The serine/threonine protein kinase Akt has recently been shown to be implicated in the pathway leading to cell survival in response to serum and growth factors in a variety of cellular systems. However, the existence of a biochemical route connecting this kinase to the large family of receptors that signal through heterotrimeric G proteins is yet to be explored. In this study, we set out to investigate whether GTP-binding protein (G protein)-coupled receptors (GPCRs) can stimulate Akt activity and survival pathways and, if so, to define the mechanism(s) whereby this class of cell surface receptors could regulate Akt function. Using ectopic expression of GPCRs in COS-7 cells as a model, we have observed that both m1 and m2 muscarinic acetylcholine receptors, representative of those GPCRs coupled to Gαi and Gαq proteins, respectively, can readily activate an epitope-tagged form of Akt kinase and prevent UV-induced apoptosis. We have also found that the pathway connecting G proteins to Akt implicates signals emanating from Gαq, Gαi, and βγ dimers, but not from Gαs or Gα12, in each case acting through a pathway that involves a phosphatidylinositol-3-OH kinase activity. Moreover, our findings suggest a role for a novel βγ-sensitive complex, p101-phosphatidylinositol-3-OH kinase-γ, in the transduction of signals leading to Akt stimulation and cell survival by GPCRs and open new avenues for research on the function of the large family of G protein-linked receptors in the regulation of anti-apoptotic pathways.

Receptors coupled to heterotrimeric GTP-binding proteins (G proteins) are the largest group of integral membrane receptors involved in the transmission of signals from the extracellular environment to the cytoplasm (1). A wide range of external stimuli, including neurotransmitters, growth factors, hormones, light, odorants, and certain taste ligands, can activate specific members of this family and promote a conformational change that is transmitted to the cytoplasmic side of the receptor protein (1). This leads to a physical interaction between the receptor and the GDP-bound G protein heterotrimer which causes the dissociation of the guanine nucleotide and the incorporation of GTP in the G protein α subunit, thus releasing the βγ heterodimer (2). In turn, GTP-bound G protein α subunits and βγ complexes initiate, independently, a wide variety of intracellular signaling pathways (3).

Although the G protein-coupled receptor (GPCR) family is involved in many functions performed by fully differentiated cells, these receptors are also expressed in most proliferating cells, and they have been implicated in embryogenesis, tissue regeneration, and growth stimulation (4). The nature of the growth regulatory pathway(s) stimulated by GPCRs has just begun to be elucidated (5). In our laboratory, we have used the ectopic expression of human muscarinic receptors for acetylcholine (mACHRs) in NIH 3T3 cells as a model system for studying mitogenic signaling through G protein-linked receptors. In this biological setting, we have shown that certain mACHR subtypes can effectively transduce mitogenic signals and, when activated persistently, induce the transformed phenotype (6). Interestingly, two recent reports have demonstrated that the activation of endogenously expressed muscarinic receptors is per se sufficient to block apoptosis in neuronal cells (7, 8). These results suggest that in addition to their role in cell growth, GPCRs might also activate yet to be defined survival pathways.

In this regard, the serine-threonine kinase Akt/PKB, which was first identified as the human homolog of a transforming oncogene (9), has been shown recently to control intracellular pathways preventing cell death in response to a variety of extracellular stimuli (10) and in a wide range of cellular systems (11, 12). The mechanism of activation of Akt by tyrosine kinase growth factor receptors has been established recently (13). However, the regulation of this intriguing kinase by GPCRs is still unclear. Initial reports showed that lysophosphatidic acid, acting through Gαi-coupled receptors, was unable to stimulate Akt activity in NIH 3T3 cells (14). Furthermore, direct activation of PKC by phorbol esters also failed to activate Akt in this fibroblast cell line (14, 15). On the other hand, recent reports have described Akt stimulation in response to GPCRs in rat epididymal fat cells (16) and human embryonic kidney 293-EBNA cells (17), albeit by a yet to be determined mechanism.

In this study, we set out to investigate whether Akt can be activated effectively by GPCRs using ectopically expressed receptors and different G protein subunits in COS-7 cells as a model system. We found that both Gαq- and Gαi-coupled GPCRs, m1 and m2 receptors, respectively, can readily activate an
epitope-tagged form of Akt kinase. We also show that both signal-transducing molecules generated upon GPCR activation, βγ complexes and α subunits, can effectively promote Akt activation in a PI3K-dependent manner. Moreover, our findings suggest a role for the novel PI3Kγ and its associated regulatory subunit, p101, in the transduction of signals leading to Akt stimulation by GPCRs. We also present evidence that GPCRs can induce survival pathways through PI3Kγ acting on Akt.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Transfection—**COS-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Cells were transfected by the DEAE-dextran technique, adjusting the total amount of DNA to 1–4 μg/platé with pcDNA3-β-galactosidase DNA, when necessary (18).

**Expression Plasmids—**An epitope-tagged Akt (pCEFL-HA-Akt) as well as the membrane-targeted mutant (pCEFL-myr-HA-Akt) were generated by inserting the coding region of Akt and myr-Akt (kindly provided by Dr. P. N. Tsichlis) into the pCEFL expression vector. Expression plasmids for m1 and m2 mAChRs, for the α, γ subunits of the PI3Kγ and their mutants, as well as expression plasmids for βα1, γα12, and γγ11 subunits of heterotrimeric G proteins were reported previously (18–20). Plasmids expressing the coding region of PI3Kγ and the p101 protein fused to the Glu-Glu tag were kindly provided by L. R. Stephens and are described elsewhere (21). Plasmids expressing the GFPα-deficient, constitutively activated forms of representative G protein α subunits (Gα12, GQ27L; Gα13, GQ205L, GαQ209L, and GαQ201C) have been described already (19, 22, 23). An expression plasmid for a chimera molecule between the extracellular and transmembrane domain of CD8 fused to the carboxyl-terminal 222 amino acids of βARK, which includes both the βγ-binding domain and the PH domain of βARK (pcDNA-CD8-βARK), has also been described recently (22, 24).

**Akt Assay and Western Blots—**Akt activity in lysates from COS-7 cells transfected with an expression vector for an epitope-tagged Akt (pCEFL-HA-Akt) was determined upon immunoprecipitation with the anti-HA-specific monoclonal antibody 12CA5 (Babco) using histone 2B (Boehringer) as substrate, essentially as described (15). Briefly, cells grown on 10-cm plates were washed once in cold phosphate-buffered saline and lysed on ice with 1 ml of lysis buffer containing protease and phosphatase inhibitors (1% Triton X-100, 10% glycerol, 137 mM NaCl, 20 mM Tris-HCl, pH 7.5, 1 μg/ml aprotinin and leupeptin, 1 μM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM disodium pyrophosphate, 20 mM Tris-HCl, pH 7.5, 1 μM aprotinin and leupeptin, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM disodium pyrophosphate, and 1 mM Na3VO4). After preclearing the samples by centrifugation, lysates were immunoprecipitated with 1 μl of anti-HA monoclonal antibody using γ-binding beads (Amersham Pharmacia Biotech) to sediment the immunocomplexes. After three 1-ml washes with lysis buffer containing protease and phosphatase inhibitors (1% Triton X-100, 10% glycerol, 137 mM NaCl, 20 mM Tris-HCl, pH 7.5, 1 μg/ml aprotinin and leupeptin, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM disodium pyrophosphate, 20 mM Tris-HCl, pH 7.5, 1 μg/ml aprotinin and leupeptin, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM disodium pyrophosphate, and 1 mM Na3VO4), and saline and lysed on ice with 1 ml of lysis buffer containing protease and phosphatase inhibitors (1% Triton X-100, 10% glycerol, 137 mM NaCl, 20 mM Tris-HCl, pH 7.5, 1 μg/ml aprotinin and leupeptin, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM disodium pyrophosphate, and 1 mM Na3VO4). After preclearing the samples by centrifugation, lysates were immunoprecipitated with 1 μl of anti-HA monoclonal antibody using γ-binding beads (Amersham Pharmacia Biotech) to sediment the immunocomplexes. After three 1-ml washes with lysis buffer, one 1-ml wash with water, and one 1-ml wash with kinase buffer (20 mM Heps, pH 7.4, 10 mM MgCl2, 10 mM MnCl2), reactions were performed for 30 min at 25°C under continuous agitation in kinase buffer containing 0.05 mg/ml histone 2B, 5 μM ATP, 1 mM dithiothreitol, and 10 μCi of [γ32P]ATP. The products of the kinase reactions were fractionated in 15% SDS-polyacrylamide gels, transferred to nylon membranes (Immobion), and exposed. Resulting autoradiograms were quantified with the use of a Molecular Dynamics densitometer. When necessary, the same membranes were analyzed subsequently by Western blot using mouse anti-HA (Babcoc, 1:500) or goat anti-Akt (C-20, Santa Cruz Biotechnology, 1:250) to visualize the endogenous protein in PC12 cells. To assess the level of expression of cotransfected proteins, 50 μl of total lysates were analyzed by Western blot using goat anti-PI3Kγ (N-16, Santa Cruz Biotechnology, 1:250), mouse anti-Glu-Glu (Babcoc, 1:500), rabbit anti-Gβγ (Santa Cruz Biotechnology, 1:250), rabbit anti-Gα12 (K-20, Santa Cruz Biotechnology, 1:500), rabbit anti-Gα13 (Upstate Biotechnologies, Inc., 1:100), rabbit anti-Gα12 (Santa Cruz Biotechnology, 1:1,000), and specific antisera against Gα13 and Gβγ as described previously (19). Bands were developed by an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech) using secondary antibodies coupled to horseradish peroxidase (Cappel).

**Apoptosis Assay—**COS-7 cells were grown on coverslips and transfected with the indicated plasmids together with an expression vector for β-galactosidase as a marker for transfection, using the LipofectAMINE-Plus reagent (Life Technologies, Inc.) following the manufacturer's instructions. Protection from UV-induced apoptosis was performed essentially as described (12). Briefly, 24 h after transfection, cells were serum starved overnight in Dulbecco's modified Eagle's medium containing 10 μg/ml Hepes and subjected subsequently to UV irradiation (120 μJ, UV-Stratalinker 1800, Stratagene). After the addition of fresh serum-free medium containing or not 1 mM carbobell, cells were maintained in the incubator, fixed 8 h later in 4% paraformaldehyde, and permeabilized with 0.01% Triton X-100. Transfected cells were identified by immunostaining for β-galactosidase expression with a mouse anti-β-galactosidase and biotinyl rhodamine-stained (Sigma, 1:200). Fragmented DNA was then visualized by the terminal deoxynucleotidyltransferase-mediated dUTP-FITC nick end labeling (TUNEL) technique using a kit from Boehringer Mannheim, following the manufacturer's instructions, except that the reaction was carried out at room temperature instead of at 37°C. The frequency of apoptosis was counted by counting a total of 100 cells in at least 5 fields/coverlip and examining them for FITC staining (TUNEL-positive) under UV light in an Axiosplan2 fluorescence microscope (Zeiss).

**RESULTS**

Stimulation of muscarinic GPCRs has been reported to induce protection from apoptosis both in cerebellar granule neurons (8) and in the pheochromocytoma cell line PC12 (7), and the Akt kinase has been implicated in survival pathways in many cell types, including PC12 and other neuroectodermal-derived cells (11, 13). Thus, we asked whether activation of endogenous muscarinic receptors would lead to Akt activation in PC12 cells. As shown in Fig. 1A, exposure of PC12 cells to the cholinergic agonist carbachol induced the rapid stimulation of the Akt phosphotransferase activity, to an extent similar to that observed in these cells in response to nerve growth factor acting on its cognate receptors.

To investigate further the molecular mechanism(s) whereby muscarinic receptors activate Akt and induce protection from cell death, we chose to use a reconstituted system, consisting of the coexpression of m1 and m2 muscarinic receptors together with an epitope-tagged Akt (HA-Akt) in COS-7 cells. Whereas m1 receptors are typical of those coupled through G proteins of the Gαq family to phospholipase C activation, m2 is known to couple through G13 to a number of effector pathways, including the inhibition of adenyl cyclases (25). In cells expressing either muscarinic receptor we observed that carbachol induced an increase in Akt activity, as judged by immune complex kinase reactions using histone 2B as a substrate. When mediated by m1 receptors, induction of histone 2B phosphorylation was evident as early as 1 min after the addition of agonist, showing an early peak at approximately 3 min after stimulation (Fig. 1B). Stimulation of m2 also caused a very rapid activation, which was evident after 1 min and reached a maximum around 15 min (Fig. 1B). Both m1- and m2-induced Akt kinase activity remained elevated for an extended period of time, decreasing to the basal activity as late as 2–3 h after treatment (data not shown).

Recent work has demonstrated that PI3K activity is required for Akt activation in the majority of the systems described to date (26). However, PI3K-independent pathways have also been described (17, 27), including those mediating Akt activation by βγ-adrenergic GPCR in epidydimal fat cells (16). In our experimental system, preincubation of cells with 50 nM wortmannin, a potent PI3K inhibitor, completely blocked both m1- and m2-mediated stimulation of Akt (Fig. 1C). Thus both Gα12 and Gβγ-coupled receptors appear to activate Akt irrespective of their G protein coupling specificity, utilizing a PI3K-dependent pathway.

As an approach to investigate which G protein(s) mediates Akt activation, we used the expression of GTPase-deficient mutationally activated forms of G protein α subunits, which can activate effector pathways by obviating the need for receptor stimulation (2). Thus, we coexpressed the epitope-tagged Akt together with GTPase-deficient mutants for Gα13, Gα12, Gαq10, and Gα121, representing each of the four known α subunits.
The cholinergic agonist carbachol activates Akt through endogenously expressed receptors in PC12 cells and through ectopically expressed m1 or m2 muscarinic receptors in COS-7 cells in a wortmannin-sensitive manner. Panel A, PC12 cells were grown in 10-cm plates to 80% confluence and serum-starved overnight before being subjected to stimulation with carbachol (cch) (1 mM, 2 min) or nerve growth factor (NGF) (50 ng/ml, 10 min). Cell lysates were then immunoprecipitated with goat anti-Akt serum, and kinase reactions were performed as described under “Experimental Procedures.” The amount of kinase activity present in each immunoprecipitate was assessed by Western blot analysis with anti-HA antibody. Autoradiograms are from a representative experiment that was repeated three times.

Fig. 2. Overexpression of α or βγ subunit of heterotrimeric G proteins results in stimulation of Akt activity. COS-7 cells were transfected with pCEFL-HA-Akt (0.5 μg/plate) together with pcDNA3-β-galactosidase vector (control), an activated form of Ras (pCEFL-AU5-RasV12, 0.5 μg/plate), or with expression vectors carrying cDNAs for the activated forms of the α subunits of Gαs, Gαi2, Gαq (panel A) or expressing β1 and γ2 G protein subunits (panel B) (0.5 μg/plate in each case). As indicated, kinase reactions and Western blot analysis were performed in anti-HA immunoprecipitates from the corresponding lysates or in 50 μl of the corresponding total lysates to confirm the expression of the different G proteins as described under “Experimental Procedures” (data not shown). The autoradiograms shown correspond to a representative experiment that was repeated three times. 32P-Labeled products as well as specific bands detected by the anti-HA antibody are indicated with an arrow. Panel C, Akt activity was measured in cells cotransfected with HA-Akt (0.5 μg/plate) and a plasmid carrying the COOH terminus of βARK fused to the CD8 receptor (CD8-βARK, 2 μg/plate) or the same amount of a vector expressing β-galactosidase as a control. Data represent the mean ± S.E. of three to five independent experiments expressed as the percentage of activation with respect to control transfected cells.

As shown in Fig. 2A, the activated mutant of Gαq was able to trigger Akt activation similar to that caused by Ras when used as control (15, 28). Activated Gαi2 also stimulated Akt, although less efficiently than Gαq, and the activated mutants of Gαs and Gαi2 had no demonstrable effect under our experimental conditions. These data indicate that Gαi2, and to a lesser extent Gαq, can mediate Akt activation by G protein-linked receptors.

When activated, GPCRs catalyze the replacement of GDP by GTP bound to the α subunit and induce the dissociation of α-GTP from βγ dimers. Although the α subunits were thought to be the sole responsible molecules for coupling receptors to second messenger-generating systems, recent work has established a critical role for βγ dimers in signal transduction (3, 19). These data prompted us to explore whether βγ dimers might also participate in signaling to Akt. We observed that cotransfected, βγ1γ2 subunits induce a remarkable increase in the phosphorylating activity of the epitope-tagged Akt, although expression of the HA-Akt was similar for each transfected cell population (Fig. 2B). In contrast, Akt was activated poorly when coexpressed with βγ1 or γ alone, or when coexpressed with β1 and an altered form of γ2 subunit, designated γ2*, which lacks the γ-isoprenylation signal and therefore fails to associate to the plasma membrane (19) (Fig. 2B). Based upon these results, we conclude that membrane-bound forms of βγ subunits of heterotrimeric G proteins can potently stimulate Akt activity in COS-7 cells. In view of these results, we next sought to explore the relative contributions of α and βγ proteins in Akt stimulation by mACHRs. To approach this question, we employed a chimeric molecule combining the extracellular and transmembrane domain of CD8 fused to the carboxyl-terminal domain of βARK which includes the high affinity βγ binding region of this kinase as described (22). This chimeric molecule targets the COOH-terminal part of βARK to the plasma mem-
bran where it is expected to bind and sequester free βγ complexes when dissociated from Ga subunits upon receptor stimulation, thus blocking βγ-dependent pathways (22). As shown in Fig. 2C, coexpression of CD8-βARK with the m2 mACHR nearly abolished the activation of Akt in response to carbachol, whereas m1-mediated Akt stimulation was only partially impaired by overexpression of this βγ-sequestering molecule. In contrast, Akt activation by other effectors such as Ras was unaffected by CD8-βARK. Taken together, these findings strongly suggest that signaling from m2 mACHR to Akt is mediated primarily by the βγ subunits of heterotrimeric G proteins, whereas m1-mediated activation is achieved via Gβγ-dependent and -independent pathways.

m1 receptors and activated Gaq efficiently stimulate phospholipase Cβ causing the hydrolysis of phosphoinositides (29). This results in the generation of two second messengers: inositol trisphosphate, which leads to the elevation of intracellular [Ca2+]i, and diacylglycerol, which activates PKC (25). However, several lines of evidence suggested that PKC does not participate in signaling from m1 and Gaq to Akt; direct activation of PKC by phorbol 12-myristate-13-acetate (100 ng/ml for 15 min) provoked no change in Akt activity, nor did specific inhibitors of PKC by phorbol 12-myristate-13-acetate (100 ng/ml for 15 min) affect m1-mediated Akt activity.

To assess further the involvement of a PI3K activity downstream of GPCR in the signaling pathway to Akt, we investigated whether the overexpression of different isoforms of PI3K was per se sufficient to induce Akt activation. As observed in Fig. 4, expression of both α and γ isoforms of PI3K triggered Akt activation poorly, although a constitutively active form of PI3Kγ (myr-PI3Kγ) (19) revealed a greater ability to stimulate Akt than each of the wild type forms. For PI3Kγ, a novel noncatalytic subunit unrelated to p85 has been identified recently (21) and named p101. It has been shown recently that coexpression of this molecule with PI3Kγ enhances its basal activity and potentiates PI3K activation by βγ subunits (21). We therefore asked whether the expression of this noncatalytic PI3K subunit p101 could potentiate the effect of PI3Kγ on Akt. As shown in Fig. 4, Akt activity increased dramatically when both proteins were expressed together. Interestingly, however, we observed that expression of p101 was able per se to induce an increase in Akt activity, to an extent comparable to that elicited by PI3Kγ alone (Fig. 4). These data suggest that p101 may activate an endogenous PI3Kγ or a PI3Kγ-like protein thereby stimulating Akt phosphorylation activity.

To assess further the biological consequences of Akt activation by GPCRs, we set out to investigate whether m1 and m2 receptors could induce survival pathways in COS-7 cells. For that, we took advantage of the recent observation that transfected COS-7 cells undergo apoptosis upon UV irradiation (12) and that Akt activation can protect cells from death in this cellular setting (12). In preliminary experiments, the ED50 for
the apoptotic effect of UV was found to be approximately 120 mJ, a dose of UV which was utilized for the subsequent assays. As shown in Fig. 5, under control conditions less than 10% of the transfected cells displayed an apoptotic phenotype and were labeled by the TUNEL reaction. However, when irradiated by UV a fraction of the untransfected and transfected cells underwent apoptosis, the former visualized as TUNEL-positive cells (FITC-stained) and the latter as both anti-b-galactosidase and TUNEL-positive cells (rhodamine- and FITC-stained, respectively), as depicted in Fig. 5B. Of interest, the addition of carbachol protected m1- and m2-transfected cells from UV-induced apoptosis, to an extent similar to that caused by transfection of an activated form of Akt, myr-Akt (Fig. 5A). In contrast, carbachol treatment produced no apparent consequences in mock-transfected or myr-Akt-transfected cells. Thus, in COS-7 cells m1 and m2 GPCRs can effectively activate survival mechanisms that are able to counteract apoptotic insults, most likely through Akt.

Based on our previous results, we wanted to study further the role of PI3K in the survival pathway stimulated by GPCRs. Initial experiments exploring the effect of wortmannin on the protective activity of m1 and m2 were inconclusive, as the role of PI3K likely through Akt.

FIG. 5. Carbachol protects COS-7 cells transfected with m1 and m2 receptors from UV-induced apoptosis. Panel A, COS-7 cells were grown onto coverslips in six-well plates and transfected with 0.5 μg/well pcDNA-β-galactosidase as a marker for transfection together with 0.5 μg/well empty expression plasmid (c), plasmids containing m1 or m2 receptors, or a myristoylated active form of Akt (pCEFL-myr-HA-Akt), as indicated. After transfection, cells were left untreated, irradiated with UV light, or stimulated with carbachol as indicated, and the percentage of apoptosis was determined by counting rhodamine-positive cells from different randomly chosen fields. Results from four to six independent experiments are shown as the mean ± S.E. Panel B, a representative field of UV-untreated (control) cells and UV-treated cells (+UV) in the presence and absence of carbachol was photographed under UV light using both rhodamine and FITC filters in an Axioplan2 fluorescence microscope under a 63 x magnification. White arrows point to cells displaying positive staining for both the marker of transfection (red) and TUNEL (green).

FIG. 6. A kinase-deficient PI3K is able to prevent the protective effect of m1 and m2 receptors on UV-induced apoptosis in COS-7 cells. COS-7 cells were grown onto coverslips in six-well plates and transfected with 0.5 μg/well pcDNA-β-galactosidase together with 0.5 μg/well plasmids containing m1 or m2 receptors or a myristoylated form of Akt (pCEFL-myr-HA-Akt). Where indicated, 1 μg of pCEFL-PI3K-K799R or empty vector as a control was included in the transfection mix. Cells were then irradiated with UV light and treated with carbachol, as indicated. The percentage of apoptotic cells was determined as described under “Experimental Procedures.” Data represent the mean ± S.E. from three independent experiments.

FIG. 6. A kinase-deficient PI3K is able to prevent the protective effect of m1 and m2 receptors on UV-induced apoptosis in COS-7 cells. COS-7 cells were grown onto coverslips in six-well plates and transfected with 0.5 μg/well pcDNA-β-galactosidase together with 0.5 μg/well plasmids containing m1 or m2 receptors or a myristoylated form of Akt (pCEFL-myr-HA-Akt). Where indicated, 1 μg of pCEFL-PI3K-K799R or empty vector as a control was included in the transfection mix. Cells were then irradiated with UV light and treated with carbachol, as indicated. The percentage of apoptotic cells was determined as described under “Experimental Procedures.” Data represent the mean ± S.E. from three independent experiments.

The multiplicity of intracellular signaling pathways activated by the large family of GPCRs has just begun to be appreciated. In particular, recent work has provided a wealth of evidence that this family of cell surface receptors can trigger biochemical routes communicating the membrane to the nucleus, thereby controlling key molecules involved in gene expression regulation (4, 5). Furthermore, these receptors can affect normal and aberrant cell growth as well as regulate antiapoptotic pathways (7, 8). In this regard, it has been shown that activation of muscarinic GPCRs in PC12 cells can induce cell survival (7). Here, we show that stimulation of PC12 cells with the cholinergic agonist carbachol can activate Akt to an extent comparable to that observed in response to nerve growth factor, a natural survival-promoting factor for these cells (30), thus suggesting that the Akt kinase might participate in the survival pathway(s) elicited by GPCRs. These cells, as many neuroectodermal derived cells, express a mixed population of muscarinic receptor subtypes (31), each exhibiting a distinct coupling selectivity, thus limiting the ability to characterize the pathway linking these receptors to Akt. However, when ectopically expressed in COS-7 cells both m1 and m2, Gα and G coupled receptors, respectively, were able to activate Akt efficiently, thus providing an experimental model where the activation of Akt by these GPCRs could be examined in a molecularly defined reconstituted system. In these cells, we found that whereas activated Gα can induce Akt activity poorly, both activated Gα and βγ complexes were potent stimulators of this serine/threonine kinase. In line with this observation, a βγ-sequestrant, CD8-βARK, nearly abolished Akt stimulation in response to m2 activation but had a more lim-
ied effect on m1-induced Akt activation. Collectively, these data indicate that whereas Gt-coupled receptors signal primarily to Akt through βγ dimers, Gq-coupled receptors utilize both βγ-dependent and -independent mechanisms, the latter likely acting through Gαq.

Of interest, PKC did not appear to link Gαq to Akt, but pharmacological and biochemical evidence supported a role for a PI3K downstream from GPCRs and heterotrimeric G protein subunits in the pathway leading to Akt, and we obtained data to suggest that the PI3Kp101 dimer is a likely candidate to communicate G proteins to Akt. This finding can help explain the seemingly contradictory results on Akt activation by GPCRs (14–17; see above) because PI3K is highly expressed in hematopoietic cells but poorly expressed in other tissues. In COS-7 cells, we can detect limited expression of PI3K (not shown) (20) which is consistent with the observation that p101 expression alone is sufficient to activate Akt, albeit to a limited extent compared with that achieved upon coexpression of PI3K and p101. Thus, in tissues and cell lines lacking PI3K, either GPCR would fail to activate Akt (14, 15) or other PI3K isoforms, such as PI3Kβ (32), might link heterotrimeric G proteins to Akt.

To examine the biological consequences of activating GPCRs in COS-7 cells, we investigated the ability of m1 and m2 receptors to protect COS-7 cells from UV-induced apoptosis. In this reconstituted system, we found that both GPCRs were able to activate cell survival pathways effectively, most likely through Akt. Furthermore, we observed that the protective effect elicited by m1 and m2 was nearly abolished by expression of a dominant-negative mutant form of PI3K, suggesting that the GPCR-PI3K pathway is biologically relevant in this cellular setting.

In summary, our findings raise the possibility of the existence of a novel pathway activating Akt and preventing apoptosis by cell surface receptors. This pathway involves extracellular ligands acting on GPCRs and the consequent activation of Gt or Gq proteins, depending on the coupling selectivity. In turn, these G proteins will release βγ subunits, and βγ dimers and activated Gαq, particularly Gαq, will then stimulate PI3Kp101 complexes or other yet to be identified PI3K isoforms, leading to Akt activation and promoting cell survival.

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