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Single Transmembrane Spanning Heterotrimeric G Protein-Coupled Receptors and Their Signaling Cascades

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***Abstract*——Heptahelical of serpentine receptors such as the adrenergic receptors are well known to mediate their actions via heterotrimeric GTP-binding proteins. Likewise, receptors that traverse the cell membrane once have been shown to mediate their biological actions by activating several different mechanisms including stimulation of their intrinsic tyrosine kinase activities or the kinase activities of other proteins. Some of these single transmembrane receptors have an intrinsic guanylyl cyclase activity and can stimulate the cyclic GMP second messenger system; however, over the last few years, several studies have shown the involvement of heterotrimeric GTP-binding proteins in mediating signals that eventually culminate in the biological actions of single transmembrane spanning receptors and proteins. These receptors include the receptor tyrosine kinases that mediate the actions of growth factors such as epidermal growth factor, insulin, insulin-like growth factor as well as receptors for atrial natiuretic hormone or the zona pellucida protein (ZP3) and integrins. In this review, the significance of the coupling of the single transmembrane spanning receptors to G proteins has been highlighted by providing several examples of the concept that signaling via these receptors may involve the activation of multiple signaling cascades.**

I. Introduction

The family of classical G protein-coupled receptors (GPCRs[1]) is also referred to as the heptahelical or ser-

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[1]Abbreviations: GPCR, G protein-coupled receptors; Gs, stimulatory G protein of adenylyl cyclase; Gi, inhibitory G protein of adenylyl cyclase; EGF, epidermal growth factor; EGFR, EGF receptor, FGF, fibroblast growth factor; FGFR, FGF receptor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; APP, amyloid precursor protein; Aβ, amyloid β peptide; ZP, zona pellucida glycoprotein; GalTase, β-1,4-galactosyltransferase; PKA, cAMP-dependent protein kinase; PI3K, phosphatidyinositol 3-kinase, MAPK, mitogen-activated protein kinase; Erk, extracellular signal-regulated kinase; NPR-C, C-type natriuretic peptide receptor, IAP/CD47, integrin-associated protein (CD47); TCR, T cell receptor; PTP-1B, protein tyrosine phosphatase-1B; IRS-1, insulin receptor substrate-1; HEK, human embryonic kidney; EDG1, endothelial differentiation gene 1; CRE, cAMP response element; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; u-PA, urokinase-type plasminogen activator; u-PAR, u-PA receptor.

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pentine receptors. As the latter names imply these receptors traverse the cell membrane seven times and have an extracellular N terminus and an intracellular C terminus. However, it has become apparent over the past several years that other receptors and proteins that are not heptahelical or serpentine also mediate some of their biological effects via activation of heterotrimeric GTP-binding proteins. To date, the role of heterotrimeric G proteins in mediating the actions of these nonclassical GPCRs such as receptors for a variety of growth factors, atrial natriuretic hormone, extracellular matrix proteins, as well as zona pellucida glycoprotein ZP3 has remained relatively unappreciated. Therefore, in this review, the activation of heterotrimeric G proteins and their role in mediating the biological actions of nonclassical GPCRs and proteins is discussed. As a prelude to this discussion, the activation and inactivation of heterotrimeric G proteins is briefly reviewed.

II. Activation and Inactivation of Heterotrimeric GTP-Binding Proteins

The heterotrimeric G proteins comprise of α, β, and γ subunits (Gα, Gβ, and Gγ). In the resting state, the α subunit is bound to GDP and is associated with the $\beta\gamma$ (G$\beta\gamma$) subunits. The GDP-bound heterotrimer is coupled to receptors and, in the case of heptahelical receptors, increases the affinity of the receptors for their ligands. Upon ligand binding, the receptor activates the trimeric G protein that it is coupled to and increases the rate of GDP-GTP exchange on the Gα subunit; the rate limiting step in the activation of G proteins is the rate of GDP-GTP exchange. Thus, the activated receptor acts as a guanine nucleotide exchange factor. The GTP-bound, active, Gα subunit dissociates from the G$\beta\gamma$ subunits; these moieties of the G protein then activate their respective effectors, enzymes such as adenylyl cyclase or phospholipase C, or ion channels (Gilman, 1987; for review, see Birnbaumer, 1992; Neves et al., 2002). This activation pathway of G proteins by receptors is shown in Fig. 1.

Upon withdrawl of ligand from the receptor, when the receptor is no longer active, the intrinsic GTPase activity of the Gα subunit hydrolyzes the GTP to GDP and the GDP-bound, inactive, Gα subunit associates with G$\beta\gamma$ subunits. In this manner, the signaling cycle of the heterotrimeric G protein is complete (Fig. 1). It should be noted that the GTPase activity of the Gα subunits may also be regulated by regulators of G proteins signaling (RGS proteins) as well as effectors (for reviews, see De Vries and Gist Farquhar, 1999 and Burchett, 2000). Moreover, effector enzymes such as adenylyl cyclases may also regulate the activation of G proteins by receptors (for review, see Patel et al., 2001). In this manner, the signaling via heterotrimeric G proteins may be fine-tuned by other proteins.

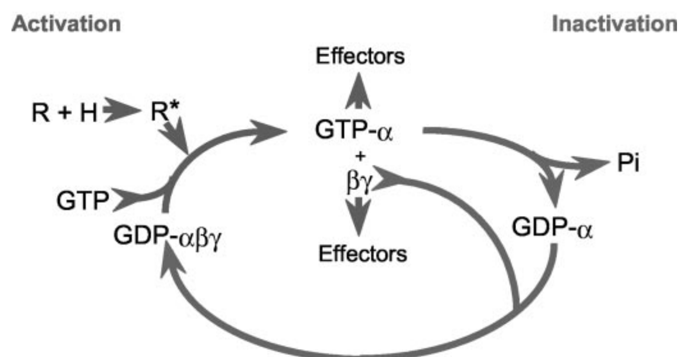

FIG. 1. Activation and inactivation cycle of heterotrimeric G proteins. Upon binding the hormone (H), the receptor (R) is activated. The activated receptor is designated R*. The active receptor increases GTP-GDP exchange on the G protein, and the active GTP-bound form of Gα dissociates from the G$\beta\gamma$ subunits (see text for details). These subunits then activate their respective effectors. The left side depicts activation of G protein and its effectors whereas the right side depicts the inactivation of G protein as detailed in the text.

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Presently, 20 forms of Gα subunits, 5 forms of Gβ subunits and 12 isoforms of Gγ subunits have been cloned and characterized (Hurowitz et al., 2000; Neves et al., 2002). The various permutations in the combinations of different forms of Gα subunit with Gβ and Gγ subunits, therefore, provide a large amount of diversity in signaling via heterotrimeric G proteins (Neves et al., 2002). The 20 forms of α subunits can be subdivided into four major groups depending upon their sequence homologies and other properties such as activation of effectors and inhibition by pharmacological agents (Neves et al., 2002). Some of the pharmacological agents that have been used to study the involvement of G proteins in signaling by receptors have been bacterial toxins. Thus, pertussis toxin obtained from *Bordella pertussis* has been shown to ADP-ribosylate the α subunits of Gi and Go proteins (Gilman, 1987; Birnbaumer, 1990). This ADP-ribosylation of Gαi and Gαo subunits functionally uncouples these G proteins from their respective receptors and thereby interrupts signaling. Similarly, cholera toxin obtained from *Vibrio cholerae* ADP-ribosylates the α subunits of Gs and Golf, and this ADP-ribosylation inhibits the GTPase activity of these G proteins (Gilman, 1987; Birnbaumer, 1990). The net result of this ADP-ribosylation is the retention of the α subunits in their active GTP-bound forms. Thus, cholera toxin increases the activity of adenylyl cyclase, the effector of both Gαs and Golf (Gilman, 1987).

III. Receptor Protein Tyrosine Kinases

The receptors for epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin, and insulin-like growth factor (IGF) belong to this family. As reviewed in detail elsewhere (Schlessinger and Ullrich, 1992; Schlessinger,

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2000), these proteins traverse the cell membrane once and consist of an extracellular ligand binding domain, a transmembrane domain, and a cytosolic domain that encompasses a protein tyrosine kinase activity. Although most of these receptors comprise a single polypeptide chain, in the case of the insulin and IGF receptor, the receptors consist of a heterotetrameric structure made up by two extracellular α chains that are connected by sulfhydryl bridges to each other and to two β chains that traverse the membrane. The intracellular domains of the two β chains contain the tyrosine kinase activity. Upon binding of their respective ligands, the receptor tyrosine kinase activity is increased, resulting in an increase in receptor autophosphorylation on tyrosine residues and the docking of phospho-tyrosine-binding proteins including, in some cases, effector proteins such as phospholipase C-γ. These docking proteins recruit other signaling proteins that may be phosphorylated and thereby initiate signaling cascades that lead to the activation of protein kinases such as the mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinase (PI3K). However, some of the pleiotropic actions of growth factors via their protein tyrosine kinase receptors also involve the activation of heterotrimeric G proteins.

A. Epidermal Growth Factor Receptor

Besides activating signaling cascades such as Erk MAPKs, the activated tyrosine-phosphorylated EGF receptor directly associates with, and following, phosphorylation on tyrosine residues activates phospholipase C-γ. In this manner, EGF can initiate the hydrolysis of phosphatidylinositol 4,5-bisphosphate leading to increase in intracellular Ca^{2+} and activation of protein kinase C (Carpenter, 1992, 2000) in certain cell types. On the other hand, in the liver, the activation of phospholipase C appears to involve other mechanisms. For instance, data from Garrison's laboratory and ours (Johnson and Garrison, 1987; Liang and Garrison, 1991, 1992; Rashed and Patel, 1991) showed that the ability of EGF to increase intracellular Ca^{2+} in liver and hepatocytes was abolished by treatment with pertussis toxin. Since pertussis toxin ADP-ribosylates and uncouples the Gi/Go family of G proteins from their receptors, it would appear that, in the liver, EGF increases phospholipase activity via activation of heterotrimeric G proteins. Indeed, the demonstration that G$\beta\gamma$ subunits can activate certain phospholipase C isoforms (Exton, 1994) would suggest that, in the liver, stimulation of the EGF receptor results in activation of Gi or Go to release activated α subunit from the G$\beta\gamma$ subunits. The activation of Gi/Go family of G proteins in the liver by EGF is also consistent with the findings of Bosch et al. (1986), which shows EGF decreases cAMP accumulation in response to glucagon. It is now well established that activated α subunit of Gi and G$\beta\gamma$ subunits inhibit certain isoforms of adenylyl cyclase (Smit and Iyengar, 1998; Patel et al., 2001).

The notion that the EGF receptor can activate signal transduction processes via heterotrimeric G proteins is also supported by additional evidence derived from the cardiac actions of EGF. Thus, in the heart, EGF increases contractility and heart rate (Nair et al., 1993). These actions of EGF are mediated by elevations in cAMP levels in cardiac myocytes (Yu et al., 1992). EGF increases cAMP levels by activating the stimulatory GTP-binding protein of adenylyl cyclase Gs and increasing the activity of adenylyl cyclase (Nair et al., 1989, 1990). The activation of adenylyl cyclase by EGF requires the intrinsic protein tyrosine kinase activity of the EGF receptor (Nair and Patel, 1993) and is attenuated by activation of protein kinase C (Nair et al., 1989). Studies designed to unravel the mechanism(s) involved in EGF receptor-mediated activation of Gs have shown that a 13 amino acid sequence in the cytosolic, juxtamembrane region of the EGF receptor is sufficient to activate the G protein (Sun et al., 1995). Moreover, phosphorylation of the protein kinase C site within this region markedly attenuates activation of Gs (Sun et al., 1995). Using a variety of approaches, it has also been shown that this cytosolic juxtamembrane region of the EGF receptor is important for the stoichiometric association with the α subunit of Gs (Gαs) (Sun et al., 1997). Notably, the region of the EGF receptor that associates with Gαs and that is involved in activation of Gs is homologous to a 15 amino acid region on the β_2-adrenergic receptor, which activates Gs (Okamoto et al., 1991b). Interestingly, the Gs-activating sequence in the β_2-adrenergic receptor includes a cAMP-dependent protein kinase (PKA) phosphosphorylation site, and the phosphorylation of this residue decreases its ability to activate Gs and increases its ability to activate Gi (Okamoto et al., 1991b). As mentioned above, the 13 amino acid Gs-activating sequence in the EGF receptor contains a protein kinase C phosphorylation site, and phosphorylation of this residue attenuates the ability of this sequence to activate Gs and to associate with Gαs (Sun et al., 1995, 1997). Clearly, there are remarkable similarities between the β_2-adrenergic receptor and the EGF receptor in terms of how they stimulate Gs and how this G protein activation is regulated by protein kinases. Recently, experimental evidence has shown that depending upon its phosphorylation state, the β-adrenergic receptor and prostacyclin receptor may activate Gs or Gi (Lawler et al., 2001; Zamah et al., 2002). Since the EGF receptor signaling via Gs is very similar to that of the β-adrenergic receptor, one wonders whether the phosphorylation of the EGF receptor on the protein kinase C site determines whether the receptor activates Gs or Gi. This may explain how the EGF receptor could be coupled to Gs in the heart and other tissues (see below) and to Gi or Go in the liver.

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Besides the interactions of the EGF receptor with Gs, it has also been demonstrated that the tyrosine kinase activity of the EGF receptor can phosphorylate $G\alpha_s$ on two tyrosine residues, and this phosphorylation of the G protein activates the protein to increase adenylyl cyclase activity (Poppleton et al., 1996). Interestingly, the association of $G\alpha_s$ with the juxtamembrane region of the EGF receptor is necessary for efficient phosphorylation of the G protein (Poppleton et al., 2000). An intriguing aspect about EGF-elicited activation of adenylyl cyclase is that this phenomenon is observed in the heart (Nair et al., 1989, 1990, 1993; Nair and Patel, 1993), parotid glands (Nakagawa et al., 1991), luteal cells (Budnik and Mukhopadhyay, 1991), and pancreatic acini (Stryjek-Kaminska et al., 1995). However, as mentioned above for the liver, EGF does not increase cAMP accumulation in other tissues or several other cell lines (Bosch et al., 1986; Yu et al., 1992). In this context, in HEK 293 cells, the expression of type V adenylyl cyclase could reconstitute the ability of EGF to increase cAMP levels (Chen et al., 1995). Notably, in the adult heart type V adenylyl cyclase is the predominant isoform that is expressed (Premont et al., 1992). Thus, the ability of EGF to stimulate adenylyl cyclase apparently depends upon the isoform of adenylyl cyclase that is expressed in any tissue or cell line. More recently, it has been demonstrated that activation of PKA in cells or treatment of purified EGF receptor with purified PKA can decrease the tyrosine kinase activity of the EGF receptor and attenuate its ability to initiate downstream signaling (Barbier et al., 1999). Hence, the ability of EGF to increase cAMP levels in cells can be modulated by the PKA-mediated negative feedback regulatory loop (Fig. 2). It should be noted, however, that activation of PKA in cells overexpressing the EGF receptor does not attenuate EGF-mediated tyrosine phosphorylation of proteins most likely because the amount of EGF receptor is far in excess of the PKA (Barbier et al., 1999).

B. Insulin and Insulin-Like Growth Factor Receptors

The insulin receptor was first proposed to be coupled to heterotrimeric G proteins by Goren et al. (1985). These authors showed that insulin-dependent lipolysis and inhibition of glucose oxidation in adipocytes were blocked by pertussis toxin. Following this original observation, there were several reports that suggested that the insulin receptor phosphorylates $G\alpha_i$ and $G\alpha o$ (O'Brien et al., 1987; Krupinski et al., 1988) and that the insulin receptor is coupled to G proteins of the Gi family (Rothenberg and Kahn, 1988; Ciaraldi and Maisel, 1989). These studies were followed by reports of G proteins associated with the insulin receptor (Jo et al., 1992, 1993) and the identification of regions within the insulin receptor that can activate heterotrimeric G proteins (Okamoto et al., 1993). More recently, several studies have examined the role of specific isoforms of the G protein α subunits in mediating the actions of insulin. Thus, decreasing the expression of $G\alpha_{i2}$ in the liver and adipose tissue results in hyperinsulinemia, glucose intolerance, and resistance to insulin (Moxham and Malbon, 1996). The insulin resistance decreases glucose transporter GLUT4 translocation to the plasma membrane in response to insulin and also decreases the activation of glycogen synthase and antilipolytic activity of insulin (Moxham and Malbon, 1996). Interestingly, the decrease in expression of $G\alpha_{i2}$ was also accompanied by

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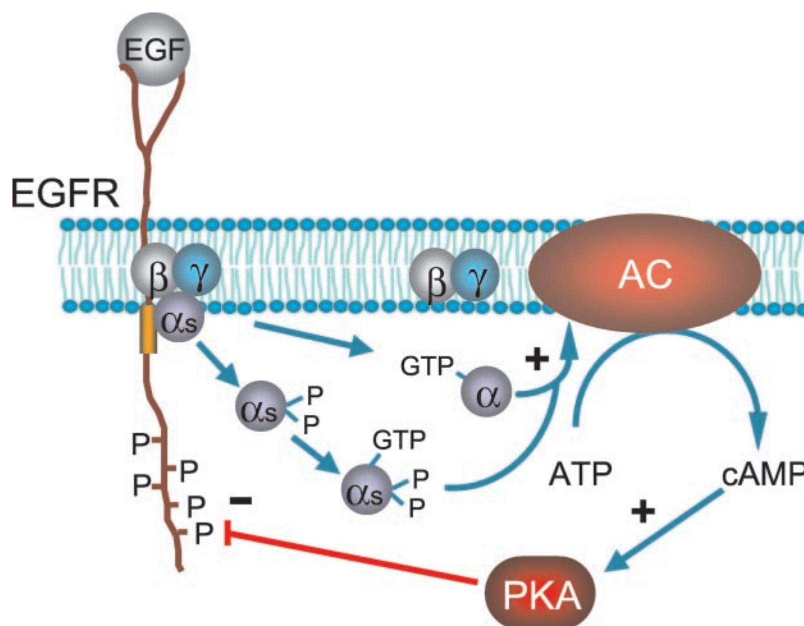

FIG. 2. Activation of Gs and stimulation of adenylyl cyclase by the EGF receptor. The direct activation of the Gs by the juxtamembrane region (orange) of the EGFR as well as activation of Gαs by tyrosine phosphorylation are shown. The feedback regulation of the EGFR by protein kinase A is also shown.

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an increase in protein tyrosine phosphatase-1B (PTP-1B) activity. PTP-1B has been shown to dephosphorylate phosphorylated tyrosine residues on the insulin receptor and the insulin receptor substrate-1 (IRS-1). Therefore, the decrease in insulin receptor autophosphorylation and tyrosine phosphorylation of IRS-1 in response to insulin in the Gαi2-deficient mice may be the result of increase in PTP-1B activity (Moxham and Malbon, 1996). Indeed, the expression of Gαi2 suppresses PTP-1B and increases sensitivity to insulin (Tao et al., 2001). Furthemore, in keeping with the notion that decreased Gαi2 activity leads to insulin resistance, the conditional expression of constitutively active Gαi2 in transgenic mice decreases the abnormalities in glucose metabolism induced by streptozotocin (Zheng et al., 1998). Recently, Song et al. (2001) have shown that the expression of constitutively active mutant (Q205L) transgene of Gαi2 in mice increased glucose transport and GLUT4 translocation in skeletal muscle and adipose tissue. The expression of the constitutively active Gαi2 also increased PI3K and Akt activities in the skeletal muscle and adipose tissue of the transgenic mice (Song et al., 2001) indicating that Gαi2 is downstream of the insulin receptor and mimics the actions of insulin.

Although the studies described above suggest that a number of the actions of insulin on glucose transport and metabolism are mediated by Gαi2, in NIH 3T3 adipocytes, insulin-elicited increase in GLUT4 translocation to membrane has been shown to be dependent on Gαq/11 (Imamura et al., 1999; Kanzaki et al., 2000). Gαq/11 was found to be associated with the insulin receptor and was phosphorylated in the presence of insulin (Imamura et al., 1999). Insulin also increased the association of Gαq/11 with p110α subunit of PI3K (Imamura et al., 1999). Moreover, a constitutively active mutant of Gαq/11, but not constitutively active Gαi2, was also able to increase basal GLUT4 translocation in NIH 3T3L1 adipocytes suggesting the involvement of Gαq/11 protein in the actions of insulin (Imamura et al., 1999; Kanzaki et al., 2000). Likewise, anti-Gαq/11, but not anti-Gαi2, also inhibited the ability of insulin to increase GLUT4 indicating that in NIH 3T3L1 adipocytes, Gαq/11 is activated by the insulin receptor. The reasons for the involvement of different G proteins, i.e., Gαq/11 in NIH 3T3L1 adipocytes versus Gαi2 in intact animals, in insulin-mediated activation of GLUT4 is presently not known. It is possible, that in the NIH 3T3L1 adipocytes maintained in culture, the insulin receptor is coupled to different G proteins. Interestingly, Imamura et al. (1999) reported that the Gαq/11 mediated activation of GLUT4 in NIH 3T3L1 adipocytes are dependent on activation of PI3K. However, the studies of Kanzaki et al. (2000) showed that although inhibition of PI3K attenuated the ability of insulin to stimulate GLUT4 translocation, Gαq/11 activation did not increase PI3K activity. The latter study concluded that insulin- elicited translocation of GLUT4 requires at least two independent signaling pathways, one involving the PI3K and the other involving the Gαq/11. The differences in the conclusions from the studies of Imamura et al. (1999) and Kanzaki et al. (2000) are not clear; however, both studies strongly support a role for Gαq/11 in insulin-induced translocation of GLUT4. More recently, studies by Dalle et al. (2001) have confirmed the role of Gαq/11 in insulin-elicited translocation of GLUT4 in NIH 3T3L1 adipocytes and in HIRcB cells. Most interestingly, like the classical G protein-coupled receptors, these authors also found an association of the insulin receptor, IGF-1 receptor (see below) and EGF receptor with β-arrestin (Dalle et al., 2001). Overall, the studies described above make a convincing argument for the involvement of heterotrimeric G proteins in mediating some of the actions of insulin. These various mechanism(s) involving insulin receptor and G proteins are schematically represented in Fig. 3.

In addition to mediating its actions via Gi and/or Gq/11 as described above, the insulin receptor can indirectly also stimulate the activity of Gs and adenylyl cyclase. Thus, chronic insulin treatment of cells has been shown to decrease the amount of β-arrestin-1, and this is accompanied by decreased association of the β-arrestin-1 with the β_2-adrenergic receptor and decreased receptor endocytosis (Dalle et al., 2002; Hupfeld et al., 2003). This results in supersensitization of the β_2-adrenergic receptors thereby increasing activation of Gs and adenylyl cyclase (Hupfeld et al., 2003). On the other hand, the insulin-mediated decrease in β-arrestin-1 by decreasing β-adrenoreceptor down-regulation inhibits the ability of agonists for these receptors to activate Erk MAPK cascade, a process that is Gi-mediated and dependent upon β_2-adrenoreceptor internalization (Dalle et al., 2002; Hupfeld et al., 2003). The paradigm described above is an example of how single transmembrane receptors like the insulin receptor can modulate the activity of G proteins other than the ones that they may be directly coupled to.

Early studies from Nishimoto's laboratory presented evidence that the IGF-II/mannose 6-phosphate receptor may also mediate its actions via heterotrimeric G proteins. Thus, in Balb/c3T3 or Chinese hamster ovary cells, IGF-II by stimulating its receptor has been shown to increase calcium influx via activation of Gi (Nishimoto et al., 1987a; Matsunaga et al., 1988; Okamoto et al., 1991a). The coupling of the IGF-II receptor to Gi was also demonstrated in reconstituted phospholipid vesicles (Nishimoto et al., 1989; Murayama et al., 1990; Okamoto et al., 1990a) and a region comprising 14 amino acids within the IGF-II receptor was identified as the G protein-activating sequence (Okamoto et al., 1990a; Okamoto and Nishimoto, 1991). Indeed, substitution of amino acids within this region changes the specificity of the coupling between IGF-II receptor and G proteins (Takahashi et al., 1993). Interestingly, the IGF-II/mannose 6-phosphate receptor does not activate G proteins

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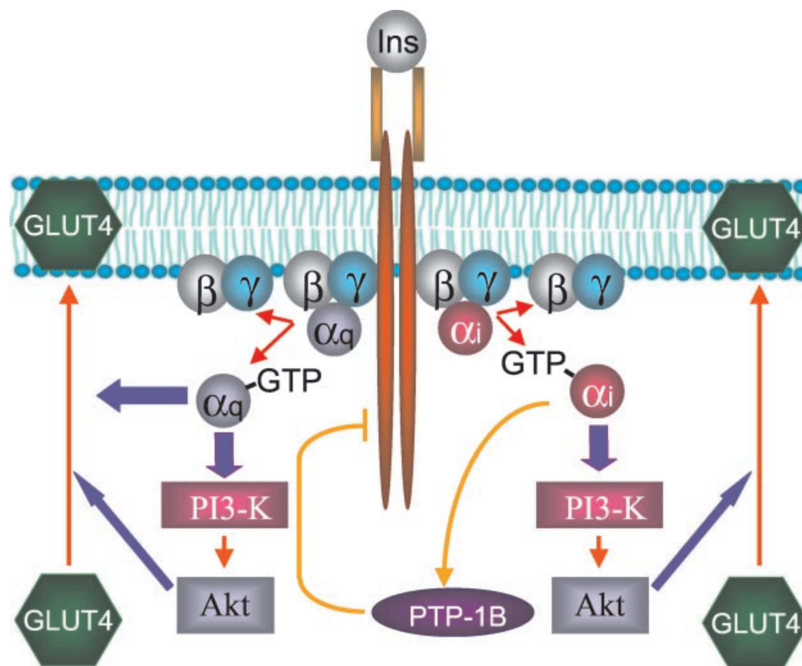

FIG. 3. Schematic of the involvement of Gαi2 and Gαq/11 in insulin-mediated GLUT4 translocation. As described in the text, the right side of the figure involving Gαi2 is derived from studies in intact animals whereas the left side depicts findings from cells in culture. The involvement of PI3K and Akt in Gαq/11-mediated actions is controversial (see text). The blue arrows indicate that other steps are involved in the processes depicted. The orange arrow indicates the interactions between levels of Gαi2 and PTP-1B that may involve changes in transcriptional or post-translational control of PTP-1B protein levels (see text). The feedback inhibition of insulin receptor autophosphorylation by PTP-1B is also shown.

when the receptor is activated by mannose 6-phosphate, but only when IGF-II is the ligand (Okamoto et al., 1990b). This suggests that the conformation of the IGF-II receptor that is activated by mannose 6-phosphate is different than the conformation that it assumes upon activation by IGF-II. Consistent with activation of Gi, the inhibitory G protein of adenylyl cylase, Nishimoto's laboratory has demonstrated that in intact cells, the activation of IGF-II receptor by IGF-II inhibits adenylyl cyclase (Ikezu et al., 1995). This study also showed that in addition to a G protein-activating region, the carboxy terminus of the IGF-II receptor also contains a PH domain that binds Gβγ subunits. Since Gβγ subunits can modulate the activity of several effectors such as phospholipase C and adenylyl cyclase (Exton, 1994; Patel et al., 2001), it is possible that by binding and sequestering the Gβγ subunits, the C terminus of the IGF-II receptor acts as a negative regulator of signaling. Support for this contention is derived from the observation that when this Gβγ binding region of the IGF-II receptor was deleted to decrease the sequestration of Gβγ subunits, the cholera toxin-stimulated activity of adenylyl cyclase in COS cells was further augmented (Ikezu et al., 1995). This is consistent with the presence of type IV adenylyl cyclase in these cells (Ikezu et al., 1995) that can be conditionally stimulated by Gβγ subunits provided that some active Gαs is present (Gao and Gilman, 1991); cholera toxin activates Gαs with a concomitant release of Gβγ subunits. The paradigm that IGF-II receptor via activation of Gαi inhibits adenylyl cyclase has also recently been confirmed in human extravillous trophoblast cells whose migration and invasiveness are stimulated by IGF-II (McKinnon et al., 2001).

Like the IGF-II receptor, the IGF-I receptor was first reported to be coupled to heterotrimeric G proteins by Nishimoto et al. (1987b). Essentially, this study showed that the IGF-I induced calcium influx and increase in DNA synthesis could be inhibited by pertussis toxin, which ADP-ribosylates the Gi/Go family of G proteins. Other reports have also suggested that several (Kanzaki et al., 1997; Poiraudeau et al., 1997; Sarbassov et al., 1997; Uehara et al., 1999), but not all (Stracke et al., 1988; Linder et al., 1994) of the actions of IGF are mediated via activation of G proteins. Recent interest in the interactions of the IGF-I receptor with heterotrimeric G proteins was rejuvenated by the demonstration that activation of extracellular signal-regulated kinases (Erks) was pertussis toxin-sensitive and mediated by G protein βγ subunits (Luttrell et al., 1995). More recent studies have shown that the IGF-I receptor actually associates with the Gαi and Gβγ subunits (Hallak et al., 2000; Dalle et al., 2001). Moreover, the activation of IGF-I receptor resulted in the release of Gβγ subunits without altering (Hallak et al., 2000) or even increasing (Dalle et al., 2001) the interactions between the receptor and Gαi. No change or increased interactions of Gαi with the IGF-I receptor would suggest that Gαi may not mediate any signals; however, in human intestinal smooth muscle cells, activation of IGF-I receptor has been shown to inhibit adenylyl cyclase in a Gαi2-dependent manner and activate Erk pathway via Gβγ subunits (Kuemmerle and Murthy, 2001). These findings clearly

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demonstrate that the association of the IGF-I receptor with Gαi2 does not alter the ability of the G protein to modulate adenylyl cyclase activity and that the adenylyl cyclase is located in close proximity of the receptor and G protein. Clearly, however, in intestinal smooth muscle cells both the α and $\beta\gamma$ subunits of Gi2 are involved in regulating signals (Kuemmerle and Murthy, 2001) that may modulate the growth of these cells. Because elevations in cAMP levels decrease growth, whereas increases in Erk activity increase growth, the coordinated and opposing actions of IGF-I on these two signaling systems, namely diminish cAMP levels and augment Erk activation, would ensure an increase in cellular growth. Although this may be sufficient to explain the growth promoting actions of IGF-I in smooth muscle cells of the intestine, it should be noted that in these cells activation of PI3K in response to IGF-I receptor activation was independent of heterotrimeric G protein activation (Kuemmerle and Murthy, 2001). This is important since PI3K via its actions on Akt and other downstream signaling mechanisms may also modulate cell survival and growth (Brazil and Hemmings, 2001; Shiojima and Walsh, 2002). Therefore, some but not all of the growth promoting actions of IGF-I may be modulated by coupling of the IGF-I receptor to Gi2.

Besides the activation of heterotrimeric Gi by IGF-I receptor, the studies of Dalle et al. (2001) revealed another interesting similarity between tyrosine kinase receptors and classical G protein-coupled receptors. These authors showed that β-arrestin-1 was associated with the IGF-I receptor, the insulin receptor and the EGF receptor. Moreover, the association between receptor tyrosine kinases and β-arrestin-1 was dependent upon activation of the receptors (Dalle et al., 2001). The role of β-arrestin-1 in IGF-I signaling is not entirely clear. However, β-arrestin-1 may serve to bring the G protein in proximity of the receptor tyrosine kinase since Gαi and β-arrestin-1 were co-immunoprecipitated (Dalle et al., 2001). Alternatively, since IGF-I receptor internalization is facilitated by β-arrestin-1 (Lin et al., 1998) and because internalization of IGF-I receptor is necessary for activation of Erk cascade (Chow et al., 1998), it is possible that β-arrestin-1 plays a pivotal role in signaling via the IGF-I receptor. Indeed, microinjection of anti-β-arrestin-1 antibodies into cells decreased IGF-I-stimulated, but not insulin-stimulated Erk activation (Dalle et al., 2001). Figure 4 schematically depicts the role of Gi in the activation of Erk and inhibition of adenylyl cyclase by the IGF-I receptor.

An important aspect about Erk cascade activation by β-arrestin-1 that needs to be noted is that in the case of certain receptors that form a stable complex with β-arrestin-1 and Erk, the activation of Erk is greater in the cytosol compared with the nucleus (Tohgo et al., 2003). This is consistent with the retention of the β-arrestin-1 in the cytosol. On the other hand, in case of receptors that form a transient complex with β-arrestin-1, a larger proportion of active Erk is observed in the nucleus (Tohgo et al., 2003). Moreover, the β-arrestin-dependent and independent activation of Erk by receptor tyrosine kinases are not necessarily redundant since the nuclear translocation by classical pathways compared with mainly cytosolic activation of Erk by β-arrestin-dependent mechanisms result in different biological endpoint (Luttrell, 2002).

C. Platelet-Derived Growth Factor and Fibroblast Growth Factor Receptors

As described for the IGF-I receptor above, the activation of Erk cascade by PDGF in airway smooth muscle

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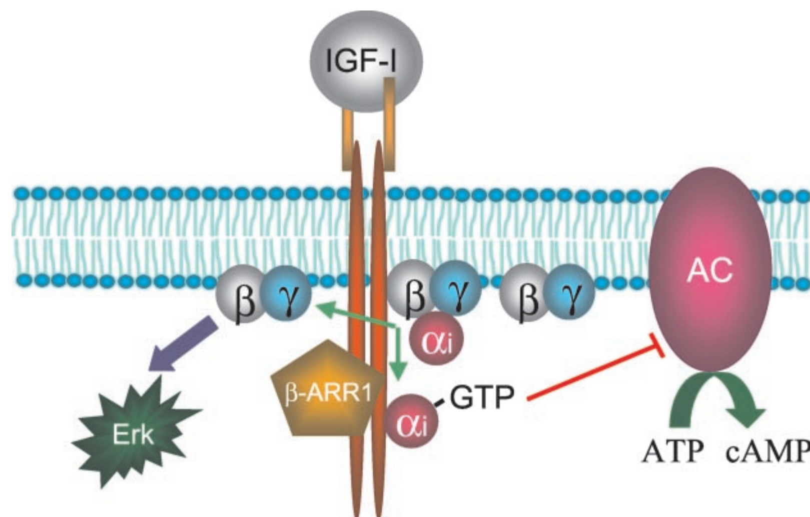

FIG. 4. The schematic depicts IGF-I-elicited activation of MAPK and inhibition of adenylyl cyclase (AC) by the $\beta\gamma$ and α subunits of Gi, respectively. The bold blue arrow indicates that several other steps (not shown here) are involved in activation of Erk by G$\beta\gamma$ subunits. As described in the text, the activated IGF-I receptor associates with β-arrestin-1 (β-ARR1), which may then play a role in receptor internalization or bring Gαi in proximity of the receptor.

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cells is mediated by G protein $\beta\gamma$ subunits, and this process can be inhibited by pertussis toxin (Conway et al., 1999). However, the activation of Erk by PDGF was dependent upon activation of $p60^{c\text{-}Src}$ and increased complex formation between Grb2 and PI3K (Conway et al., 1999). Interestingly, pertussis toxin inhibited both the activation of $p60^{c\text{-}Src}$ and Grb2/PI3K complex formation by PDGF (Conway et al., 1999). Apparently, the phosphorylation of Grb2 adapter protein Gab1 by the activated PDGF receptor is also mediated via Gi, and this phosphorylation is necessary for the formation of Gab1/Grb2/PI3K complex (Rakhit et al., 2000). More recently, using HEK cells overexpressing the PDGF-β receptors and the product of endothelial differentiation gene 1, EDG1, Pyne's group has shown that the PDGF-β receptor and EDG1 are associated with each other (Alderton et al., 2001). Since EDG1 is a classical G protein-coupled receptor whose agonist is sphingosine-1-phosphate, Pyne and coworkers (Alderton et al., 2001) propose that this type of complex between receptor tyrosine kinases and classical G protein-coupled receptors may provide the means to couple heterotrimeric G proteins to single transmembrane receptors. Although attractive, the acceptance of this model must await the demonstration of an association between classical G protein-coupled receptors and receptor tyrosine kinases without the overexpression of either protein. Similar to the studies of Dalle et al. (2001) with the IGF-I receptor (see above), Alderton et al. (2001) also observed the presence of G protein-coupled receptor kinase 2 (Grk2) and β-arrestin-1 in the EDG1/PDGF receptor complex. However, unlike the findings of Dalle et al. (2001), the amount of β-arrestin-1 in the EDG1/PDGF receptor immunocomplex (Alderton et al., 2001) was not altered in the presence or absence of PDGF. The precise reasons for this difference are not known but may be related to the overexpression of EDG1 receptors that may render some of the receptors to be constitutively active.

To date, only one report has provided evidence for an interaction of the FGF receptor to heterotrimeric G proteins. Krieger-Brauer et al. (2000) have shown that activation of FGF receptors by basic FGF activates Gs to increase the dissociation of Gαs and G$\beta\gamma$ subunits. The Gαs activates adenylyl cyclase whereas the G$\beta\gamma$ subunits inhibits NADPH-dependent H_2O_2 generation (Krieger-Brauer et al., 2000). The activation of adenylyl cyclase by the FGF receptors via Gs is reminiscent of the findings described above for the EGF receptor whereas the utilization of the G$\beta\gamma$ subunits to simultaneously activate another signaling pathway is similar to the findings with IGF-I in intestinal smooth muscle cells (Kuemmerle and Murthy, 2001) (see above). However, whether the FGF receptor activates Gs directly or indirectly via another protein or receptor is unknown.

IV. Other Single Transmembrane Receptors and Proteins Coupled to Heterotrimeric G Proteins

A. Amyloid Precursor Protein

Among the various spliced isoforms, the 695 amino acid long amyloid precursor protein (APP) is preferentially expressed in neurons. In patients with familial Alzheimer's disease, missense mutations of Val642 have been discovered (Hardy, 1992). Although mutations at Val642 have been shown to result in the secretion of a longer form (Aβ1–42) of Aβ amyloid from cleavage of APP (Suzuki et al., 1994), the pathology of Alzheimer's disease does not appear to be related to Aβ1–42 accumulation (LaFerla et al., 1995). Instead, because expression of the familial Alzheimer's disease associated Val642 mutants of APP result in cell death (Yamatsuji et al., 1996a,b), it is thought that this property of Val642 mutants is the cause of neurodegeneration in Alzheimer's disease. This coupled with the fact that the APP protein is a single transmembrane protein that resembles cell surface receptors (Schubert et al., 1991; Ferreira et al., 1993) has generated interest in the possible signaling mechanisms that this protein may activate within cells. To this end, a significant amount of evidence suggests that APP by interacting with and activating Go can induce cell death. Thus, Nishimoto's laboratory showed that a sequence encompassed by His657-Lys676 in APP695 binds to Go and activates the G protein (Nishimoto et al., 1993). Since the APP does not have a ligand, it may be argued that the protein may not activate Go. However, several lines of evidence suggest that APP may act as a G protein-coupled receptor. First, the apoptosis mediated by Val642 mutants of APP can be abolished by treatment of cells with pertussis toxin (Yamatsuji et al., 1996b). Since pertussis toxin ADP ribosylates and blocks the activation of Gi/Go by receptors, it would appear that APP couples to Go or Gi. This was supported by the observation that a monoclonal antibody against APP increased the ability of APP to activate Go in isolated lipid vesicles that contained reconstituted APP and Go (Okamoto et al., 1995). The ability of an antibody to increase the ability of APP to activate Go is consistent with APP having a receptor-like function. Third, the three naturally occurring FAD mutations (V642I, V642F, V642G) at Val642 position in APP are much more apoptotic than any other substitutions of this residue (Yamatsuji et al., 1996b) and act as constitutively active receptors to stimulate Go (Okamoto et al., 1996). Fourth, as described by in vitro studies (Nishimoto et al., 1993), the V642I mutant of APP requires the region His657–676 to activate Go (Yamatsuji et al., 1996a) further lending credence to the claim that this short region in APP is the Go-activating sequence. The portion of Go that contacts the APP protein has been shown to be the last 5 amino acids in the Gαo subunit. Hence, the expression of a Gαs/Gαo chimera that contained the last 5 amino acids of Gαo in NK1 cells ex-

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pressing V642F mutant of APP was able to permit the activation of the chimeric Gαs protein and increase cAMP response element (CRE) activity (Ikezu et al., 1996). This is consistent with the notion that the C terminus of G protein α subunits interact with receptors that activate the G proteins. The C terminus of other G protein α subunits did not permit interactions between APP and G protein (Ikezu et al., 1996) and in contrast to the findings with chimeric Gαs, the activation of nonchimeric Gαo by the V642F mutant of APP decreased CRE activity in NK1 cells (Ikezu et al., 1996). Depending upon the isoforms of adenylyl cyclases that are expressed in the NK1 cells, the activation of Go may decrease CRE activity in several ways. First, Gαo has been shown to inhibit certain isoforms (types V and VI) of adenylyl cyclase (Sunahara et al., 1996). Second, the G$\beta\gamma$ subunits released upon Go activation may also inhibit the activity of type I adenylyl cyclase (Patel et al., 2001). Whatever the precise mechanism, the resultant decrease in cAMP levels would diminish CRE activity. However, the studies of Giambarella et al. (1997a) suggest that in NK1 cells activated Gαo does not decrease cAMP levels. Therefore, the inactivation of CRE may occur via a cAMP-independent mechanism.

To determine whether the Gαo or G$\beta\gamma$ subunits of Go were involved in inducing apoptosis following activation of the G protein by mutant APP, Nishimoto's laboratory performed several systematic studies with separate G protein subunits. In sum, these findings showed that G$\beta\gamma$ subunits and not Gαo were involved in APP-mediated apoptosis (Giambarella et al., 1997b).

It should be noted that heterotrimeric G proteins may mediate the actions of other proteins that may be involved in apoptosis of cells associated with Alzheimer's disease. For instance, while the studies of Wolozin et al. (1996) have confirmed the involvement of Go/Gi in mutant APP-induced cell death in PC12 cells, these studies also implicated the involvement of presenilin 2, in Gi/Go activation. Presenilin 2 is a protein that is functionally related to APP. Hence, antisense oligodeoxynucleotide that decreases presenilin 2 expression inhibits APP-induced cellular apoptosis (Dewji and Singer, 1996), placing presenilin 2 downstream of APP protein. Interestingly, mutations of presenilin 2 that are found in FAD induce apoptosis via a mechanism that involves the activation of Gi/Go proteins (Wolozin et al., 1996). The predicted secondary structure of presenilin 2 resembles that of seven transmembrane G protein-coupled receptors. This coupled with the fact that presenilin 2 may be downstream of APP in the apoptotic pathway (Dewji and Singer, 1996) raises the possibility that APP may activate Go by association with and/or activation of presenilin 2. This scenario would be akin to the PDGF receptor EDG1 interaction mentioned above. However, the in vitro findings from Nishimoto's laboratory that show a direct interaction between APP and Go (reviewed above) would refute this suggestion.

Go/Gi may also be involved in apoptosis induced by the Aβ peptide, which is released upon proteolytic cleavage of APP. Several studies have shown that Aβ can induce cell death (Selkoe, 2001), and this effect of Aβ peptide can be blocked by pertussis toxin (Rymer and Good, 2001; Wei et al., 2002). Given the reports that Aβ may bind the APP protein (Lorenzo et al., 2000), β-amyloid peptide-binding protein (Kajkowski et al., 2001), and p75^{NTR} (Yaar et al., 1997, 2002) and induces cellular apoptosis, it is tempting to speculate the Aβ peptide is a ligand to these proteins that act as receptors and signal downstream via activation of Go/Gi. This possibility needs to be experimentally addressed.

B. C-Type Natriuretic Peptide Receptor

As the name implies, the natriuretic peptide receptors bind peptides that induce natriuresis from the kidney. The first natriuretic peptide to be discovered was atrial natriuretic peptide (ANP), which is secreted upon atrial distention (de Bold et al., 1981). This was followed by the discovery of brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) (Sudoh et al., 1988, 1989; Brenner et al., 1990). ANP and BNP oppose the actions of vasopressin, endothelins, and the renin-angiotensin system (Brenner et al., 1990; Ruskoaho, 1992). In addition, the natriuretic peptides have also been shown to be important in regulating cardiac development (Walther et al., 2002). ANP, BNP, and CNP bind to three types of natriuretic peptide receptors designated as the A-type (NPR-A), B-type (NPR-B), and the C-type (NPR-C) natriuretic peptide receptor (Fuller et al., 1988; Chang et al., 1989; Chinkers et al., 1989; Schulz et al., 1989a,b). Among these, NPR-A and NPR-B receptors are single transmembrane guanylyl cyclases whose ability to synthesize cGMP is markedly augmented upon binding their ligands ANP and BNP, respectively (Schulz et al., 1989a,b). On the other hand, the single transmembrane NPR-C that binds ANP, BNP, and CNP was originally thought to be involved in the clearance of atrial natriuretic peptide from the circulation (Porter et al., 1988).

Earlier studies from Cantin's group suggested that in the aorta, adrenal cortex, and pituitary, ANP decreases the activity of adenylyl cyclase via activation of Gi (Anand-Srivastava et al., 1984, 1985a,b, 1987). Later, with the discovery of specific peptides that selectively bind the different types of NPRs, the Cantin group suggested that the inhibition of adenylyl cyclase by ANP was mediated by activation of NPR-C (Anand-Srivastava et al., 1990). Since the NPR-C contains a short (37 amino acid long) cytosplasmic domain, Anand-Srivastava et al. set out to determine if this region of NPR-C could inhibit adenylyl cyclase activity in a Gi-dependent manner. Indeed, the inhibition of adenylyl cyclase in a Gi-dependent manner can be mimicked by a peptide corresponding to the 37 amino acid long cytoplasmic domain of the NPR-C (Anand-Srivastava et al., 1996) and by smaller regions within this domain (Pagano and

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Anand-Srivastava, 2001). The ability of NPR-C to inhibit adenylyl cyclase has also been suggested to be responsible for inhibition of cyclooxygenase 2 induction (Kiemer et al., 2002), astrocyte proliferation (Levin and Frank, 1991) as well as a decrease in the production and release of endothelin from endothelial cells (Hu et al., 1992). Thus, the NPR-C represents another single transmembrane receptor that can couple with G proteins and modulate signaling in cells.

C. Zona Pellucida Glycoprotein Receptor

The process of egg fertilization involves several steps including cell recognition in a species-specific manner, signaling, and exocytosis steps (Wassarman, 1999). Sperm recognition by the egg coat requires an interaction between specific cell surface proteins on both cell types. The zona pellucida (ZP) glycoprotein coat on mammalian oocytes comprises three proteins ZP1, ZP2, and ZP3. Among these, *O*-linked oligosaccharide ligand on ZP3 on the egg coat component is responsible for binding to β-1,4-galactosyltransferase (GalTase) on the free-swimming sperm (Wassarman, 1999). This GalTase/ZP3 interaction is then followed by activation of a pertussis toxin-sensitive, heterotrimeric G protein (Gong et al., 1995) that induces the acrosome reaction during which hydrolytic enzymes are released to permit the penetration of the sperm through the zona pellucida. Recently, Shi et al. (2001) have confirmed the earlier findings of Gong et al. (1995) that the GalTase on the sperm surface binds ZP3 and induces egg activation. Interestingly, in the studies of Shi et al. (2001), mutation of one residue on β-1,4-galactosyltransferase, which uncouples it from G proteins also resulted in the loss of cortical granule exocytosis. These studies clearly demonstrate that the egg/sperm interaction process that occurs early in fertilization is mediated by a single transmembrane receptor (GalTase) that is coupled to a heterotrimeric G protein.

D. Integrins and Associated Proteins

Another family of receptors that has recently been shown to signal via G proteins is the extracellular protein receptor family, integrins. In smooth muscle cells, the function of $\alpha 2\beta 1$ integrin to increase chemotaxis toward collagen is augmented by integrin-associated protein (IAP/CD47) (Wang and Frazier, 1998). IAP/CD47 is a five transmembrane spanning protein that is activated by a sequence RFYVVMWK on the C-terminal cell binding domain of thrombospondin 1 (Gao et al., 1996a,b). This protein is, therefore, also referred to as the thrombospondin receptor. Smooth muscle cells from IAP/CD47 deficient (IAP$^{-/-}$) animals do not migrate in response to a peptide agonist (4N1K) of IAP/CD47 whereas migration of normal smooth muscle cells is significantly enhanced by the IAP/CD47-activating peptide (Wang et al., 1999). The 4N1K-stimulated migration was inhibited by treatment of cells with pertussis toxin, suggesting the involvement of a Gi/Go family of G protein in the process. Consistent with this notion, 4N1K caused a decrease in cAMP accumulation (Wang et al., 1999). Interestingly, 4N1K also decreased Erk activation, and pertussis toxin treatment of cells increased Erk activation suggesting that a tonic negative regulation was alleviated (Wang et al., 1999). Gαi has also been demonstrated to associate with IAP/CD47 (Frazier et al., 1999). These and other data suggest that IAP/CD47 stimulates $\alpha 2\beta 1$ integrin-elicited migration of smooth muscle cells via Gi-mediated inhibition of Erk activity and suppression of cellular cAMP accumulation (Wang et al., 1999).

Integrins may also facilitate the coupling of other receptors to G proteins. For instance, it has been reported that the first extracellular loop of the heptahelical P2Y$_2$ purinergic receptor has an integrin-binding RGD domain that permits interactions with integrins (Erb et al., 2001). The disruption of this interaction with integrins by site-directed mutagenesis of the RGD domain also abolishes the ability of the P2Y$_2$ receptor to activate Go (Erb et al., 2001). However, the ability of the mutant receptor to activate Gq was not altered (Erb et al., 2001). Since, IAP/CD47 associates with and activates Gi/Go (reviewed above), it is possible that the activated P2Y$_2$ receptor via its interactions with integrins may utilize IAP/CD47 to activate Go. In this manner, integrins and IAP/CD47 may facilitate other receptors including classical G protein-coupled receptors such as the P2Y$_2$ receptor to activate Gi or Go.

More recently, studies by Meyer et al. (2000) have shown that twisting or shear stress-mediated activation of integrins in bovine endothelial cells in culture and in NIH 3T3 cells results in activation of the cAMP/protein kinase A signaling pathway via activation of Gs and stimulation of adenylyl cyclase. Although the molecular details of how the integrins activate Gs remain unknown, the studies of Meyer et al. (2000) make a first step toward understanding how mechanical forces regulate cAMP levels in cells. An interesting aspect about integrin signaling is that the engagement of integrins also activates certain receptor tyrosine kinases such as the EGF receptor (Yamada and Even-Ram, 2002). In fact, extracellular protein-stimulated cellular migration is enhanced in the presence of active EGF receptor expression in cells (Li et al., 1999). Inasmuch as the receptor tyrosine kinases activate heterotrimeric G proteins (reviewed above), one wonders whether these receptor tyrosine kinases are involved in integrin-mediated activation of G proteins. This possibility warrants further examination.

E. T Cell Receptors

The T cell receptor (TCR) is a multisubunit complex consisting of clonotypic α and β chains that interact with CD3-γ, δ, ϵ chains as well as the TCR ζ chain. The binding of antigen-presenting cells to the TCR initiates

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a number of signaling events that culminate in T cell proliferation and/or differentiation. Several studies have therefore been performed on the mechanism of T cell activation by TCR. The reader is referred to recent reviews on the variety of signals that are initiated by the TCR (van Leeuwen and Samelson, 1999; Davis, 2002; Griffiths and Penninger, 2002a,b; Kane et al., 2002). However, only the activation of heterotrimeric G proteins by TCR/CD3 complex will be discussed here.

Very early on, it was discovered that cholera toxin inhibits the TCR- mediated increase in inositol trisphosphate production, increase in cytosolic-free Ca^{2+}, and proliferation of T cells upon TCR activation (Imboden et al., 1986; Nel et al., 1988). Since cholera toxin ADP ribosylates the α subunit of Gs, it was assumed that modulation of Gs activity by TCR was somehow involved in downstream signaling. However, later studies showed that cholera toxin blunted TCR-mediated events by decreasing the number of TCR on the cell surface (Sommermeyer et al., 1990). This example underscores the need for appropriate controls to facilitate the interpretation of data with toxins that modulate G protein activity.

Despite the problems with cholera toxin and Gs involvement in TCR activation noted above, several studies have implicated heterotrimeric G proteins as downstream mediators of TCR functions. Thus, Cenciarelli et al. (1992) demonstrated that a G protein was involved in the tyrosine phosphorylation of the ζ chain of the TCR. This conclusion was arrived at from results of studies with GTP analogs that showed that GTPγS stimulated and GDPβS inhibited TCRζ chain phosphorylation; however, the nature of the G proteins (Ras family versus heterotrimeric) was not identified. Similarly, Ohmura et al. (1992) also reported that a 68-kDa GTP-binding protein was associated with the TCR complex. This GTP-binding protein was not sensitive to pertussis toxin or cholera toxin (Ohmura et al., 1992) and its identity remains unknown. Interestingly, the TCRζ chain itself can bind GTP and GDP (Peter et al., 1992), and certain lysine residues in this protein were identified to be crucial for this function. Thus, it is possible that the effects of GTP analogs on TCRζ chain phosphorylation reported by Cenciarelli et al. (1992) did not involve a G protein but rather were mediated by binding of the GTP analogs directly to the ζ chain.

Perhaps the strongest evidence suggesting the involvement of heterotrimeric G proteins in TCR activation is derived from two studies. The first of these demonstrated that the TCR can activate Gαq/11 family members (Stanners et al., 1995). Moreover, the CD3 ϵ subunit associates with these G proteins, and activation of Gα11 increases phospholipase Cβ activity (Stanners et al., 1995). Thus, TCR-mediated increase in intracellular Ca^{2+} may result from stimulation of phospholipase Cβ activity and increase in inositol 1,4,5-trisphosphate levels. Interestingly, the tyrosine kinase inhibitors, genistein and tyrphostin, attenuated the activation of the G protein in response to anti-CD3-mediated activation of the TCR/CD3 complex (Stanners et al., 1995). These findings would suggest that tyrosine kinase activation is necessary for G protein activation. Moreover, the expression of a functionally inactive mutant of Gα11 inhibited TCRζ chain phosphorylation (Stanners et al., 1995) demonstrating that the initial suggestions of Cenciarelli et al. (1992) concerning the involvement of a heterotrimeric G protein in phosphorylation of the TCRζ chain may indeed be correct. More recently, Lippert et al. (2000) showed that treatment of T lymphocytes with pertussis toxin inhibited cell proliferation, interleukin-2 production, and CD25 expression in response to CD3 activation. Moreover, the expression of inactive Gαi2 inhibited interleukin-2 production in response to CD3 activation (Lippert et al., 2000), suggesting that this G protein is involved in TCR/CD3 complex-activated interleukin-2 production. The fact that interleukin-8 in an autocrine manner can increase interleukin-2 production in a Gαi2-dependent manner (Lippert et al., 2000) suggests that the G protein may be involved downstream of the TCR/CD3 complex, perhaps at the level of chemokine receptor-G protein coupling.

V. Concluding Remarks

As reviewed above, it is clear that a large number of receptors and proteins that do not belong to the classical family of seven transmembrane G protein-coupled receptors can associate with and/or activate heterotrimeric G proteins. The spectrum of these receptors spans from receptor tyrosine kinases to proteins involved in Alzheimer's disease (APP), or binding of natriuretic peptides, oocyte fertilization, and even receptors that bind the extracellular matrix proteins. Table 1 provides a summary of the G proteins that are directly or indirectly stimulated by the various proteins reviewed here. Notably, as mentioned under the different subsections, each

TABLE 1

Summary of the G proteins activated by single transmembrane receptors and other proteins reviewed above. The direct and indirect interactions of proteins with heterotrimeric G proteins are shown.

Single Transmembrane Receptor or Protein	G Protein Activated by Direct Interactions	G Protein Activated by Indirect Interactions
EGF receptor[a]	Gs	
Insulin receptor[a]	Gi/Go, Gq/11	
IGF-I receptor	Gi/Go, G$\beta\gamma$	
IGF-II receptor	Gi, G$\beta\gamma$	
PDGF receptor		Gi via EDG1
FGF receptor	Gs[b]	Gs[b]
APP	Go	Go via presenilin
CNP	Gi	
ZP3	Gi/Go	
IAP/CD47	Gi	
Integrins		Go via $P2Y_2$ receptors Gs[b]
TCR (CD3ϵ)	Gq/11	Gi via interleukin 8[b]

[a] Receptors that have been shown to phosphorylate the α subunits of the G proteins that they interact with.
[b] Interactions for which the mechanisms (direct or indirect) are not clear.

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of these proteins may also activate other signaling processes that are G protein-independent such as phosphorylation of proteins on tyrosine residues by receptor tyrosine kinases. The added ability to signal via heterotrimeric G proteins permits the various receptors to increase the diversity of signals that they can mediate and also increase the complexity of their biological actions. For instance, receptors that activate Gs or Gi and therefore stimulate or inhibit adenylyl cyclase and subsequently PKA may also modulate their own ability to activate the Erk cascade. It has been shown that cAMP and PKA via Rap1 can decrease the activity of Raf-1 (also known as c-Raf) but increase the activity of B-Raf (see (Stork and Schmitt, 2002) for review). Therefore, activation of PKA via Gs in cells that express Raf-1 may decrease the ability of EGF or FGF to activate the Erk cascade but in cells expressing B-Raf, Gs activation may augment the ability of EGF or FGF to activate Erk. Conversely, in cells expressing Raf-1, activation of Gi may decrease cAMP content and, therefore, PKA activity and augment the ability of receptors that stimulate Gi to activate the Erk cascade. This is only one of many mechnisms by which the signaling to Erk cascade can be modulated by PKA or cAMP. For a complete review on different mechanisms by which the Erk pathway may be regulated by cAMP and PKA, the reader is referred to a recent article by Stork and Schmitt (2002).

Although some of the molecular details of how some of the receptors and proteins reviewed above activate the respective heterotrimeric G proteins have been delineated, there are yet other proteins in this family whose actions at the molecular level remain to be determined. Given the importance and diverse nature of the receptors in this family, research in this field is sure to attract much more attention and should prove to be a fruitful endeavor for the next several years.

One of the concepts that warrants further investigation is that the receptors or proteins that mediate their actions via G proteins may do so by engaging other receptors such as the APP/prenesilin, integrin/P2Y_2 receptor and PDGF/EDG1 interactions reviewed above. This concept needs to be carefully investigated keeping in mind that a given receptor may engage heterotrimeric G proteins by interacting with receptors of not only the seven transmembrane family but also those described above that belong to the single transmembrane family. Thus, integrins by transactivating receptor tyrosine kinases such as EGF receptor (Prenzel et al., 2000) may activate heterotrimeric G proteins whereas APP may use presenilin 2 to do the same. Moreover, receptors such as the urokinase-type plasminogen activator receptor (u-PAR) that do not have a cytoplasmic domain and are tethered to cell membrane via a glycosyl-phosphatidylinositol moiety (Behrendt et al., 1991; Fazioli and Blasi, 1994) can also modulate cell migration in a pertussis toxin-sensitive manner implicating the involvement of Gi or Go protein in the actions of u-PA (Degryse et al., 1999). The exact mechanism of action of u-PAR-mediated activation of Gi/Go proteins is not known; however, since u-PAR interacts with integrins (Wei et al., 1996; Degryse et al., 1999), it is possible that the coupling to Gi/Go somehow involves integrins and IAP/CD47 akin to the scenario with the P2Y_2 receptor discussed above. This possibility needs to be further explored. In this same context, studies also need to be performed to make sure that the activation of certain single transmembrane receptors do not lead to production of autocrine factors such as prostanoids that then bind to their heptahelical receptors to activate G proteins. The elucidation of the mechanisms involved is surely going to show that a given receptor may mediate its final biological action via activation of a number of signaling cascades, thus emphasizing the concept that a single receptor may initiate a network of signaling rather than a simple linear signaling process.

Finally, it is becoming increasingly clear that cytokine receptors, ion channels, and the heptahelical receptors can also form complexes with and transactivate the receptor tyrosine kinases such as the EGF receptor (Prenzel et al., 2000; Pyne et al., 2003). Thus by transactivating receptor tyrosine kinases, the activation of these proteins may also indirectly engage signaling via G proteins, which they do not directly couple with, further adding diversity to the biological endpoints.

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