# Mammalian G Proteins and Their Cell Type Specific Functions

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**Wettschureck, Nina, and Stefan Offermanns.** Mammalian G Proteins and Their Cell Type Specific Functions. *Physiol Rev* 85: 1159–1204, 2005; doi:10.1152/physrev.00003.2005.—Heterotrimeric G proteins are key players in transmembrane signaling by coupling a huge variety of receptors to channel proteins, enzymes, and other effector molecules. Multiple subforms of G proteins together with receptors, effectors, and various regulatory proteins represent the components of a highly versatile signal transduction system. G protein-mediated signaling is employed by virtually all cells in the mammalian organism and is centrally involved in diverse physiological functions such as perception of sensory information, modulation of synaptic transmission, hormone release and actions, regulation of cell contraction and migration, or cell growth and differentiation. In this review, some of the functions of heterotrimeric G proteins in defined cells and tissues are described.
I. INTRODUCTION

All cells possess transmembrane signaling systems that allow them to receive information from extracellular stimuli like hormones, neurotransmitters, or sensory stimuli. This fundamental process allows cells to communicate with each other. All transmembrane signaling systems share two basic elements, a receptor which is able to recognize an extracellular stimulus as well as an effector which is controlled by the receptor and which can generate an intracellular signal. Many transmembrane signaling systems like receptor tyrosine kinases incorporate these two elements in one molecule. In contrast, the G protein-mediated signaling system is relatively complex consisting of a receptor, a heterotrimeric G protein, and an effector. This modular design of the G protein-mediated signaling system allows convergence and divergence at the interfaces of receptor and G protein as well as of G protein and effector. In addition, each component, the receptor, the G protein as well as the effector can be regulated independently by additional proteins, soluble mediators, or on the transcriptional level. The relatively complex organization of the G protein-mediated transmembrane signaling system provides the basis for a huge variety of transmembrane signaling pathways that are tailored to serve particular functions in distinct cell types. It is probably this versatility of the G protein-mediated signaling system that has made it by far the most often employed transmembrane signaling mechanism. In this review we summarize some of the biological roles of G protein-mediated signaling processes in the mammalian organism which are based on their cell type-specific function. Although we have tried to cover a wide variety of cellular systems and functions, the plethora of available data forced us to restrict this review. Particular emphasis is placed on cellular G protein functions that have been studied in primary cells or in the context of the whole organism using genetic approaches.

A. Basic Principles of G Protein-Mediated Signaling

More than 1,000 G protein-coupled receptors (GPCRs) are encoded in mammalian genomes. While most of them code for sensory receptors like taste or olfactory receptors, ~400–500 of them recognize nonsensory ligands like hormones, neurotransmitters, or paracrine factors (53, 185, 519, 534, 649). For more than 200 GPCRs, the physiological ligands are known (Table 1). GPCRs for which no endogenous ligand has been found are “orphan” GPCRs (376, 389, 688).

Upon activation of a receptor by, e.g., its endogenous ligand, coupling of the activated receptor to the heterotrimeric G protein is facilitated. Multiple site-directed mutagenesis experiments have been performed on G protein-coupled receptors, and they have revealed various cytoplasmic domains of the receptors that are involved in the specific interaction between the receptors and the G protein. However, despite the determination of the structure of rhodopsin at atomic resolution (504), it is still not clear how specificity of the receptor-G protein interaction is achieved and how a ligand-induced conformational change in the receptor molecule results in G protein activation (177, 212, 213, 565, 674).

The heterotrimeric G protein consists of an α-subunit that binds and hydrolyzes GTP as well as of a β- and a γ-subunit that form an undissociable complex (233, 255, 475). Several subtypes of α-, β-, and γ-subunits have been described (Table 2). To dynamically couple activated receptors to effectors, the heterotrimeric G protein undergoes an activation-inactivation cycle (Fig. 1). In the basal state, the βγ-complex and the GDP-bound α-subunit are associated, and the heterotrimer can be recognized by an appropriate activated receptor. Coupling of the activated receptor to the heterotrimer promotes the exchange of GDP for GTP on the G protein α-subunit. The GTP-bound α-subunit dissociates from the activated receptor as well as from the βγ-complex, and both the α-subunit and the βγ-complex are now free to modulate the activity of a variety of effectors like ion channels or enzymes. Signaling is terminated by the hydrolysis of GTP by the GTPase activity, which is inherent to the G protein α-subunit. The resulting GDP-bound α-subunit reassociates with the βγ-complex to enter a new cycle if activated receptors are present. For recent excellent reviews on basic structural and functional aspects of G proteins, see References 49, 83, 361, and 526.

While the kinetics of G protein activation through GPCRs has been well described for quite a while, only recently has the regulation of the deactivation process been understood in more detail. Based on the observation that the GTPase activity of isolated G proteins is much lower than that observed under physiological conditions, the existence of mechanisms that accelerate the GTPase activity had been postulated. Various effectors have indeed been found to enhance GTPase activity of the G protein α-subunit, thereby contributing to the deactivation and allowing for rapid modulation of G protein-mediated signaling (23, 45, 348, 571). More recently, a family of proteins called “regulators of G protein signaling” (RGS proteins) has been identified, which is also able to increase the GTPase activity of G protein α-subunits (272, 481, 550). There are ~30 RGS proteins currently known, which have selectivities for G protein α-subfamilies. The physiological role of RGS proteins is currently under investigation. Besides their role in the modulation of G protein-mediated signaling kinetics, they also influence the specificity of the signaling process and in some cases may have effector functions.
### TABLE 1. Physiological ligands of G protein-coupled receptors

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<th>Receptor</th>
<th>Coupling to G Protein</th>
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<td>Gq/11</td>
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<td></td>
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<td>Gq/11, Gi/o</td>
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<td>GABA_B1 (binding), GABA_B2 (signaling)</td>
<td>Gq/11</td>
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<td>Gi/o</td>
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<td>GPRC6A</td>
<td>Gi/o,Gq/11</td>
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<td><strong>Biogenic Amines</strong></td>
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<td>Neuropeptide Y (NPY) etc.</td>
<td>Y1, Y2, Y4, Y5, Y6</td>
<td>Gi-11</td>
<td>582</td>
</tr>
<tr>
<td>Nerotensin</td>
<td>NTS1, NTS2</td>
<td>Gi-11</td>
<td>582</td>
</tr>
<tr>
<td>Opioids (μ-endorphin, Met/Leu-enkephalin, dynorphin A, nociceptin/orphanin FQ)</td>
<td>ORL1</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Orexin A/B</td>
<td>OX1, OX2</td>
<td>Gi-11</td>
<td>582</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>OT</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Parathyroid hormone (related peptide)</td>
<td>PTH/PTHrP</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Prokineticin-1,2</td>
<td>PK-R1, PK-R2</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Prolactin-releasing peptide</td>
<td>PrRP (GPR10)</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Relaxin, insulin-like 3</td>
<td>LGR7, LGR8</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Secretin</td>
<td>Secretin</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>SST1, SST2, SST3, SST4, SST5</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Substance P (SP)</td>
<td>NK2</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Thyrotropin (TSH)</td>
<td>TSH</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Thyrotropin-releasing hormone (TRH)</td>
<td>TRH-1, TRH-2</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Urotensin II</td>
<td>UT-II (GPR14)</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Vasoactive intestinal polypeptide (VIP), PACAP</td>
<td>VPAC1, VPAC2, PAC1</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>V1a, V1b, V2</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Proteases (the new NH2-terminal domain produced by proteolytic cleavage serves as a tethered ligand)</td>
<td></td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Thrombin and others</td>
<td>PAR-1, PAR-3, PAR-4</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
<tr>
<td>Trypsin and others</td>
<td>PAR-2</td>
<td>Gi-11, Gi-12, Gi-13</td>
<td>582</td>
</tr>
</tbody>
</table>
The interaction of G proteins with the inner side of the plasma membrane is facilitated by lipid modifications of both the α-subunit as well as of the γ-subunit of the βγ-complex (97, 448, 589, 728). Recent data provide evidence that heterotrimeric G proteins of the G_i family are also involved in receptor-independent processes (52, 413), which appear to be critically involved in the positioning of the mitotic spindle and the attachment of microtubules to the cell cortex (234). These processes also involve a group of proteins that carry a so-called GoLoco motif which functions as a guanine nucleotide dissociation inhibitor (684).

### TABLE 1—Continued

<table>
<thead>
<tr>
<th>Sensory Stimuli</th>
<th>Receptor</th>
<th>Coupling to G Protein</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>~500 nm (max. absorption)</td>
<td>Rhodopsin (11-cis-retinal)</td>
<td>G_i, G_o</td>
<td>717</td>
</tr>
<tr>
<td>~426 nm (max. absorption)</td>
<td>Blue-opsin (11-cis-retinal)</td>
<td>G_i, G_o</td>
<td>471</td>
</tr>
<tr>
<td>~530 nm (max. absorption)</td>
<td>Green-opsin (11-cis-retinal)</td>
<td>G_i, G_o</td>
<td>471</td>
</tr>
<tr>
<td>~560 nm (max. absorption)</td>
<td>Red-opsin (11-cis-retinal)</td>
<td>G_i, G_o</td>
<td>471</td>
</tr>
<tr>
<td>~425–480 nm (max. absorption)</td>
<td>Melanopsin (11-cis-retinal)</td>
<td>G_g11?</td>
<td>436, 505, 530</td>
</tr>
<tr>
<td>Odorants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1 group (few in human, ~150 in mouse)</td>
<td>V1R1 + V1R3</td>
<td>G_g11?</td>
<td>457, 477, 730</td>
</tr>
<tr>
<td>V2 group (none in human, ~150 in mouse)</td>
<td>V1R2 + V1R3</td>
<td>G_g11?</td>
<td>94, 457, 463</td>
</tr>
<tr>
<td>Taste</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bitter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odorants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pheromones</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the reference column, reviews are cited whenever possible to limit the number of references.

The functional versatility of the G protein-mediated signaling system is based on its modular architecture and on the fact that there are numerous subtypes of G proteins. The α-subunits that define the basic properties of a heterotrimeric G protein can be divided into four families, Gαo, Gαq/Gαo, Gαi/Gα11, and Gα12/Gα13 (Table 2). Each family consists of various members that often show very specific expression patterns. Members of one family are structurally similar and often share some of their functional properties. The βγ-complex of mammalian G proteins is assembled from a repertoire of 5 G protein β-subunits and 12 γ-subunits (Table 2). While β1- to β4-subunits form a tight complex with γ-subunits which can only be separated under denaturing conditions, the β5-subunit interaction with γ-subunits is comparably weak (347, 543). The β5-subunit is an exception in that it can also be found in a complex with a subgroup of RGS proteins (689). The βγ-complex was initially regarded as a more passive partner of the G protein α-subunit. However, it has become clear that βγ-complexes freed from the G protein α-subunit can regulate various effectors (112). These βγ-mediated signaling events include the regulation of ion channels (488), of particular isoforms of adenylyl cyclase and phospholipase C (169, 615), as well as of phosphoinositide-3-kinase isoforms (641). With a few exceptions, the ability of different βγ-combinations to regulate effector functions does not dramatically differ (112).

Most receptors are able to activate more than one G protein subtype. The activation of a G protein-coupled receptor therefore usually results in the activation of several signal transduction cascades via G protein α-subunits as well as through the freed βγ-complex. The pattern of G proteins activated by a given receptor determines the cellular and biological response, and activated receptors that lead to functionally similar or identical cellular effects usually activate the same G protein subtypes. The G protein receptor interaction in general does not occur in an absolutely specific or in a completely promiscuous manner. Some receptors appear to interact only with certain G protein subforms, and in some cellular systems, the compositions of defined G protein-mediated signaling pathways can be very specific. However, there are some characteristic patterns of receptor-G protein coupling that have been described for the majority of receptors (Fig. 2).

The G proteins of the G_i/G_o family are widely expressed and especially the α-subunits of G_{11}, G_{12} and G_{13} have been shown to mediate receptor-dependent inhibition of various types of adenylyl cyclases (615). Because the expression levels of G_i and G_o are relatively...
Activation of Gi/Go is therefore believed to be the major coupling mechanism that results in the activation of the effects of Go, which is particularly abundant in the lapping functions. In contrast to other G proteins, the subforms suggests that they may have partially overlapping functions. In contrast to other G proteins, the effects of Go, which is particularly abundant in the nervous system, appears to be primarily mediated by its βγ-complex. Whether Ga_o can regulate effectors directly is currently not clear. A less widely expressed member of the Ga_o/Ga_o family is Ga_11 (438), which in contrast to G_i and G_o is not a substrate for PTX. Ga_11 is expressed in various tissues including the nervous system and platelets. It shares some functional similarities with G_i-type G proteins but has recently been shown to interact specifically with various other proteins including Rap1GAP and certain RGS proteins (438). Several α-subunits like gustducin and transducins belong to the Ga_o family is G_i/G_o family close to G_o. The function of members of the Ga_o family has often been studied using a toxin from Clostridium botulinum (pertussis toxin; PTX) which is able to ADP-ribosylate most of the members of the Ga_o family close to their COOH termini. COOH-terminally ADP-ribosylated Ga_o is unable to interact with the receptor. Thus PTX treatment results in the uncoupling of the receptor and G_o. The structural similarity between the 3 Ga_i subforms suggests that they may have partially overlapping functions. In contrast to other G proteins, the effects of G_o, which is particularly abundant in the nervous system, appears to be primarily mediated by its βγ-complex. Whether Ga_o can regulate effectors directly is currently not clear. A less widely expressed member of the Ga_o/Ga_o family is Ga_11 (438), which in contrast to G_i and G_o is not a substrate for PTX. Ga_11 is expressed in various tissues including the nervous system and platelets. It shares some functional similarities with G_i-type G proteins but has recently been shown to interact specifically with various other proteins including Rap1GAP and certain RGS proteins (438).

The Ga_11 family of G proteins couples receptors to β-isoforms of phospholipase C (169, 538). The α-subunits of Ga_q and Ga_11 are almost ubiquitously expressed while the

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene</th>
<th>Expression</th>
<th>Effector(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Subunits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ga_1</td>
<td>GNASS</td>
<td>Ubiquitous</td>
<td>AC (all types) ↑</td>
</tr>
<tr>
<td>Ga_2</td>
<td>(GNAS1)</td>
<td>Neuroendocrine</td>
<td>AC ↑</td>
</tr>
<tr>
<td>Ga_11</td>
<td>(GNAL)</td>
<td>Olfactory epithelium, brain</td>
<td>AC ↑</td>
</tr>
<tr>
<td>Ga_o</td>
<td>(GNAS2,3)</td>
<td>Widely distributed</td>
<td>AC (types II,III,IV,VII) ↓ (directly regulated)</td>
</tr>
<tr>
<td>Ga_12</td>
<td>(GNAN1)</td>
<td>Widespread distribution via Gi/Go</td>
<td></td>
</tr>
<tr>
<td>Ga_13</td>
<td>(GNAN2)</td>
<td>Ubiquitous</td>
<td></td>
</tr>
<tr>
<td>Ga_14</td>
<td>(GNAN3)</td>
<td>Widely distributed</td>
<td></td>
</tr>
<tr>
<td>Ga_15</td>
<td>(GNAN4)</td>
<td>Neuroanatomical, platelets</td>
<td></td>
</tr>
<tr>
<td>Ga_16</td>
<td>(GNAM16)</td>
<td>Taste cells, brush cells</td>
<td></td>
</tr>
<tr>
<td>Ga_17</td>
<td>(GNAN7)</td>
<td>Retinal rods, taste cells</td>
<td></td>
</tr>
<tr>
<td>Ga_18</td>
<td>(GNAN8)</td>
<td>Retinal cones</td>
<td></td>
</tr>
<tr>
<td>β-Subunits</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>b_1</td>
<td>GNBI</td>
<td>Widely, retinal rods</td>
<td>PLC-β1 ↓</td>
</tr>
<tr>
<td>b_2</td>
<td>GNBB</td>
<td>Widely distributed</td>
<td>PLC-β1 ↓</td>
</tr>
<tr>
<td>b_3</td>
<td>GNBC</td>
<td>Widely, retinal cones</td>
<td>PLC-β1 ↓</td>
</tr>
<tr>
<td>b_4</td>
<td>GNBC</td>
<td>Widely distributed</td>
<td>PLC-β1 ↓</td>
</tr>
<tr>
<td>b_5</td>
<td>GNBC</td>
<td>Mainly brain</td>
<td></td>
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<tr>
<td>γ-Subunits</td>
<td></td>
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</tr>
<tr>
<td>γ_i</td>
<td>GNGT1</td>
<td>Retinal rods, brain,</td>
<td></td>
</tr>
<tr>
<td>γ_1</td>
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<td>Retinal cones, brain</td>
<td></td>
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<tr>
<td>γ_2</td>
<td>GNG2</td>
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<td></td>
</tr>
<tr>
<td>γ_3</td>
<td>GNG3</td>
<td>Brain, blood</td>
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</tr>
<tr>
<td>γ_4</td>
<td>GNG4</td>
<td>Brain and other tissues</td>
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</tr>
<tr>
<td>γ_5</td>
<td>GNG5</td>
<td>Widely</td>
<td></td>
</tr>
<tr>
<td>γ_6</td>
<td>GNG6</td>
<td>Widely</td>
<td></td>
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<tr>
<td>γ_7</td>
<td>GNG7</td>
<td>Ofactory/vomeronasal epithelium</td>
<td></td>
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<tr>
<td>γ_8</td>
<td>GNG8</td>
<td>Widely</td>
<td></td>
</tr>
<tr>
<td>γ_9</td>
<td>GNG9</td>
<td>Widely</td>
<td></td>
</tr>
<tr>
<td>γ_10</td>
<td>GNG10</td>
<td>Widely</td>
<td></td>
</tr>
<tr>
<td>γ_11</td>
<td>GNG11</td>
<td>Widely</td>
<td></td>
</tr>
<tr>
<td>γ_12</td>
<td>GNG12</td>
<td>Widely</td>
<td></td>
</tr>
<tr>
<td>γ_13</td>
<td>GNG13</td>
<td>Brain, taste buds</td>
<td></td>
</tr>
</tbody>
</table>

AC, adenyl cyclase; PDE, phosphodiesterase; PLC, phospholipase C; GIRK, G protein-regulated inward rectifier potassium channel; VDCC, voltage-dependent Ca^2+ channel; PI-3-K, phosphatidylinositol 3-kinase; GRK, G protein-regulated kinase; RhoGEF, Rho guanine nucleotide exchange factor.
other members of this family like Ga14 and Ga15/16 (Ga15 being the murine, Ga16 the human ortholog) show a rather restricted expression pattern. Receptors that are able to couple to the Gq/G11 family do not appear to discriminate between Gq and G11 (490, 660, 696, 705). Similarly, there is obviously no difference between the abilities of both G protein α-subunits to regulate phospholipase C β-isozymes. While Gaq and Gα11 both are good activators of β1-, β3-, and β4-isozymes of phospholipase C (PLC), the PLC β2-isozyme is a poor effector for both (538). The biological significance of the diversity among the Gaq gene family is currently not clear. While the importance of Gq and G11 in various biological processes has been well established, the roles of Ga14 and Ga15/16, which show very specific expression patterns, are not clear. Mice carrying inactivating mutations of the Ga14 and Ga15 genes have no or very minor phenotypical changes (132; H. Jiang and M. I. Simon, personal communication). In contrast, mice lacking Gaq or both Gaq and Gα11 have multiple defects (489, 494, 497) (see below).

The G proteins G12 and G13, which are often activated by receptors coupling to Gq/G11, constitute the G12/G13 family and are expressed ubiquitously (139, 607). The analysis of cellular signaling processes regulated through G12 and G13 has been difficult since specific inhibitors of these G proteins are not available. In addition, G12/G13-coupled receptors usually also activate other G proteins. Most information on the cellular functions regulated by G12/G13 therefore came from indirect experiments employing constitutively active mutants of Gα15/Gα13. These studies showed that G12/G13 can induce a variety of signaling pathways leading to the activation of various downstream effectors including phospholipase A2, Na+/H+ exchanger, or c-jun NH2-terminal kinase (139, 193, 276, 523, 607). Another important cellular function of G12/G13 is their ability to regulate the formation of actomyosin-based structures and to modulate their contractility by increasing the activity of the small GTPase RhoA (79). Activation of RhoA by Gα12 and Gα13 is mediated by a subgroup of guanine nucleotide exchange factors (GEFs) for Rho which include p115-RhoGEF, PDZ-RhoGEF, and LARG (194, 236, 618). While the RhoGEF activity of PDZ-RhoGEF and LARG appears to be activated by both Gα12 and Gα13, p115-RhoGEF activity is stimulated only by Gα13. Recently, an interesting link between G12/G13 and cadherin-mediated signaling was described, both Gα12 and Gα13 interact with the cytoplasmic domain of some type I and type II class cadherins, causing the release of β-catenin from cadherins (434, 435). Various other proteins including Bruton’s tyrosine kinase, the Ras GTGase-activating protein Gap1m, radixin, heat shock protein 90, AKAP110, protein phosphatase type 5, or Hax-1 have also been shown to interact with Gα12 and/or Gα13 (309, 359, 485, 532, 638, 709).

The ubiquitously expressed G protein Gs couples many receptors to adenylyl cyclase and mediates receptor-dependent adenylyl cyclase activation resulting in increases in the intracellular cAMP concentration. The α-subunit of Gs, Gαs, is encoded by GNAS, a complex imprinted gene that gives rise to several gene products due to the presence of various promoters and splice variants (Fig. 3). In addition to Gαs, two transcripts encoding XLαs and Nesp55 are generated by promoters upstream of the Gαs promoter. While the chromogranin-like protein Nesp55 is structurally and functionally not related to Gαs, XLαs is structurally identical to Gαs but has an extra long NH2-terminal extension that is encoded by a specific first exon (329). In contrast to Gαs, XLαs has a limited expression pattern being mainly expressed in the adrenal gland, heart, pancreatic islets, brain, and the pars intermedia of the pituitary (509). However, XLαs shares with Gαs the ability to bind to βγ-subunits and to mediate receptor-dependent stimulation of cAMP production (33, 339). Interestingly, the first exon of the Gnasxl gene encodes another protein termed ALEX (338), which is able to interact with the XL domain of XLαs and to inhibit its activity (188, 338). Interestingly, Nesp55 and XLαs are differentially imprinted. While the promoter of Nesp55 is DNA-methylated on the paternally inherited allele resulting in the expression only from the maternally inherited allele, the promoter driving XLαs expression is methyl-
ated on the maternal allele, and XLαs is only expressed from the paternal allele (244, 245, 515). Several other transcripts like Nespas of which some are believed to be untranslated show ubiquitous expression and are derived from the paternal allele due to differentially methylated promoter regions (243, 294, 396, 619). In contrast, the

FIG. 2. Typical patterns of receptor/G protein coupling. Although there are many exceptions, three basic patterns of receptor-G protein coupling have been found which critically define the cellular response after ligand-dependent receptor activation. α2, α2-adrenergic receptor; D1-5, dopamine receptor subtypes 1 to 5; GIRK, G protein-regulated inward rectifier potassium channel; 5-HT1-2, serotonin receptor subtypes 1 and 2; M1-5, muscarinic acetylcholine receptor subtypes 1 to 5; mGluR1-7, metabotropic glutamate receptor subtypes 1 to 7; PLC-β, phospholipase C-β; PI-3-K, phosphoinositide-3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; Rho-GEF, Rho-guanine nucleotide exchange factor; TP, thromboxane A2 receptor; IP, prostacyclin receptor.

FIG. 3. Model of the GNAS gene complex with some of its transcripts. Some of the transcripts generated from the maternal and paternal allele are shown on the top and bottom, respectively. Open boxes indicate noncoding sequences; closed boxes indicate coding sequences. Exon 3 of the GNAS gene (hatched box) is alternatively spliced out giving rise to long and short forms of Gαs. Promoters active on the maternal and paternal allele are indicated by arrows. While Nesp is only expressed from the maternal allele, XLαs and Nespas are expressed from the paternal allele. The GNAS promoter is biallelically active; however, in a few tissues only the maternal allele is expressed (see text). Several other transcripts of the GNAS gene complex have been described; however, their function is unclear. Exon sequences are shown in black and white for coding and noncoding sequences, while transcripts are shown in gray and white for coding and noncoding sequences.
promoter driving the expression of $\alpha_s$ has been shown to be biallelically active and to lack differential methylation (85, 244, 245, 733). However, in a few tissues such as the renal proximal tubules, the thyroid, pituitary, and ovaries, the paternal $\alpha_s$ expression is silenced by an as yet undefined mechanism (209, 242, 394, 414, 723).

II. CARDIOVASCULAR SYSTEM

A. Autonomic Control of Heart Function

Cardiac regulation by the sympathetic system is mediated by $\beta$-adrenergic receptors that are coupled primarily to $G_s$ (Fig. 4). cAMP produced in response to $G_s$ activation directly modulates the gating of hyperpolarization-activated, cyclic nucleotide-gated channels and activates protein kinase A (PKA) which in turn phosphorylates several proteins involved in excitation-contraction coupling including L-type $Ca^{2+}$ channels, phospholamban, or troponin I (44). These cellular changes are believed to underlie the well-known effects of sympathetic cardiac activation including positive chronotropic, dromotropic, lusitropic, and inotropic effects (545). Transgenic overexpression of the short form of $G_s$ ($G_s$-$S$) in the murine heart had no effect on the basal cardiac function but resulted in an enhanced efficacy of $\beta_1$-adrenoceptor $G_s$ signaling, and chronotropic and inotropic responses to catecholamines were increased (299). Once $G_s$-overexpressing mice become older, they develop clinical and pathological signs of cardiomyopathy (300). These pathological processes are accompanied by a lack of normal heart rate variability as well as of protective desensitization mechanisms (635, 650). The development of cardiomyopathy after prolonged overexpression of $G_s$ is in line with the current concept of the pathophysiological mechanisms underlying the development of chronic heart failure. The insufficient cardiac output characteristic for heart failure typically goes along with an increased sympathetic tone resulting in chronic catecholamine stimulation of cardiomyocytes, which is believed to be deleterious (71). Although the $\beta_1$-adrenoceptor is the predominant subtype expressed in cardiomyocytes, also $\beta_2$-adrenoceptors are expressed in the heart (545). Interestingly, there is increasing evidence that $\beta_1$- and $\beta_2$-adrenoceptors play different roles in catecholamine-induced cardiomyopathy. Mice overexpressing human $\beta_2$-adrenoceptors have only slightly altered cardiac function and appear to have normal life expectancy (259, 260, 443, 544) while mice overexpressing $\beta_1$-adrenoceptors develop severe hypertrophy and die of heart failure (162). The $\beta_1$- and $\beta_2$-adrenoceptors also differ with regard to their signal transduction. While $\beta_1$-adrenergic receptors are $G_s$ coupled, $\beta_2$-adrenoceptors are also able to couple to $G_i$-type G proteins (700, 701) (Fig. 4). The additional activation of $G_i$ via $\beta_2$-adrenergic receptors may explain the observed differences in signaling induced via $\beta_1$- and $\beta_2$-adrenergic receptors (116, 125, 232, 405, 725, 736). This led to the hypothesis that $\beta_2$-adrenoceptor stimulation exerts some sort of protection against cardiac hypertrophy and failure, especially under conditions of chronic activation of the $\beta$-adrenergic system and that this is due to signaling via $G_i$. The well-documented upregulation of $G_i$ in human heart failure (174, 482) may be a mechanism to counteract deleterious $G_s$-mediated signaling. The potential cardioprotective role of $G_i$ is also supported by studies in mice. While the overexpression of $\beta_2$-adrenergic receptors in normal cardiomyocytes is well tolerated, mice which lack in addition the major $G_i$-subunit, $G_{i2}$, die within a few days after birth (180). Mice that overexpress $\beta_2$-adrenoceptors in cardiomyocytes and which carry only one intact $G_{i2}$ gene allele develop more pronounced cardiac hypertrophy and earlier heart failure compared with $\beta_2$-adrenoceptor transgenic animals with normal $G_{i2}$ levels.

![Fig. 4. Role of heterotrimeric G proteins in mediating autonomic control of heart function by the sympathetic and parasympathetic system. $\beta_1/\beta_2$, $\beta_1$- and $\beta_2$-adrenergic receptor; $M_2$, muscarinic receptor; $I_f$, pacemaker channel; GIRK, G protein-regulated inward rectifier potassium channel; VDCC, voltage-dependent calcium channel; PKA, protein kinase A.](http://physrev.physiology.org/10.1152/physrev.00241.2004)
The muscarinic acetylcholine (M₂) receptor that is coupled to G\(\alpha_q/G\beta\gamma\) G proteins mediates the parasympathetic regulation of the heart (Fig. 4). The negative chronotropic and dromotropic effects of the parasympathetic system are believed to result from the G₁₃-mediated inhibition of adenyl cyclase, resulting in an inhibition of the cAMP production as well as by the activation of G protein-regulated inward rectifier potassium channels (GIRK) by \(\beta\gamma\)-subunits released from activated G\(\alpha/G\beta\gamma\) (601). The atrial GIRK consists of Kir3.1 and Kir3.4 subunits. Mice lacking either of the two channel subunits have normal basal heart rates but show reduced vagal and adenosine-mediated slowing of heart rate and markedly reduced heart rate variability, which is thought to be determined by the vagal tone (47, 680). The involvement of G\(\beta\gamma\) complexes in regulation of GIRK channels has been well established using electrophysiological and biochemical approaches (349, 398, 679). Mice in which the amount of functional G\(\beta\gamma\) protein was reduced by more than 50% in cardiomyocytes also show an impaired parasympathetic heart rate control (207). The central role of G\(\alpha_q\) in inhibitory regulation of heart rate and atrioventricular conduction has led to attempts to treat cardiac arrhythmias by atrioventricular nodal gene transfer of G\(\alpha_{q_{12}}\) in a model of persistent atrial fibrillation in swine (146). While wild-type G\(\alpha_{q_{12}}\) did not change basal heart rate, a constitutively active mutant of G\(\alpha_{q_{12}}\) resulted in a significant decrease in heart rate. When tested for their effects in a model for tachycardia-induced cardiomyopathy, the condition was significantly improved by wild-type G\(\alpha_{q_{12}}\) and even more by constitutively active G\(\alpha_{q_{12}}\) (37). In addition to the stimulatory regulation of potassium channels, muscarinic regulation of heart function also involves inhibition of voltage-dependent L-type Ca\(^{2+}\) channels via an unknown mechanism. In mice lacking the \(\alpha\)-subunit of G\(\beta\gamma\), inhibitory muscarinic regulation of cardiac L-type Ca\(^{2+}\) channels was abrogated, although G\(\alpha_q\) represents only a minor fraction of all G proteins in the heart (639). Interestingly, mice which lack the \(\alpha\)-subunit of G\(\beta\gamma\) (G\(\alpha_{q_{12}}\)) also show a severely affected inhibitory regulation of L-type Ca\(^{2+}\) channels via muscarinic M₂ receptors (101, 468). This suggests that both G proteins, G\(\alpha_q\) and G\(\alpha_{q_{12}}\), are involved in the regulation of cardiac L-type Ca\(^{2+}\) channels.

B. Myocardial Hypertrophy

Myocardial hypertrophy is the chronic adaptive response of the heart to injury or increased hemodynamic load. It is characterized by increased cardiomyocyte size and protein content, as well as altered gene expression, recapitulating an embryonic phenotype (109, 301). Such pathological myocardial hypertrophy was shown to be associated with increased cardiac mortality (191, 285, 535), raising the question whether prevention of pathological hypertrophy is beneficial or not (191). Several mechanosensitive mechanisms involving stretch-activated ion channels, integrins or Z-disc proteins were suggested to mediate myocardial hypertrophy in response to pressure overload (191, 285, 535). In addition, GPCR agonists like norepinephrine/phenylephrine, angiotensin II, or endothelin-1 were shown to induce a hypertrophic phenotype in cultured rat embryonic cardiomyocytes (4, 341, 560, 581). These ligands are known to activate G\(q/G_{11}\)-coupled receptors, such as the \(\alpha_1\)-adrenergic receptor, the angiotensin AT₁ receptor, or the endothelin ET\(_A\) receptor (362, 561, 592). Activation of G\(\alpha_q\) by Pasteurella multocida toxin (559) or expression of wild-type G\(\alpha_q\) (5, 362) induces the hypertrophic phenotype in cultured cardiomyocytes, while inhibition of G\(q/G_{11}\) by the RGS domain of GIRQ2 inhibited agonist-induced hypertrophy (423). In vivo, cardiac-restricted expression of wild-type (128) or constitutively active G\(\alpha_q\) (437) results in cardiac hypertrophy. In addition, in vivo overexpression of typically G\(q/G_{11}\)-coupled receptors (444, 474) or their downstream effectors (65, 454, 656) induces hypertrophy. Conversely, in vivo inhibition of G\(q/G_{11}\) by overexpression of RGS4, a GTPase-activating G protein for G\(q/G_{11}\) and G\(\alpha/G\beta\gamma\) (547), or by overexpression of the COOH terminus of G\(\alpha_q\) (10) results in a reduced hypertrophic response, and cardiomyocyte-specific inactivation of the genes encoding G\(\alpha_q/G_{11}\) completely abrogates the hypertrophic response elicited by pressure overload (677). Interestingly, an impaired hypertrophic response due to inhibition of G\(q/G_{11}\)-mediated signaling does not negatively influence long-term cardiac function (166), suggesting that hypertrophy in response to pressure overload is not necessarily required to maintain cardiac function. In addition, pressure overload-induced myocardial hypertrophy, the G\(q/G_{11}\)-mediated signaling pathway was also implicated in the pathogenesis of diabetic cardiomyopathy. G\(\alpha_q\) levels and PKC activity were shown to be enhanced in the streptozotocin-induced diabetic rat heart (714), and heart specific overexpression of RGS4 protected mice against different models of diabetic cardiomyopathy. In contrast, heart-specific expression of a RGS-resistant G\(\alpha_q\) caused sensitization towards diabetic cardiomyopathy (235). The downstream signaling processes in G\(q/G_{11}\)-mediated hypertrophy are complex and not fully understood (Fig. 5). Intracellular Ca\(^{2+}\) mobilization in response to activation of G\(q/G_{11}\)-coupled receptors promotes Ca\(^{2+}/\text{calmodulin} (\text{CaM})\)-dependent activation of calcineurin, which in turn mediates dephosphorylation and nuclear translocation of transcription factors of the NFAT (nuclear factor of activated T cells) family. Although activation of the calcineurin/NFAT signaling pathway is clearly sufficient to induce myocardial hypertrophy, it is not completely clear whether inhibition of this signaling pathway prevents hypertrophy (for review, see Refs. 190, 191). In addition, a variety of other effectors have been implicated in myo-
cardiac hypertrophy, such as protein kinase C (PKC) isoforms, mitogen-activated protein (MAP) kinases, the phosphatidylinositol (PI) 3-kinase/Akt/GSK-3 pathway or small GTPases (for review, see Refs. 148, 191, 285, 535).

GPCRs known to mediate myocardial hypertrophy can also activate \( G_{12}/G_{13} \) family G proteins, resulting in the activation of downstream effectors including the calcineurin/NFAT pathway, PKC isoforms, MAP kinases, the PI-3-kinase/Akt/GSK-3 pathway or small GTPases. CaM, calmodulin; DAG, diacylglycerol; IP\(_3\), inositol 1,4,5-trisphosphate; MAPK, mitogen-activated protein kinases; NFAT, nuclear factor of activated T cells; PI-3-K, phosphoinositide-3-kinase; PIP\(_2\), phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC-\( \beta \), phospholipase C-\( \beta \).

C. Smooth Muscle Tone

Smooth muscle tone is controlled by the phosphorylation state of the regulatory light chain (MLC\(_{20}\)) of myosin II (for review, see Refs. 269, 593, 594). MLC\(_{20}\) is phosphorylated by the Ca\(^{2+}\)/CaM-dependent myosin light chain kinase (MLCK), leading to enhanced velocity and force of actomyosin cross-bridging. Dephosphorylation of MLC\(_{20}\) is mediated by myosin phosphatase, an enzyme that is negatively regulated by the Rho/Rho-kinase pathway. Thus increased contractility can be achieved through Ca\(^{2+}\)-mediated MLCK activation and through Rho-dependent inhibition of MLC\(_{20}\) dephosphorylation. A variety of transmitters and hormones regulate smooth muscle tone through GPCRs (Fig. 6). Typical vasoconstrictor receptors, such as the angiotensin AT\(_1\) receptor, the endothelin ET\(_A\) receptor, or the \( \alpha_1 \)-adrenergic receptor, act on \( G_q/G_{11} \)-coupled receptors (159, 215, 724) to enhance intracellular Ca\(^{2+}\) concentration, leading to MLCK activation. Increased intracellular Ca\(^{2+}\) levels are not only due to IP\(_3\)-mediated Ca\(^{2+}\) release from the sarcoplasmic reticulum, but also to Ca\(^{2+}\) influx through calcium channels or voltage-gated Ca\(^{2+}\) channels (for review, see Refs. 269, 593). In addition, many \( G_q/G_{11} \)-coupled receptors have been shown to activate RhoA, thereby contributing to Ca\(^{2+}\)-independent smooth muscle contraction (593, 594). Smooth muscle specific overexpression of a COOH-terminal \( G_q \) peptide, which is believed to inhibit the receptor/G protein interaction, ameliorates hypertension induced by long-term treatment with phenylephrine, serotonin, or angiotensin II (331). Mice lacking RGS2, a GTPase activating G protein which accelerates the inactivation of \( G_q/G_{11} \), suffer from hypertension (261). Interestingly, it was recently shown that the nitric oxide/cGMP cascade, which constitutes the main relaxant pathway in smooth muscle cells, negatively regulates \( G_q/G_{11} \) signaling by cGMP kinase-mediated phosphorylation and activation of RGS2 (625). However, in addition to this peripheral vascular mechanism, an increased sympathetic tone might contribute to elevated arterial blood pressure in RGS2-deficient mice (227).

In vitro, most \( G_q/G_{11} \)-coupled vasoconstrictor receptors also activate \( G_{12}/G_{13} \) family G proteins, like the receptors for endothelin-1, vasopressin, angiotensin II (215, 257), thrombin (411), or thromboxane A\(_2\) (491, 492). Constitutively active forms of \( G_{12} \) and \( G_{13} \) induced a pronounced, RhoA-dependent contraction in cultured vascular smooth muscle cells, and receptor-mediated contractions were strongly inhibited by dominant negative forms of \( G_{12} \) and \( G_{13} \) (215). These data suggest that also the associated embryonic gene program (179). However, no in vivo data on the role of \( G_{12}/G_{13} \) in myocardial hypertrophy are available.
G₁₂/G₁₃-mediated signaling pathway is involved in the regulation of smooth muscle tone, most likely by modulating the activity of myosin phosphatase via Rho/Rho-kinase. In accordance with this, inhibition of Rho-kinase was shown to normalize blood pressure in humans and experimental animals (426, 636). The relative contribution of Gq/11/Ca²⁺/H₁₁₀₀₁⁻mediated and G₁₂/G₁₃/Rho/Rho-kinase-mediated signaling to regulation of vascular smooth muscle tone is not clear. However, data obtained in visceral smooth muscle suggested that Gq/G₁₁ conveys a fast, transient response, while G₁₂/G₁₃ mediates a sustained, tonic contraction (257).

Vascular smooth muscle relaxation is mediated by a variety of mechanisms, one of them being the activation of Gₛ-coupled receptors like the adenosine A₂ receptors, β₂-adrenergic receptors, or prostaglandin receptor subtypes IP, DP, and EP₂. How the subsequent increase in cAMP levels reduces smooth muscle tone is not understood. In vitro data suggest that the relaxant effect is partially due to a PKA-mediated MLCK phosphorylation, which decreases the enzyme's affinity for the Ca²⁺/CaM complex, but the physiological relevance of this signaling pathway is unclear. Possible other substrates for PKA are heat shock protein 20, RhoA, or myosin phosphatase (for review, see Ref. 269). cAMP has also been suggested to cross-activate cGMP kinase I in vascular or airway smooth muscle (32, 390), but this hypothesis has been questioned by the finding that vessels from cGKI-deficient mice relax normally in response to cAMP (518). In addition, cAMP-independent mechanisms of Gₛ-mediated relaxation involving large-conductance, Ca²⁺-activated K⁺ (MaxiK, BK) channels have been proposed (624).

The role of Gₛ-mediated signaling in vascular smooth muscle tone seems to differ between different vessel types. An inhibitory effect of PTX on norepinephrine-induced contractility was reported in rat tail artery (516, 600) but was absent in aorta (517). High blood pressure in spontaneously hypertensive rats is preceded by increased expression of Gi proteins (18, 19), and PTX treatment delayed the onset of hypertension (387), suggesting that decreased cAMP levels play a role in the pathogenesis of this model of hypertension. Enhanced signaling via a PTX-sensitive G protein was reported in immortalized B lymphoblasts from patients with essential hypertension (520), and this was attributed to a C825T polymorphism in the gene coding for Gβ₃, a constituent of the Gi heterotrimer (586). The C825T polymorphism was suggested to be associated with an increased risk of hypertension, obesity, and arteriosclerosis in some (for review, see Ref. 585) but not in all studies (283, 616, 617). However, the significance of genetic association studies in general remains controversial (20, 199, 291).

Very similar to vascular smooth muscle, also airway smooth muscle tone is mainly regulated by Gₛ/G₁₁ family...
G proteins, which mediate bronchoconstriction, and Ga family G proteins, which mediate bronchodilatation. Acetylcholine released from postganglionic parasympathetic nerves controls resting tone mainly via the Gq/G11-coupled M3 receptor subtype (90), but also other Gq/G11-coupled receptors are expressed in airway smooth muscles, like the H1 histamine receptor (133, 222), the leukotriene CysLT1 receptor (314), the B2 bradykinin receptor (421, 630), the ETB endothelin receptor (216, 241, 441), and others. Airway hyperreactivity in the A/J mouse strain was suggested to be due to enhanced agonist affinity and increased G protein coupling efficiency of the M3 muscarinic receptor (205), and Gq protein was shown to be upregulated in antigen-induced airway hyperresponsive rats (106). Mice lacking the α-subunit of Ga showed impaired metacholine-induced airway responses and lacked the typical increase in metacholine sensitivity after allergen sensitization and reexposure (55). Not much is known about the role of Gα12 and Gα13 in airway smooth muscle tone regulation. The fact that repetitive antigen challenge significantly increases the expression of these proteins in airway smooth muscle suggests a role in allergic asthma (105, 108), but direct evidence for an involvement of Gα12/Gα13 is still lacking. Ga-coupled receptors play an important role in the relaxation of contracted airway smooth muscle most prominently the β2-adrenergic receptor, but also the prostaglandin E2 receptor EP2 (512) or the prostacyclin IP receptor (41) (for review, see Ref. 628). The Gq family of G proteins contributes to the regulation of airway smooth muscle contractility mainly by inhibiting the relaxant effects of Ga. The inhibitory effect of PTX on acetylcholine-induced bronchoconstriction is negligible in normal rats, but significant in rats suffering from antigen-induced airway hyperresponsiveness (107). In these mice, Gαq protein is upregulated in bronchial smooth muscle cells, suggesting that the relative contribution of Gq-mediated constriction is increased in antigen-challenged airway smooth muscle (107).

D. Platelet Activation

Platelets are small cell fragments that circulate in the blood and adhere at places of vascular injury to the vessel wall where they become activated resulting in the formation of a platelet plug that is responsible for primary hemostasis. Platelets can also become activated under pathological conditions, e.g., on ruptured atherosclerotic plaques leading to arterial thrombosis. Platelet adhesion and activation is initiated by their interaction with adhesive macromolecules like collagen and von Willebrand factor (vWF) at the subendothelial surface (303, 554). While collagen is able to induce firm adhesion of platelets to the subendothelium (666), the recruitment of additional platelets to the growing platelet plaque requires the local accumulation of diffusible mediators that are produced or released once platelet adhesion has been initiated, and some level of activation through platelet adhesion receptors has occurred (3). These mediators include ADP/ATP and thromboxane A2 (TXA2), which are secreted or released from activated platelets as well as thrombin, which is produced on the surface of activated platelets. These platelet stimuli have in common their action through G protein-coupled receptors. While ADP induces the activation of Gq and G13 via P2Y1 and P2Y12 receptors (197, 354), the activated TXA2 receptor (TP) couples to Gq and G12/G13 (337, 492) (Fig. 7). G protein-coupled protease-activated receptors (PARs) that are activated by thrombin are functionally coupled to Gq, G12/G13, and in some cases to Gq (121). In response to these secondary mediators of platelet activation, platelets immediately undergo a shape change reaction during which they become spherical and extrude pseudopodia-like structures.

FIG. 7. Role of heterotrimeric G proteins in mediating platelet activation by soluble mediators like ADP, thromboxane A2 (TXA2), thrombin, and epinephrine. Major roles are played by the G proteins Gq, G12, and Gq which couple receptors to the indicated effector molecules. The subsequent signaling processes eventually lead to platelet responses like shape change, degranulation, and aggregation (for details, see text). TP, TXA2 receptor; PAR, protease-activated receptor; P2Y1/P2Y12, purinergic receptors; α2A, α2A-adrenergic receptor; RhoGEF, Rho guanine nucleotide exchange factor; PLC-β2/3, phospholipase C-β2/3; PI3-K, phosphoinositide-3-kinase; PI3P, phosphatidylinositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; PI4P, phosphatidylinositol 3,4,5-trisphosphate.
structures. In addition, the glycoprotein IIb/IIIa (integrin αIIbβ3) undergoes a conformational change resulting in binding of fibrinogen/vWF and subsequent platelet aggregation. Finally, the formation and release of TxA2, thrombin, and ADP is further stimulated. Thus secondary mediators increase through G protein-coupled receptors their own formation resulting in an amplification of their effects, and eventually all G protein-mediated signaling pathways induced via these receptors become activated. The multiple positive feedback mechanisms operating during platelet activation have obscured the exact analysis of the roles individual G protein-mediated signaling pathways play during the platelet activation process. Progress has recently been made using genetic mouse models in understanding the role of individual G protein-mediated signaling pathways during platelet activation.

The requirement of Gq-mediated signaling for agonist-induced platelet activation has been demonstrated by the phenotype of Goq-deficient platelets, which fail to aggregate and to secrete in response to thrombin, ADP, and TxA2 due to a lack of agonist-induced phospholipase C activation. This dramatic phenotype found in Goq-deficient platelets is due to the fact that platelets lack Gα11 (313), which is in most other cells coexpressed with Goq and can compensate Gq deficiency. Mice lacking Goq have increased bleeding times and are protected against collagen/epinephrine-induced thromboembolism (494). Although Gq-mediated signaling appears to be absolutely required for platelet activation, there is clear evidence that also Gq type G proteins need to be activated to induce full activation of integrin αIIbβ3. In mice lacking the α-subunit of Gq2, the response of ADP that acts through the Gq-coupled P2Y12 Receptor is reduced (304). However, also the effects of mediators like thrombin and TxA2, which primarily signal through Gq and G12/G13 were found to be inhibited in platelets lacking Goq2 (304, 712). This supports the view that platelet activation by thrombin and thromboxane A2 requires in part the action of secondary mediators like ADP, which are released after activation of Gq-mediated signaling pathways through TxA2 and thrombin receptors. An important role of the Gq-mediated signaling pathway in platelet activation is also suggested by studies in platelets lacking the Gq-coupled P2Y1 receptor or after pharmacological blockade of P2Y1 (170, 251, 310, 379, 568). These platelets do not aggregate in response to low and intermediate concentrations of ADP unless Gq-mediated signaling is induced via activation of another receptor. Similarly, platelets lacking P2Y12 or in which P2Y12 was pharmacologically blocked did not aggregate in response to ADP unless the Gq-mediated pathway was activated via a different receptor (181, 568). Thus there is clear evidence that Gq and Gq synergize to induce platelet activation. It is currently not clear how Gq contributes to integrin αIIbβ3 activation in platelets, but a decrease in cAMP levels is unlikely to be involved (129, 529, 569, 712).

Another member of the Gq family of heterotrimeric G proteins, Gz, has been implicated in platelet activation induced by epinephrine acting on α2-adrenergic receptors. In contrast to ADP, TxA2, and thrombin, epinephrine is alone not able to fully activate mouse platelets. However, it is able to potentiate the effect of other platelet stimuli. In Gαz-deficient platelets, the inhibitory effect of epinephrine on adenylyl cyclase and epinephrine-potentiating effects were strongly impaired while the effects of other platelet activators appear to be unaffected (713).

Despite the central role of Gq in platelet activation, it was recently demonstrated that induction of Gq- and G12/G13-mediated signaling pathways is sufficient to induce integrin αIIbβ3 activation (149, 483). Interestingly, in Gα13-deficient platelets, but not in Gαq-deficient platelets, the potency of various stimuli including TxA2, thrombin, and collagen to induce platelet shape change and aggregation is markedly reduced (455). These defects are accompanied by a defect in the activation of RhoA and a delayed phosphorylation of the myosin light chain as well as by an inability to form stable platelet thrombi under high shear stress conditions (455). In addition, mice carrying platelets that lack Gα13 have an increased bleeding time and are protected against the formation of arterial thrombi induced in a carotid artery thrombosis model (455). These data indicate that in addition to Gq and Gi also G13 is crucially involved in the signaling processes mediating platelet activation via G protein-coupled receptors both in hemostasis and thrombosis. These findings also indicated that G13-mediated signaling is not only involved in the response of platelets to relatively low stimulus concentrations that induce platelet shape change but is also required for normal responsiveness of platelets at higher stimulus concentrations. A reduced potency of platelet activators in the absence of G13-mediated signaling becomes in particular limiting under high flow conditions that lead to a rapid clearance of soluble stimuli from the site of platelet activation and formation of mediators. In addition, the defective activation of RhoA-mediated signaling in the absence of G13 appears to contribute to the observed defect in the stabilization of platelet aggregates under high shear stress ex vivo as well as in vivo. In fact, RhoA-mediated signaling has been suggested to be required for platelet aggregation under high shear conditions as well as for the irreversible aggregation of platelets in suspension (450, 570).

These studies have clearly shown that three G proteins are major mediators of platelet activation via G protein-coupled receptors: Gq, G12, and G13. However, even in the absence of either Gαq, G12, or G13 some platelet activation can still be induced, while in the absence of both Gαq and Gα13, platelets are unresponsive to thrombin, TxA2, or ADP. This indicates that the activation of Gq-mediated signaling alone is not sufficient to induce any platelet activation (456). The optimal activation of plate-
III. ENDOCRINE SYSTEM AND METABOLISM

The endocrine system consists of a variety of glands and other structures that produce, store, and secrete hormones directly into the systemic circulation, thereby controlling electrolyte and water homeostasis, metabolism, growth, reproduction, etc. GPCRs contribute to endocrine functions in a twofold way: 1) by mediating hormonal end organ effects and 2) by controlling hormone secretion itself. Hormone secretion, as well as secretion from neuronal or exocrine cells, typically involves elevation of cytosolic Ca\(^{2+}\) and/or cAMP (for review, see Refs. 160, 738). In most secretory cells, Ca\(^{2+}\) influx through voltage-operated Ca\(^{2+}\) channels is the dominant mode of regulation, like in adrenal chromaffin cells (160), while in other cells, such as anterior pituitary gonadotropes, Ca\(^{2+}\) mobilization from internal stores is the critical step (633). In yet another endocrine cell, such as lactotrophs, both increased intracellular Ca\(^{2+}\) levels and cAMP production contribute to secretion (130, 186, 620). Accordingly, with rare exceptions, activation of G\(_s\) and/or G\(_{q}/G_{11}\) family G proteins enhances secretion regardless of the endocrine cell type involved.

A. Hypothalamo-Pituitary System

Hormone release from the anterior pituitary is tightly controlled by hypothalamic releasing hormones and release inhibiting factors, all of which act through GPCRs. The receptors for corticotropin-releasing hormone (263) and growth hormone-releasing hormone (GHRH) (588) primarily act through G\(_s\), while receptors for gonadotropin-releasing hormone (445), thyrotropin-releasing hormone (211, 720), and the many prolactin-releasing factors (186) mainly act through G\(_q/G_{11}\) family G proteins, and only partly through G\(_s\). In addition to their secretagogue effects, hypothalamic releasing hormones regulate hormone synthesis and cell proliferation (81, 430, 531, 583, 587, 647). Anterior pituitary secretion and proliferation is not only stimulated by the classical hypothalamic releasing hormones, but also by a variety of other factors, such as the gastrointestinal peptide hormone ghrelin, which enhances growth hormone (GH) secretion via the predominantly G\(_q/G_{11}\)-coupled growth hormone secretagogue receptor GHS-R (343, 588), or members of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily, which exert secretagogue effects on a variety of pituitary cell types via their G\(_s\)-coupled receptors (579). The in vivo relevance of G\(_s\) family G proteins in anterior pituitary function was studied in mice and in patients with inactivating or activating G\(_s\) mutants. Somatotroph-specific overexpression of cholera toxin, which irreversibly activates G\(_s\) by ADP ribosylation, caused somatotroph hyperplasia, increased GH levels and gigantism in mice (82). In humans, activating mutations of GNAS can be found in \(-40\%\) of GH-producing pituitary tumors (363, 406), as well as in \(-10\%\) of nonfunctioning pituitary adenomas (406, 632, 685). These activating mutations of GNAS encode substitutions of either Arg-201 or Gln-227, two residues that are critical for the GTPase reaction (187, 223, 363, 406). Such activating somatic GNAS mutations are not necessarily restricted to the pituitary, but are often part of the McCune-Albright syndrome, which is defined by the trias fibrous dysplasia of bone, café-au-lait skin pigmentation, and endocrine hyperfunctions of variable degree (for review, see Refs. 599, 669). Endocrine hyperfunction is due to constitutive activation of G\(_s\) signaling in other endocrine glands, leading to adrenal hyperplasia with Cushing syndrome (60, 182), precocious puberty (138, 574), or hyperthyroidism (see sect. mC). In melanocytes, increased G\(_s\) activity mimics the activity of melanocyte stimulating hormone, leading to typical café-au-lait hyperpigmentation (334).

Heterozygous inactivating GNAS mutations result in Albright hereditary osteodystrophy (AHO), a congenital disorder characterized by obesity, short stature, brachydactyly, subcutaneous ossifications, and neurobehavioral deficits of variable severity (for review, see Refs. 13, 366, 599, 669). In addition to these defects, patients with maternally inherited mutations show multihormone resistance (termed pseudohypoparathyroidism type Ia, PHP1a) in tissues with a paternally imprinted GNAS allele, such as proximal tubules of the kidney, thyroid, or ovaries (209, 242, 414, 723). In these tissues, the effects of G\(_s\)-coupled hormone receptors, like those for parathyroid hormone, thyroid stimulating hormones, or the gonadotropins, are impaired. Clinically, this results in variable degrees of hypocalcemia and hyperphosphatemia, hypothyroidism (see also sect. mC), and delayed or incomplete sexual development and reproductive dysfunction in women (13, 366, 599, 669). These abnormalities of the reproductive system are easily explained by malfunction of receptors for follicle-stimulating hormone and luteinizing hormone. In addition, at least in mice, the G\(_s\)-coupled orphan receptor GPR3 is crucially involved in the maintenance of meiotic arrest in oocytes (432, 433).

The phenotype of humans heterozygous for an inactivating GNAS mutation is partly reproduced in mice carrying a targeted disruption of Gnas exon 2. In these
animals, PTH resistance was only found if the mutation was maternally inherited, and only these animals showed reduced Gαq expression in the renal cortex (723). In humans, renal PTH resistance without Albright hereditary osteodystrophy (PHP Ib) can also be due to other GNAS mutations, such as a mutant which results in a biallelic paternal imprinting phenotype (395), or a mutant unable to interact with the PTH receptor (697). Yet another GNAS mutation causes impaired signaling via the PTH and TSH receptors, but enhanced signaling via the likewise Gαq-coupled receptor for luteinizing hormone, leading to enhanced testosterone production. This paradoxical combination of gain and loss of function is explained by the fact that the underlying GNAS mutation results in a constitutively active form of Gαq which, however, is temperature sensitive. The mutant is stable only at the relatively low temperature in the testis, but rapidly degraded at 37°C, leading to Gαq deficiency (287). With respect to pituitary function, patients with inactivating GNAS mutations show variable degrees of GHRH resistance (415), GH deficiency (210), or hypoprolactinemia (88). In accordance with the important role of Gαq family G proteins in lactotrophs and somatotrophs, hypothalamic inhibiting hormones, like dopamine or somatostatin, act through Gαq-coupled receptors (311, 536).

Releasing hormone secretion itself is influenced by GPCRs, and several former orphan receptors were recently shown to positively regulate releasing hormone secretion. Kisspeptins for example, a family of peptides derived from the metastasis suppressor gene Kiss-1, were shown to enhance hypothalamic gonadotropin-releasing hormone secretion via the GPR54 receptor (137, 220, 472), and genetic inactivation of GPR54 in mice or mutation in humans causes hypogonadotropic hypogonadism (137, 196, 220, 575). The peptide hormone ghrelin induces pituitary growth hormone release not only directly via activation of GHS-R on somatotroph cells, but also acts as a releasing factor for hypothalamic GHRH (343). Both GPR54 and GHS-R are known to activate Gq/11 family G proteins (343, 345), suggesting that releasing hormone release is controlled by the same mechanisms as pituitary hormone release. In line with this notion, mice lacking both Gαq alleles and one Gα11 allele selectively in the nervous system show severe somatotroph hypoplasia with dwarfism due to reduced hypothalamic GHRH production, which is probably secondary to impaired GHS-R signaling (676).

### B. Pancreatic β-Cells

The tight regulation of blood glucose levels is mainly achieved by the on-demand release of insulin from pancreatic β-cells. High glucose levels result in enhanced intracellular glucose metabolism with ATP accumulation and consecutive closure of ATP-sensitive K+ channels, leading to the opening of voltage-operated Ca2+ channels and Ca2+-mediated insulin exocytosis (27, 119). In addition to the ATP-dependent mechanism of insulin release, several GPCRs have been shown to either amplify or to inhibit glucose-induced insulin release (for review, see Refs. 161, 364, 558), and these receptors and their respective ligands play an important role in the regulation of islet function by, e.g., the autonomous system (for review, see Ref. 7). Neuropeptides and hormones that potentiate insulin secretion mainly act though Gq-coupled receptors, like glucose-dependent insulinotropic polypeptide, secretin, cholecystokinin, PACAP, glucagon, vasoactive intestinal polypeptide, or glucagon-like peptide-1 (GLP-1) (for review, see Refs. 161, 418, 542). The potentiating effect of Gq on glucose-induced insulin release (576, 608, 609) might either be mediated by phosphorylation of voltage-operated Ca2+ channels (295) or through the opening of nonselective cation channels (275). Transgenic expression of a constitutively active Gαq mutant in mouse β-cells caused increased islet cAMP production and insulin secretion, but these changes were only detectable in the presence of phosphodiesterase inhibitors, suggesting that increased Gαq activity is normally compensated by up-regulation of cAMP degrading enzymes like phosphodiesterases (408). Conversely, activation of receptors coupled to Gi or Gr, like the α2-adrenergic receptor or receptors for somatostatin, neuropeptide Y, prostaglandin E2, or galanin, inhibits insulin secretion in a PTX-sensitive manner (319, 328, 365, 514, 542).

Not only Gα family members, but also Gq/G11 family G proteins, can mediate potentiation of glucose-induced insulin release. Acetylcholine released from postganglionic parasympathetic nerves or muscarinic agonists act through the Gq/G11-coupled M3 receptor (58, 157) to enhance insulin release during the cephalic phase of insulin secretion (8, 447, 691). This effect was shown to depend on PLC activation and consecutive inositol 1,4,5-trisphosphate (IP3)-mediated intracellular Ca2+ elevation (46, 469, 726) and PKC activation (22). The exact pathways leading to increased insulin secretion are not clear, but activation of L-type Ca2+ channels (58), modulation of ATP-sensitive K+ channels (469), activation of CaM-kinase II (427, 537), or enhanced plasma membrane Na+ permeability (254) have been suggested. In addition to acetylcholine, a variety of other local mediators act through Gq/G11-coupled receptors to enhance insulin release, like cholecystokinin via the CCK1 receptor (652), bombesin via the BB2 receptor (521, 646), arginine vasopressin via the V1b receptor (415), GH deficiency (210), or hypoprolactinemia (319, 447, 691). Fatty acids such as palmitate potentiate insulin secretion mainly act through Gαq-coupled receptors, like glucose-dependent insulinotropic polypeptide, secretin, cholecystokinin, PACAP, glucagon, vasoactive intestinal polypeptide, or glucagon-like peptide-1 (GLP-1) (for review, see Refs. 161, 418, 542). The potentiating effect of Gq on glucose-induced insulin release (576, 608, 609) might either be mediated by phosphorylation of voltage-operated Ca2+ channels (295) or through the opening of nonselective cation channels (275). Transgenic expression of a constitutively active Gαq mutant in mouse β-cells caused increased islet cAMP production and insulin secretion, but these changes were only detectable in the presence of phosphodiesterase inhibitors, suggesting that increased Gαq activity is normally compensated by up-regulation of cAMP degrading enzymes like phosphodiesterases (408). Conversely, activation of receptors coupled to Gi or Gr, like the α2-adrenergic receptor or receptors for somatostatin, neuropeptide Y, prostaglandin E2, or galanin, inhibits insulin secretion in a PTX-sensitive manner (319, 328, 365, 514, 542).

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to mediate these effects. The former orphan GPCR GPR40 was shown to mediate the effects of saturated and unsaturated fatty acids (C>6) on intracellular Ca\(^{2+}\) mobilization in pancreatic β-cells (70, 296, 346). These effects were not PTX sensitive, suggesting that G\(_q/G_{11}\)-mediated intracellular Ca\(^{2+}\) mobilization was involved (70, 296). Another recently deorphanized GPCR probably coupled to G\(_q/G_{11}\) is GPR120, which was suggested to be involved in fatty acid-induced release of GLP-1 from intestinal cells, thereby contributing to GLP-1-mediated insulin release (265).

C. Thyroid Gland/Parathyroid Gland

Thyroid stimulating hormone (TSH) regulates thyroid cell proliferation as well as thyroid hormone synthesis and release through the G protein-coupled TSH receptor (508). In vitro, the TSH receptor was shown to couple to all four G protein families (15, 16, 368), but the major signal transduction pathway in vivo seems to be the G\(_s/cAMP\) cascade, which was shown to activate iodide organification, thyroid hormone production, secretion, and thyroid cell mitogenesis (153–155, 546). In TSH receptor-deficient mice, direct stimulation of adenylyl cyclase restores the ability to concentrate and organify iodide, suggesting that expression of the sodium-iodide symporter is controlled by G\(_s\) (417). In vitro, overexpression of constitutive active G\(_s\) in a thyroid cell line (462) or activation of G\(_s\) by transgenic expression of cholera toxin in the mouse thyroid (727) caused hyperplasia and increased hormone secretion. In line with this, spontaneous activating mutations of GNAS can cause thyroid cell hyperfunction in humans, leading to hyperthyroidism, goiter, and benign adenoma (175, 406, 424, 502). Malignant transformation of thyroid cells has also been observed (165, 611, 711), but seems to require additional mutational or epigenetic events (115). Much more frequent than activating GNAS mutations are the activating mutations of the TSH receptor itself (508), which mainly lead to constitutive activation of the G\(_s/cAMP\) cascade, or, in some cases, to activation of both G\(_s\) and G\(_q/G_{11}\)-coupled pathways (48, 643). Since the paternal GNAS allele is partly imprinted in thyroid cells (209, 394), inactivating GNAS mutants inherited from the maternal side cause TSH resistance with mild TSH resistance can also be observed in patients with PHP1B due to a GNAS mutation with paternal specific epigenotype of both exon 1A regions (34, 394, 395). In addition to adenylyl cyclase activation, TSH stimulates PLC activity (344, 369, 644), but the TSH concentrations needed for PLC stimulation are 100-fold higher than those needed for adenylyl cyclase stimulation (643).

The parathyroid gland controls Ca\(^{2+}\) homeostasis through parathyroid hormone (PHT), which enhances Ca\(^{2+}\) (re)absorption in gut and kidney, as well as Ca\(^{2+}\) release from bone. High extracellular Ca\(^{2+}\) concentrations activate the G protein-coupled extracellular Ca\(^{2+}\) sensing receptor (CaR), leading to inhibition of PTH production and secretion in parathyroid cells (for review, see Refs. 74, 268, 661). Activation of the CaR causes a PTX-insensitive (240) stimulation of PLC-β isoforms, with consecutive increments in inositol phosphates, DAG, and intracellular Ca\(^{2+}\) levels (73, 75, 478). In addition, an activation of phospholipases A\(_2\) and D (333) as well as a PTX-sensitive suppression of cAMP formation was observed (98). These studies suggested that the CaR couples both to G\(_q/G_{11}\) and G\(_i/G_{α}\) family G proteins, and this notion was supported by the finding that CaR activation induced incorporation of radiolabeled GTP into G\(_α_q\) and G\(_α_i\) in Madin-Darby kidney (MDCK) cells, which endogenously express low levels of the CaR (25). The fact that increased intracellular Ca\(^{2+}\) levels result in decreased, not increased, hormone release is quite exceptional, and parathyroid cells are, besides renin-secreting juxtaglomerular cells (572), the only endocrine cells showing such inverse coupling. However, the molecular mechanism underlying Ca\(^{2+}\)-mediated inhibition of PTH secretion is not understood (for review, see Refs. 74, 86, 268, 661).

D. Regulation of Carbohydrate and Lipid Metabolism

Normal blood glucose levels are maintained both by regulating the activity of enzymes involved in carbohydrate metabolism and by controlling glucose uptake into peripheral tissues. Insulin is a major regulator of both processes, but also a variety of GPCR agonists, like catecholamines and glucagon, contribute to glucose homeostasis. In hepatocytes, activation of G\(_s\)-coupled receptors like the β\(_2\)-adrenergic receptor or glucagon receptors causes PKA-mediated phosphorylation of key enzymes which regulate glycogen synthesis, glycogen breakdown, glycolysis, or gluconeogenesis (167, 168). Together, these changes lead to enhanced hepatic glucose release. Comparable changes can be induced by activation of G\(_q/G_{11}\)-coupled receptors like the α\(_1\)-adrenergic receptor or receptors for vasopressin and angiotensin, and these effects are probably mediated by Ca\(^{2+}\)/calmodulin-dependent alteration of enzymatic activity (167, 168). In the periphery, glucose uptake into skeletal muscle and adipocytes is mediated by translocation of glucose transporter subtype 4 (GLUT4) from intracellular vesicles to the plasma membrane (665). A variety of GPCRs have been demonstrated to modify insulin-induced GLUT4 translocation, and even a direct interaction between the insulin receptor and heterotrimeric G proteins was suggested (412). Heterozygous GNAS-deficient mice show, in addition to other metabolic abnormalities (for effects on lipolysis, see below), an
increased sensitivity towards insulin, which was attributed to enhanced insulin-dependent glucose uptake into the skeletal muscle (104, 721). In line with this, transgenic expression of a constitutively active mutant of Gαs (GαsQ227L) in fat, liver, and skeletal muscle decreased glucose tolerance (284), suggesting that Gαs-mediated signaling negatively regulates insulin-induced GLUT4 translocation. In contrast, G11 family G proteins seem to facilitate insulin effects. Pretreatment of isolated adipocytes and soleus muscle with PTX results in reduced insulin-stimulated glucose uptake (111, 325), and mice in which Gαs was downregulated in liver and adipose tissue using an antisense RNA approach show insulin resistance with hyperinsulinemia, decreased glucose tolerance, and insulin resistance (459–461). In the latter mice, both insulin-induced GLUT4 translocation to the plasma membrane and activation of glycogen synthase and antilipolytic mediators were impaired (461). The decrease in Gαs levels was accompanied by an increase in protein tyrosine phosphatase-1B (PTP-1B) activity, an enzyme known to dephosphorylate phosphorylated tyrosine residues on the insulin receptor and on insulin receptor substrate-1. This suggests that the inhibition of insulin signaling in mice with reduced Gαs levels is due to disinhibition of PTP-1B (461). Mice expressing a constitutively active mutant of Gαs (GαsQ205L) in fat, liver, and skeletal muscle displayed reduced fasting blood glucose levels and increased glucose tolerance (103). Adipocytes from these mice showed enhanced insulin-induced glucose uptake and GLUT4 translocation, as well as increased PI 3-kinase and Akt activities (596). In addition, PTP-1B is suppressed in Gαs overexpressing mice (626), and streptozotocin-induced diabetic changes are ameliorated (732). Up to now it is not clear at which levels the insulin receptor-mediated pathway interacts with the G11-mediated pathway. It was suggested that G11/Gαs family G proteins physically interact and are phosphorylated by the activated insulin receptor (for review, see Ref. 510). In addition, G11/Gαs might be involved in insulin-mediated autophosphorylation of the insulin receptor (351).

Data from 3T3 L1 adipocytes strongly point to an involvement of Gq/G11 family G proteins in basal and insulin-induced GLUT4 translocation. Overexpression of wild-type or constitutively active Gαq increased basal GLUT4 translocation (290, 326), while microinjection of Gαq/α11 antibodies or RGS2 protein inhibited insulin-induced GLUT4 translocation (290, 326). In line with these findings, inhibition of GRK2, a negative regulator of Gq/G11 signaling, increased insulin-stimulated GLUT4 translocation, while adenovirus-mediated overexpression of GRK2 reduced translocation as well as 2-deoxyglucose uptake (637). The exact mechanisms underlying Gq/G11-mediated GLUT4 translocation are not fully understood. Several Gq/G11-coupled receptors are able to stimulate glucose uptake via GLUT4 translocation, such as receptors for endothelin-1 (ET-1), norepinephrine, platelet-activating factor, or bradykinin (335, 336, 698). Microinjection of an anti-Gαq/α11 antibody or of RGS2 protein causes inhibition of ET-1-induced GLUT4 translocation (289). Of note, chronic ET-1 treatment inhibits insulin-stimulated glucose uptake and GLUT4 translocation in 3T3-L1 adipocytes, and decreased tyrosine phosphorylation of insulin receptor substrates was suggested to mediate this heterologous desensitization (292). The Gq/G11-mediated effect on GLUT4 translocation was shown to be PI 3-kinase dependent in some (289, 290) but not in all studies (59, 326). Recently, a role for the ADP ribosylation factor 6 and the Ca2+-activated tyrosine kinase Pyk2 was suggested (59, 371, 506).

Other examples for G protein-regulated metabolic processes are adipocyte lipolysis and lipogenesis. Activation of Gq-coupled receptors like those for catecholamines, ACTH, glucagonos, TSH, or PTH enhances lipolysis via PKA-dependent phosphorylation of hormone-sensitive lipase (274, 401, 606). In adipocytes from patients heterozygous for an inactivating GNAS mutation, the lipolytic effect in response to epinephrine is impaired, and this was suggested to contribute to obesity observed in AHO patients (87). Heterozygous disruption of Gnas exon 2 in mice causes not only enhanced insulin sensitivity (721), but also a variety of other metabolic defects that depend on the parental origin of the inherited mutation (722). While mice with a maternally inherited inactivating mutation of the Gnas gene are obese and hypometabolic, paternally inherited Gnas mutations cause abnormal leanness with decreased serum, liver, and muscle triglycerides, and lipid oxidation in adipocytes is enhanced (104, 722). Since these mice have increased urine norepinephrine excretion, it was suggested that increased sympathetic stimulation of adipocytes caused the changes in lipid metabolism (104, 722). The reason for the restriction to paternally inherited mutations is not completely clear. Deletion of Gnas exon 2 does not only affect the expression of Gαs, but also of alternative gene products like XLαs (Fig. 3) (245, 667). An involvement of XLαs in the hypermetabolic phenotype seems likely for three reasons. First, XLαs is only expressed from the paternal allele (104) and is therefore well suited to explain a paternally inherited phenotype. Second, XLαs-deficient mice show a phenotype similar to paternally exon 2-deficient mice (522). Third, mice with paternal inheritance of a Gnas exon 1 deletion, which does not affect XLαs expression, do not show the respective phenotype (668). Interestingly, studies in XLαs-deficient mice suggest that Gαs and XLαs can exert opposing effects on whole body metabolism (522, 668). In addition to Gαs family G proteins, also the Gq/G11 family was implicated in the regulation of lipolysis. Expression of antisense RNA to Gαq in liver and white fat caused hyperadiposity, which was suggested to
be attributed to an impaired lipolytic response towards α1-adrenergic agonists (198).

Inhibition of adipocyte lipolysis is mediated by the insulin receptor or by activation of Gα-coupled receptors like the α2-adrenergic receptor, receptors for adenosine or prostaglandin (401) or the recently deorphanized receptor for nicotinic acid, GPR109A (HM74a/PUMA-G) (634). Gαi was suggested to be directly involved in the antilipolytic effect of insulin, since insulin-dependent inhibition of lipolysis and activation of glucose oxidation in adipocytes were shown to be PTX sensitive (219).

IV. IMMUNE SYSTEM

A. Leukocyte Migration/Homing

Directed cell movement in response to an increased concentration of chemoattractant underlies the correct targeting of leukocytes to lymphatic organs during antigen surveillance and also allows them to migrate to sites of infection and/or inflammation (for review, see Refs. 653, 694). Known lymphocyte chemoattractants either belong to the large family of chemokines (355, 500) or to the lysophospholipid family, like sphingosine-1-phosphate (SIP) or lysophosphatidic acid (LPA) (123, 221, 282). Both chemokine and lysophospholipid receptors are GPCRs. While chemokine receptors primarily act through Gi family G proteins (355), lysophospholipid receptors activate Gi1, G12/G13, and Gq/G11 family G proteins depending on type and activation state of the respective cell (377, 584, 612).

Inactivation of Gi family G proteins by PTX pretreatment strongly impairs lymphocyte migration in vitro (28, 598) and causes defective homing to spleen, lymph nodes, and Peyer’s patches in vivo (31, 124, 507, 662), suggesting that Gi family G proteins are involved in these processes. In line with this, inactivation of Gi by transgenic expression of the S1 subunit of PTX in murine thymocytes resulted in accumulation of mature T cells in the thymus, with greatly reduced levels of T cells in peripheral lymphatic organs (91, 92). To investigate the role of Gi-mediated signaling in more detail, mouse lines carrying inactivating mutations of Gα1 subtypes were generated. Both Gαi2 and Gαi3 are expressed in the murine thymus (91), but only inactivation of Gαi2 mimicked the phenotype of PTX expressing mice with respect to thymic accumulation of mature T cells. Neither Gαi2- nor Gαi3-deficient mice showed defects in T cell homing to the periphery (552), suggesting that the homing defects induced by PTX probably result from the combined inactivation of Gα2 and Gα5.

Despite the predominant role of Gi signaling in chemokine-induced lymphocyte migration, an involvement of Gq/G11 family G proteins was suggested by a variety of in vitro studies (11, 21, 353, 590, 716). In contrast to other tissues, hematopoietic cells do not only express the α-subunits Gαq and Gα11, but also Gα15, which corresponds to human Gα16 (17, 683). Mice deficient for Gα11, Gα15, or both Gαq and Gα15 were normal under basal conditions and after antigenic challenge, and only a minor signaling defect in response to complement C5a was found in Gα15-deficient macrophages (132).

The G12/G13 effector RhoA was repeatedly shown to be involved in the regulation of lymphocyte adhesion and migration (367, 631), but direct evidence for an involvement of the G12/G13 family is still lacking. Indirect evidence for a role of G13 in lymphocyte migration comes from studies in Lsc-deficient mice. The murine Rho-specific guanine nucleotide exchange factor Lsc (p115RhoGEF in humans) is expressed exclusively in hematopoietic cells and couples Gα13 to the activation of RhoA (236). Lsc-deficient mice show impaired agonist-induced actin polymerization and motility, as well as abnormal B-cell homing and altered T- and B-cell proliferation (214). Defective migration was also observed in mice lacking the proton-sensing receptor G2A (465), which was shown to couple to Gα13 (316). G2A-deficient macrophages (659) and T cells (533) showed reduced migration towards lysophosphatidylcholine (LPC), while G2A overexpression in a macrophage cell line enhanced migration towards LPC (533, 715). How LPC effects are affected by the absence of G2A is currently not clear (690). In the latter cells, LPC-induced chemotaxis was inhibited by overexpression of dominant negative mutants of Gαq/G11 or Gα12/Gα13, as well as expression of RGS domains or GRK2 (specific for Gαq/G11) or of p115RhoGEF (specific for G12/G13), while PTX treatment was without effect (715).

Also neutrophils respond to a variety of chemoattractants, such as N-formyl-Met-Leu-Phe (fMLP), C5a, platelet activating factor (PAF), or the chemokine interleukin (IL)-8, with polarization and directed migration. This effect was shown to be PTX sensitive (43, 217, 577, 598) and to be mainly mediated via βγ-subunits (479, 480) (Fig. 8). Lentiviral-mediated knockdown of different G protein subunits in a macrophage cell line revealed that complement C5a-induced migration critically depends on Gβ2, but not on Gβ1, Gαq, or Gαi3 (286). Intracellular effectors of Gβγ in neutrophils are the γ isoform of PI 3-kinase (PI3Kγ) and the β2- and β3-isosforms of phospholipase C (PLC-β2, -β3). While neutrophils from mice lacking PLC-β2 and -β3 show normal, or even enhanced, chemotactic responses (388), migration of PI3Kγ-deficient neutrophils was severely impaired (266, 388, 566). Moreover, PI3Kγ-deficient neutrophils failed to accumulate at sites of inflammation in a septic peritonitis model (266), suggesting that PI3Kγ mediates chemotactic responses both in vitro and in vivo. In addition to PI3Kγ, RhoA activity seems to be required for fMLP-induced neutrophil chemokinesis and chemotaxis (review in Ref. 484), and espe-
FIG. 8. Gα family G proteins are centrally involved in neutrophil migration and activation and mediate their effects via the β2-and β3-isofoms of phospholipase C (PLC-β2/β3), phosphoinositide 3-kinases (PI-3-K), or the RacGEF P-Rex1. Btk, Bruton’s kinase; DAG, diacylglycerol; IP3, inositol 1,4,5-trisphosphate; PIP2, phosphatidylinositol 4,5-bisphosphate; PI(3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; PKC, protein kinase C.

\[ \text{Chemoattractant} \rightarrow \text{chemokine} \rightarrow \text{G} \rightarrow \text{activation of Gi defined “frontness” by activating PI3K and Rac, whereas activation of G12/G13 defined “backness” by inducing RhoA-dependent actomyosin interaction} \]

B. Immune Cell Effector Functions

Activation of immune cells by antigen contact initiates complex signaling cascades leading to proliferation, differentiation, or initiation of effector functions like cytokine production, mediator release, phagocytosis, etc. Although these functions are not directly G protein mediated, G proteins might have important modulatory roles (312, 400).

In neutrophils, chemoattractant-induced O2− formation is mediated via Gα-coupled receptors (39) and is strongly impaired both in neutrophils from mice lacking PLC-β2 and -β3 (388, 695, 699) and in PEBKγ-deficient neutrophils (266, 388, 566). PI3K-mediated formation of phosphatidylinositol 3,4,5-trisphosphate (PIP3) was shown to activate the small GTPase Rac, which contributes to neutrophil migration by actin polymer formation in the leading edge (540, 653) and to O2− formation by NADPH oxidase activation (144). Recently, a new Rac GEF termed P-Rex1 was shown to mediate Rac activation in response to PIP3 and Gβγ in neutrophils (672).

In lymphocytes, the balance between the Th1-driven cellular immunity and Th2-driven humoral response is mainly shaped by the cytokine pattern present during T helper cell activation. PTX has long been known to promote Th1 responses (246, 270, 464), and this was suggested to be due to a negative regulation of IL-12 production by Gαi, especially in dendritic cells (246, 279). Mice lacking Gα12 develop a diffuse inflammatory colitis resembling ulcerative colitis in humans (277, 552), and adenocarcinomas secondary to chronic inflammation were often observed (552). In these mice, levels of proinflammatory Th1-type cytokines and of IL-12 were increased (277), and these changes clearly preceded the onset of bowel inflammation (497, 498). In addition, antigen presenting cells like CD8α+ dendritic cells showed a highly increased basal production of IL-12 (246), suggesting that colitis in these mice is a Th1-driven disease and that production of proinflammatory Th1 cytokines is constitutively suppressed through a Gα-mediated pathway. Accordingly, activation of Gα-mediated signaling opposes these effects. Cholera toxin, which activates Gα by ADP-ribosylation, can be used as an adjuvant to promote Th2 responses (419, 687, 707), and systemic administration of cholera toxin during induction of Th1-based autoimmune diseases can shift the immune response to the nonpathogenic Th11 phenotype (610). In general, the Gα family was suggested to attenuate proinflammatory signaling, but direct evidence is lacking. In vitro, application of cAMP (62, 321, 671) or activation of typically Gα-coupled receptors, like those for vasoactive intestinal polypeptide or PACAP (135, 201), was shown to inhibit immune cell functions. Immune cells from mice lacking the Gα-coupled adenosine A2a receptor respond with enhanced cytokine transcription and NFκB activation to activation of Toll-like receptors (404), and activation of Gα by cholera toxin inhibits signaling in T cells (476, 595) or natural killer cells (678).

The relevance of Gαq/G11 and Gα12/G13 family G proteins in lymphocyte activation and proliferation is unclear. In vitro studies suggested an involvement of both families in the activation of Bruton’s tyrosine kinase, a protein that is required for normal B-cell development and activation (42, 309, 402). The Gαq/G11 family, especially human Gαq16 has been implicated in T-cell activation in a variety of in vitro studies (392, 603, 734), but the physiological relevance of these findings is unclear. In vivo, no obvious immunological defects were observed in Gα11−/−, Gα15−/−, or Gα15−/−;Gαq−/− mice (132). However, after antigenic challenge, Gαq-deficient mice showed impaired eosinophil recruitment to the lung, probably due to an impaired production of granulocyte macrophage colony stimulating factor GM-CSF by resident airway leu-
kocytes (56). Indirect evidence for a role of Gq/G11 and/or G12/G13 in T-cell activation comes from RGS2-deficient mice, which show impaired T-cell proliferation and IL-2 production after T-cell receptor stimulation, as well as defective antiviral immunity in vivo (499). Inactivation of the murine TxA2 receptor, which is typically coupled to Gq/G11 and/or G12/G13 family G proteins (337, 492), caused a lymphoproliferative syndrome due to prolonged T-cell/DC interaction (317). Genetic inactivation of the proton-sensitive G2A receptor (465), which was shown to activate G12/G13, causes a late-onset autoimmune syndrome in mice (372), suggesting that these G proteins may be involved in the regulation of lymphocyte activation.

V. NERVOUS SYSTEM

G proteins play multiple roles in the nervous system as most neurotransmitters also activate G protein-coupled metabotropic receptors to modulate neuronal activity. In contrast to ionotropic receptors, GPCRs that are present presynaptically and postsynaptically mediate comparatively slow responses. Only a few well-studied examples are described below.

A. Inhibitory Modulation of Synaptic Transmission

The regulation of neurotransmitter release at presynaptic terminals is an important mechanism underlying the modulation of synaptic transmission in the nervous system. Inhibitory regulation of neurotransmitter release is mediated by various GPCRs like α2-adrenoceptors, μ- and δ-opioid receptors, GABAR receptors, adenosine A1, or endocannabinoid CB1-receptors. These receptors have in common that they couple to G proteins of the Gq/G0 family. A major mechanism by which these G proteins mediate the inhibition of transmitter release is the inhibitory modulation of the action potential-evoked Ca2+ entry to the presynaptic terminal which is required to trigger neurotransmitter release. N- and P/Q-type calcium channels that are concentrated at nerve terminals as well as R-type calcium channels have been shown to be inhibited via Gq/G0-coupled receptors (145). This inhibition is due to the interaction of the G protein βγ-complex with the α1-subunit of Ca2,1.1–2.3 (89, 420). The β-subunit of the channel as well as strong depolarization can reduce this inhibition (61, 439). Most Gβγ combinations are similarly effective in inhibiting channels of the Ca2, family (202, 288, 557). There is some evidence that βγ-mediated inhibition of Ca2,2 channels is not the only mechanism through which GPCRs mediate inhibition of neurotransmitter release (449). Also, G protein-coupled inwardly rectifying K+ channels (GIRKs) are localized at presynaptic terminals; however, their physiological role in regulation of transmitter release from presynaptic terminals is less clear (156, 524). GIRKs are well-established effectors for G protein βγ-subunits (113, 356, 398, 718), and most Gβγ combinations appear to be similarly effective in activating GIRKs (681, 708). There is also increasing evidence that the Gβγ-mediated inhibition of neurotransmitter release at the presynaptic terminus involves mechanisms downstream of the regulation of Ca2+ (51). This may involve the direct interaction between Gβγ and the core vesicle fusion machinery as suggested by the observation that Gβγ can directly bind to SNARE proteins like syntaxin and SNAP25 as well as cysteine string protein (CSP) (50, 51, 305, 410). Evidence exists that presynaptic Ca2+ channels, syntaxin1, and the α-subunit of G0 are components of a functional complex at the presynaptic nerve terminal release site (384, 602).

Both Gq-type G proteins as well as G0 are highly abundant in the nervous system. Mice lacking the α-subunit of G0 are smaller and weaker than their littermates and have a greatly reduced life expectancy (308, 639). In addition, these animals have tremors and occasional seizures, and they show an increased motor activity with an extreme turning behavior. Gαo-deficient mice have also been shown to be hyperalgesic (308). When neuronal cells from Gαo-deficient mice were analyzed by electrophysiological methods for the regulation of GIRKs and voltage-dependent Ca2+ channels through GPCRs, it was found that the recovery kinetics after agonist washout were much slower in the absence of Galpha. However, current modulation via various receptors was as effectively as in wild-type cells (225, 308). This indicates that other G proteins especially Gq-type G proteins that are activated by the same receptors can compensate for G0 deficiency.

B. Modulation of Synaptic Transmission by the Gq/G11-Mediated Signaling Pathway

In the nervous system, the G proteins Gq and G11 are widely expressed (622), and they are involved in multiple pathways that modulate neuronal function. The modulation of synaptic transmission has best been described at the parallel fiber (PF)-Purkinje cell (PC) synapse in the cerebellum. At the PF-PC synapse, Gq/G11 mediate the effects of metabotropic glutamate group 1 receptors (mGluR1). The Gq/G11-mediated IP3-dependent transient increase in the dendritic Ca2+ concentration, which does not require changes in the membrane potential (178, 621), is important for the induction of long-term depression (LTD) at the PF-PC synapse (452). LTD in turn is believed to be one of the cellular mechanisms underlying cerebellar motor learning (66). Interestingly, while Gαq-deficient mice develop an ataxia with clear signs of motor coordination deficits, Gα11-deficient animals show only very subtle defects in motor coordination (237, 489). A detailed comparison of both mouse lines showed that Ca2+ re-
sponses to mGluR1 activation were absent in Gaq-deficient mice, whereas they are indistinguishable between wild-type and Ga11-deficient animals (237). However, synaptically evoked LTD was decreased in Ga11-deficient animals, but to a lesser extent than in Gaq-deficient mice. The predominant role of Ga in Purkinje cells can be explained by the higher expression compared with Gai (489). In fact, quantitative single-cell RT-PCR analysis showed that Purkinje cells express at least 10-fold more Gai than Ga11 (237).

Lack of Gai also results in a defect in the regression of supernumerary climbing fibers innervating Purkinje cells in the third postnatal week. This process is believed to be due to a defect in the modulation of the PF-PC synapse. Similar cerebellar phenotypes as in Gai-deficient mice have been described in mice lacking the mGluR1 (9, 323) as well as in mice lacking the β2-isof orm of PLC, which is predominantly expressed in the rostral cerebellum (324, 453). Interestingly mGluR1, Gai, and PLC-β4 can be found colocalized in dendritic spines of PCs (324, 622, 664), suggesting that they are components of a signaling cascade involved in the modulation of the PF-PC synapse.

Interestingly, hippocampal synaptic plasticity is equally impaired in Gai- and Ga11-deficient mice (451). However, mGluR1-dependent long-term depression in the hippocampal CA1-region was absent in Gai-deficient mice but appeared to be unaffected in mice lacking Ga11 (340). Similarly, suppression of slow afterhyperpolarizations via muscarinic and metabotropic glutamate receptors was nearly abolished in Gai-deficient mice but was unchanged in mice lacking Ga11 (350).

Besides the voltage-dependent, G protein-mediated inhibition of calcium channels via Gβγ (see above), functional studies in calyx-type nerve terminals and in sympathetic neurons have identified a voltage-insensitive inhibition of calcium currents via metabotropic receptors that is believed to involve Gq/G11 (322, 449). How Gq/G11 activation can lead to inhibition of voltage-dependent calcium channels is not clear. However, recent evidence suggests that the depletion of phosphatidylinositol 4,5-bisphosphate (PIP2), the substrate of PLC plays a role (200). There is also evidence that activation of Gq/G11-mediated signaling on the postsynaptic site is involved in the induction of retrograde signaling via the endocannabinoid system. Gq/G11-coupled receptors like group I mGluRs as well as muscarinic M1 receptors can lead to the activation of the retrogradely acting cannabinoid system by stimulating the formation of endocannabinoids (136, 189, 380, 409).

C. Roles of Gz and Gol in the Nervous System

Gz is the ubiquitous G protein that couples receptors in a stimulatory fashion to adenylyl cyclases. However, in a few tissues the G protein Gol is the predominant G protein that couples receptors to adenylyl cyclase. Apart from the olfactory system (see below), Gol expression levels exceed those of Gz, also in a few other defined brain regions like the nucleus accumbens, the olfactory tubercle, and the striatum (40, 120, 737). In the striatum, Gol appears to be critically involved in dopamine (D1) and adenosine (A2) receptor-mediated effects (258, 737). Data from various laboratories show that in the striatum, the dopamine D1 receptor, Gol, and adenylyl cyclase type V as well as the G protein γ7-subunit are coexpressed. Strikingly, mice lacking either of these signaling components show clear signs of motor abnormalities (40, 298, 573, 703). Mice lacking adenylyl cyclase V show an attenuated D1-receptor/Golf-mediated adenylyl cyclase activation in the striatum (298). Gγ7-deficient mice have strongly reduced levels of striatal Gol and activation of adenylyl cyclase via dopamine receptors is abolished in the striatal cells (573). Thus, in rodents, striatal D1 receptors appear to activate adenylyl cyclase V through a G protein containing Gol and γ7. In humans, it has recently been shown that Gol expression is markedly diminished in the putamen of patients with Huntington disease, while the putamen of patients with Parkinson disease showed significantly increased levels of Gol and Gγ7 (120).

The G protein Gz is a member of the Gz/Golf family. It shares with other Gz/Golf family members the ability to inhibit adenylyl cyclase (176, 267). It is expressed primarily in brain, retina, adrenal medulla, and platelets. Gz-deficient mice are viable and have no major phenotypical abnormalities. However, Gz deficiency results in an abnormally organized response to certain psychoactive drugs (253, 713). This includes a considerably pronounced cocaine-induced increase in locomotor activity as well as a reduction in the short-term antinociceptive effects of morphine (713). In addition, Gz-deficient animals develop significantly increased tolerance to morphine (374). Interestingly, the behavioral effects of catecholamine reuptake inhibitors that are used as antidepressant drugs were abolished in mice lacking Gz (713). While mice lacking Gz clearly indicate that Gz plays a role in the nervous system and is involved in various drug responses, the function of Gz on a cellular level in the nervous system remains unclear.

VI. SENSORY SYSTEMS

G protein-mediated signal transduction processes mediate the perception of many sensory stimuli. Odors, light, and especially sweet and bitter taste substances act directly on GPCRs that modulate the activity of primary sensory cells via often very specialized heterotrimeric G proteins.
A. Visual System

The human retina contains two types of photoreceptor cells, rods and cones. While rods mediate mainly achromatic night vision and contain one type of GPCR, rhodopsin, cones are responsible for chromatic day vision and contain three GPCRs (opsins), which are sensitive to different parts of the visible light spectrum. Rhodopsin and opsins are coupled to different but closely related heterotrimeric G proteins. Rod-transducin (Gt-r) and cone-transducin (Gt-c), which mediate the effects of rhodopsins and opsins, respectively, couple these receptors in a stimulatory manner to cGMP-phosphodiesterase (PDE) by binding and sequestering the inhibitory γ-subunit of the retinal type 6 PDE (PDE6). Activation of PDE lowers cytosolic cGMP levels leading to a decreased open probability of cGMP-regulated cation channels in the plasma membrane, which eventually results in hyperpolarization of the photoreceptor cells (24). In mice lacking Gαt-r, the majority of retinal rods do not respond to light any more, and these animals develop mild retinal degeneration with increasing age (84). To ensure an adequate time resolution of the light signal, the transducin-mediated signaling process initiated by light-induced receptor activation requires an efficient termination mechanism. At least three proteins contribute to the rapid deactivation of transducin by increasing its GTPase activity: RGS9 and Gβγ5L, which form a complex, and the γ-subunit of the transducin effector cGMP-PDE (24). In mice lacking Gβγ5, the levels of RGS9 and other G protein γ-like (GGL) domain RGS proteins in various tissues including retina are drastically reduced, suggesting that the formation of the RGS9 Gβγ complex is required for expression and function of RGS9 (100). Animals lacking Gβγ5 or RGS9 show a strongly impaired termination of transducin-mediated signaling (99, 352, 407).

The light-induced hyperpolarization of photoreceptor cells results in a decreased release of glutamate. Glutamate released from photoreceptor cells acts on two classes of second-order neurons in the retina, one that depolarizes in response to glutamate most likely via ionotropic glutamate receptors (OFF bipolar cells) and another that hyperpolarizes (ON bipolar cells). ON bipolar cells are in the absence of light inhibited by glutamate released from rods and cones, and this effect is mediated by the metabotropic glutamate receptor mGluR6. Light-induced hyperpolarization of rods results in decreased glutamate release and disinhibition of ON bipolar cells due to a decreased activation of mGluR6. In mice lacking mGluR6 or the Gαo splice variant Gαo1, the modulation of ON bipolar cells in response to light is abolished (142, 143, 425), indicating that Gαo is the principle mediator of glutamate-induced inhibition of ON bipolar cells, which occurs especially in the absence of light. The retina-specific RGS protein Ret-RGS1 is localized in the dendritic tips of ON bipolar cells together with mGluR6 and Gαo1 and may play a critical role in increasing the deactivation of Gαo1 resulting in an acceleration in the rising phase of the light response of the ON bipolar cells. This mechanism has been suggested to match the kinetics of ON bipolar cell activation to that of the OFF bipolar cells that arises directly from ligand-gated channel activation by glutamate (141).

B. Olfactory/Pheromone System

Chemosensation by the olfactory system is based on the expression of a huge variety of GPCRs specifically in the olfactory epithelium. Individual olfactory sensory neurons appear to often express only one olfactory receptor, and olfactory sensory neurons expressing one receptor type converge to the same subgroup of glomeruli in the olfactory bulb (457, 548). Rodents have ~1,000 different olfactory receptors, whereas the number in humans is considerably smaller and has been estimated to be ~350 (457). Despite this remarkable diversity on the level of the receptor, the olfactory GPCRs appear to employ the same G protein-mediated signal transduction pathway in olfactory sensory neurons. The G protein Gαolf is centrally involved in signaling by olfactory receptors in response to odorant stimuli, and Gαolf-deficient mice are anosmic exhibiting dramatically reduced electrophysiological responses to all odors tested (40). Gαolf, a G protein related to Gαs, couples olfactory receptors in the cilia to adenylyl cyclase III, resulting in the increased formation of cAMP. cAMP then activates a cyclic nucleotide-gated (CNG) cation channel consisting of three different subunits, CNGA2, CNGA4, and CNGB1. The increase in cellular Ca2+ activates a Ca2+-activated Cl− channel that further depolarizes the cell membrane (457, 548). Most Gαolf-deficient pups die a few days after birth due to insufficient feeding. Rare surviving animals exhibit inadequate maternal behavior resulting in the death of all pups born to Gαolf-deficient mothers. This indicates that normal nursing and mothering behavior in rodents is greatly dependent on an intact olfactory system (40). The fundamental role of this signaling pathway is underscored by the anosmic phenotype found not only in mice lacking Gαolf but also adenylyl cyclase (693) as well as in mice lacking the subunits of the olfactory CNG channel (30, 76, 731).

A second olfactory system called the accessory olfactory system or the vomeronasal system exists in most mammals (152, 330). The peripheral sensory structure of this system, the vomeronasal organ, is localized at the bottom of the nasal cavity. The vomeronasal system responds topheromones that mediate defined effects on individuals of the same species and modulate social, aggressive, reproductive, and sexual behaviors. In the vomeronasal organ, two families of GPCRs, which in mice...
behavioral and anatomical abnormalities in G protein family is expressed in a different population of sweet, umami, and bitter detection. The T2 receptors and T2 receptors, have been implicated in families of candidate mammalian taste receptors, T1 receptors directly on ion channels (391). During recent years, two through GPCRs, whereas salty and sour tastants act qualities sweet, bitter, and amino acid (umami) signal.

C. Gustatory System

Unlike the olfactory system, chemosensation by the gustatory system involves only in part G protein-mediated signal transduction mechanisms. The taste qualities sweet, bitter, and amino acid (umami) signal through GPCRs, whereas salty and sour tastants act directly on ion channels (391). During recent years, two families of candidate mammalian taste receptors, T1 receptors and T2 receptors, have been implicated in sweet, umami, and bitter detection. The T2 receptors are a group of ~30 GPCRs that are specifically expressed in taste buds of the tongue and that are linked to bitter taste in mice and humans (6, 78, 94, 429). The T1 receptor family consists of only three GPCRs and has been shown to form heterodimers. T1R1 and T1R3 form a receptor that responds to amino acids carrying the umami taste (477), and T1R2 forms heterodimers with T1R3 that functions as a sweet receptor (386, 477). Studies in knockout mice support a role of T1R2/T1R3 as a sweet receptor and of T1R1/T1R3 as an umami receptor. In addition, T1R3 may form homodimers that can also mediate some sweet tastes, and there is evidence that also a splice variant of the mGluR4 glutamate receptor may be involved in umami sensation (95, 96, 127, 730). The signal transduction mechanisms used by different G protein-coupled taste receptors are less clear. Gustducin, a G protein mainly expressed in taste cells, is believed to be able to couple receptors to phosphodiesterase resulting in a decrease of cyclic nucleotide levels. Mice lacking the α-subunit of gustducin show impaired responses to bitter, sweet, as well as umami tastes (247, 555, 692). However, the residual bitter and sweet taste responses in Gαgust-deficient mice and the finding that expression of a dominant negative mutant of α-gustducin reduces this responsiveness even further indicates that α-gustducin is not the only α-subunit involved in sweet, bitter, and umami signal transduction (416, 556). In addition, α-gustducin-deficient mice expressing the α-subunit of rod-transducin as a transgene driven by the α-gustducin promoter partially recovered responses to sweet and bitter compounds (247). However, in mice lacking the α-subunit of rod-transducin, responses to bitter and sweet compounds were normal, whereas the responses to various umami compounds were impaired (249). Thus gustducin is involved in sweet, bitter, and umami taste detection, whereas rod-transducin mediates part of umami taste sensation. Both gustducin and transducin are able to activate phosphodiesterase, thereby leading to decreased cGMP levels, disinhibition of cyclic nucleotide-inhibited channels, and calcium influx. In addition, bitter tastants have also been shown to activate PLC-β2 through Gβγ subunits (probably Gβ2γ13) released from gustducin or other G proteins (416), and PLCβ2 has recently been shown to be involved in the activation of transient receptor potential M5 (TRPM5) cation channels, which cause depolarization of the cells (729). Interestingly, mice lacking PLC-β2 or TRPM5 are completely insensitive not only to bitter tastants but also to sweet and umami (729), indicating that the G protein/PLC-β2/TRPM5 signaling cascade is centrally involved in the sensation of these three taste qualities. However, other signal transduction pathways have been suggested, and it is not clear how gustducin or transducin functions are related to those of PLCβ2 and TRPM5.

VII. DEVELOPMENT

Although heterotrimeric G proteins are expressed throughout the prenatal development of the mammalian organism, only a limited number of studies have so far...
addressed the role of G proteins in development. Most insights into the developmental role of G protein-mediated signaling pathways came from studies on mouse mutants lacking individual G protein α-subunits. Some null mutations like those of the genes encoding Gα12, Gα13, or Gαq/Gα11 are embryonic lethal (493, 495, 723) and clearly indicate an essential role during development.

A. G13-Mediated Signaling in Embryonic Angiogenesis

Both G12 and G13 have been shown to induce cytoskeletal rearrangements in a Rho-dependent manner (79, 563). Lack of Gα13 in mice results in embryonic lethality at midgestation. At this stage, mouse embryos express both Gα12 and Gα13. Gα13-deficient mouse embryos show a defective organization of the vascular system, which is most prominent in the yolk sac and in the head mesenchyme (493). Vascularization of blood vessel formation through the differentiation of progenitor cells into endothelial cells was not affected by the loss of Gα13. However, angiogenesis which includes sprouting, growth, migration, and remodeling of existing endothelial cells, was severely disturbed, and the maintenance of the integrity of newly developed vessels appeared to be defective in Gα13-deficient embryos. Chemokinetic effects of thrombin, which acts through protease-activated receptors (PARs), were completely abrogated in fibroblasts lacking Gα13, indicating that Gα13 is required for full migratory responses of cells to certain stimuli. Interestingly, approximately one-half of the embryos that lack the protease-activated receptor 1 (PAR-1) also die at midgestation with bleeding from multiple sites (118). This phenotype of embryos lacking PAR-1 which is expressed in endothelial cells, can be rescued by a PAR-1 transgene whose expression is driven by an endothelial-specific promoter (226). This clearly indicates that PAR-1 function is required for proper vascular development. The more severe embryonic defect of Gα13 compared with PAR-1-deficient embryos suggests that Gα13 function is not restricted to protease-activated receptor signaling.

The defects observed in Gα13-deficient embryos and cells occurred in the presence of Gα12, and loss of Gα12 did not result in any obvious defects during development. Interestingly, Gα12-deficient mice that carry only one intact Gα13 allele also die in utero (228). This genetic evidence indicates that Gα13 and its closest relative, Gα12, fulfill at least partially nonoverlapping cellular and biologic functions, which are required for proper development.

B. Gq/G11-Mediated Signaling During Embryonic Myocardial Growth

The Gαq/Gα11-mediated signaling pathway appears to play a pivotal role in the regulation of the physiological myocardial growth during embryogenesis. This is demonstrated by the phenotype of mice lacking both Gαq and Gα11. These mice die at embryonic day 11 due to a severe thinning of the myocardial layer of the heart (495). Both the trabecular ventricular myocardium as well as the subepicardial layer appeared to be underdeveloped. There are several Gq/G11-coupled receptors that may be involved in the regulation of cardiac growth at midgestation. Inactivation of the gene encoding the Gq/G11-coupled serotonin 5-HT2B receptors in mice resulted in cardiomyopathy with a loss of ventricular mass due to a reduction in the number and size of cardiomyocytes (473), and lack of both endothelin A (ETA) and endothelin B (ETB) receptors, which can signal through Gq/G11, resulted in midgestational cardiac failure (710). Most likely there is some degree of signaling redundancy with several inputs into the Gq/G11-mediated signaling pathway, and only deletion of both the Gq and the Gα11 gene results in severe phenotypic defects during early heart development. Interestingly, one intact allele of the Gq or the Gα11 gene was sufficient to overcome the early developmental block in heart development. However, newborn mice that have only one intact Gq or Gα11 allele show an increased incidence of cardiac defects ranging from septal defects to univentricular hearts (495).

C. Neural Crest Development

Signaling through Gq/G11 has been implicated in the proliferation and/or migration of neural crest cells. ET-1 and the Gq/G11-coupled endothelin A (ETα) receptor are essential for normal function of craniofacial and cardiac neural crest. ET-1 and ETα receptor-deficient mice die shortly after birth due to respiratory failure (114, 357, 358). Severe skeletal abnormalities could be observed in their craniofacial region, including a homeotic transformation of mandibular arch-derived structures into maxillary-like structures as well as absence of auditory ossicles and tympanic ring (503, 553). A hypomorphic phenotype similar to, but less severe than ETα or ET-1 null mice could be observed in Gαq (−/−);Gα11 (−/+), mice (495). In Gαq/Gα11 double-deficient mice studied at embryonic day 9.5, a expression of ET-1-dependent transcription factors like Dlx3, Dlx6, dHAND, and eHAND was ablated (297), a finding similar to those made in mice lacking ETα or ET-1 (114, 629). This suggests that in the neural crest-derived pharyngeal arch mesenchyme, a signaling pathway involving ET-1, ETα receptors, and Gαq/Gα11 is operating. ET-1, which is expressed in the pharyngeal endoderm binds to ETα receptors in neural crest cells of the pharyngeal arches of embryonic day 9.5 embryos (114). Gq/G11 then couples ETα receptors via phospholipase C-β activation to the activation of the expression of genes encoding transcription factors including Dlx3.
However, several hypermorphic alleles of the G
neural crest cells has not been studied directly so far. in ET-3-induced differentiation of cutaneous and enteric
tivities indicating that ETA receptor-mediated neural crest
development requires a certain amount of Gαq/Gαi11. It is
also possible that Gαi11 is expressed at lower levels than
Gαq in the neural crest-derived mesenchyme of the pha-
ryngeal archs, or that the ETα receptor couples preferen-
tially to Gαq.

The ET-3 and ETB receptor system has been shown
to be involved in the development of neural crest cells
taking part in the formation of epidermal melanocytes as
well as the myenteric ganglia of the distal colon. In mice lacking ET-3 or the ETB receptor, this results in white-
spotted hair and skin color as well as a dilation of the
proximal colon (38, 710). These defects are very similar to
those present in humans suffering from multigenic Hirschsprung disease, which in various cases has been
shown to be caused by mutations in ETβ or ET-3 genes
(158, 204). Whether Gq/G11-mediated signaling is involved
in ET-3-induced differentiation of cutaneous and enteric
neural crest cells has not been studied directly so far.
However, several hypermorphic alleles of the Gαq and
Gαi11 genes have recently been found in mouse mutants
with an aberrant accumulation of pigment-producing me-
lanocytes (302, 642). Genetic evidence was provided that
the action of Gαq/Gαi11 depended on the ETB receptor.

VIII. CELL GROWTH AND TRANSFORMATION

During recent years it has become obvious that
GPCRs and heterotrimeric G proteins play important
roles in the regulation of cell growth and that some G
proteins can induce cellular transformation (140, 229,
549).

A. Constitutively Active Mutants of Gαq/Gαi11

Transforming mutants of Gαq/Gαi11 have not been
found in human tumors. However, their potential onco-
genic function has been studied in cell lines by expres-
sion of constitutively active forms like Gαq/Q209L or
Gαi11/Q209L. These studies indicated that activated Gαq/311
lead to transformation of fibroblasts when expressed
at low levels (318) but induces growth inhibition and
apoptosis when expressed at higher levels (140). Interest-
ingly, various Gq/G11-coupled receptors have been shown
to possess highly transforming activity. This has very
early been shown for the 5-HT2c serotonin receptor as
well as for the M1, M3, and M6 muscarinic receptors,
which are able to transform fibroblasts in the presence of
a receptor agonist (231, 315). It is well known that various
Gq/G11-coupled neuropeptide receptors like those for gas-
trin-releasing peptides, galanin, neuromedin B, vasopres-
sin, and others are involved in the autocrine and paracrine
stimulation of proliferation in small cell lung cancer cells
(250). This suggests that endogenous Gq/G11-coupled re-
ceptors can be tumorigenic in the presence of excess
ligands. In vitro experiments indicate that constitutively
active Gq/G11-coupled receptors can enhance mitogenesis
and tumorigenicity (14), and the virally encoded KSHV-G
protein-coupled receptor, which is a constitutively active
Gq/G11-coupled receptor, has been shown to behave as a
viral oncogene and angiogenesis activator and appears to
be involved in Kaposi sarcoma progression (26, 29, 458).

B. The Oncogenic Potential of Gαs

Gs-mediated increases in cAMP can induce growth
inhibition in some tissues while in others it can have a
transforming potential. The gsp oncogene encodes a
GTPase-deficient mutant of Gαs and has been found in up
to 30% of thyroid toxic adenomas and in a few thyroid
carcinomas. A constitutively active mutant of Gαs has
also been found in GH-secreting pituitary adenomas as
well as in the McCune-Albright syndrome (172, 599).
Interestingly, responses to constitutively active Gαs are cell
type specific. In some cells, an increase in cAMP
and consecutive activation of PKA can inhibit Raf kinase and
prevent transformation of cells (102). In contrast, in vari-
ous neuronal and neuroendocrine cells, Gαs-mediated
cAMP formation activates cell growth (164, 551). The
transforming potential of Gαs obviously depends on the
cellular context that links Gαs-mediated cAMP formation
to inhibition or stimulation of ERK (604). While various
kinases upstream of ERK have been involved in cAMP/
PKA-induced ERK regulation, the determinants of the net
proliferative effects of cAMP remain elusive (604). A
proposed role of the cAMP-activated Epac/Rap1 pathway in
cAMP-mediated ERK activation has been questioned
(163).

The potential of Gs-mediated signaling to induce tu-
mor formation in endocrine cells is also underlined by
the fact that constitutively active mutants of various Gs-cou-
pled receptors have been detected in human tumors. Up
to 80% of hyperfunctioning human thyroid adenomas and
a minority of differentiated thyroid carcinoma have been
shown to contain constitutively active TSH receptors
(507, 508). Similarly, mutations resulting in constitutive
activation of the luteinizing hormone receptor have been
shown to cause hyperplastic growth of Leydig cells in a
form of familial male prepubertal puberty (578) as well as
in Leydig cell tumors (393).
C. G1-Mediated Cell Transformation

The gip2 oncogene that encodes an active mutant of Gα12 has been found in adrenal cortical tumors as well as in human ovarian sex cord stromal tumors (406). It is a matter of debate how frequent these Gα12 mutations occur in these tumors. Expression of constitutively active Gα12 in fibroblast cell lines leads to cell transformation, an effect which has not been completely understood yet, but may result from the derepression of the Ras/ERK pathway in response to a decrease in cellular cAMP levels (446, 640). Depending on the cellular system, G1-mediated release of free βγ-subunits may also be involved in the growth-promoting effect of Gα12 (122).

D. Cellular Growth Induced by Gα12/Gα13

The G protein α-subunit Gα12 was identified as an oncogene present in soft tissue sarcoma (93). This oncogene, termed gep, encoded the wild-type form of Gα12. At the same time, the potent transforming ability of constitutively active Gα12 and Gα13 could be shown in various systems (307, 645, 655, 703, 704). While no activating mutation of Gα12 or Gα13 has been found in human tumors, increased expression levels of both proteins have been detected in various human cancers (230). Both Gα12 and Gα13 can induce activation of the small GTPase RhoA via regulation of a subgroup of RhoGEF proteins (195, 236). Interestingly, RhoA has been found to be overexpressed in various tumor types (2, 192, 320), and Rho GTPases have been involved in carcinogenesis and cancer progression (385, 564). Recently, an interesting link between G12/G13 and cadherin-mediated signaling was described (327, 434, 435). Activated Gα12 and Gα13 can interact with the cytoplasmic domain of some type I and type II class cadherins, like E-cadherin, N-cadherin, or cadherin-14, causing the release of β-catenin from cadherins and its relocalization to the cytoplasm and nucleus, where it is involved in transcriptional activation. In addition, activated Gα12 can block cadherin-mediated cell adhesion in various cells including breast cancer cells (434). This downregulation of cadherin-mediated cell adhesion and the release of β-catenin from cadherins may be a mechanism by which Gα12/Gα13 induces cellular transformation and metastatic tumor progression.

IX. CONCLUDING REMARKS

The field of heterotrimeric G proteins was launched more than 30 years ago when the involvement of guanine nucleotides in the hormonal stimulation of adenyl cyclase was discovered. Since then, the field has enormously expanded, and G protein-mediated signal transduction has turned out to be the most widely used transmembrane signaling system in higher organisms. It consists of hundreds of receptors that signal to dozens of G proteins and effectors. The modular nature of this signal transduction system with multiple components enables cells to assemble vast, combinatorially complex signaling units that allow different cells in different contexts to respond adequately to extracellular signals. There is probably not a single cell in a mammalian organism that does not employ several G protein-mediated signaling pathways. Often these pathways integrate the information conveyed by several receptors recognizing different ligands. Only in recent years did we gain a more systematic insight into the role of individual G proteins on a cellular and supracellular level. However, many aspects of G protein-mediated signaling, especially under in vivo conditions, still remain to be elucidated. While many components of the system have been identified, new approaches are required to determine the exact composition of individual signaling units and to define their exact cellular localization. This will probably lead to the identification of even more proteins and nonprotein factors that modulate G protein-mediated signaling. An increasing use of in vivo models is necessary to test the significance of signaling mechanisms described in vitro under normal conditions as well as in disease states. The parallel application of genetic, genomic, and of new proteomic approaches will be required to continue to define how the G protein-mediated signaling system works on a molecular, cellular, and systemic level. Such an integrated view will provide the basis for a full understanding of the physiological and pathophysiological role of G protein-mediated signaling and will allow the full exploitation of this multifaceted signaling system as a target for pharmacological interventions.

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Gene trapping of G protein beta subunits results in neurodegeneration.


CELLULAR FUNCTIONS OF G PROTEINS


