REVIEW ARTICLE

Signalling functions and biochemical properties of pertussis toxin-resistant G-proteins

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Pertussis toxin (PTX) has been widely used as a reagent to characterize the involvement of heterotrimeric G-proteins in signalling. This toxin catalyses the ADP-ribosylation of specific G-protein α subunits of the G_i family, and this modification prevents the occurrence of the receptor–G-protein interaction. This review focuses on the biochemical properties and signalling of those G-proteins historically classified as ‘PTX-resistant’ due to the inability of the toxin to influence signalling through them. These G-proteins include members of the G_q and G_{12} families and one G_i family member, i.e. G_z . Signalling pathways controlled by these G-proteins are well characterized only for G_q family members, which activate specific isoforms of phospholipase C, resulting in increases in intracellular calcium and activation of protein kinase C (PKC), among other responses.

While members of the G_{12} family have been implicated in processes that regulate cell growth, and G_z has been shown to inhibit adenylate cyclase, the specific downstream targets to these G-proteins *in vivo* have not been clearly established. Since two of these proteins, $G_{12\alpha}$ and $G_{z\alpha}$, are excellent substrates for PKC, there is the potential for cross-talk between their signalling and G_q -dependent processes leading to activation of PKC. In tissues that express these G-proteins, a number of guanine-nucleotide-dependent, PTX-resistant, signalling pathways have been defined for which the G-protein involved has not been identified. This review summarizes these pathways and discusses the evidence both for the participation of specific PTX-resistant G-proteins in them and for the regulation of these processes by PKC.

INTRODUCTION TO G-PROTEIN α SUBUNITS

Intracellular communication most often entails the release of soluble, hydrophilic, signalling molecules by one cell and the binding of these molecules by cell-surface receptors of another cell. This binding of signalling molecules sets off a complex cascade of events that ultimately results in a cellular response. Heterotrimeric guanine-nucleotide-binding regulatory proteins (G-proteins) are a major cog in the machinery that a cell utilizes to transduce many of these extracellular signals into intracellular responses. The role of G-proteins in signal transduction has been intensively investigated and extensively reviewed [1–5]. However, the properties and signalling functions of the subgroup of G-proteins that are resistant to modification by the bacterial agent pertussis toxin (PTX) have not been extensively reviewed (although see [109]). Since this toxin is often the first tool used in efforts to characterize a G-protein-dependent signalling process, we felt that a comprehensive review in this area would be useful.

G-proteins reside predominantly at the intracellular face of the plasma membrane, where they can interact with both upstream (receptor) and downstream (effector) components of distinct signalling systems. These proteins are trimeric molecules, consisting of subunits designated α , β and γ . Multiple isoforms of each subunit have been identified in mammalian cells, with about 20 different α , five β and ten γ subunits having been reported to date [3,6–9]. G-proteins accomplish their remarkable task by the superposition of a cycle of nucleotide exchange and GTP hydrolysis upon subunit dissociation and reassociation. When a given agonist-bound receptor interacts with a particular G-

protein trimer in the inactive (GDP-bound) state, this contact drives the release of GDP from the $G\alpha$ subunit. GTP binding leads to a conformational change in $G\alpha$ that decreases its affinity for both $G\beta\gamma$ and the receptor. This results in dissociation of the complex into receptor, $G\alpha$ -GTP and $G\beta\gamma$ components. Additionally, the receptor has a decreased affinity for its agonist in this state and releases the ligand. Either the activated $G\alpha$ (GTP-bound) or $G\beta\gamma$, or both, can then interact with specific effector molecule(s) to modulate the production of soluble intracellular molecules termed second messengers [3–5]. Some examples of effectors include adenylate cyclase [10], GMP-phosphodiesterase [11], phosphatidylinositol-specific phospholipase C (PI-PLC) [12] and ion channels [13,14]. The signal is terminated by the intrinsic GTPase activity of the $G\alpha$ subunit, which hydrolyses the bound GTP to GDP, and the cycle is completed as the resultant $G\alpha$ -GDP re-associates with $G\beta\gamma$. The re-formed G-protein oligomer can begin the cycle anew by interacting again with a liganded receptor. A different class of GTP-binding proteins, monomeric proteins often referred to as ‘small-molecular mass G-proteins’, can also play important roles in signal transduction via different mechanisms [15–18], but they will not be discussed further here.

Historically, G-protein α subunits are viewed as the functional component of the G-protein because of their ability both to bind guanine nucleotides and to interact with receptors and effectors, although it is now clear that the $G\beta\gamma$ complex also plays a role in the latter functions [19–25]. Nevertheless, heterotrimeric G-proteins are classified according to the identity of their unique $G\alpha$ subunit. Although sequence heterogeneity among $G\alpha$ sub-

Abbreviations used: CTX, cholera toxin; GAP, GTPase-activating protein; JNK, Jun kinase; mGluR, metabotropic glutamate receptor; PKC, protein kinase C; PLC, phospholipase C; PI-PLC, phosphatidylinositol-specific PLC; PTX, pertussis toxin; RGS, regulators of protein signalling; TRH, thyrotropin-releasing hormone; TXA₂, thromboxane A₂; VDCC, voltage-dependent calcium channels.

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units allows one to divide them into four major subfamilies, termed G_s , G_i , G_q and G_{12} , they share some common features. Each member of the $G\alpha$ family contains a single guanine-nucleotide-binding site and possesses an intrinsic GTPase activity. The crystal structures of $G_{12}\alpha$ and G_{i1} [26,27] and earlier biochemical data (see [28] for a review) indicate that four regions comprise the nucleotide-binding pocket of $G\alpha$ subunits. The amino acid sequences in these four regions are highly conserved, although differences in these regions are likely to account for the characteristic nucleotide exchange and GTP hydrolysis properties of each $G\alpha$ subunit.

$G\alpha$ subunits contain site(s) for NAD^+ -dependent ADP-ribosylation catalysed by cholera toxin (CTX) and/or PTX. These bacterial toxins have been very useful tools in identifying the involvement of G-proteins in signalling systems. CTX catalyses the ADP-ribosylation of a conserved arginine residue on $G\alpha$ proteins, resulting in inhibition of both GTPase activity and interaction with $G\beta\gamma$ [29]. Members of the G_s subfamily, and $G_{12}\alpha$, are substrates for CTX. Interestingly, despite the fact that only these $G\alpha$ s are good substrates for CTX, the conserved arginine residue that is the site for CTX-catalysed ADP-ribosylation is highly conserved among $G\alpha$ subunits. In poor CTX substrates this residue is presumably not accessible to the toxin. Evidence for this comes from studies showing that some of these G-proteins can be efficiently modified by CTX if complexed with an agonist-bound receptor in the absence of exogenous GTP. These conditions generate a nucleotide-free G-protein associated with the receptor, and this form is thought to be accessible to CTX. This phenomenon has been clearly demonstrated for two members of the G_i subfamily, G_{i2} and G_{i3} [30–35]. Preliminary evidence also suggests that activation of $G_{12}\alpha$ converts it into a CTX substrate (T. A. Fields and P. J. Casey, unpublished work).

PTX catalyses the ADP-ribosylation of a cysteine residue at position –4 from the C-terminus of most $G\alpha$ subunits in the G_i family, which results in the inhibition of receptor–G-protein coupling. This toxin was initially used to differentiate guanine-nucleotide-dependent inhibition of adenylate cyclase (through G_i), which is PTX-sensitive, from stimulation of adenylate cyclase (through G_s), which is PTX-resistant [36,37]. Over time, however, it has become clear that many other G-protein-dependent signalling processes are also resistant to PTX. Since the initial use of PTX to identify the involvement of G-proteins in signalling, biochemical and molecular approaches have led to the identification and sequence determination of two additional subfamilies of $G\alpha$ proteins, i.e. G_q and G_{12} , that lack the cysteine residue that is the PTX site and are therefore candidates for participating in PTX-resistant signalling events [38–41]. Additionally, a member of the G_i subfamily, G_{12} , has been identified that differs from its brethren in that it lacks the cysteine residue that is the PTX site [42,43]. $G_{12}\alpha$ and the G_q and G_{12} subfamilies will be the subject of the remainder of this review, and we will refer to these proteins as ‘the PTX-resistant G-proteins’, even though literally this phrase also includes members of the G_s subfamily. We discuss what is known of the biochemistry and the signalling functions of the PTX-resistant G-proteins, and additionally point out those PTX-resistant signalling processes for which the G-protein involved has not been identified.

BIOCHEMICAL PROPERTIES OF PTX-RESISTANT G-PROTEINS

The PTX-resistant G-proteins are fairly ubiquitously expressed in tissues, except for $G_{12}\alpha$, which is predominantly expressed in neurons, platelets and chromaffin cells [42–44], $G_{14}\alpha$, which is predominantly expressed in spleen, lung, kidney and testis [40],

and $G_{15}\alpha$ and $G_{16}\alpha$, which are expressed in cells of myeloid and lymphoid lineage [40,41]. The biochemical properties of several PTX-resistant G-proteins, namely $G_{12}\alpha$, $G_q\alpha$, $G_{12}\alpha$ and $G_{13}\alpha$, have been characterized by many different investigators. The results are summarized in Table 1, which compares the biochemical properties of these and other G-proteins. One striking observation is that the intrinsic GTPase activities of the PTX-resistant G-proteins tend to be lower than those of the other G-proteins. This is most apparent in the case of $G_{12}\alpha$, whose k_{cat} value for GTP hydrolysis is as much as 200-fold lower than those of $G_s\alpha$ and $G_i\alpha$ at 30 °C [45,46], although k_{cat} values determined for $G_{12}\alpha$ and $G_{13}\alpha$ are only 2–4 times that of $G_{12}\alpha$ [47,48]; the rate constant for $G_q\alpha$ is about 15 times that of $G_{12}\alpha$ [49].

The structural basis for this sluggish GTPase activity undoubtedly lies in the sequences of these G-proteins. One sequence that probably contributes is the so-called ‘GAGES’ region of the first nucleotide-binding domain. $G_{12}\alpha$ and members of the G_q subfamily have amino acid substitutions in this conserved motif (see Table 2). Mutations in the analogous regions of $G_s\alpha$ and the small G-protein Ras lead to a marked inhibition of the GTPase activities of these two proteins [28,50]. In solved crystal structures, these residues comprise the phosphate-binding region that loops around the α - and β -phosphates of GTP [26,27]. A direct role for this region in GTP hydrolysis has not been discerned, but the glutamate residue in this motif does form an ionic bond with Arg-178 in G_{i1} to help trap guanosine 5'-[thio]triphosphate within the interdomain cavity in this protein [27], and the backbone amide of the analogous residue in $G_{12}\alpha$ makes a direct contact with the bridging oxygen between the β - and γ -phosphates [26]. Other regions may also contribute to the diminished GTPase activity of these proteins; this is particularly evident for $G_{12}\alpha$, since this G-protein possesses low GTPase activity despite the absence of substitutions in the GAGES motif.

The low rate of GTP hydrolysis by PTX-resistant G-proteins is also similar to that seen for the small G-proteins in the Ras family, which possess k_{cat} values for GTP hydrolysis as low as 0.02 min⁻¹ at 37 °C [51]. In the presence of specific proteins termed GTPase-activating proteins (GAPs), however, GTP hydrolysis for these small G-proteins can be stimulated several-thousand-fold (for reviews, see [17–18]). Since PTX-resistant G-proteins hydrolyse GTP so slowly, it is not surprising that there exist GAPs that accelerate their hydrolytic activity also. The effector for $G_q\alpha$, PLC- β 1, can stimulate the GTPase activity of $G_q\alpha$ by ~50-fold [52]. The effectors of some PTX-sensitive G-proteins have also been shown to have GAP activity. In the visual system, where rapid turnover in signalling is required, the effector for $G_t\alpha$, i.e. cGMP phosphodiesterase, stimulates the GTPase activity of $G_t\alpha$ [53,54]. Full efficacy of the cGMP-phosphodiesterase-dependent GAP activity apparently requires the presence of another unknown factor that exists on rod outer segment membranes [55–57]. There is also indirect evidence that the L-type Ca²⁺ channel, a candidate effector for $G_o\alpha$, can stimulate the GTPase activity of this G-protein [58–60].

Recently, a member of the RGS family (which stands for ‘regulators of G-protein signalling’), termed RGS10, has been shown to stimulate the GTPase activity of $G_{12}\alpha$ [61]. The GAP activity of RGS proteins is not limited to PTX-resistant G-proteins, however. In fact, RGS10, GAIP and RGS4 have all been shown to act as GAPs specifically for members of the G_i subfamily, of which $G_{12}\alpha$ is the only PTX-resistant member [61–63]. While the physiological function of the RGS family of proteins is unknown, genetic evidence from *Saccharomyces cerevisiae* and *Candida elegans* indicates that these proteins are involved in the attenuation of G-protein signalling processes (see [64] for a review). The existence of GAPs for both PTX-resistant

Table 1 Comparison of the biochemical properties of mammalian G-proteins

First-order rate constants were determined in the cited studies. N.T., not tested.

Subfamily	G α	Nucleotide binding, hydrolysis [44,45,47,49,69,231]		PKC substrate [70,71,75,77]	Toxin substrate	Attached lipid(s)†	Distribution
		k_{off} (min ⁻¹)	k_{cat} (min ⁻¹)				
G _i	G ₂	0.02 (30 °C)	0.05 (30 °C)	Very good	None	S-Palmitoyl, <i>N</i> -myristoyl	Brain, adrenal medulla, platelets
	G _{i1}	0.026 (30 °C)	2.4 (20 °C)	Poor	PTX	S-Palmitoyl, <i>N</i> -myristoyl	Nearly ubiquitous
	G _{i2}	0.072 (30 °C)	2.7 (20 °C)	Poor	PTX	S-Palmitoyl, <i>N</i> -myristoyl	Ubiquitous
	G _{i3}	0.025 (30 °C)	1.8 (20 °C)	N.T.	PTX	S-Palmitoyl, <i>N</i> -myristoyl	Nearly ubiquitous
	G ₀	0.19 (20 °C)	2.2 (20 °C)	Poor	PTX	S-Palmitoyl, <i>N</i> -myristoyl	Brain, others
	G _t	*	3.6 (22 °C)	Poor	PTX, CTX	<i>N</i> -Myristoyl and variants	Retina
G _s	G _s (45 kDa)	0.16 (20 °C)	-3.5 (20 °C)	Poor	CTX	S-Palmitoyl	Ubiquitous
G ₁₂	G ₁₂	0.01 (30 °C)	0.1–0.2 (30 °C)	Very good	None	S-Palmitoyl	Ubiquitous
	G ₁₃	0.01–0.02 (30 °C)	>0.2 (30 °C)	Poor	None	S-Palmitoyl	Ubiquitous
G _q	G _q	*	0.8 (30 °C)	N.T.	None	S-Palmitoyl	Nearly ubiquitous

* These G-proteins will not appreciably exchange nucleotide in the absence of an activated receptor.

† Fatty acyl chains other than palmitate can also be found in the thioester linkage.

Table 2 Comparison of selected sequences and function of mammalian G-proteins

The numbers listed for the G α -sequences represent the positions of the amino acids in that protein. The C in large print in the C-terminal sequences of the G_i proteins is the cysteine residue that is the site for PTX-catalysed ADP-ribosylation. 'GAGES' refers to the motif in the first nucleotide-binding domain of G-proteins. Abbreviations and symbols: AC, adenylate cyclase; PDE, phosphodiesterase; ↓, inhibits; ↑, stimulates.

Subfamily	G α	Signalling	'GAGES' sequence	C-terminal sequence	Refs.
G _i	G ₂	↓AC, ?other	GTSN SGK STI (40–49)	IQNNLK YIG LC (345–355)	42,47,123
	G _{i1}	↓AC	GAGESG K STI	IKNNLK D CGLF	232,233
	G _{i2}	↓AC	GAGESG K STI	IKNNLK D CGLF	233
	G _{i3}	↓AC	GAGESG K STI	IKNNLK D CGLY	233
	G ₀	↓Ca ²⁺ channels, others	GAGESG K STI	IKNNLR D CGLY	234,237
	G _t	↑cGMP-PDE	GAGESG K STI	IKENLK D CGLF	236,235
G _s	G _{gust}	↑PDE	GAGESG K STI	IKENLK D CGLF	239,240
	G _s	↑AC	GAGESG K STI	QRMHL PQ YELL	232,241
	G _{olf}	↑AC	GAGESG K STI	QRMHL PQ YELL	242
G ₁₂	G ₁₂	?Stimulate cell growth	GAGESG K STF	LQENLK D IMLQ	39,109
	G ₁₃	?Stimulate cell growth	GAGESG K STF	LHDNL K QLMLQ	39
G _q	G _q	↑PLC- β	GTGESG K STF	LQLNL K EYNLV	38,96,99,100
	G ₁₁	↑PLC- β	GTGESG K STF	LQLNL K EYNLV	38
	G ₁₄	↑PLC- β	GTGESG K STF	LQLN R EFNLV	40
	G ₁₅	↑PLC- β	G P GESG K STF	LARYL D EINLL	40
	G ₁₆	↑PLC- β	G P GESG K STF	LARYL D EINLL	41

and -sensitive trimeric G-proteins suggests that, *in vivo*, modulation of GTPase activity allows the cell another means by which to regulate G-protein signalling.

Basal rates of nucleotide exchange are also distinctive for the PTX-resistant G-proteins; this is especially true for G α . Nucleotide exchange on G α is very slow even at optimal (low micromolar) Mg²⁺ concentrations ($k = 0.02 \text{ min}^{-1}$ at 30 °C), and this rate is suppressed to nearly zero by millimolar Mg²⁺ concentrations [44]. In contrast, high Mg²⁺ concentrations stimu-

late nucleotide exchange on many other G-proteins [1]. The mechanism by which Mg²⁺ suppresses nucleotide exchange on G α is not clear, but it is known that the effect is exerted at the GDP-dissociation step [44]. The suppression of nucleotide exchange by Mg²⁺ seen with G α is qualitatively similar to that seen for Ras proteins [65]. It has also been shown that unsaturated lipids such as arachidonate suppress nucleotide exchange on G α [66]. The IC₅₀ for each lipid corresponds to its critical micellar concentration. Available data indicate that the mechanism of this suppression involves the binding of the negatively charged surface of the acidic lipid micelles to the nucleotide-free form of G α , which is then irreversibly inactivated. This type of protein–lipid interaction is not without precedent. In Ras signalling, unsaturated acidic lipids inhibit the GTPase activation of Ras *in vitro* by binding to its GAP [67,68]. While this lipid effect on nucleotide exchange is specific for G α among heterotrimeric G-proteins examined to date, its biological significance remains to be established. The nucleotide-exchange properties of the other PTX-resistant G-proteins are also somewhat distinctive. G₁₂ α and G₁₃ α exchange nucleotides more slowly ($k = 0.01\text{--}0.02 \text{ min}^{-1}$) than most other G-proteins (see Table 1) [47,48], and G_q α will not exchange nucleotides *in vitro* in the absence of an activated receptor [49]. The latter property of G_q α is not unique to that protein, however, As G_i α also exchanges guanine nucleotides poorly in the absence of its receptor, rhodopsin [69].

Another potentially important biochemical property of at least two PTX-resistant G-proteins is their ability to be phosphorylated. G α and G₁₂ α have been shown to be excellent *in vitro* substrates for protein kinase C (PKC) [70,71]. Members of all three major subtypes of PKC (i.e. classical, calcium-independent and atypical) are able to phosphorylate these two G-proteins [71]. Activation of PKC in intact platelets by agents such as thrombin, thromboxane A₂ (TXA₂) analogues and phorbol esters leads to rapid and near-stoichiometric phosphorylation of G α [70,72]. The primary site of G α -phosphorylation has been mapped by both protein chemistry and mutational analysis to Ser-27 near the N-terminus [70,73]. While the biological significance of this phosphorylation is unclear, phosphorylation of both G α and G₁₂ α blocks their interaction with G $\beta\gamma$ [71,74], suggesting that this phosphorylation is a

Figure 1 Model for the potential roles of α subunit phosphorylation in signalling by G_z and G_{12}

Simultaneous activation of G_z/G_{12} - and PKC-controlled processes could result in rapid modulation of signalling through these G-proteins by the following mechanism. Activation of either G-protein by an appropriate agonist (A)-bound receptor (R) would result in GTP binding and subsequent subunit dissociation. The GTP-bound α subunit would then interact with an as-yet-unidentified effector (E). The intrinsic GTPase activity of the α subunit, which could be increased by the interaction between it and the effector, would convert it into the GDP-bound form that would dissociate from the effector. Once dissociated, the α subunit could be readily phosphorylated by activated PKC. The phosphorylated α subunit is rendered incapable of reassociating with $G\beta\gamma$, and a subsequent interaction with the activated receptor is prevented. This could result in either attenuation of signalling through the G-protein or potentiation of signalling by creating a pool of free $G\beta\gamma$, or both.

regulatory mechanism for attenuating signalling through these $G\alpha$ subunits by preventing subunit reassociation (Figure 1). Alternatively, the prevention of subunit reassociation could potentiate signalling by creating a pool of free $G\beta\gamma$ that could continue to modulate effector activity. Several PTX-sensitive G-proteins have also been reported to be substrates for PKC-catalysed phosphorylation [75–80]. Although the available data are not as convincing as for $G_z\alpha$ and $G_{12}\alpha$, these observations suggest that phosphorylation might be a general mechanism for regulating G-protein activity.

A biochemical property that PTX-resistant G-proteins share with other G-proteins is their covalent modification by lipids. Like most other $G\alpha$ proteins identified to date, PTX-resistant G-proteins can be modified by palmitate, a 16-carbon saturated fatty acid [81–83]. Palmitate is post-translationally linked to one or more cysteine residues near the N-terminus through a thioester linkage; this modification is reversible and may be regulated [84]. Mutational analyses with $G_q\alpha$ (as well as $G_s\alpha$) have suggested that palmitoylation is essential both for membrane association and for signalling function [61,82]. However, other data have indicated that it is not the palmitate but rather the cysteine residues that serve as the site for palmitoylation in $G_q\alpha$ and that are critical for function [85]. For $G_z\alpha$, palmitoylation may not be required for signalling, since an altered form that has a cysteine-to-alanine mutation at the palmitoylation site appears to inhibit adenylate cyclase more efficaciously than wild-type $G_z\alpha$ when transfected into HEK 293 cells [86]. The potential roles of palmitoylation in G-protein signalling have been reviewed [87–90]. In addition to being palmitoylated, $G_z\alpha$ is, like the other members of the G_i subfamily to which it is related, co-trans-

lationally modified by myristate, a 14-carbon saturated fatty acid that is covalently attached to an N-terminal glycine residue through an amide linkage. This stable modification is critical for membrane localization; myristoylation also increases the affinity of G_i family members for $G\beta\gamma$ subunits [91–95].

SIGNALLING THROUGH PTX-RESISTANT G-PROTEINS

Since the initial observation that many G-protein-dependent signalling processes are PTX-resistant, a number of investigators have focused their attention on not only identifying the PTX-resistant G-proteins but also characterizing the specific signalling pathways in which each participates. The following discussion summarizes the various approaches of investigation, the results of which have significantly enhanced our understanding of the signalling functions of these G-proteins.

G_q signalling

Signalling through G_q family G-proteins has been well characterized over the past 5 years, beginning with the observation that $G_q\alpha$ directly stimulates PLC- β isoenzymes in *in vitro* assays [96–98]. Other members of the G_q subfamily (see Table 2) can regulate PLC- β activity as well, although there are differences among the members of this subfamily in the potency and specificity for the various PLC isoenzymes [99–102]. In addition, it appears that $G_{15}\alpha$ and $G_{16}\alpha$ can be activated by a greater variety of receptors than can $G_q\alpha$, $G_{11}\alpha$ or $G_{14}\alpha$ [103]. G_q -subfamily-mediated regulation of PLC- β activation has been reviewed elsewhere [104,105]. While there are also several reports

that link G_q activation to the mitogen-activated protein (MAP) kinase cascade, available evidence suggests that the involvement is indirect, i.e. via PLC activation [106–108].

G_{12} signalling

The targets of the G_{12} subfamily members are not well defined, and signalling through these proteins has been the subject of a recent mini-review [109]. The thrombin receptor has been shown to activate both $G_{12}\alpha$ and $G_{13}\alpha$ in platelet membranes [110]. One functional consequence of the activation of G_{12} is modulation of Na^+/H^+ exchange [111,112]. Transfection of COS-1 cells with mutationally activated G_{12} or G_{13} stimulated amiloride-sensitive Na^+/H^+ exchange 2–5-fold [111]. Stimulation of Na^+/H^+ exchange has also been demonstrated in cells transfected with either mutationally activated $G_{13}\alpha$ or a $G_{13}\alpha$ – $G_{12}\alpha$ -chimaera that could be activated by G_i -coupled receptors [112]. Recent evidence suggests that $G_{13}\alpha$ stimulates Na^+/H^+ exchange through both Cdc42/MEKK- and RhoA-dependent pathways [113]. In addition, the finding that the bradykinin-mediated inhibition of a voltage-dependent calcium current could be blocked by the intracellular application of $G_{13}\alpha$ -specific antisera suggests an involvement in this process [114].

G_{12} proteins have also been implicated in pathways that regulate cell growth. Expression of constitutively activated $G_{12}\alpha$ in 1321 astrocytoma cells increased AP-1-mediated transcriptional activation [115]; thrombin-stimulated DNA synthesis was also shown to be G_{12} -dependent by blocking this effect with G_{12} -specific antisera [115]. Mutationally activated $G_{12}\alpha$ and $G_{13}\alpha$ have potent transforming potential in NIH 3T3 and Rat 1 fibroblasts [116–119]. The mechanism for this is unclear, as no disturbances in cAMP, cytosolic Ca^{2+} , phosphotyrosine-containing proteins, PLC activity or phospholipase D activity were noted in cells transformed with mutant $G_{12}\alpha$ [118]. These data were corroborated by the observation that neither purified $G_{12}\alpha$ nor purified $G_{13}\alpha$ influenced the activities of adenylate cyclase (types I, II or V), PLC (β_1 , β_2 , β_3 or δ -1), phospholipase D or phosphoinositide 3-kinase in *in vitro* assays [47,48]. An increase in phospholipase A_2 activity was observed in $G_{12}\alpha$ -transformed cells, but there is as yet no evidence that $G_{12}\alpha$ directly activates this enzyme.

Other recent studies have implicated G_{12} subfamily members in a number of diverse cellular processes. Transfection of mutationally activated $G_{12}\alpha$ or $G_{13}\alpha$ into NIH 3T3 cells activated the Jun kinase (JNK)/stress-activated protein kinases in a Ras-dependent manner [120]. In contrast, a recent report has found that JNK activation by $G_{13}\alpha$ was Ras-independent [113]. The involvement of G-protein-coupled receptors in regulating cell responses through interactions with small G-proteins and JNK has recently been reviewed [121]. G_{12} proteins have also been linked to the regulation of the actin cytoskeleton. Mutationally activated G_{12} proteins induce actin polymerization and focal adhesion when transfected into Swiss 3T3 cells [122]. This phenomenon was blocked by botulinum toxin, suggesting that the response was dependent on the small G-protein Rho. $G_{12}\alpha$ and $G_{13}\alpha$ may also be involved in thrombin and thromboxane A_2 signalling in platelets, as both G-proteins showed increased incorporation of azidoanilido-GTP in response to these agonists [110].

G_z signalling

The limited pattern of expression of G_z and its distinguishing biochemical properties (see above) make identification of its signalling function an intriguing question. As with the G_{12} family, much of what is known about G_z signalling has been

Table 3 Effectors and receptors linked to G_z

These receptors have been demonstrated to activate G_z . The methods employed (generally transfection or reconstitution) are described in the text. Abbreviations and symbols: A, adrenergic; AC, adenylate cyclase; Ad, adenosine; D, dopamine; 5HT, 5-hydroxytryptamine; fMLP, *N*-formylmethionyl-leucylphenylalanine; i.c.v., intracerebroventricular; LPA, lysophosphatidic acid; M, muscarinic; melatonin; O, opioid; -R, receptor; N.R., not reported; ↓, inhibits; ↑, stimulates.

Response	Receptor or agonist	Method	Cell or tissue	Refs.
↓AC (endogenous)	D ₂ -D-R, α_2 -AR, A ₁ -AdR, C5a-R, fMLP-R, LPA-R, Mel-R, δ -O-R, κ -O-R	Transfection	HEK 293, NIH 313	123,127,129
↑AC-II (via $G\beta\gamma$ release)	μ -O-R, fMLP-R, Mel-R	Transfection	HEK 293	129–131
↑PLC (via $G\beta\gamma$ release)	δ -O-R	Transfection	Ltk ⁻ fibroblasts	125
Anti-nociception	μ -O-R	I.c.v. injection	Rat brain	136
NR	M ₂ -M-R	I.c.v. injection	NR	133
NR	5HT-1A-R	Co-infection	Sf9 cell membranes	134

uncovered from heterologous expression studies in intact cells. Co-transfection of $G_z\alpha$ into HEK 293 cells with D₂-dopamine, α_2 -adrenergic, A₁-adenosine or lysophosphatidic acid receptors produces PTX-resistant inhibition of stimulated adenylate cyclase upon activation of these receptors [123]. Furthermore, transfection of cells with mutationally activated $G_z\alpha$ (Q205L) produced constitutive inhibition of adenylate cyclase [123]. The ability of $G_z\alpha$ to inhibit adenylate cyclase directly was demonstrated by *in vitro* reconstitution experiments in which guanosine 5'-[γ -thio]triphosphate-liganded $G_z\alpha$ inhibited types I and V adenylate cyclases [47]. It is interesting to note that, like $G_z\alpha$, the type I isoform of adenylate cyclase is most highly expressed in the nervous system; however, expression of type V is found in heart, brain and other tissues (see [10,124] for reviews).

A variety of co-transfection studies have indicated that G_z can couple to a number of receptors that ordinarily activate G_i proteins. In a series of articles, Wong and colleagues have co-transfected $G_z\alpha$ along with a number of receptors into immortalized cell lines and measured the participation of G_z by assessing PTX-insensitive inhibition of adenylate cyclase in the transfectants [125–131]. In another study, Bourne and co-workers demonstrated that C-terminal five amino acids of $G_z\alpha$ are sufficient to specify the interaction of $G_z\alpha$ with G_i -coupled receptors [134]. The results from many separate studies that demonstrate the activation of $G_z\alpha$ by various receptors are summarized in Table 3. Collectively, this work clearly demonstrates that G_z can interact with receptors and can couple these receptors to inhibition of a particular effector, namely adenylate cyclase.

Other approaches have linked G_z to G_i -coupled receptors. Parker et al. [133] found that, when recombinant $G_z\alpha$ was reconstituted into phospholipid vesicles with M₂-muscarinic receptors, muscarinic agonists could stimulate nucleotide exchange on $G_z\alpha$. Also, the co-expression of $G_z\alpha$ and 5HT-1A receptors in Sf9 cells imparts guanosine 5'-[β,γ -imino]triphosphate sensitivity to the binding of a variety of agonists of the receptor [134]. These co-expression experiments were also performed with other members of the G_i family ($G_{i1}\alpha$, $G_{i2}\alpha$, $G_{i3}\alpha$ and $G_{o}\alpha$), each of which coupled to the 5HT-1A receptor equally well. Thus, from this work and the co-transfection experiments, it appears that G_i family members are similar *in vitro* with regard to their ability to interact with similar receptors.

G_{α} has been linked to inositol phosphate production; however, the studies involved did not distinguish between direct stimulation of PLC by G_{α} and indirect stimulation by release of a pool of $G\beta\gamma$ [125]. In another study, injection of rat GH3 cells with a mixture of antisense oligonucleotides corresponding to coding regions within G_{α} , $G_{11\alpha}$ and G_{α} attenuated the ability of the cells both to produce inositol phosphates in response to thyrotropin-releasing hormone (TRH) and to open L-type Ca^{2+} channels in response to a second challenge by TRH [135]. Inhibitors of PKC also blocked the channel activation induced by a second application of TRH. This approach, however, did not distinguish between effects mediated by suppression of any one of these three G-proteins.

G_{α} signalling has also been examined in whole animals. Administration of G_{α} antisense oligodeoxynucleotides to rats via intracerebroventricular injection for 5 days resulted in a reduction in the expression of G_{α} in periaqueductal grey matter (40%), striatum (41%), thalamus (16%) and hypothalamus (54%), as estimated by immunoblot analysis [136]. These animals displayed a relative diminution of anti-nociceptive effects induced by μ -, but not δ -, opioid agonists. Similar results were reported from a series of studies in which antisera directed against G_{α} were administered by intracerebroventricular injection to rats [137–139]; however, the mechanism by which injection of antisera might affect the expression of G_{α} is unclear.

G_{α} , like the G_{12} proteins, may be involved in signalling that regulates cell growth. Transfection of Swiss 3T3 cells with the constitutively active Q205L variant of G_{α} showed that the presence of this mutant G_{α} induced a transformed phenotype, i.e. showing a faster growth rate, focus formation, anchorage-independent growth in soft agar and increased incorporation of [3 H]thymidine in the absence of growth factors [140]. Transfection of this activated G_{α} into NIH 3T3 cells also produced an altered phenotype, although the alterations were less extensive. Changes in adenylate cyclase activity were not detected in the transformed cells, and the mechanism by which G_{α} transformed these cells was not discerned. This ability of constitutively active G_{α} to transform cells is similar to that seen with the related PTX-sensitive protein $G_{1\alpha 2}$ [141]. Mutationally activated $G_{1\alpha 2}$ (R179C) has been detected in human ovarian and adrenal cortical tumours [142]; additionally, similar mutations of G_{α} have been found in human pituitary and thyroid tumours [144]. It is possible, then, that mutations in G-protein α subunits, including G_{α} , may be involved in human carcinogenesis (see [143] for a review). However, in a limited search of human endocrine tumours, mutations in G_{α} have not been found [144].

ADDITIONAL PTX-RESISTANT PATHWAYS THAT ARE CANDIDATES FOR REGULATION BY G-PROTEINS

The following discussion of signalling pathways in mammalian cells will focus on native signalling in platelets and neurons, since these tissues express not only the nearly ubiquitous G_i and G_{12} family proteins, but also G_{α} . Many of the agonists mentioned activate multiple pathways, and the predominant mode of action of many is through activation of PTX-sensitive G-proteins; however, the discussion will be limited to evidence for guanine-nucleotide-sensitive, PTX-resistant (and CTX-resistant) events. In many of the pathways discussed, the identity of the G-protein involved has not been determined. Many of the data that are discussed are summarized in Table 4. Those signalling events that are also influenced by activation of PKC will be highlighted, since, as discussed above, PKC phosphorylates G_{α} and $G_{12\alpha}$, and this modification may play a role in down-regulating or otherwise modulating signalling through these G-proteins [71,74].

Table 4 Pertussis toxin-resistant pathways in representative tissues

This table summarizes some PTX-resistant signalling pathways in neurons and several other cells that express G_{α} . The cell lines listed are as follows: NG108-15, SHSY5Y and SH-EP, derived from neuroblastoma; PC12, derived from pheochromocytoma; GH3 and 7315c, derived from pituitary tumour; RBL-2H3, derived from mast cells; HEL, human erythroleukaemia. Abbreviations used: AA, arachidonic acid; ATII, angiotensin II; α_1 - or α_2 -AR, adrenergic receptor; BK, bradykinin; CCK, cholecystokinin; ET, endothelin; EP, prostaglandin E; 5HT $_2$ - or 5HT $_3$ -R, 5-hydroxytryptamine (serotonin) receptors; GABA γ -aminobutyric acid; M muscarinic; NK1, neurokinin 1; NT, neurotensin; PAF, platelet-activating factor; P $_{2Y}$ -R, purinergic receptor $_{2Y}$; -R, receptor; SP substance P; $\uparrow$, stimulates; $\downarrow$, inhibits.

Response	Receptors	Cell or tissue	Refs.
$\uparrow$ PLC	mGluR, 5-HT-R, NK-3-R, α_1 -AR	Purkinje cells; dorsolateral septal, cerebral cortical, hippocampal, myenteric AH and hypothalamic neurons	162,168,170,174, 176,177,185
$\uparrow$ PLC	BK-R, ET-R, ATII-R, M-R, P $_{2Y}$ -R	SH-EP, SHSY5Y, NG108-15 cells	179–183
$\uparrow$ PLC	Thrombin R*, TXA $_2$ -R	Platelets	150–152,154,155
$\uparrow$ [Ca $^{2+}$] (via PLC?)	Thrombin R, PAF-R, P $_{2Y}$ -R	HEL cells	160
$\uparrow$ PLC	CCK-R	Substantia nigra neurons	175
$\uparrow$ AA release	α_1 -AR	Spinal cord neurons	210
$\uparrow$ PLC	P $_{2Y}$ -R, BK-R, NT-R	Superior colliculus, dorsal root ganglion, substantia nigra neuron	171–173
$\uparrow$ PLC	THR-R, EP-R, M-R, IgE-R	Pituitary lactotrophs, GH3 cells, 7315c cells, chromaffin cells, PC12 cells, RBL-2H3 cells	211,212,215–218, 224,227,229
$\uparrow$ K $^{+}$ channel	P $_{2Y}$ -R	Inferior colliculus, hippocampal and striatal neurons	205–209
$\downarrow$ K $^{+}$ channel	SP-R, M-R	Nucleus basalis and pyramidal neurons	204–208
$\downarrow$ VDCC	M-R† α_2 -AR†, SP-R (NK1-R)	Sympathetic and hippocampal neurons	198–201

* The sensitivity of thrombin stimulation of PLC to PTX has varied in the hands of different investigators.

† The inhibition of VDCC upon stimulation of the muscarinic receptor and α_2 -adrenergic receptor was partially resistant to PTX.

Platelets

Signalling in platelets has been extensively studied, and several PTX-resistant, G-protein-dependent, pathways have been identified (see [145] for a review). Platelets are a particularly good place to search for signalling events that potentially involve PTX-resistant G-proteins, because they express members of the G_i and G_{12} subfamilies, as well as high levels of G_{α} (~20–25 pmol/mg of membrane protein). However, data from studies involving PTX treatment of platelets can be difficult to interpret, because these cells are relatively resistant to treatment with the toxin [145]. Consequently, conclusions regarding the involvement of G-proteins in platelet signalling have often been conflicting.

One pathway that has been identified in most cases as being PTX-resistant in platelets is the PLC/inositol phosphate pathway [146]. A number of agonists activate this pathway, but two agonists, thrombin and thromboxane A $_2$ (TXA $_2$), are especially interesting, since treatment of platelets with these agents has been shown to stimulate the PKC-dependent phosphorylation of G_{α} [72]. Thrombin initiates several signalling cascades in platelets, including the PLC/inositol phosphate pathway [147]. The addition of thrombin stimulates GTPase activity in platelet membranes that is partially resistant to PTX [148,149]. Thrombin stimulates PLC-dependent production of inositol phosphates, but the sensitivity of this process to PTX varies [150–152]. Treatment of platelets with TXA $_2$ analogues results in PTX-

resistant phosphoinositide turnover and does not influence the PTX-catalysed ADP-ribosylation of G_i [151,152]. Interestingly, TXA_2 has also been shown to inhibit adenylate cyclase, but this observation has not been made by others, and the sensitivity of this phenomenon to PTX was not documented [153].

The activation of PKC has been demonstrated to perturb signalling through thrombin and TXA_2 . Pretreating platelets with PKC-activating phorbol esters results in the inhibition of PTX-resistant increases in intracellular calcium (presumably from increased inositol phosphate production) in response to either thrombin or TXA_2 [154,155]. One group correlated this PKC-dependent inhibition with the concomitant phosphorylation of G_{i2} , thus implicating this G-protein in thrombin and TXA_2 signalling [155]. However, this does not preclude the involvement of the PTX-resistant G-proteins, particularly since, as mentioned above, activation of the PLC/inositol phosphate cascade by thrombin and TXA_2 has been shown to be, at least in part, PTX-resistant. Recent studies have demonstrated the direct involvement of some of the PTX-resistant G-proteins in this pathway. Affinity-purified antisera directed against the C-terminus of $G_{q\alpha}$ inhibited thrombin-stimulated GTPase activity in platelet membranes, while a G_z -specific antiserum had little effect [156]. Similar studies with TXA_2 in platelet membranes showed identical results, i.e. a G_z -specific antiserum, but not one directed against G_z , inhibited TXA_2 -stimulated GTPase activity [157]. Also, using the photoreactive GTP analogue azidoanilido-GTP, it was demonstrated that thrombin and TXA_2 can activate not only the PTX-resistant $G_{q\alpha}$ but also $G_{12\alpha}$ and $G_{13\alpha}$ in platelet membranes [110]. If either agonist had activated the other PTX-resistant G-protein, i.e. $G_{z\alpha}$, this method would probably have been unable to detect it, since $G_{z\alpha}$ does not bind azidoanilido-GTP appreciably [168].

Thrombin has also been linked to PTX-resistant signalling in HEL cells, which are related to platelets in that they share phenotypic characteristics with megakaryocytes [159] and express low levels of $G_{z\alpha}$ (T. A. Fields and P. J. Casey, unpublished work). Treatment of these cells with thrombin generated a PTX-resistant increase in intracellular calcium [160]. This effect was also seen with platelet-activating factor, ATP and prostaglandin I_2 analogues. However, these data are difficult to interpret, since Brass et al. [161] showed that the increase in intracellular calcium in HEL cells in response to thrombin was sensitive to PTX.

Neurons

There are number of signalling pathways in neurons that are resistant to PTX treatment. As with platelets, one PTX-resistant pathway that has been characterized is the PLC/inositol phosphate cascade. Metabotropic glutamate receptors (mGluRs) have been implicated in this pathway in several specific areas of the mammalian nervous system. In Purkinje cells, mGluR agonists stimulated a PTX-resistant, inositol phosphate-dependent increase in intracellular calcium [162]. These cerebellar neurons express high levels of $G_{z\alpha}$ [163] and of mGluRs, especially mGluR1 [164,165]. The expression and signalling of the various mGluR subtypes has been reviewed elsewhere [166]. Interestingly, in cerebellar granule neurons, which do not express $G_{z\alpha}$, mGluR agonists generated increases in intracellular calcium that were PTX-sensitive [167]. Zheng and Gallagher [168,169] demonstrated that treatment of neurons from the rat dorsolateral septal nucleus with mGluR agonists evoked a slow, PTX-resistant, depolarization that was probably dependent on PLC activation. mGluR agonists also suppressed neurotransmitter release at nerve terminal autoreceptors via the PLC/inositol phosphate pathway, and this suppression was partially PTX-

resistant [170]. Other agonists have been shown to activate a PTX-resistant PLC/inositol phosphate response in neurons, including adenosine [171], bradykinin [172], neurotensin [173], neurokinin [174], cholecystokinin [175] and 5-hydroxytryptamine [176,177]. Additionally, agonists of the D_2 dopamine receptor can activate a PTX-resistant PLC/inositol phosphate response in NG108 cells when the receptor is transfected into them [178], demonstrating that this receptor is capable of coupling to PTX-resistant G-proteins.

PKC activation has been shown to play a role in many of these pathways. In the neuroblastoma SHSY5Y cell line, muscarinic, bradykinin, endothelin and angiotensin II receptor stimulation generated phosphoinositide turnover that was insensitive to PTX, and the response was inhibited by prior PKC activation with phorbol esters [179,180]. In another neuroblastoma cell line, NG108-15, similar effects were seen with bradykinin receptor, purinoreceptor (P2) and endothelin-1 receptor stimulation. The responses were inhibited by PKC activation; the rapid desensitization was shown to involve both receptor-related and post-receptor mechanisms [181–184]. A similar phenomenon was observed in cultured neurons, i.e. PKC activation attenuated the α_1 -adrenergic stimulation of phosphoinositide turnover [185]. However, the stimulation of PLC in this example was only partially resistant to PTX. The 5-hydroxytryptamine-mediated stimulation of phosphoinositide turnover mentioned above evoked an outward current in hippocampal neurons that was blocked by prior treatment with phorbol 12-myristate 13-acetate [176]. The effect, however, was not consistently observed. The PKC-dependent phosphorylation of G-proteins may be involved in this down-regulation, but other downstream targets for PKC may also be important, as was demonstrated for neurotensin activation of PLC [186].

Since members of the G_q family of G-proteins are known to activate PLC directly [96–98,102], these G-proteins have been assumed to be involved in neuronal PTX-resistant PI-PLC activation. However, experiments examining the identity of the G-protein involved in these pathways have not been conclusive. One of the receptors, mGluR1, has been shown to activate PLC via G_{11} and G_{14} when expressed in *Xenopus* oocytes [187]; however, Ueda et al. [188] demonstrated that mGluR1 could also activate PLC via G_i in *Xenopus* oocytes (probably by release of $G\beta\gamma$). Gutowski et al. [189] demonstrated that an antiserum that recognizes the C-terminus of members of the G_q family attenuated bradykinin-stimulated phosphoinositide turnover in NG108-15 cell members; $G_{z\alpha}$ -specific antisera were not tested. Kwatra et al. [190] demonstrated that the substance P receptor, which has been implicated in PTX-resistant PLC activation, couples to $G_{q\alpha}$ when reconstituted in phospholipid vesicles. These data support the idea that members of the G_q family of G-proteins are involved in neuronal phosphoinositide turnover, but do not exclude the possibility that other PTX-resistant G-proteins may be involved as well.

PTX-resistant G-proteins have also been implicated in the regulation of a number of neuronal ion channels (see Table 4). The G-protein-dependent regulation of ion channels is a complex topic that has been reviewed elsewhere [13,14]. Membrane-delimited G-protein regulation of channels, i.e. regulation that is independent of soluble intermediates, is often interpreted as evidence of a direct interaction between the channel and a G-protein, although there is little solid evidence for truly direct interactions [14]. The following examples will refer to membrane-delimited regulation.

Although G-protein regulation of voltage-dependent Ca^{2+} channels (VDCC) is generally PTX-sensitive [191–197], several reports have demonstrated the involvement of PTX-resistant G-

proteins in the inhibition of these channels. Treatment of sympathetic neurons with muscarinic, substance P or α_2 -adrenergic receptor agonists inhibited VDCC in a complex, partially PTX-resistant, G-protein-dependent, manner [198–200]. There is also a report of glutamate agonists eliciting G-protein-dependent inhibition of VDCC in hippocampal neurons [201]. This inhibition was resistant to PTX, but the control in this experiment (acetylcholine) also elicited PTX-resistant inhibition under the conditions of the experiment, so the results are difficult to interpret. The PTX-resistant inhibition of VDCC may also be influenced by PKC activation. Swartz et al. [202,203] found that glutamate-, γ -aminobutyric acid, adenosine-, muscarinic-, α -adrenergic- and luteinizing-hormone-releasing-hormone-mediated inhibition of VDCC was attenuated by PKC activation. Although PTX sensitivity was not assessed in these experiments, as noted above, glutamate and α -adrenergic agonist-mediated inhibition of VDCC is partially PTX-resistant.

Neuronal K^+ channels can also be influenced by PTX-resistant G-proteins. Several groups have demonstrated that substance P-mediated inhibition of K^+ channels is PTX-resistant [199,204]. A purine receptor agonist evoked a PTX-resistant potassium current in neurons from the hippocampus, striatum and inferior colliculus [205,206]. The current in the striatum, however, was probably the result of indirect activation, since PKC inhibitors blocked the response to purinergic agonists [207]. Muscarinic stimulation of pyramidal neurons suppressed a K^+ channel current and this response was resistant to PTX, while inhibition of VDCCs in response to the same agonist remained sensitive to PTX [208].

PTX-resistant G-proteins have been implicated in some other neuronal responses. One study showed that γ -aminobutyric acid-, 5-HT₃- and 5-HT₂-receptor-mediated stimulation of electrical activity in the rat medial prefrontal cortex was resistant to PTX [209]. The mechanism of this response was not determined. Additionally, the sensitivity of these responses to guanine nucleotides was not assessed. Also, Kanterman et al. [210] showed that stimulation of dissociated spinal cord neurons with α_1 -adrenergic agonists elicited PTX-resistant arachidonate release, while stimulation of PLC in this system was PTX-sensitive. The authors proposed that direct stimulation of phospholipase A₂ by a PTX-resistant G-protein might play a role.

Other tissues

There are several other tissues that express PTX-resistant G-proteins, including G_z α , in which PTX-resistant pathways have been identified. RBL-2H3 cells, which are derived from mast cells and which express low levels of G_z α (T. A. Fields and P. J. Casey, unpublished work), respond to antigen stimulation of the IgE receptor with increased phosphoinositide turnover in a PTX-resistant manner [211]. The participation of G_z α in this pathway was implied in further work, which correlated the dexamethasone-induced down-regulation of G_z α expression with an inability to respond to antigen stimulation [212]. In another study, stimulation of platelet-activity factor receptors transfected into these cells induced PTX-resistant phosphoinositide turnover [213]. However, signalling through the platelet-activating factor receptor in native haematopoietic cells is generally PTX-sensitive [214].

Several PTX-resistant signalling pathways have been investigated in pituitary cells. A PTX-resistant increase in phosphoinositide turnover in cultured pituitary cells in response to TRH was reported [215,216]. Similar results were observed on TRH stimulation of the pituitary tumour cell lines GH3 and 7315c [217,218]. As discussed above, there is evidence that TRH

stimulation of phosphoinositide turnover in GH3 cells could involve G_z α , but the experiments performed did not distinguish between G_q α and G_z α [135]. There is, however, one study directly implicating G_q α in TRH-stimulated PLC activation. Hsieh and Martin [219] blocked TRH-stimulated phosphoinositide turnover in membranes from GH3 cells by prior treatment with G_q α -specific antisera. Other work has implied the involvement of G_q family members in the pituitary PLC/inositol phosphate pathway by demonstrating that PLC activation down-regulates expression of these G-proteins [220]. Other experiments involving cells of pituitary origin demonstrated that dopamine can apparently inhibit growth through ill-defined mechanisms. Senogles [221] reported that dopamine D₂ agonist treatment of a pituitary cell line transfected with the D₂ receptor inhibited cell growth. The inhibition required PKC and was resistant to PTX treatment. These observations seem to contradict earlier work in which treatment of a similar cell line also inhibited DNA synthesis in a fashion that was sensitive to PTX [222]. PTX-resistant inhibition of adenylate cyclase in pituitary cells has also been reported. Treatment of pituitary cell membranes with adenosine led to inhibition of vasoactive intestinal peptide-stimulated adenylate cyclase, and this inhibition was unaltered by prior treatment with PTX [215]. However, adenosine can inhibit adenylate cyclase through an allosteric 'P' site on the enzyme, and the PTX-resistant inhibition in this study was attributed to this mechanism.

In the adrenal medulla, subtypes of prostaglandin E₂ receptors on chromaffin cells couple to a variety of second messenger pathways, some of which are PTX-resistant [223]. One important pathway for some subtypes of the receptor for prostaglandin E₂ is the PTX-resistant stimulation of phosphoinositide turnover in chromaffin cells [224,225]. Although the receptor has been co-purified and cross-linked with a PTX-resistant G-protein [226], subtypes of this receptor generally activate the PTX-sensitive G_i [223]. Multiple splice variants of this receptor probably account for its ability to couple to several different G-proteins [227].

The ability of chromaffin cells to secrete catecholamines is also regulated in part by G-proteins. Dahmer and Senogles [228] recently demonstrated that treatment of chromaffin cells with D₁- but not D₂-selective dopamine agonists evoked inhibition of secretion that was PTX-resistant. In PC12 cells, which are derived from a pheochromocytoma tumour of the rat adrenal medulla, activation of muscarinic receptors led to a PTX-resistant stimulation of PLC [229]. Muscarinic stimulation of phospholipase D was also PTX-resistant in this cell line [230].

CONCLUSIONS

The PTX-resistant G-proteins share some biochemical features that are highlighted in this review. We have attempted to summarize the substantial body of work that has emerged from investigations of the function of these G-proteins. Among the PTX-resistant G-proteins, signalling pathways are well understood only for the G_q subfamily. While signalling through G₁₂ is poorly understood, recent evidence linking this family of proteins to several pathways that regulate cell growth and differentiation has been presented. Other studies with G₁₂ family members implicate these proteins in regulating Na⁺/H⁺ exchange, activating the JNK/SAPK pathway, and influencing the actin cytoskeleton. What is known of signalling through the enigmatic G_z protein has also been discussed. Interestingly, mutationally activated G_z α , like activated G₁₂ α or G₁₃ α , can generate a transformed phenotype in some cells. Most of the work with G_z α has focused on heterologous expression with receptors in non-native conditions. These studies have demonstrated that G_z can be activated by a number of receptors, most of which normally

activate the related protein G_i , and that activated $G_z\alpha$ can inhibit at least two subtypes of adenylate cyclase. However, since $G_z\alpha$ is not a substrate for PTX, and since adenylate cyclase inhibition is generally PTX-sensitive *in vivo* or *in situ*, the question of whether G_z functions to inhibit adenylate cyclase in tissues remains an open one. Since $G_z\alpha$ and $G_{12}\alpha$ are both substrates for PKC, their signalling could be intimately linked to G_q signalling. A survey of PTX-resistant signalling pathways in tissues that express both $G_z\alpha$ and G_{12} family members suggests that a variety of receptors, as well as PLC and several ion channels, are potential components of these signalling systems.

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