**β-Arrestin1 Mediates Insulin-like Growth Factor 1 (IGF-1) Activation of Phosphatidylinositol 3-Kinase (PI3K) and Anti-apoptosis*\**

Thomas J. Povsic‡§, Trudy A. Kohout†, and Robert J. Lefkowitz**

From the ‡Division of Cardiology and the §Howard Hughes Medical Institute, Department of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710 and **Neurocrine Biosciences Incorporated, San Diego, California 92121

β-arrestins (1 and 2) are widely expressed cytosolic proteins that play central roles in G protein-coupled receptor signaling. β-arrestin1 is also recruited to the insulin-like growth factor 1 (IGF-1) receptor, a receptor tyrosine kinase, upon agonist binding. Here we report that, in response to IGF-1 stimulation, β-arrestin1 mediates activation of phosphatidylinositol 3-kinase in a pathway that leads to the subsequent activation of Akt and anti-apoptosis. This process is independent of both G, and ERK activity. The pathway fails in mouse embryo fibroblasts lacking both β-arrestins and is restored by stable transfection of β-arrestin1. Remarkably, this pathway is insensitive to chemical inhibition of IGF-1 receptor tyrosine kinase activity. These results suggest that, in addition to their roles in G protein-coupled receptor signaling, β-arrestins couple the IGF-1 receptor tyrosine kinase to the phosphatidylinositol 3-kinase system and suggest that this mechanism is operative independently of the tyrosine kinase activity of the receptor.

β-arrestin isoforms 1 and 2 are widely expressed cytosolic proteins that are recruited to, and mediate desensitization of, G-protein-coupled receptors (GPCRs)† upon agonist binding. Although β-arrestins were initially described as negative regulators of GPCR function, more recent work has shown that β-arrestin recruitment to agonist-occupied receptors also leads to activation of a variety of signaling pathways (1, 2). For example, β-arrestin recruitment to agonist-bound receptors facilitates their clathrin-mediated endocytosis while acting as a scaffold to facilitate activation of c-Jun amino-terminal kinase 3 (3) and ERK (4).

Although the preponderance of literature ties arrestsins to GPCR-mediated signaling, β-arrestin1 is also recruited to at least two related receptor tyrosine kinases, the IGF-1 and insulin receptors, in an agonist dependent manner (5, 6). The receptor for IGF-1, a classical receptor tyrosine kinase, binds β-arrestin1 in a ligand-dependent manner and, in a process reminiscent of GPCR-stimulated β-arrestin recruitment, this facilitates clathrin-mediated receptor internalization, MAP kinase activation, and the stimulation of cellular proliferation (5).

IGF-1 plays central roles in controlling cellular metabolism, differentiation, proliferation, and apoptosis. Similarly to many receptor tyrosine kinases, the IGF-1 receptor mediates many of these effects via activation of phosphatidylinositol 3-kinases (PI3Ks), a conserved group of lipid kinases that play vital roles in the regulation of many fundamental cellular processes including cellular proliferation, chemotaxis (7), regulation of cell size (8), cellular adhesion (9, 10), glucose metabolism (11–13), activation of immunological responses (14), and protection from apoptosis (15). Given the central roles played by PI3Ks in cellular responses to receptor tyrosine kinase stimulation, we sought to assess the roles, if any, played by β-arrestins in IGF-1-mediated activation of PI3K.

**EXPERIMENTAL PROCEDURES**

**Creation of Double Knockout (DKO) and B1 Cell Lines—**Mouse embryonic fibroblasts lacking β-arrestin (16) were grown in Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. To establish genetically identical cell lines differing only in the level of β-arrestin1 expression, cells lacking β-arrestin were transfected with pcDNA3.1-Zeo (Invitrogen) or pcDNA3.1-β-arrestin1-FLAG (17) using LipofectAMINE® to create DKO and B1 cells, respectively. Stably transfected lines were selected and maintained in growth medium containing 0.3 mg/ml Zeocin (Invitrogen).

**Staining for β-Arrestins**—Cells cultured in 6-well plates in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum were incubated in medium containing 10% fetal bovine serum, medium lacking serum, or medium lacking serum but supplemented with IGF-1 (5 nM) for a period of 12–16 h. Assays were always conducted when cells were <50% confluent, as confinement was found to impact the effect of IGF-1. Cells were trypsinized and counted, and equal numbers of cells were used for each analysis. Apoptosis was determined according to the manufacturer’s instructions using a Roche Applied Science cell death detection kit, which quantitatively measures fragmentation of histone-bound DNA. Each experiment was performed at least in triplicate, and results are reported in units of mAU/min. Apoptosis was also measured using annexin staining. Cells were treated as described above, trypsinized, incubated with fluorescein isothiocyanate-annexin-V (Pharmingen), and the percentage of apoptotic cells was quantitated by flow cytometry.

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Membranes were solubilized in lysis buffer (137 mM NaCl, 10 mM Tris for 3 min) to remove nuclear and organelle material, followed by Tween 20, and immunoblots were probed for PO4ERK using an antibody specific for β-arrestin1 and β-arrestin2. WT, wild type, B, DKO and B1 cells were stimulated for 3 min with IGF-1 (5 nM), and PI3Kα activity was determined. Cell membranes were isolated by differential centrifugation as detailed under Experimental Procedures. PI3Kα was immunoprecipitated using an anti-p110α antibody, and the phosphorylation of phosphatidylinositol was quantitated after TLC on silica plates using an Amersham Biosciences PhosphorImager. Results represent the mean fold increase ± S.D. in PI3Kα activity, n = 3. *p < 0.05 significant for comparison of DKO vs. IGF-1; #p < 0.01 for comparison of B1 cells vs. IGF-1, (one-sample t tests).

Assays of Akt Activity—Subconfluent cells were incubated in serum-free medium for 60 min and then stimulated with IGF-1 (5 nM) for 3 min. Cells were lysed using radioimmune precipitation assay buffer containing 1 mM sodium vanadate and protease inhibitors. Akt was immunoprecipitated from cell lysates normalized for protein content by BCA analysis (Pierce) using anti-Akt beads (Cell Signaling), and the phosphorylation of a glycoprotein synthase kinase 3β (GSK-3β) substrate was assayed according to the manufacturer’s instructions (Akt assay kit; Cell Signaling). Cell lysates were also subjected to SDS-PAGE, transferred to nitrocellulose membranes, blocked in 5% milk in Tris-buffered saline/Tween 20 (TBS-T), and immunoblots were probed for PO4Akt using an antibody to Akt phosphorylated at Ser-473 (Cell Signaling), or Thr-308 (Cell Signaling). Identical results were obtained using either antibody or assays of Akt activity. Because Akt activity correlated with the detection of PO4Akt by an immunoblot of cell lysates, immunoblotting for PO4Akt was used in subsequent experiments to assay Akt activation. In control experiments to assay the effectiveness of pertussis toxin, cells under identical conditions were stimulated with IGF-1 (5 nM) or oleoyl-t-a-lysophosphatidic acid (LPA; 10 μM) for 5 min, and cell lysates were subjected to SDS-PAGE, transferred to nitrocellulose membranes, blocked in 5% milk in Tris-buffered saline/Tween 20, and immunoblots were probed for PO4ERK using an antibody specific for phosphorylated MAP kinase (Cell Signaling). Immunoblots were visualized using ECL (Biosciences).

Assay of PI3K Activity—After incubation in serum-free medium (60 min), cells were stimulated with IGF-1 (5 nM) for 3 min. Cells were washed, suspended in KHEM (50 mM KCl, 50 mM Hepes, 10 mM EGTA), and ATP (5 mM) for 3 min, and cell lysates were subjected to PAGE and immunoblotting with an antibody specific for β-arrestin and β-arrestin. WT, wild type, B, DKO and B1 cells were stimulated for 3 min with IGF-1 (5 nM), and PI3Kα activity was determined. Cell membranes were isolated by differential centrifugation as detailed under Experimental Procedures. PI3Kα was immunoprecipitated using an anti-p110α antibody, and the phosphorylation of phosphatidylinositol was quantitated after TLC on silica plates using an Amersham Biosciences PhosphorImager. Results represent the mean fold increase ± S.E. in PI3Kα activity, n = 3. *p = not significant for comparison of DKO vs. IGF-1; #p < 0.01 for comparison of B1 cells vs. IGF-1.

FIG. 1. IGF-1 stimulates membrane PI3Kα activity in β-arrestin1-containing B1 cells, but not in β-arrestin DKO cells. A, DKO mouse fibroblasts express no β-arrestin, whereas B1 cells express β-arrestin1 at endogenous levels (16). Cells were lysed using radioimmune precipitation assay buffer containing 1 mM sodium vanadate and protease inhibitors. Akt was immunoprecipitated from cell lysates normalized for protein content by BCA analysis (Pierce) using anti-Akt beads (Cell Signaling), and the phosphorylation of a glycoprotein synthase kinase 3β (GSK-3β) substrate was assayed according to the manufacturer’s instructions (Akt assay kit; Cell Signaling). Cell lysates were also subjected to SDS-PAGE, transferred to nitrocellulose membranes, blocked in 5% milk in Tris-buffered saline/Tween 20 (TBS-T), and immunoblots were probed for PO4Akt using an antibody to Akt phosphorylated at Ser-473 (Cell Signaling), or Thr-308 (Cell Signaling). Identical results were obtained using either antibody or assays of Akt activity. Because Akt activity correlated with the detection of PO4Akt by an immunoblot of cell lysates, immunoblotting for PO4Akt was used in subsequent experiments to assay Akt activation. In control experiments to assay the effectiveness of pertussis toxin, cells under identical conditions were stimulated with IGF-1 (5 nM) or oleoyl-t-a-lysophosphatidic acid (LPA; 10 μM) for 5 min, and cell lysates were subjected to SDS-PAGE, transferred to nitrocellulose membranes, blocked in 5% milk in Tris-buffered saline/Tween 20, and immunoblots were probed for PO4ERK using an antibody specific for phosphorylated MAP kinase (Cell Signaling). Immunoblots were visualized using ECL (Biosciences).
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Fig. 3. IGF-1-stimulated activation of Akt and anti-apoptosis is dependent on β-arrestin-mediated activation of PI3K but is not influenced by inhibition of ERK or G\(_i\) signaling. A, B1 and DKO cells were incubated with wortmannin (100 nM) or LY294002 (10 μM) for 30 min in serum-free medium. After stimulation with IGF-1 (5 nM), cell lysates were subjected to PAGE and blotted for Akt phosphorylated at Ser473. Shown is a representative (n = 3) immunoblot for PO4Akt. B, B1 cells were incubated in medium supplemented with 10% fetal bovine serum (white column), serum-free medium containing wortmannin (100 nM; black column), serum-free medium supplemented with IGF-1 (5 nM; stippled column), or serum-free medium supplemented with IGF-1 (5 nM) and wortmannin (100 nM; shaded column) for a period of 16 h. After the incubation period, cells were trypsinized and counted, and equal numbers of cells were used for each analysis. Apoptosis was quantitated using an ELISA assay for DNA fragmentation. Results are reported as mAU/min units and represent the mean ± S.E. of 10 independent determinations. #, *", p < 0.0001 for comparison of serum-starved cells ± IGF-1; *, p < 0.0001 for comparison of IGF-1 ± wortmannin treatment. C, DKO and B1 cells were incubated with or without pertussis toxin (100 ng/ml) overnight in serum-free medium and then stimulated with IGF-1 (5 nM) for 5 min. Cell lysates were subjected to PAGE and blotted for Akt phosphorylated at Ser473. Shown is a representative (n = 3) immunoblot for PO4Akt and PO4ERK. D, B1 cells were incubated in medium supplemented with 10% fetal bovine serum (white column), serum-free medium (black column), serum-free medium supplemented with IGF-1 (5 nM; stippled column), serum-free medium containing pertussis toxin (100 ng/ml; hatched column), or serum-free medium supplemented with IGF-1 (5 nM) and pertussis toxin (100 ng/ml; shaded column) for a period of 16 h. After the incubation period, cells were trypsinized and counted, and equal numbers of cells were used for each analysis. Apoptosis was quantitated using an ELISA assay for DNA fragmentation. Results are reported as mAU/min units and represent the mean ± S.E. of 10 independent determinations. #, *", p < 0.0001 for comparison of serum-starved cells ± IGF-1; *, p = 0.0002 for comparison of pertussis toxin treated cells ± IGF-1 treatment. E, B1 cells were incubated in medium supplemented with 10% fetal bovine serum (white column), serum-free medium (black column), serum-free medium supplemented with IGF-1 (5 nM; stippled column), serum-free medium containing PD98059 (10 μM; hatched column), or serum-free medium supplemented with IGF-1 (5 nM) and PD98059 (10 μM; shaded column) for a period of 16 h. After the incubation period, cells were trypsinized and counted, and equal numbers of cells were used for each analysis. Apoptosis was quantitated using an ELISA assay for DNA fragmentation, and each experiment was run in triplicate. Results are reported as mAU/min units and represent the mean ± S.E. of three experiments. #, *", p < 0.0004 for comparison of serum-starved cells ± IGF-1 treatment; *, p = 0.01 for comparison of PD98059 treated cells ± IGF-1 treatment.

RESULTS AND DISCUSSION

Separation of wortmannin treatment/H11006 assay for DNA fragmentation. Results are reported as mAU/min units and represent the mean ± S.E., n = 5. #, *", p = not significant for comparison of wortmannin treatment ± IGF-1; *, p < 0.0001 for comparison of IGF-1 ± wortmannin treatment. Shown is a representative (n = 3) immunoblot for PO4Akt and PO4ERK. D, B1 cells were incubated in medium supplemented with 10% fetal bovine serum (white column), serum-free medium (black column), serum-free medium supplemented with IGF-1 (5 nM; stippled column), serum-free medium containing pertussis toxin (100 ng/ml; hatched column), or serum-free medium supplemented with IGF-1 (5 nM) and pertussis toxin (100 ng/ml; shaded column) for a period of 16 h. After the incubation period, cells were trypsinized and counted, and equal numbers of cells were used for each analysis. Apoptosis was quantitated using an ELISA assay for DNA fragmentation. Results are reported as mAU/min units and represent the mean ± S.E. of 10 independent determinations. #, *", p < 0.0001 for comparison of serum-starved cells ± IGF-1; *, p = 0.0002 for comparison of pertussis toxin treated cells ± IGF-1 treatment. E, B1 cells were incubated in medium supplemented with 10% fetal bovine serum (white column), serum-free medium (black column), serum-free medium supplemented with IGF-1 (5 nM; stippled column), serum-free medium containing PD98059 (10 μM; hatched column), or serum-free medium supplemented with IGF-1 (5 nM) and PD98059 (10 μM; shaded column) for a period of 16 h. After the incubation period, cells were trypsinized and counted, and equal numbers of cells were used for each analysis. Apoptosis was quantitated using an ELISA assay for DNA fragmentation, and each experiment was run in triplicate. Results are reported as mAU/min units and represent the mean ± S.E. of three experiments. #, *", p < 0.0004 for comparison of serum-starved cells ± IGF-1 treatment; *, p = 0.01 for comparison of PD98059 treated cells ± IGF-1 treatment.

Separated using SDS-PAGE. Immunoblotting was performed using PY20HRP antibody (BD Transduction Laboratories).

Statistical Analysis—All comparisons are Student’s two-valued t tests unless otherwise specified.
tivity is observed in DKO cells. Total PI3K activity, as assayed in whole cell lysates, is also increased in B1 cells (1.81 ± 0.11-fold induction; p < 0.0002), but not in DKO cells (1.17 ± 0.08-fold induction; p = not significant; n = 9; data not shown).

Generation of phosphatidylinositol 3,4,5-triphosphate by PI3Kα leads to membrane recruitment and activation of Akt, the first discovered downstream target of PI3K (21). Akt activation proceeds via interaction of membrane-bound phosphatidylinositol 3,4,5-triphosphate with the PH domain of Akt, with recruitment of Akt to the plasma membrane where it is phosphorylated by PDK1 on Thr-308 as well as by an unknown kinase on Ser-473 (21, 22). To assess whether β-arrestin1-mediated PI3Kα activation results in activation of Akt, we compared the effect of IGF-1 on Akt phosphorylation and activation in B1 and DKO cells (Fig. 2A). After 60 min of serum starvation, no detectable phosphorylated Akt was observed in either B1 or DKO fibroblasts. Replicating the pattern of activation observed with PI3Kα, IGF-1 stimulation results in robust phosphorylation and activation of Akt in B1, but not DKO, cells. Control blots indicate that Akt is expressed at equal levels in these cell types (Fig. 2A, middle section). Identical results were obtained upon measurement of Akt activity as assayed by phosphorylation of an Akt substrate peptide or by using an immunooassay specific for Akt Thr-308 phosphorylation (data not shown). Although DKO and B1 cells were obtained from a common precursor, we wished to exclude the remote possibility that IGF-1 signaling had become defective in DKO cells. After stimulation with IGF-1, the tyrosine phosphorylation profile of cell lysates of DKO and B1 cells was compared. Under conditions in which Akt phosphorylation was observed only in the B1 cell line (Fig. 2A, top section, PO4Akt blot), IGF-1-stimulated tyrosine phosphorylation was observed in both cell lines (Fig. 2A, bottom section, PO4Tyr blot), suggesting that both cell lines contain a functional receptor. We conclude that, in response to IGF-1 stimulation, β-arrestin1 mediates activation of PI3Kα at the plasma membrane, thereby effecting activation of Akt.

IGF-1 plays a vital role in regulating apoptosis in virtually all cell types, a role reflected by the importance of IGF-1 signaling in transformation, malignancy, and modulating cell survival (15, 23). To determine whether β-arrestin-mediated activation of PI3K and Akt is important to this role of IGF-1 signaling, we compared the ability of IGF-1 stimulation to protect subconfluent B1 and DKO from apoptosis induced by serum withdrawal.

As shown in Fig. 2B, serum withdrawal leads to an increase in apoptosis in both B1 and DKO cells as measured using an assay of DNA fragmentation. Although IGF-1 (5 nM) dramatically inhibits apoptosis in B1 cells, no such effect is observed in DKO cells, suggesting that β-arrestin1 may play an important role in mediating anti-apoptotic signaling in response to IGF-1 stimulation. To confirm these findings, measurement of cell surface annexin was used as an index of apoptosis and yielded identical results (data not shown). Interestingly, DKO cells undergo apoptosis at higher rates upon withdrawal of serum. It is possible that this reflects the presence of minute quantities of growth factors, including IGF-1, that signal via β-arrestin-dependent mechanisms to inhibit apoptosis.

In addition to activation of PI3K, IGF-1 stimulation activates the MAP kinase ERK via the G protein G12 (6, 24, 25). Accordingly, we used chemical inhibitors of PI3K, MEK (an upstream kinase of ERK), and G12 to determine the relative importance of each of these IGF-1-stimulated pathways in β-arrestin1-mediated activation of Akt and anti-apoptosis. Inhibition of PI3K...
with wortmannin or LY294002 blocks IGF-1-stimulated activation of Akt in B1 cells, whereas IGF-1 again fails to activate Akt in DKO cells (Fig. 3A). Irreversible PI3K inhibition using wortmannin also blocks IGF-1-stimulated anti-apoptotic signaling in B1 cells (Fig. 3B), establishing the central role of PI3K in β-arrestin-1-mediated Akt activation and anti-apoptosis. In contrast, inhibition of G<sub>i</sub> signaling with pertussis toxin has no effect on either IGF-1-stimulated Akt activation (Fig. 3C) or the anti-apoptotic effects of IGF-1 (Fig. 3D) in B1 cells, but it does inhibit activation of ERK by either IGF-1 (Fig. 3C, bottom section) or oleoyl-L-α-lysophosphatidic acid (data not shown) by over 65% (24). Inhibition of ERK activation with PD98059, an inhibitor of the upstream kinase MEK, also has no effect on IGF-1-stimulated anti-apoptosis (Fig. 3E). Together, these findings suggest that β-arrestin1 plays a specific role in IGF-1-stimulated activation of PI3K, a pathway required for activation of Akt and responsible for the anti-apoptotic effect of IGF-1.

Stimulation of the IGF-1 receptor results in activation of its intrinsic receptor tyrosine kinase activity, leading to receptor tyrosine auto-phosphorylation as well as to tyrosine phosphorylation of adaptors such as the IRS proteins and Shc (19, 26). These steps are thought to be vital to the recruitment of class IA PI3Ks, which occurs via interaction between the SH2 domains of the regulatory subunits of PI3K and tyrosine-phosphorylated sites on the receptor or on IRS and other adaptor proteins (27). To determine the importance of IGF-1 receptor tyrosine kinase activity for β-arrestin1-mediated activation of PI3K/Akt, we examined the effect of AG1024, a specific inhibitor of IGF-1 receptor tyrosine kinase activity, on IRS-1 phosphorylation and on the activation of Akt in B1 cells.

After a brief incubation in serum-free medium, no phosphorylated IRS-1 or Akt was detected in B1 cells (Fig. 4, lanes 1 and 3). IGF-1 stimulation leads to robust tyrosine phosphorylation of IRS-1 and activation of Akt (Fig. 4, lane 2). In the presence of AG1024, IGF-1-stimulated tyrosine phosphorylation of IRS-1 is abolished (Fig. 4, top section, lane 4), indicating that IGF-1 receptor tyrosine kinase activity is responsible for phosphorylation of IRS-1 and that AG1024 effectively abolishes this activity.

Surprisingly, AG1024 has no effect on IGF-1-stimulated activation of Akt (Fig. 4, bottom section, lane 4). This result suggests that, upon IGF-1 stimulation, β-arrestin1-mediated activation of PI3K/Akt in B1 cells proceeds independently of IGF-1 receptor kinase activity and IRS-1 phosphorylation.

β-arrestin strongly binds the non-receptor tyrosine kinase Src and recruits Src to agonist-occupied receptors, a process important in the activation of MAP kinase cascades in response to β₂-adrenergic receptor activation (28). More recently Src phosphorylation of Akt has been shown to be required for Akt activation (29–31). Given these findings, we considered that β-arrestin-mediated recruitment of Src to the IGF-1 receptor might serve as an alternative signaling mechanism to agonist activation of intrinsic receptor tyrosine kinase activity.

To test this hypothesis, we assessed the ability of two specific inhibitors of Src tyrosine kinase activity, PP2 and SU6656, to block IGF-1-stimulated activation of Akt in B1 cells. Both PP2 (65% inhibition) and SU6656 (75% inhibition) effectively block IGF-1-stimulated activation of Akt in B1 cells (Fig. 5A), indicating that Src activity is required for Akt activation as has been described previously (29–31). These inhibitors did not block activation of PI3K in response to IGF-1 stimulation (Fig. 5B), suggesting that the role of Src is downstream of PI3K and that it is therefore not involved in β-arrestin1-mediated PI3K activation.

The classical paradigm for IGF-1 receptor signaling involves sequential activation of its intrinsic receptor tyrosine kinase activity, receptor auto-phosphorylation, as well as phosphorylation of adaptor proteins, recruitment and activation of PI3K via SH2-PO₄-Yyr interactions, and activation of Akt, leading to anti-apoptosis (Fig. 6, left side) (26, 32). We have discovered a novel, previously unappreciated pathway in which β-arrestin1 mediates activation of PI3K in response to IGF-1 stimulation (Fig. 6, right side). In common with the classical IGF-1 signaling paradigm, β-arrestin1-mediated IGF-1 signaling results in activation of PI3K and Akt. In contrast to the classical pathway, the β-arrestin-mediated pathway does not require IGF-1 receptor tyrosine kinase activity. This pathway also does not require the G protein G<sub>i</sub>, or the activation of the MAP kinase ERK, which has been shown to possess anti-apoptotic signaling capabilities in other cell lines.

The precise nature of the interactions involved in this new pathway has yet to be fully delineated. Both β-arrestin1 and β-arrestin2 play a role in scaffolding a variety of signaling pathways (3, 4). Given that β-arrestin1 is recruited to IGF-1 receptors upon agonist stimulation, one possibility is that β-arrestin1 directly recruits and scaffolds PI3Kα and/or Akt; however, attempts to immunoprecipitate PI3Kα with β-arrestin1 in cellular systems, at both endogenous and over-expressed levels, have failed to demonstrate a direct interaction (data not shown).

β-arrestin1-mediated activation of PI3K/Akt in response to thrombin, a protease that activates a classical seven-membrane spanning receptor, has been described recently (33). A role for β-arrestin in this signaling pathway was imputed based on interference studies in which overexpression of a mutated (“dominant negative”) β-arrestin resulted in decreased activation of PI3K/Akt in response to thrombin stimulation. This
pathway does not seem to proceed via trans-activation of the epidermal growth factor or the platelet-derived growth factor receptor (34) and also has no effect on thrombin-stimulated cellular proliferation. Strikingly, stimulation of the PAR-1 receptor results in rapid tyrosine phosphorylation of both the IGF-1 receptor and the adaptor protein IRS-1 (35, 36). Taken together, these data raise the possibility that thrombin-stimulated, β-arrestin-dependent activation of Akt might occur via activation of the IGF-1-signaling pathway. This could occur via trans-activation of the receptor in a manner reminiscent of that described for the angiotensin-epidermal growth factor receptor system (4), with subsequent β-arrestin-mediated signaling downstream of the IGF-1 receptor as was observed in the current study. Alternatively, agonist-mediated β-arrestin recruitment to the thrombin GPCR might lead to recruitment and activation of Src or other tyrosine kinases, which might then phosphorylate downstream targets, leading to activation of PI3K and Akt (35, 36).

The activation of PI3K is a key step that couples the activation of receptor tyrosine kinases to downstream effects. The data presented here are the first to implicate β-arrestin in the activation of PI3K in response to stimulation of a receptor tyrosine kinase. This signaling mechanism has several novel aspects as well as significant implications for receptor biology. We demonstrate that this step leads to activation of Akt and plays a vital role in the important anti-apoptotic effects of IGF-1. This pathway proceeds independently of the tyrosine kinase activity of the receptor, presenting a novel signaling motif whereby β-arrestin, perhaps by serving a scaffolding function with a non-receptor kinase, bypasses the requirement for activation of intrinsic receptor tyrosine kinase activity (Fig. 6). Perhaps of greater significance, our findings provide evidence that GPCRs and receptor tyrosine kinases act via overlapping mechanisms and that β-arrestin bridges the signaling capabilities of these two classes of receptors. This further raises the possibility that β-arrestins might play more widespread roles in receptor tyrosine kinase signaling than have been appreciated previously.

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