

REVIEW ARTICLE

# Heterotrimeric G-proteins and their role in opioid receptor function

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**ABSTRACT** Heterotrimeric G-proteins are signal transducers of heptahelical receptors. They consist of  $\alpha$  and  $\beta\gamma$  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 ( $\mu$ ,  $\delta$ ,  $\kappa$  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.

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**KEY WORDS**

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## 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  $\alpha$ ,  $\beta$ , and  $\gamma$ . Traditionally, the type of the  $\alpha$  subunit is used to define the G-protein oligomer. To date, 23 distinct  $\alpha$  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.  $G_s$ , while others are more or less specialized, for example,  $G_o$  for brain tissue or  $G_{t1}$  and  $G_{t2}$  for retinal rods and cones, respectively. G-protein  $\beta\gamma$  subunits are enzymes with inherent GTPase activity. They are also subject to several cotranslational and posttranslational modifications.  $\beta$ ,  $\gamma$ , and

$\alpha_z$  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  $\beta$  subunits and increasing their affinity for  $\alpha$  dimers (Linder et al. 1991). In addition to this irreversible lipid modification, some  $\beta$  subunits, e.g.  $\beta_5$ , 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,  $G_s$  undergoes substantial depalmitoylation, which may be further increased by cholera toxin. Inactivation of the  $G_s$  subunit is associated with repalmitoylation, which inhibits the interaction of this subunit with other regulatory proteins, e.g. G-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  $\alpha$  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  $G_o$  and  $G_i$  subunits can be modified in this way, resulting in uncoupling from the receptor by inhibiting the activation of the  $\alpha$  subunit. Cholera toxin specifically ADP-ribosylates an arginine residue in  $\alpha_{11}$ ,  $\alpha_{12}$  and  $\alpha_{13}$ , leading to inhibited GTPase activity, that hence to constitutive activation of those  $\alpha$  subunits (Hepler and Gilman 1992). There are also several possible sites for phosphorylation.

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Five (35-37 kDa) and 12 (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 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  $\beta$  subunit interacts with the  $\alpha$  subunit, while the  $\alpha$  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 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 G 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 G subunits is close to this, there is not a one to one correspondence between them, and no RGS specific for  $G_s$  and  $G_{12}$  has so far been identified. The GTPase-activating domain acts catalytically: a single molecule of RGS can accelerate the GTPase activity of 4-6 G subunits. They not only provide enhancement of the enzymatic activity for most of the G 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  $G_{12}$  (Snow et al. 1998). The RGS11/ $G_{12}$  complex is a selective regulator of  $G_{12}$ .

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

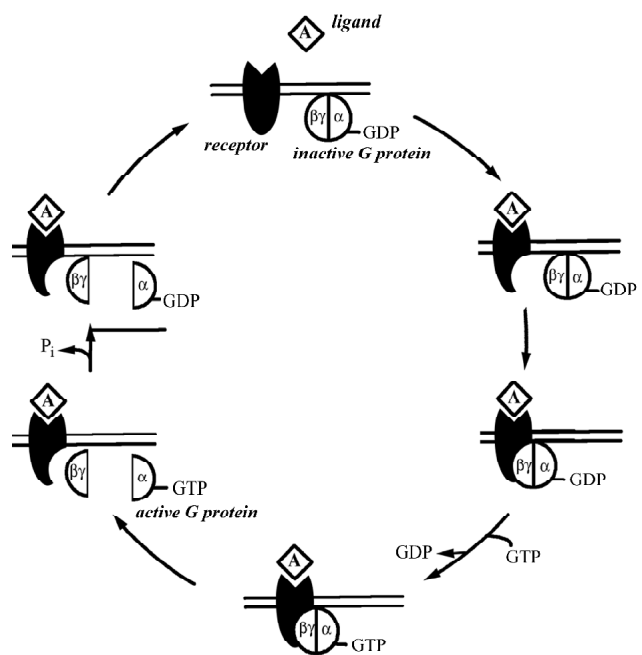
of  $\alpha$  and  $\beta\gamma$  subunits leads to bifurcation of the signal; and on the third level, G-protein subunits can activate several effector molecules before reassociation (Milligan 1996).

### G-protein-effector interaction

Recent results show that, upon activation of a G-protein, both  $\alpha$  and  $\beta\gamma$  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  $\alpha$ -GTP and the  $\beta\gamma$  dimer, each of them capable of activating effectors. The effect is terminated by the inherent GTPase activity of the  $\alpha$  subunit and the reassociation of  $\alpha$ -GDP with  $\beta\gamma$ . 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<sub>i</sub> and the inhibitory control of G<sub>o</sub> (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 b i 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,  $\mu$ ,  $\kappa$  and  $\delta$  (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  $\alpha$  subunits and effectors interacting with them

Subtype	Expression	Effectors
$\alpha_{s1}$ (2 forms)*	Ubiquitous	Adenylyl cyclase (all types)
$\alpha_{sL}$ (2 forms)*	Ubiquitous	Ca <sup>2+</sup> channel (L-type)
$\alpha_{olf}$	Olfactory epithelium	Adenylyl cyclase (type V)
$\alpha_{gust}$	Taste buds, gut	?
$\alpha_{t-r}$	Retinal rods	cGMP phosphodiesterase
$\alpha_{t-c}$	Retinal cones	
$\alpha_{i1}$	Widely	Adenylyl cyclase (types I, III, V, VI)
$\alpha_{i2}$	Ubiquitous	K <sup>+</sup> channel
$\alpha_{i3}$	Nearly ubiquitous	Ca <sup>2+</sup> channels (Types L and N)
$\alpha_{o1}$	Neuronal and neuroendocrine	Adenylyl cyclase ?
$\alpha_{o2}$	Neuronal and neuroendocrine	
$\alpha_z$	Neuronal, platelets	
$\alpha_q$	Ubiquitous	Phospholipase-C (4 1 3 > 2)
$\alpha_{11}$	Ubiquitous	
$\alpha_{14}$	Kidney, lung, spleen	
$\alpha_{15}$ (mouse)	Hematopoetic cells	
$\alpha_{16}$ (human)		
$\alpha_{12}$	Ubiquitous	?
$\alpha_{13}$	Ubiquitous	?

G-protein  $\alpha$  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  $\mu$  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  $\mu/\delta$ , exhibit distinct ligand binding and signaling characteristics. Additional signaling features and regulation occur when  $\delta$  or  $\mu$  opioid receptors form heterodimers with  $\alpha_2$ -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  $\alpha_2$  receptors,  $\mu$  opioid receptors undergo isoproterenol-mediated endocytosis. Conversely, the  $\alpha_2$  receptors in these cells undergo etorphine-mediated endocytosis. However, the coexpression of  $\mu$  opioid receptors with  $\alpha_2$  receptors blocks opioid- and isoproterenol-mediated endocytosis. Ligand-specific regulation of the endocytosis has been detected for homooligomers of the receptor. Homodimer formation is reduced by increasing concentrations of agonists, such as DADLE, DPDPE or etorphine, while morphine is ineffective. Depolymerization

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  $\delta$  receptors, the third extracellular loop is likewise important for ligand selectivity (Quock et al. 1999). Identification of the specific residues in the  $\delta$  receptor involved in agonist and antagonist binding may facilitate the further development of therapeutically useful opioids since  $\delta$  agonists have minimal abuse potential and do not cause respiratory depression, two major side-effects of the use of  $\mu$  receptor-selective agonists. Nonetheless,  $\delta$  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  $\delta$  receptors as compared to  $\mu$  and  $\kappa$  receptors

**Table 2.** Mammalian G-protein  $\beta$  and  $\gamma$  subunits and effectors interacting with them

Subtype	Expression	Effectors
1	Ubiquitous	Adenylyl cyclase (type I)
2	Ubiquitous	Adenylyl cyclase (types II, IV)
3	Ubiquitous	Phospholipase-C
4 <sup>+</sup>	Ubiquitous	( $\beta_3 \beta_2 \beta_1 > 4$ )
5 <sup>5s</sup>	Mainly brain	K <sup>+</sup> channel
5L		Retina Ca <sup>2+</sup> channels
		Receptor kinases (types 2, 3)
		Phospholipase-A <sub>2</sub> ?
6 <sup>+</sup>	Retinal rods	Phosphoinositide 3-kinase ?
7	Mainly brain	?
8	Mainly brain	?
9	Mainly brain	?
10	Ubiquitous	?
11	Widely distributed	?
12 <sup>+</sup>	Retinal cones	?
	Widely distributed	?
	Widely distributed	?
	Ubiquitous	?

combinations apparently not formed are  $\beta_2 \beta_1$  and  $\beta_2 \beta_{11}$ ; tissue-specific combinations are  $\beta_1 \beta_1$  for retinal rods and  $\beta_3 \beta_8$  for retinal cones. <sup>+</sup> Splice variants. <sup>\*</sup> These 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 receptor subtypes, i.e.  $\mu_1$  and  $\mu_2$  (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; Fabin et al. 1996).

Opioid binding is modulated by a number of reagents.  $\text{Na}^+$  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.  $\text{Na}^+$  or  $\text{Mg}^{2+}$  results only in quantitative changes in the thermodynamic parameters. In the presence of the GTP analog Gpp(NH)p, or Gpp(NH)p +  $\text{Na}^+$  or Gpp(NH)p +  $\text{Na}^+$  +  $\text{Mg}^{2+}$ , 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 (Fabin 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 -

**Table 3.** Opioid receptor pharmacology

Receptor	Biochemical effects	Physiological effects
$\mu$	cAMP inhibition Stimulation of $\text{IP}_3$ formation $\text{Ca}^{2+}$ channel inhibition $\text{K}^+$ channel stimulation increase of intracellular $\text{Ca}^{2+}$	Analgesia Sedation Immunosuppression
$\mu_1$		Supraspinal analgesia Prolactin release Acetylcholine turnover feeding
$\mu_2$		Spinal analgesia GH release stimulation Respiratory depression Inhibition of GI transit GPI motility decrease Inhibition of GI transit
Morphine-6 -glucuronide		Analgesia Dysphoria Spinal analgesia Diuresis, sedation Rabbit <i>vas deferens</i> bioassay
$\kappa_1$	Inhibition of cAMP accumulation Inhibition of PI hydrolysis $\text{Ca}^{2+}$ channel inhibition $\text{K}^+$ channel stimulation	Pharmacology unknown
$\kappa_2$	Inhibition of cAMP accumulation $\text{K}^+$ channel stimulation	Hyperalgesia (early) Supraspinal analgesia (later) Analgesia (spinal, supraspinal) Mouse <i>vas deferens</i> bioassay Dopamine turnover inhibition GH release stimulation GI motility decrease GI motility decrease
$\delta$ KOR-3/ORL-1	Inhibition of cAMP accumulation $\text{K}^+$ channel stimulation Inhibition of cAMP accumulation $\text{K}^+$ channel stimulation increase of intracellular $\text{Ca}^{2+}$	
$\sigma_1$		
$\sigma_2$		

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

adrenergic receptor kinase (ARK; 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  $\mu$  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 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 ( $\mu$ ,  $\delta$ , 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.

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