranes are suitable for the investigation of this opioid receptor type, since they contain a high proportion of κ receptors as compared to μ and δ receptors

Table 2. Mammalian G-protein b and g subunits and effectors interacting with them

Subtype	Expression	Effectors
β_1	Ubiquitous	Adenylyl cyclase ↓ (type I)
β_2	Ubiquitous	Adenylyl cyclase ↑ (types II, IV)
β_3	Ubiquitous	Phospholipase-Cβ ↑
β_4	Ubiquitous	(β3 ≥ β2 ≥ β1 > β4)
β_{5S}*	Mainly brain	K^+ channel ↑
β_{5L}*		Retina Ca^{2+} channels ↓
		Receptor kinases (types 2, 3) ↑
		Phospholipase-A_2 ↑ ?
γ_1+	Retinal rods	Phosphoinositide 3-kinase ↑ ?
γ_2	Mainly brain	?
γ_3	Mainly brain	?
γ_4	Mainly brain	?
γ_5	Ubiquitous	?
γ_7	Widely distributed	?
γ_8+	Retinal cones	?
γ_{10}	Widely distibuted	?
γ_{11}+	Widely distributed	?
γ_{12}	Ubiquitous	?

βγ combinations apparently not formed are $\beta_2\gamma_1$ and $\beta_2\gamma_{11}$; tissue-specific combinations are $\beta_1\gamma_1$ for retinal rods and $\beta_3\gamma_8$ for retinal cones. * Splice variants. + These γ subunits are farnesylated; all others are geranylgeranylated. The data was taken from Weiland et al. (1997).

(Simon et al. 1984). Frog brain membranes also contain κ receptor subtypes, i.e. κ_1 and κ_2 (Benyhe et al. 1990; Wollemann et al. 1993). A detailed characterization of these binding sites in ligand binding studies indicated that they might couple to G-proteins (Benyhe et al. 1991; Rottmann et al. 1994; Boz et al. 2000).

Another mode of investigation of ligand-receptor interactions considers energetic aspects. Thermodynamic analysis provides a means of determining the underlying driving forces of binding and intermolecular interactions; such information can not be easily obtained by other techniques. Thus, conformational changes or protein-protein associations should provoke characteristic thermodynamic behavior. With this approach, it has been established that opioid agonist binding is mainly entropy driven, while opioid antagonist binding is exothermic, and therefore enthalpy driven (Nicolas et al. 1982; Hintzemann et al. 1985; Zeman et al. 1987; Borea et al. 1988; F bi n et al. 1996).

Opioid binding is modulated by a number of reagents. Na^+ and GTP decrease agonist binding without affecting antagonist binding. Divalent cations also differentiate agonist and antagonist binding (Szűcs et al. 1987; Benyhe et al. 1989 and references therein). These agents are also to be required for the functional coupling of opioid receptors to inhibitory G-proteins (Blume et al. 1979; Childers 1991; Johansson et al. 1992). By means of thermodynamic investigations, additional information can be expected about this signal transduction step. Na^+ or Mg^{2+} results only in quantitative changes in the thermodynamic parameters. In the presence of the GTP analog Gpp(NH)p, or Gpp(NH)p + Na^+ or Gpp(NH)p + Na^+ + Mg^{2+}, the affinity of dihydromorphine binding decreases dramatically, which might reflect functional uncoupling of the receptor-ligand complex and G-proteins. These altered molecular interactions are also indicated by the curvilinear van't Hoff plot and entropy increase (F bi n et al. 1996).

Consequences of repeated ligand administration

The chronic use of opiates results in drug addiction, including tolerance to and dependence on the drug; besides its scientific importance, this phenomenon has a great social impact. Despite intensive research in this field, the precise molecular mechanism that accounts for it is unknown.

In biochemical terms, the long-term presence of the agonist generally leads first to desensitization, which means that the receptor is unable to activate effector molecules in consequence of the uncoupling of the receptor from the transducer G-protein. The reason for this is the phosphorylation of the receptor by specific kinases, such as β-

Table 3. Opioid receptor pharmacology

Receptor	Biochemical effects	Physiological effects
μ	cAMP inhibition Stimulation of IP_3 formation Ca^{2+} channel inhibition K^+ channel stimulation increase of intracellular Ca^{2+}	Analgesia Sedation Immunosuppression
μ_1		Supraspinal analgesia Prolactin release Acetylcholine turnover feeding
μ_2		Spinal analgesia GH release stimulation Respiratory depression Inhibition of GI transit GPI motility decrease
Morphine-6β-glucuronide		Inhibition of GI transit
κ		Analgesia Dysphoria
κ_1	Inhibition of cAMP accumulation Inhibition of PI hydrolysis Ca^{2+} channel inhibition K^+ channel stimulation	Spinal analgesia Diuresis, sedation Rabbit *vas deferens* bioassay
κ_2	Pharmacology unknown	Pharmacology unknown
κ_3	Inhibition of cAMP accumulation	
KOR-3/ORL-1	K^+ channel stimulation Inhibition of cAMP accumulation	Hyperalgesia (early) Supraspinal analgesia (later)
δ	Inhibition of cAMP accumulation K^+ channel stimulation increase of intracellular Ca^{2+}	Analgesia (spinal, supraspinal) Mouse *vas deferens* bioassay Dopamine turnover inhibition GH release stimulation
δ_1		GI motility decrease
δ_2		GI motility decrease

The data was taken from Standifer and Pasternak (1997) with modifications. GH: growth hormone, GI: gastrointestinal, GPI: guinea pig ileum.

adrenergic receptor kinase (βARK; Arden et al. 1995) or calcium/calmodulin-dependent protein kinase II (Koch et al. 1997). This occurs on a minute time scale. Desensitization is usually followed by sequestration and internalization of the receptor into endosomal vesicles. This is still a minute to hour-long procedure. Proteins in the endosomal vesicles can be recycled to the cell surface or degraded in lysosomes. On a longer time scale, down-regulation of the receptor can occur, meaning reduction of the total (surface and intracellular) receptor number. This certainly involves much more complicated regulatory steps in the gene expression, translation and/or degradation of the certain protein. The above-mentioned steps might give rise to the pharmacological phenomenon of tolerance, meaning that the same dose of the drug becoming ineffective in evoking a given response on repeated administration, or conversely, an even larger dose of drug is necessary to achieve the same magnitude of effect. The term dependence refers more to physiological (or somatic) and psychological aspects of addiction, the former characterized by withdrawal symptoms on the cessation of drug administration, and the latter by drug-seeking behavior. The different anatomical correlates and molecular mechanisms responsible for the opiate dependence have been reviewed by Nestler (1992, 1994, 1996).

As mentioned above in the discussion of the possibility of receptor oligomerization, opioid receptors are regulated in a ligand-specific manner (Burford et al. 1998; Keith et al. 1998; Allouche et al. 1999; Li et al. 1999). For example, the agonists DAMGO and endomorphin-1, but not morphine, caused μ receptor internalization, even though they were similar in activating individual G-proteins. Since endocytosis is associated with functional desensitization of receptor-mediated signal transduction, the differential effects of opiate drugs on this regulatory mechanism may be of great physiological importance. Whistler et al. (1999) suggest that the ability of a drug to induce opioid receptor endocytosis is an independent functional property of agonists, and they introduce the RAVE factor (relative activity versus endocytosis) as a measure of this. If the peptide agonist DAMGO is defined as having a RAVE value of 1, morphine has an approximately 4 times greater RAVE value, showing that its relative ability to signal is much higher than its relative ability to induce receptor endocytosis. They also hypothesized that, in contrast with the prevailing hypothesis, the failure of morphine-activated receptors to uncouple from G-protein and endocytose appropriately might be responsible for the high tolerance induced by this alkaloid.

However, not only receptors take part in the manifestation of tolerance and dependence, but also other elements of the signal transduction pathway. Although most emphasis has been placed on analysis of the internalization and redistribution of GPCRs, it has also been recognized that sustained agonist treatment of cells can result in alterations in both the cellular distribution and levels of G-proteins activated by the relevant GPCR (Nestler et al. 1989; Terwilliger et al. 1991; Van Vliet et al. 1993; Selley et al. 1997). Exposure of cells to agonists of receptors linked to G-proteins can the result in up- or downregulation of cellular levels or redistribution of G-proteins from membranes to the cytosol. Agonist-induced reductions in G-protein levels have been observed for members of each of the G_s, G_i and G_q families of G-proteins, are likely to be dependent upon the level of receptor expression or the brain area investigated, and are generally restricted to the G-protein(s) with which the receptor interacts. The mechanisms responsible vary with cell type and include both second messenger-dependent and -independent enhanced protein degradation. An agonist-induced reduction in cellular G-protein levels can provide one mechanism for the development of sustained heterologous desensitization (for a review, see Milligan 1993). Selective upregulation of certain G-proteins after chronic opioid treatment has also been reported (Escriba et al. 1994; Manji et al. 1997). The distinct pattern of changes in G-protein subtypes detected after morphine administration might represent different stages of the cellular adaptation to the continuous presence of the drug and might reflect different roles of the G-protein subtypes in this process. These data fit into the scheme of drug regulation of neuronal gene expression suggested by Nestler (1992, 1994), where one main group of genes targeted by the drug effect is that encoding G-proteins. The altered gene expressions of several components of the cell signaling system, such as adenylyl cyclase (Avidor-Reis et al. 1996; Rivera and Gintzler 1998), protein kinase-C (PKC; Ventayol et al. 1997), G-protein coupled receptor kinase (Ozaita et al. 1998) and protein phosphatases (Bernstein and Welch 1998), contribute to the neuronal plasticity.

Conclusions

Data obtained by the use of molecular biological tools prove that heterotrimeric G-proteins are not merely simple on/off switches of effector functions, but also active integrators of different intracellular processes. They provide several stages for the fine-tuned regulation of the cellular functions induced by different extracellular stimuli. The number of known members of the RGS family will definitely increase rapidly in the future, probably including proteins with effects different from the activation of GTP hydrolysis. Having several protein-protein interaction domains, G-proteins can regulate the compositions of molecular complexes formed after ligand-receptor activation, recruiting components known previously to belong to separated signaling pathways.

As we begin to understand the detailed molecular mechanisms involved in the signaling of opioid receptors, the complexity is becoming increasingly evident. The heterogeneity of the receptors (μ, δ, κ and several subtypes in these classes) and of the signal transducer G-proteins interacting

with them is further complicated by the variety of downstream elements of different signaling cascades. This wide array of interactions and regulatory effects might provide the basis of the unique properties of the opioid ligands in inducing heavy addiction in drug users. The clue to the prevention of the manifestation of tolerance in the clinical use of opioids or the successful therapy of opioid-dependent patients lies in identification of the particular signaling complexes implicated in the post-receptor events.

References

Ahnert-Hilger G, Schafer T, Spicher K, Grund C, Schultz G, Wiedenmann B (1994) Detection of G-protein heterotrimers on large dense core and small synaptic vesicles of neuroendocrine and neuronal cells. Eur J Cell Biol 65(1):26-38.

Allouche S, Polastron J, Hasbi A, Homburger V, Jauzac P (1999) Differential G-protein activation by alkaloid and peptide opioid agonists in the human neuroblastoma cell line SK-N-BE. Biochem J 342(Pt 1):71-78.

Arden JR, Segredo V, Wang Z, Lameh JH, Sadee W (1995) Phosphorylation and agonist-specific intracellular trafficking of an epitope-tagged mu-opioid receptor expressed in HEK 293 cells. J Neurochem 65:1636-1645.

Avidor-Reis T, Nevo I, Levy R, Pfeuffer T, Vogel Z (1996) Chronic opioid treatment induces adenylyl cyclase V superactivation. Involvement of G-betagamma. J Biol Chem 271:21309-21315.

Bem WT, Yeung SJ, Belcheva M, Barg J, Coscia CJ (1991) Age-dependent changes in the subcellular distribution of rat brain μ-opioid receptors and GTP binding regulatory proteins. J. Neurochem 57:1470-1477.

Benyhe S, Farkas T, Wollemann M (1989) Effects of sodium on [^{3}H]ethylketocyclazocine binding to opioid receptors in frog brain membranes. Neurochem Res 14:205-210.

Benyhe S, Szűcs M, Varga E, Simon J, Borsodi A, Wollemann M (1989) Cation and guanine nucleotide effects on ligand binding properties of mu and delta opioid receptors in rat brain. Acta Biochim Biophys Hung 24:69-81.

Benyhe S, Varga E, Hepp J, Magyar A, Borsodi A, Wollemann M (1990) Characterization of kappa$_1$ and kappa$_2$ opioid binding sites in frog (Rana esculenta) brain membrane preparation. Neurochem Res 15:899-904.

Bernstein MA, Welch SP (1998) Inhibition of protein phosphatases alters the expression of morphine tolerance in mice. Eur J Pharmacol 341:173-177.

Birnbaumer L (1992) Receptor-to-effector signaling through G proteins: Roles for βγ dimers as well as α subunits. Cell 71:1069-1072.

Blume AJ, Lichtshtein D, Boone G (1979) Coupling of opiate receptors to adenylate cyclase: requirement for Na^+ and GTP. Proc Natl Acad Sci USA 76:5626-5630.

Bomsel M, Mostov K (1992) Role of heterotrimeric G proteins in membrane traffic. Mol Biol Cell 3:317-328.

Borea PA, Bertelli GM, Gilli G (1988) Temperature dependence of the binding of μ, δ and κ agonists to the opiate receptors in guinea-pig brain. Eur J Pharmacol 146:247-252.

Bourne HR, Sanders DA, McCormic F (1990) The GTPase superfamily: a conserved switch for diverse cell functions. Nature 348:125-132.

Bourne HR, Sanders DA, McCormic F (1991) The GTPase superfamily: a conserved structure and molecular mechanism. Nature 349:117-127.

Boz B, Farkas J, T th G, Wollemann M, Szűcs M, Benyhe S. (2000) Receptor binding and G-protein activation by new Met5-enkephalin-Arg6-Phe7 derived peptides. Life Sci 66:1241-1251.

Brett J, Gerlach H, Nawroth P, Steinberg S, Godman G, Stern D (1989) Tumor necrosis factor/cachectin increases permeability of endothelial cell monolayers by a mechanism involving regulatory G proteins. J Exp Med 169:1977-1991.

Burchett SA (2000) Regulators of G protein signaling: a bestiary of modular protein binding domains. J Neurochem 75:1335-1351.

Burford NT, Tolbert LM, Sad e W (1998) Specific G protein activation and μ-opioid receptor internalization caused by morphine, DAMGO and endomorphin I. Eur J Pharmacol 342:123-126.

Carrasco MA, Sierralta J, Mazancourt P (1994) Characterization and subcellular distribution of G-proteins in highly purified skeletal muscle fractions from rabbit and frog. Arch Biochem Biophys 310:76-81.

Chen Y, Mestek A, Liu J, Hurley JA, Yu L (1993) Molecular cloning and functional expression of rat μ-opioid receptor from rat brain. Mol Pharmacol 44:8-12.

Childers SR (1991) Opioid receptor-coupled second messenger systems. Life Sci 48:1991-2003.

Collins S, Caron MG, Lefkowitz RJ (1992) From ligand binding to gene expression: new insights into the regulation of G-protein-coupled receptors. Trends Biochem Sci 17:37-39.

Colombo MI, Mayorga LS, Nishimoto I, Ross EM, Stahl PD (1994) Gs regulation of endosome fusion suggests a role for signal transduction pathways in endocytosis. J Biol Chem 269(21):14919-14923.

Cvejic S, Devi LA (1997) Dimerization of the delta opioid receptor: implication for a role in receptor internalization. J Biol Chem 272:26959-26964.

Escriba PV, Sastre M, Garcia-Sevilla JA (1994) Increased density of guanine nucleotide-binding proteins in the postmortem brains of heroin addicts. Arch General Psychiatry 51:494-501.

Evans CJ, Keith DE, Morrison H, Magendzo K, Edwards RH (1992) Cloning of a delta opioid receptor by functional expression. Science 258:1952-1955.

F bi n G, Szab C.A, Boz B, Greenwood J, Adamson P, Deli MA, Jo F, Krizbai IA, Szűcs M (1998) Expression of G-protein subtypes in cultured cerebral endothelial cells. Neurochem Int 33:179-185.

F bi n G, Benyhe S, Farkas J, Szűcs M (1996) Thermodynamic parameters of opioid binding in the presence and absence of G-protein coupling. J Rec and Signal Transd Res 16:151-168.

Gaveriaux-Ruff C, Peluso J, Befort K, Simonin F, Zilliox C, Kieffer BL (1997) Detection of opioid receptor mRNA by RT-PCR reveals alternative splicing for the delta- and kappa-opioid receptors. Brain Res Mol Brain Res 48:298-304.

Gilman AG (1987) G proteins: transducers of receptor-generated signals. Ann Rev Biochem 56:615-649.

Goodman RH (1990) Regulation of neuropeptide gene expression. Annu Rev Neurosci 13:111-117.

Green JM, Zhelesnyak A, Chung J, Lindberg FP, Sarfati M, Frazier WA, Brown EJ. (1999) Role of cholesterol in formation and function of a signaling complex involving alphavbeta3, integrin-associated protein (CD47), and heterotrimeric G proteins. J Cell Biol 146:673-682.

Gudermann T, Sch neberg T, Schultz G (1997) Functional and structural complexity of signal transduction via G-protein-coupled receptors. Annu Rev Neurosci 20:399-427.

Hall A (1990) The cellular functions of small GTP-binding proteins. Science 249:635-639.

Helms JB (1995) Role of heterotrimeric GTP binding proteins in vesicular protein transport: indications for both classical and alternative G protein cycles. FEBS Lett 369:84-88.

Hepler JR, Gilman AG (1992) G-proteins. Trends Biochem Sci 17:383-387.

Hintzemann R, Murphy M, Curell J (1985) Opiate receptor thermodynamics: agonist and antagonist binding. Eur J Pharm 108:171-177.

Holz GG, Turner TJ (1998) Pertussis toxin-sensitive GTP-binding proteins characterized in synaptosomal fractions of embryonic avian cerebral cortex. Comp Biochem Physiol 119B:201-211.

Hoyer J, Popp R, Meyer J, Galla HJ, Gogelein H (1991) Angiotensin II, vasopressin and GTP[gamma-S] inhibit inward-rectifying K+ channels in porcine cerebral capillary endothelial cells. J Membr Biol 123:55-62.

Johansson L, Persson H, Rosengren E (1992) The role of Mg^{2+} on the formation of the ternary complex between agonist, β-adrenoceptor, and

G_s-protein and an interpretation of high and low affinity binding of β-adrenoceptor agonists. Pharmacol Toxicol 70:192-197.

Jordan BA, Trapaidze N, Gomes I, Nivarthi R, Devi LA (2001) Oligomerization of opioid receptors with beta 2-adrenergic receptors: a role in trafficking and mitogen-activated protein kinase activation. Proc Natl Acad Sci USA 98:343-348

Jordan BA, Cvejic S, Devi LA (2000) Opioids and their complicated receptor complexes. Neuropsychopharmacology 23:S5-S18

Kaziro Y, Itoh H, Kozasa T, Nakafuku M, Satoh T (1991) Structure and functions of signal-transducing GTP-binding proteins. Annu Rev Biochem 60:349-400.

Keith DE, Anton B, Murray SR, Zaki PA, Chiu PC, Lissin DV, Monteillett-Agius G, Stewart PJ, Evans C,, von Zastrow M (1998) μ-opioid receptor internalization: opiate drugs have differential effects on a conserved endocytic mechanism in vivo and in vitro. Mol Pharmacol 53:377-384.

Kieffer BL, Befort K, Gaveriaux-Ruff C, Hirth CG (1992) The delta-opioid receptor: Isolation of a cDNA by expression cloning and pharmacological characterization. Proc Natl Acad Sci USA 89:12048-12052.

Koch T, Schulz S, Schroder H, Wolf R, Raulf E, Hollt V (1998) Carboxyl-terminal splicing of the rat mu opioid receptor modulates agonist-mediated internalization and receptor resensitization. J Biol Chem 273:13652-13657.

Koch T, Kroslak T, Mayer P, Raulf E, Hollt V (1997) Site mutation in the rat mu-opioid receptor demonstrates the involvement of calcium/calmodulin-dependent protein kinase II in agonist-mediated desensitization. J Neurochem 69:1767-1770.

Ktistakis NT, Linder ME, Roth MG (1992) Action of brefeldin A blocked by activation of a pertussis-toxin-sensitive G protein. Nature 356:344-346.

Laduron PM (1994) From receptor internalization to nuclear translocation. New targets for long-term pharmacology. Biochem Pharmacol 47:3-13.

Laduron PM (1992) Genomic pharmacology: more intracellular sites for drug action. Biochem Pharmacol 44:1233-1242.

Lanoix J, Roy L, Paiement J (1989) Detection of GTP-binding proteins in purified derivatives of rough endoplasmic reticulum. Biochem J 262:497-503.

Laugwitz K-L, Offermanns S, Spicher K, Schultz G (1993) Mu- and delta-opioid receptors differentially couple to G-protein subtypes in membranes of human neuroblastoma SH-SY5Y cells. Neuron 10:233-242.

Li J-G, Luo J-Y, Krupnick JG, Benovic JL, Liu-Chen L-Y (1999) U50,488H-induced internalization of the human κ opioid receptor involves a β-arrestin- and dynamin-dependent mechanism. J Biol Chem 274:12087-12094.

Linder ME, Pang IH, Duronio RJ, Gordon JI, Sternweis PC, Gilman AG (1991) Lipid modifications of G protein subunits: Myristoylation of $G_{o\alpha}$ increases its affinity for βγ. J Biol Chem 271:8772-8778.

Manji HK, Chen G, Potter W, Kosten TR (1997) Guanine nucleotide binding proteins in opioid-dependent patiens. Biol Psychiatry 41:130-134.

Martin WR, Eades CC, Thompson JA, Gilbert PE, Huppler RE (1976) The effect of morphine and nalorphine-like drugs in the nondependent and morphine dependent chronic spinal dog. J Pharmacol Exp Ther 197:517-532.

McKenzie FR, Milligan G (1990) Delta-opioid-receptor-mediated inhibition of adenylate cyclase is transduced specifically by the guanine-nucleotide-binding protein Gi2. Biochem J 267:391-398.

Milligan G (1993) Agonist regulation of G protein levels and distribution: mechanisms and functional implications. Trends Pharmacol Sci 14:413-418.

Milligan G (1996) The stoichiometry of expression of protein components of the stimulatory adenylyl cyclase cascade and the regulation of information transfer. Cell Signal 8:87-95.

Montminy MR, Gonzalez GA, Yamamoto KK (1990) Regulation of cAMP-inducible genes by CREB. Trends Neurosci 13:184-188.

Morishita R, Nakayama H, Isobe T, Matsuda T, Hashimoto Y, Okano T, Fukada Y, Mizuno K, Ohno S, Kozawa O, Kato K, Asano T (1995) Primary structure of a γ subunit of G protein, γ_{12}, and its phosphorylation by protein kinase C. J Biol Chem 270:29469-29475.

Mumby SM, Heukeroth RO, Gordon JI, Gilman AG (1990) G protein a subunit expression, myristoylation and membrane association in COS cells. Proc Natl Acad Sci USA 87:728-732.

Nestler EJ, Erdos JJ, Terwilliger R, Duman RS, Tallman JF (1989) Regulation of G proteins by chronic morphine in the rat locus coeruleus. Brain Res 476:230-239.

Nestler EJ (1996) Under siege: The brain on opiates. Neuron 16:897-900.

Nestler EJ (1994) Molecular neurobiology of drug addiction. Neuropsychopharmacol 11:77-87.

Nestler EJ (1992) Molecular mechanisms of drug addiction. J Neurosci 12:2439-2450.

Nicolas P, Hammonds Jr, RG, Gomez S, Li CH (1982) β-Endorphin: Thermodynamics of the binding reaction with rat brain membranes. Arch Biochem Biophys 217:80-86.

Offermanns S, Schultz G, Rosenthal W (1991) Evidence for opioid receptor-mediated activation of the G-proteins, G_o and G_{i2}, in membranes of neuroblastoma x glioma (NG108-15) hybrid cells. J Biol Chem 266:3365-3368.

Ozaita A, Escriba PV, Ventayol P, Murga C, Mayor F Jr, Garcia-Sevilla JA (1998) Regulation of G protein-coupled receptor kinase 2 in brains of opiate-treated rats and human opiate addicts. J Neurochem 70:1249-1257.

Pierce KL, Luttrell LM, Lefkowitz RJ (2001) New mechanisms in heptahelical receptor signaling to mitogen activated protein kinase cascades. Oncogene 20:1532-1539.

Pimplikar SW, Simons K (1993) Regulation of apical transport in epithelial cells by a Gs class of heterotrimeric G protein. Nature 362:456-458.

Quock RM, Burkey TH, Varga E, Hosohata Y, Hosohata K, Cowell SM, Slate CA, Ehlert FJ, Roeske WR, Yamamura HI (1999) The delta-opioid receptor: molecular pharmacology, signal transduction, and the determination of drug efficacy. Pharmacol Rev 51:503-532.

Raub TJ (1996) Signal transduction and glial cell modulation of cultured brain microvessel endothelial cell tight junctions. Am J Physiol 271:C495-503.

Ray K, Kunsch C, Bonner LM, Robishaw JD (1995) Isolation of cDNA clones encoding eight different human G protein γ subunits, including three novel forms designated the γ_4, γ_{10}, and γ_{11} subunits. J Biol Chem 270:21765-21771.

Raynor K, Kong H, Law S, Heerding J, Tallent M, Livingston F, Hines J, Reisine T (1996) Molecular biology of opioid receptors. NIDA Res Monogr 161:83-103.

Rivera M, Gintzler AR (1998) Differential effect of chronic morphine on mRNA encoding adenylyl cyclase isoforms: relevance to physiological sequela of tolerance/dependence. Brain Res Mol Brain Res 54:165-169.

Robinson MS, Kreis TE (1992) Recruitment of coat proteins onto Golgi membranes in intact and permeabilized cells: effects of brefeldin A and G protein activators. Cell 69:129-138.

Rottmann M, Benyhe S, Szűcs M (1994) Guanine nucleotide and cation modulation of [^{3}H]ethylketocyclazocine binding in frog brain membranes. Regul Peptides S23-S25.

Seasholtz TM, Majumdar M, Brown HJ (1999) Rho as a mediator of G protein-coupled receptor signaling. Mol Pharmacol 55:949-956.

Selbie LA, Hill SJ (1998) G-protein-coupled-receptor cross-talk: the fine tuning of multiple receptor-signalling pathways. Trends Pharmacol Sci 19:87-98.

Selley DE, Nestler EJ, Breivogel CS, Childers SR (1997) Opioid receptor coupled G-proteins in rat locus coeruleus membranes: decrease in activity after chronic morphine treatment. Brain Res 746:10-18.

Simon J, Szűcs M, Benyhe S, Borsodi A, Zeman P, Wollemann M (1984) Solubilization and characterization of opioid binding sites from frog (Rana esculenta) brain. J Neurochem 43:957-963.

Snow BE, Krumins AM, Brothers GM, Lee SF, Wall MA, Chung S, Mangion J, Arya S, Gilman AG, Siderovski DP (1998) A G protein

gamma subunit-like domain shared between RGS11 and other RGS proteins specifies binding to Gbeta5 subunits. Proc Natl Acad Sci USA 95:13307-13312

Standifer, KM, Pasternak, GW (1997) G proteins and opioid receptor-mediated signalling. Cell Signal 9:237-248.

Schulz S, Schreff M, Koch T, Zimprich A, Gramsch C, Elde R, Hollt V (1988) Immunolocalization of two mu-opioid receptor isoforms (MOR1 and MOR1B) in the rat central nervous system. Neurosci 82: 613-622.

Surrat CK, Johnson PS, Moriwaki A, Seidleck BK, Blaschak CJ, Wang JB, Uhl GR (1994) μ opiate receptor. Charged transmembrane domain amino acids are critical for agonist recognition and intrinsic activity, J Biol Chem 269:20548-20553.

Szűcs M, Coscia CJ (1992) Differential coupling of opioid binding sites to guanosine triphosphate binding regulatory proteins in subcellular fractions of rat brain. J Neurosci Res 31:565-572.

Szűcs M, Spain JW, Oetting GM, Moudy AM, Coscia CJ (1987) Guanine nucleotide and cation regulation of μ, δ and κ opioid receptor binding: Evidence for differential postnatal development in rat brain. J Neurochem 48:1165-1170.

Terwilliger RZ, Beitner-Johnson D, Sevarino KA, Stanley NC, Nestler EJ (1991) A general role for the adaptations in G proteins and cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. Brain Res 548:100-110.

Toki C, Oda K, Ikehara Y (1989) Demonstration of GTP-binding proteins in rat liver Golgi fraction. Biochem Biophys Res Comm 164:333-338.

Van Vliet BJ, Van Rijswijk ALCT, Wardeh G, Mulder AH, Schoffelmeer ANM (1993) Adaptive changes in the number of Gs- and Gi- proteins underlie adenylyl cyclase sensitization in morphine-treated rat striatal neurons. Eur J Pharmacol 245:23-29.

Ventayol P, Busquets X, Garcia-Sevilla JA (1997) Modulation of immunoreactive protein kinase C-alpha and beta isoforms and G proteins by acute and chronic treatments with morphine and other opiate drugs in rat brain. Naunyn Schmiedeberg's Arch Pharmacol 355:491-500.

Vogel SS, Chin GJ, Schwartz JH, Reese TS (1991) Pertussis toxin-sensitive G proteins are transported toward synaptic terminals by fast axonal transport. Proc Natl Acad Sci USA 88:1775-1778.

Watson AJ, Katz A, Simon MI (1994) A fifth member of the mammalian G protein β-subunit family. J Biol Chem 269:22150-22156.

Watson N, Linder ME, Druey KM, Kehrl JH, Blumer KJ (1996) RGS family members: GTPase activating proteins for heterotrimeric G-protein α-subunits. Nature 383:172-175.

Wedegaertner PB, Bourne HR (1994) Activation and depalmitoylation of Gsα. Cell 77:1063-1070.

Weiland T, Schulze R, Jakobs KH (1997) Heterotrimeric guanine nucleotide binding proteins: structure and function. In Wirtz KWA, ed., Molecular mechanisms of signalling and membrane transport. NATO ASI Series, Vol. H 101.

Whistler JL, Chuang HH, Chu P, Jan LY, von Zastrow M (1999) Functional dissociation of mu opioid receptor signaling and endocytosis: implications for the biology of opiate tolerance and addiction. Neuron 23:737-746.

Wollemann M, Benyhe S, Simon J (1993) The kappa-opioid receptor: evidence for different subtypes. Life Sci 52:599-611.

Yasuda K, Raynor K, Kong H, Breder CD, Takeda J, Reisine T, Bell GI (1993) Cloning and functional comparison of κ and δ opioid receptors from mouse brain. Proc Natl Acad Sci USA 90:6736-6740.

Zarbin MA, Palacios JM, Wamsley JK, Kuhar MJ (1983) Axonal transport of beta-adrenergic receptors. Antero- and retrogradely transported receptors differ in agonist affinity and nucleotide sensitivity. Mol Pharmacol 24:341-348.

Zarbin MA, Wamsley JK, Kuhar MJ (1990) Anterograde transport of opioid receptors in rat vagus nerves and dorsal roots of spinal nerves: pharmacology and sensitivity to sodium and guanine nucleotides. Exp Brain Res 81:267-278.

Zastrov MV, Keith DE, Evans CJ (1993) Agonist-induced state of the opioid receptor that discriminates between opioid peptides and opiate alkaloids. Mol Pharmacol 44:166-172.

Zazopoulos E, De Cesare D, Foulkes NS, Mazzucchelli C, Lamas M, Tamai K, Lalli E, Fimia G, Whitmore D, Heitz E, Sassone-Corsi P (1997) Coupling signal transduction to transcription: the nuclear response to cAMP. In Wirtz KWA, ed., Molecular mechanisms of signalling and membrane transport. NATO ASI Series, Vol. H 101.

Zeman P, T th G, Kvetnansky R (1997) Thermodynamic analysis of rat brain opioid mu-receptor-ligand interaction. Gen Physiol Biophys 6:237-248.