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Commentary

GTP-binding proteins in cell survival and demise: the emerging picture in the pancreatic β-cell

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Abstract

It is widely believed that guanine nucleotide-binding regulatory proteins (G-proteins) play central roles as ''molecular switches'' in a variety of cellular processes ranging from signal transduction to protein and vesicle trafficking. To achieve these regulatory functions, G-proteins form complexes with a wide range of effector molecules whose activities are altered upon interaction with the G-protein. These effector molecules can be either soluble or membrane bound, and it is likely that some are localized to secretory granules where they direct the movement, docking, and fusion of granules during exocytosis. The effector molecules regulated by G-proteins are diverse and include phospholipases, protein kinases, protein phosphatases, ion channels, adenylate cyclases, cytoskeletal elements, as well as secretory vesicle and plasma membrane-associated fusion-proteins. The majority of studies performed in the pancreatic β-cell have focused on the role of G-proteins in the regulation of insulin secretion, whereas very little attention has been focused on their potential involvement in other cellular processes. Such studies have identified and implicated both heterotrimeric (comprising α, β, and γ subunits) and monomeric (low molecular mass) G-proteins in the regulation of insulin secretion, but intriguing recent evidence has also begun to emerge which favors the view that they may be involved in the maintenance of β-cell viability. In the present commentary, we will review this evidence and discuss the current understanding of the role of G-proteins in the life and death of the β-cell.

Keywords: G-proteins; Apoptosis; Pancreatic β-cell; Diabetes mellitus

1. Introduction

The expression of heterotrimeric and monomeric G-proteins in β-cells has been confirmed by a variety of methods including western blotting, immunocytochemistry, and reverse transcriptase–polymerase chain reaction. These approaches have revealed the presence of a wide range of members of the G-protein family including isoforms of G_i, G_o, G_s, G_{olf}, G_t, and G_q as well as Rab, Rac, Rho, and Cdc42 [1–3]. Functional studies have confirmed that at least some of these molecules are involved in the physiological control of insulin secretion [1–5], and it has been shown that depletion of intracellular GTP (*via* the use of specific inhibitors of inosine monophosphate dehydrogenase) leads to a marked reduction in the insulin secretory response, suggesting that this nucleotide plays a permissive role in nutrient-induced insulin secretion [6]. In addition, it has been well established that modulation of the activity of heterotrimeric G-proteins (by cholera and PTXs) is associated with disruption of signal transduction by cell surface receptors and results in altered patterns of insulin release [1–3]. Furthermore, the use of specific inhibitors of post-translational modification of G-proteins (e.g. lovastatin, an inhibitor of protein isoprenylation) has revealed a requirement for such modifications in the control of insulin secretion [7]. Finally, using a further range of specific bacterial toxins to selectively modify and inactivate monomeric G-proteins (see further), it has now been possible to assign specific roles to some of these in nutrient- and calcium-mediated insulin secretion [4]. Thus, there is abundant evidence that G-proteins are pivotal to the control of insulin secretion.

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Abbreviations: PTX, pertussis toxin; GEF, guanine nucleotide exchange factor; GRF, guanine nucleotide releasing factor; NO, nitric oxide; cAMP, cyclic AMP; cGMP, cyclic GMP; IL-1β, interleukin; MPA, mycophenolic acid; COX-2, cyclooxygenase-2; PDE, phosphodiesterase; PGE_2, prostaglandin E_2; PKB, protein kinase B; LPA, lysophosphatidic acid.

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2. Regulation of G-protein function

Knowledge about the regulation of G-proteins is still evolving, and the field has reached a bewildering level of complexity. One aspect of their biochemistry that has become clear, however, is that a major mode of regulation occurs at the level of GDP dissociation and that this, in turn, regulates the activation state of G-proteins. In the GDP-liganded form, G-proteins are essentially inactive, but they become activated upon dissociation of GDP in exchange for a molecule of GTP. In the case of trimeric G-proteins, this step is promoted by the binding of an agonist to the extracellular domain of a relevant G-protein-coupled receptor. However, since there is no evidence for direct activation of classical cell surface receptors by nutrient insulin secretagogues in β-cells, it follows that, if trimeric G-proteins are involved in the actions of these compounds, then soluble, intracellular regulators of GDP/GTP binding must be sought [1,8]. Some of these may be biologically active lipids, but there is also evidence that nitric oxide (NO) could be a diffusible regulator of G-proteins [9].

The activation of monomeric G-proteins is achieved by different means and is facilitated (or dampened) by a family of intracellular proteins that bind to and alter their ability to hydrolyze GTP and to interact with membranes and effectors [1]. These factors belong to the class of GTP/GDP exchange factors (GEFs), and they can operate either as GDP dissociation stimulators (GDSs; when they promote GDP release and facilitate GTP binding) or as GDP dissociation inhibitors (GDIs; when they inhibit GDP release). Other regulatory proteins include GTPase activating proteins (GAPs), which increase the rate of hydrolysis of GTP and thereby terminate the activation process [1]. Of these various peptide factors, few have been characterized in the β-cell. We have reported the localization of a putative guanine nucleotide exchange factor (GEF) in the β-cell and have also demonstrated the regulation of GEF activity by lipid mediators, including arachidonic acid, lysophosphatidic acid (LPA), and phosphatidic acid [10], although the identity of the G-protein regulated by this GEF has not been defined. In addition, the expression of specialized GEFs that may be involved in the control of Rab3 functionality [11] and the mediation of some effects of cyclic AMP (cAMP) [12] has also been reported. The presence of calcium-sensitive GEFs has also been observed and, by analogy with effects in other cells, it is likely that one of these, GRF-1, may negatively modulate the Ras signaling pathway [13]. Using western blot analysis, a GRF-1-like protein has been identified recently in HIT cells,[1] and this may provide a link between trimeric and monomeric G-proteins since Ras GRF-1 is regulated by the βγ subunits of trimeric G-proteins in some cells [14,15]. This finding is of particular importance since it suggests the existence of mechanisms that may allow the pathways regulated by trimeric and monomeric G-proteins to interact in β-cells. Since both may play critical roles in the regulation of cell viability (see further), it is possible that dysfunction of the pancreatic β-cell (mediated by cytokines, for example) could arise by disruption of the normal cross-talk between them. In this context, a provocative commentary by Hall [16] drew attention to the increasing range of mechanisms by which a level of cross-talk can be achieved between trimeric and monomeric G-proteins. This subject has been almost entirely overlooked in studies of β-cell function but could prove to be of critical importance for the control of cell death.

[1] Tannous M, Mattingly RR and Kowluru A, unpublished observation.

3. Role of G-proteins in the apoptotic demise of the β-cell

Cell death can occur by several mechanisms, but much research has shown that necrosis and apoptosis are the two principal forms [17–20]. Necrosis results from physical injury and occurs in an uncontrolled manner, whereas apoptosis is a deliberate and genetically controlled cellular response to specific stimuli. The characteristic features of apoptosis and necrosis are different. Necrosis is represented by cytoplasmic organelle destruction and loss of plasma membrane integrity, whereas apoptosis is associated with plasma membrane blebbing, loss of microvilli, chromatin condensation, and nuclear DNA fragmentation [17–20]. A variety of stress stimuli, such as TNFα, Fas ligand, growth factor withdrawal, anti-cancer agents, oxidative stress, heat shock, and exposure to ionizing radiation and ultraviolet light, are all known to be capable of inducing apoptosis [17–20]. In addition, the process can be induced in β-cells by exposure to certain cytokines, including those (e.g. interleukin-1β; interferon-γ) released by cells present in the islet infiltrates in type 1 diabetes [21–26].

During the course of our investigation of the roles of low molecular weight G-proteins in pancreatic β-cells, we observed that long-term inactivation of specific G-proteins (e.g. by inhibition of post-translational modification) resulted in apoptotic β-cell death [27]. Moreover, we also found that manipulation of the activation state of trimeric G-proteins could increase cell death [28,29]. Thus, it appears that islet G-proteins may play a role in the maintenance of cell viability, and it is possible that cytokine treatment could lead to selective disruption of one or more of these pathways. In this context, it is interesting to note that several recent reports have implicated the involvement of specific G-proteins in cytokine- or ceramide-mediated apoptosis in different cell types [30–50]. Very little is known about the regulatory roles of G-proteins in the apoptosis of pancreatic β-cells following exposure to cytokines, and we consider that this is a fertile area for further research. As a stimulus to this, the following sections will present an overview of the status of current

research concerning the involvement of G-proteins (trimeric and monomeric) in the apoptotic demise of the β-cell.

3.1. Heterotrimeric G-proteins as mediators of β-cell death

Heterotrimeric G-proteins have now been implicated in the control of cell viability in a range of cell types, although the pathways involved remain poorly characterized [51–55]. In the majority of cases, G-protein activation has been associated with an increase in cell death, suggesting the existence of G-protein-dependent pathways by which the execution phase of apoptosis is promoted. There is evidence that such a mechanism also operates in pancreatic β-cells since the global G-protein activator sodium fluoride (NaF) and the peptide activator mastoparan are cytotoxic to both clonal β-cells and to primary islet cells [28,55]. At least part of this cytotoxicity derives from induction of apoptosis, as evidenced by DNA fragmentation, chromatin condensation, and loss of phosphatidylserine asymmetry in the plasma membrane of NaF-treated β-cells [29]. Interpretation of these data, however, is complex since both agents exert multiple effects in cells. In the case of mastoparan, there is only minimal evidence to support a role for a G-protein in mediating an apoptotic mode of cell death, but for NaF the evidence is stronger. For example, the chelating agent desferrioxamine attenuates the response to NaF, consistent with G-protein involvement [28]. Desferrioxamine has a high affinity for Al^{3+} and chelates this ion very effectively, thereby preventing formation of the $[AlF_4]^-$ complex that is absolutely required for G-protein activation.

The factors involved in the cytotoxic activity of NaF may be several, but one response that occurs in a number of cell types is an elevation of cAMP levels, resulting from activation of G_s [56,57]. Thus, it is possible that β-cells treated with NaF may maintain elevated levels of cAMP and that this could be responsible for the enhanced rate of cell death. In support of this, it has been shown that prolonged exposure to the membrane permeant analogue of cAMP, dibutyryl-cAMP, is associated with an increase in β-cell death [28]. Furthermore, treatment of clonal BRIN-BD11 β-cells with the adenylate cyclase activator forskolin has also been found to potentiate the pro-apoptotic effects of serum withdrawal [58]. These results suggest that chronic elevation of islet cell cAMP levels can be detrimental to cell function and cause increased apoptosis. In support of this, recent data have indicated that the ability of the survival factor IGF-1 to maintain β-cell viability derives, in part, from its ability to lower cAMP levels by activation of a specific isoform of cAMP- phosphodiesterase (PDE) (PDE3) in β-cells [58]. The mechanism by which long-term elevation of cAMP causes β-cells to undergo apoptosis has not been disclosed and warrants further study. In this context, it is known that cAMP can elicit responses that are independent of protein kinase A (PKA), and it is interesting to note that islet cells have been shown recently to express GEFs that are regulated by cAMP [12]. cAMP-GEFs couple changes in the concentration of cAMP to the activation state of monomeric G-proteins, and it is conceivable that they may form part of a cross-talk pathway by which heterotrimeric G-proteins (acting *via* cAMP) are able to regulate a subgroup of low molecular weight G-proteins that, in turn, control cell death (Fig. 1).

One further area in which direct trimeric G-protein involvement has been cited relates to the actions of interleukin (IL)-1β in the β-cell. This concept has been the subject of controversy since some authors have failed to find evidence for G-protein involvement in the actions of IL-1β [59,60]. However, others have reported marked effects [61]. In particular, Rabuazzo *et al.* [61] suggested that ADP-ribosylation of islet G-proteins with either cholera or PTXs leads to an attenuation of the ability of IL-1β to inhibit insulin secretion and to induce NO formation. We have been unable to observe any effect of pertussis toxin (PTX) on IL-1β-induced NO production in RINm5F cells,[2] but this may reflect differences between clonal β-cells and primary islets. In islets, Rabuazzo *et al.* suggest that IL-1β signaling *via* the NO pathway may involve one or more G-proteins that can serve as substrates for cholera and PTXs. Although contentious, this proposal is consistent with a subsequent report that prostaglandin E_2 (PGE_2) may be responsible for some of the inhibitory actions of IL-1β in islet cells [25]. Since IL-1β induces the expression of cyclooxygenase-2 (COX-2) in islet cells (Fig. 2) and this enzyme generates prostaglandins (including PGE_2) from arachidonic acid, it follows that PGE_2 levels are likely to be increased in and around islets exposed to IL-1β. This is significant since it is known that PGE_2 can regulate islet G-proteins, as evidenced by our own studies that described the presence of a high affinity, PGE_2-sensitive GTPase in the secretory granules of normal islets and clonal HIT-T15 cells [62]. We also showed that the carboxymethylation of G-protein γ-subunits is increased in a PGE_2-dependent manner [63]. When considered in the light of the observation that PGE_2 causes inhibition of insulin secretion by a PTX-sensitive mechanism, these findings may account for some of the responses observed by Rabuazzo *et al.* in IL-1β-treated islets [61].

3.2. Monomeric G-proteins as mediators of β-cell death

A number of monomeric G-proteins that are expressed in pancreatic β-cells [1], including Cdc42, Rho, Ras, and Rac, have been implicated in the effector pathways of apoptosis [30–50]. Some are believed to promote the activation of mitogen-activated protein kinases, in particular the c-*Jun* N-terminal kinase or stress-activated

[2] Elliott J and Morgan NG, unpublished observations.

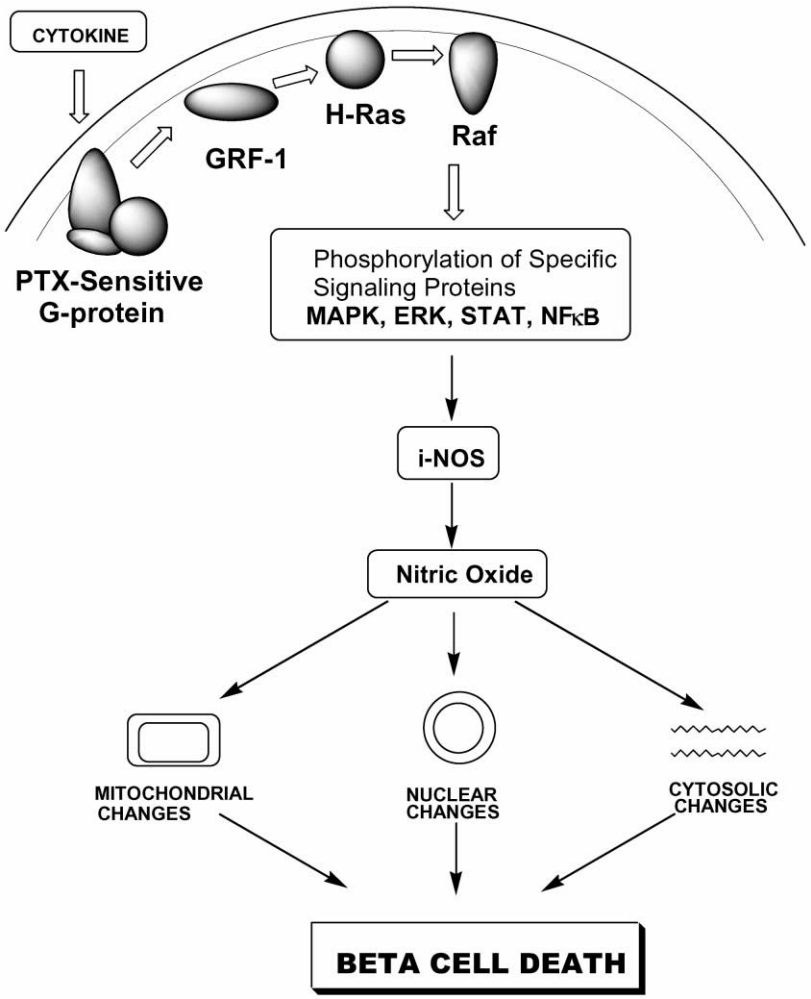

Fig. 1. Possible transduction mechanisms involving G-proteins in cytokine-mediated β-cell demise. This model predicts that IL-1-induced dysfunction and demise of the pancreatic cell is under the control of both trimeric and monomeric G-proteins. Exposure of isolated β-cells to cytokines leads to activation of H-Ras involving the intermediacy of GEFs, such as the GRF-1. Activated Ras recruits Raf-1 to the membrane, which triggers a series of signaling events including activation of the phosphorylation of specific signaling proteins, resulting in increased expression of inducible nitric oxide synthase (iNOS) and nitrite release. This leads to alterations in cellular metabolism at the cytosolic, mitochondrial, and nuclear levels, leading to cellular dysfunction and demise of the effete β-cell. Abbreviations: MAPK, mitogen-activated protein kinase; ERK, extracellular receptor kinase; STAT, signal tranducers and activators of transcription; and NFκB, nuclear factor-κB.

protein kinase (JNK/SAPK), and evidence indicates that this event is mediated by a group of secondary effectors, such as the p21Rac/Cdc42-activated kinases. The latter enzymes have not yet been characterized in the β-cell, although it is known that functional JNK/SAPK is expressed. Studies by Gulbins *et al.* [31] have shown that the ability of Fas and ceramide to cause apoptosis may involve members of the Rho subfamily of G-proteins, especially Rac and Ras, in human leukemic Jurkat cells. These authors correlated the activation of Rho family G-proteins with the JNK/p38 kinase cascade and argued that activation of the G-proteins is a key component of this cascade since Fas and ceramide failed to promote apoptosis when the G-proteins were inactivated [31,33,34]. Interestingly, activation of the Rho family G-proteins was followed by their translocation to the cytoskeletal fraction, although the functional significance of this observation has not been established. Even so, these data strongly suggest that activation of specific Rho subfamily G-proteins may be a prerequisite for the induction of cell death by agents that cause ceramide generation. This is significant since there is also a general consensus that Rho subfamily G-proteins are involved in cytokine-mediated apoptosis, suggesting the existence of common, G-protein-mediated, effector mechanisms in the ceramide- and cytokine-activated pathways.

It is now important to determine the exact loci at which monomeric G-proteins exert their modulatory effects, and some initial hints have been obtained. For example, Rho G-proteins can stimulate arachidonic acid generation (presumably *via* activation of cytosolic phospholipase A_2) and thereby cause the activation of sphingomyelinase, which, in turn, generates ceramides from sphingomyelin [35]. This would be a pro-apoptotic effect. However, controversy exists since these data contrast with the observations of Gomez *et al.* [36], who have suggested that activation of

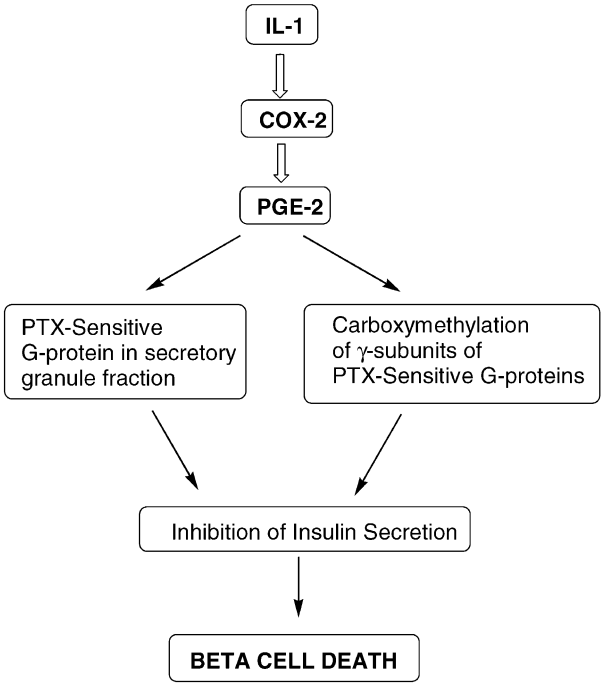

Fig. 2. Possible G-protein-mediated signal transduction mechanisms in IL-mediated β-cell dysfunction involving PGE_2. Exposure of IL-1 leads to increased expression of COX-2 and subsequent generation of PGE_2, which, in turn, has been shown to modulate the functions of a PTX-sensitive G-protein(s) in the pancreatic β-cell. Even though the exact identity of these proteins is unknown at the moment, they might participate in the inhibition of glucose-stimulated insulin secretion and subsequent dysfunction and demise of the pancreatic β-cell.

Rho prevents apoptosis by increasing the expression of Bcl-2 in murine T cells. This may reflect a cell-specific difference in response, but it also raises the possibility that, depending upon the prevailing conditions, Rho G-proteins can function either as *suppressors* or as *inducers* of apoptosis.

Despite the increasing evidence that is accumulating in other cell types, to our knowledge there are only two studies in which the roles of monomeric G-proteins in β-cell death have been specifically investigated. In the first of these, Li *et al.* [27] demonstrated that long-term depletion of intracellular GTP resulted in the apoptotic death of normal rat islets and clonal β-cells. These authors explored the possibility that dysfunction of G-proteins was responsible for this effect and observed that inhibition of protein isoprenylation by lovastatin resulted in a marked reduction in DNA, total protein, and insulin content, and that this was associated with a reduced cell number and with loss of viability. These effects of lovastatin were prevented by co-provision of mevalonic acid, which bypasses the blockade of hydroxy methyl glutaryl CoA-reductase induced by the drug, and restores the capacity for protein isoprenylation. Biochemical evidence confirmed that lovastatin treatment resulted in the apoptosis of HIT-T15 β-cells, and provision of exogenous mevalonic acid reversed this response. These data suggest (but do not prove) that a functionally competent G-protein is required to prevent the induction of apoptosis in β-cells. As such, this situation is analogous to that seen in islets treated with PTX, where stimulus-induced apoptosis is also enhanced, consistent with the involvement of an anti-apoptotic G-protein [28,29]. Since the γ-subunits of trimeric G-proteins are isoprenylated, it might be considered that the susceptibility to apoptosis conferred by lovastatin could relate to the loss of functional competence of a specific trimeric G-protein rather than to inactivation of a low molecular weight G-protein, under these conditions. To clarify these issues further, the effects of clostridial toxin-B on MPA- or lovastatin-induced β-cell death have been examined. This toxin blocks the function of Cdc42, Rac, and Rho by inducing their glycosylation, but it does not glycosylate the γ-subunit of trimeric G-proteins [3]. HIT cells treated with the toxin alone did not lose viability, although the cells exhibited characteristic morphological changes (rounding-up), confirming that Rho functionality had been altered. When these cells were then exposed to lovastatin or MPA, cell death was increased significantly, consistent with the involvement of Rho G-proteins in β-cell survival.

In a more recent study [64], two variants of *Clostridium sordellii* lethal toxin were used in combination with specific chemical inhibitors of Ras function (manumycin and damnacanthal), and it was demonstrated that Ras activation may be necessary for IL-1β-induced NO release from HIT cells. Additional data supporting this hypothesis are provided in Table 1. These data provide the first experimental evidence that Ras may be involved in cytokine-mediated NO release in β-cells and suggest that Ras may be required for IL-1β-induced dysfunction of the β-cell [64].

Table 1
Data implicating a role for Ras in IL-induced NO release from HIT-T15 cells[a]

Probe	Effects on function	Target G-proteins	Effects on NO release
LT-82	Inhibition by glucosylation	Ras, Rap, Rac, Ral, but not Cdc42	Inhibition
LT-9048	Inhibition by glucosylation	Ras, Cdc42, Rac, Rap, but not Ral	Inhibition
Toxin-B	Inhibition by glucosylation	Rac, Cdc42, Rho	No effect
C3-exotoxin	Inhibition by ribosylation	Rho	No effect
CNF-1	Activation by deamidation	Rho	No effect
Damnacanthal	Inhibition of function	Ras	Inhibition
Manumycin	Inhibition of farnesylation	Ras	Inhibition

[a] See reference [64] for additional details.

4. Roles for G-proteins in the mitogenesis and survival of the β-cell

In addition to their possible role as mediators of islet cell apoptosis, there is also clear evidence that heterotrimeric G-proteins can exert anti-apoptotic functions in the β-cell [28,29]. This is most evident from studies in which cells have been treated with PTX to inactivate the $G_{i/o}$ family of heterotrimeric G-proteins. Under such circumstances, the actions of agents that are pro-apoptotic are enhanced markedly. This was first observed for clonal β-cells treated with NaF, but similar observations have been made subsequently in cells treated with IL-1β or chemical NO donors [29]. In addition, it is also clear that an equivalent response occurs in both rat and human islets [65], suggesting that the effect is not restricted to transformed cells. On the basis of these observations, it appears that a member of the G_i or G_o class of G-proteins plays a role in antagonizing the activation of apoptosis in β-cells. When this antagonistic action is prevented (consequent upon ADP-ribosylation by PTX), then the sensitivity of the cells to pro-apoptotic stimuli is heightened. One important feature of this pathway is that it functions even when cells are incubated in the absence of an agonist capable of activating G_i/G_o-coupled receptors. Thus, it follows that there must be a constitutive level of activation of this pathway in β-cells that is sufficient to oppose the actions of pro-apoptotic agents. Indeed, if the G_i/G_o pathway is further activated (e.g. by addition of noradrenaline or somatostatin), this does not lead to any additional attenuation of apoptosis (see footnote 3), implying that the constitutive level of activation exerts maximum inhibition.

There is, however, an alternative explanation for these phenomena that should not be overlooked—notably, that the critical PTX substrate involved in the anti-apoptotic response is not G_i or G_o. In this context, it has been demonstrated that β-cells also express G_t (transducin [66]), and it is known that G_t can serve as a substrate for PTX [67]. The function of G_t in the β-cell has not been established but, in the retina, it regulates the activity of a cGMP-PDE. In retinal photoreceptors, activation of G_t leads to increased cGMP-PDE activity and causes a rapid decline in cGMP levels [68]. There is very little information on the expression of cGMP-PDE isoforms in β-cells, and it is unclear whether a G_t-regulated activity is present. However, it is clear that cGMP can affect the viability of β-cells since, in common with the situation in cardiac myocytes, elevation of cGMP leads to β-cell death [26,69,70]. Thus, one area that is ripe for further study concerns the relationship between G_t and cGMP in islets and the possible involvement of PTX in regulating this pathway.

A further important feature of the response of islet cells to PTX is that the agent has no effect on viability when cells are incubated in the presence of the toxin alone [28,29]. Thus, it can be deduced that PTX treatment does not alter the actions of a pathway involved in the maintenance of viability under resting conditions. Rather, it appears that the toxin interacts with signaling mechanisms that oppose the induction of apoptosis only when a pro-apoptotic stimulus has been applied. One obvious possibility that might account for the effects of PTX on islet cell viability is that they are mediated by a rise in cAMP. Thus, it has been demonstrated that PTX treatment leads to a modest elevation of cAMP levels in β-cells (by blockade of the tonic inhibitory control of adenylate cyclase exerted by G_i [2]), and it cannot be excluded that activation of PKA or cAMP-GEFs may mediate the response under these conditions. However, an alternative mechanism has been proposed recently, based on evidence that the response to PTX is abolished by the presence of tyrosine kinase inhibitors [29]. This suggests that a PTX-sensitive G-protein may regulate the state of tyrosine phosphorylation of a key target protein that is central to the onset of apoptosis in the β-cell. This is an attractive hypothesis since it is clear that, in many cells, survival signals are regulated by tyrosine phosphorylation (e.g. *via* the receptors for insulin and IGF-1), and there is also evidence that src-family tyrosine kinases can mediate the actions of G-proteins on apoptosis [71]. Although, at present, the putative target phosphoprotein has not been identified, a working hypothesis can be constructed (Fig. 3). In this scenario, it is envisaged that the actions of a G-protein-regulated, pro-apoptotic, tyrosine kinase activity are opposed by the constitutive activation of a PTX-sensitive G-protein that, in turn, mediates the dephosphorylation of a

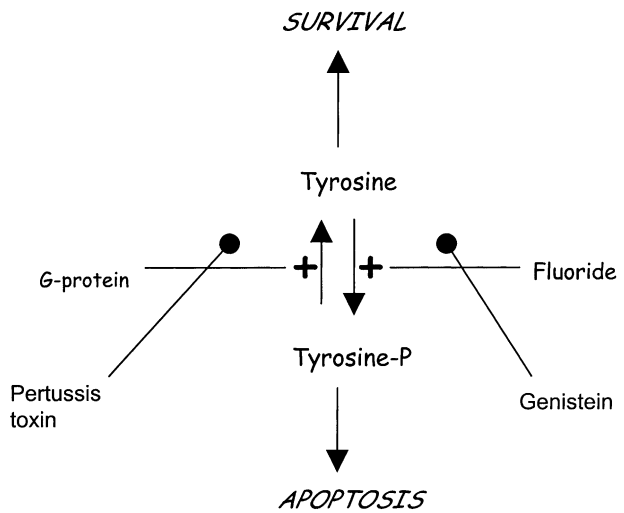

Fig. 3. Hypothesis to explain the involvement of PTX-sensitive G-proteins in the control of β-cell viability. In this model, β-cell survival is regulated by the phosphorylation state of one or more critical tyrosine residues. Increased tyrosine phosphorylation leads to apoptosis in a genistein-sensitive manner. The constitutive activation of a PTX-sensitive G-protein opposes tyrosine phosphorylation and maintains cell survival. Blockade of the activity of this G-protein by treatment of cells with PTX leads to enhancement of the pro-apoptotic effects of agents acting through the kinase pathway.

critical substrate. On this basis, it would be logical to assume that the PTX-sensitive G-protein is likely to control a tyrosine phosphatase enzyme and, although this has not been demonstrated in β-cells, it is clear that such mechanisms exist in other cell types [72,73].

One point at which tyrosine kinase pathways converge with those regulated by PTX-sensitive G-proteins in cells is at the level of a serine/threonine kinase, protein kinase B (PKB) (also termed Akt). This enzyme exists in several isoforms and has been implicated in mediating the anti-apoptotic actions of growth factors and certain G-protein-coupled receptors [74]. Thus, it represents an attractive candidate as one site for regulation of apoptosis by heterotrimeric G-protein-dependent mechanisms. Interestingly, the control of PKB by G-proteins is almost certainly not mediated by the α-subunit but, rather, by βγ [75,76]. Release of specific βγ heterodimers from the holoprotein leads to the association of these subunits with phosphatidylinositol-3-kinase (PI-3-K) and causes activation of this enzyme. This, in turn, induces the recruitment and activation of an intermediate kinase (PDK) leading to the phosphorylation of PKB at a specific serine residue (Ser-473). Once phosphorylated, PKB is active and, in this form, it promotes cell survival [77]. The mechanisms operating downstream of PKB are still being defined but are likely to involve the phosphorylation of BAD in order to block its interaction with Bcl-2. As a result, Bcl-2 remains in an uncomplexed state, and its anti-apoptotic function persists [78]. Thus, any mechanism by which PKB activation is achieved is likely to result in a reduction in the extent of cell death, and it is possible that the constitutive activation of PTX-sensitive G-proteins within the β-cell provides sufficient βγ subunits to maintain the activity of this pathway.

The presence of PKB in islet cells has been confirmed in a number of studies and blockade of PI-3-K activation has been associated with a reduction in PKB phosphorylation, leading to a marked decline in the survival of cultured islet cells [79]. It is not yet known whether PTX can regulate the phosphorylation state of PKB in islet cells, but this has been demonstrated in other cell types where it has been correlated with heightened sensitivity to pro-apoptotic stimuli [80]. In view of these results, it seems likely that investigation of the relationship between ADP-ribosylation of islet G-proteins by PTX and the regulation of PKB phosphorylation (and activity) will be a fruitful area for further research. Furthermore, since there is evidence [81] that PKB activity can be regulated by monomeric G-proteins (including Ras), the possibility that it may also be involved in the control of apoptosis by these molecules should be considered.

Although, this article has focused principally on the roles of G-proteins in the regulation of β-cell death, it is important to emphasize that a growing body of evidence has also implicated G-proteins in the control of DNA synthesis and mitogenesis in these cells. We have reviewed [6] this evidence previously, but of particular relevance to the current considerations are studies by Dunlop *et al.* [82]. These authors employed neonatal rat islets to demonstrate the reduced activation of specific G-proteins (e.g. Ras) after depletion of GTP by MPA and showed that this was accompanied by marked attenuation of glucose- and LPA-induced mitogenesis [83]. Several intermediary steps involved in the signaling cascade of LPA and glucose (including ERK1 phosphorylation and Cdk4 activation) were also attenuated in GTP-depleted cells. In separate work [27], depletion of GTP was shown to inhibit β-cell mitogenesis in clonal β-cells, as reflected by decreased [^{3}H]thymidine incorporation into DNA. Thus, it is possible that G-proteins may play reciprocal roles that allow them to regulate both growth and death in β-cells.

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