β₁-adrenergic receptors, expressed at high levels in the human heart, have a carboxyl-terminal ESKV motif that can directly interact with PDZ domain-containing proteins. Using the β₁-adrenergic receptor carboxyl terminus as bait, we identified the novel β₁-adrenergic receptor-binding partner GIPC in a yeast two-hybrid screen of a human heart cDNA library. Here we demonstrate that the PDZ domain-containing protein, GIPC, co-immunoprecipitates with the β₁-adrenergic receptor in COS-7 cells. Essential for this interaction is the Ser residue of the β₁-adrenergic receptor carboxyl-terminal ESKV motif. Our data also demonstrate that β₁-adrenergic receptor stimulation activates the mitogen-activated protein kinase, ERK1/2. β₁-adrenergic receptor-mediated ERK1/2 activation was inhibited by pertussis toxin, implicating Gₛ, and was substantially decreased by the expression of GIPC. Expression of GIPC had no observable effect on β₁-adrenergic receptor sequestration or receptor-mediated cAMP accumulation. This GIPC effect was specific for the β₁-adrenergic receptor and was dependent on an intact PDZ binding motif. These data suggest that GIPC can regulate β₁-adrenergic receptor-stimulated, Gₛ-mediated, ERK activation while having no effect on receptor internalization or Gₛ-mediated cAMP signaling.

β₁-Adrenergic receptors (βARs)¹ play a critical role in the regulation of cardiovascular function (1). There are three subtypes of βAR, β₁AR, β₂AR, and β₃AR. Both the β₁AR and β₂AR subtypes are expressed in the heart and appear to regulate cardiac function through similar intracellular signaling pathways (2). These receptors are members of the seven transmembrane spanning receptor superfamily and are thought to signal largely through heterotrimeric G proteins; primarily Gₛ. Activation of Gₛ stimulates adenylyl cyclase increasing intracellular cAMP. Increased cAMP activates cAMP-dependent protein kinase (PKA), which is believed to mediate many of the cellular responses associated with βAR activation (3, 4).

Despite similarities in signaling, there are several striking physiological differences between the β₁AR and β₂AR subtypes (5, 6). For example, β₁AR and β₂AR subtypes play opposing roles in regulating cardiac myocyte apoptosis: stimulation of the β₁AR increases apoptosis whereas stimulation of the β₂AR inhibits apoptosis (7, 8). β₂AR-induced apoptosis is implicated in the transition from cardiac hypertrophy to heart failure (9, 10). The differential regulation of cardiac cell survival by these βAR subtypes has been partially explained by the ability of the β₁AR to couple to the pertussis toxin (PTX)-sensitive G protein, Gₛ (11). β₂ARs in human and mouse cardiomyocytes have been shown to activate both Gₛ and G₁, whereas β₁AR stimulation has, thus far, only been shown to activate Gₛ (12). Activation of the β₂AR, stimulates a Gₛ-mediated PI3K-Akt-dependent cell-survival signaling pathway and prevents cardiomyocytes from undergoing Gₛ-mediated apoptosis (11). Recent studies suggest that Gₛ switching by the β₂AR is the consequence of PKA-mediated receptor phosphorylation (13, 14). β₂AR/Gₛ coupling has also been implicated in receptor-mediated ERK activation (13, 15). β₂AR-stimulated ERK1/2 activation has been reported in cultured HEK-293 and COS-7 cells, and in isolated cardiac myocytes (16, 17). This β₂AR-stimulated ERK1/2 activation is mediated by β₂γ subunits of PTX-sensitive G proteins (Gₛ) through a pathway involving the non-receptor tyrosine kinase c-Src, small G protein Ras and Raf-1 kinase (15, 18). The mechanism(s) of β₂AR-stimulated ERK activation is somewhat more complex. A number of groups have reported that the β₂AR is unable to stimulate ERK activation, conclusion based on the perceived inability of the β₂AR to couple to Gₛ (19, 20). However, recent data from cardiac myocytes suggest that the β₂AR can activate ERK and p38 in a Gₛ-dependent manner; even though the β₂AR is less potent in stimulating ERK activation than the β₁AR (11, 21). The mechanisms behind the observed differential activation of ERK are still unknown.

Recent data suggest that βARs may transduce signals that are both dependent and independent of heterotrimeric G proteins (22, 23). Well known receptor-interacting proteins, such as β-arrestins and G protein-coupled receptor kinases (GRKs), which were thought previously only to regulate receptor desensitization, have been implicated in signaling pathways that may be G protein-independent (24–26). Another G protein-independent mechanism underlying βAR-mediated cell signaling has recently been demonstrated. Both β₁ARs and β₂ARs have carboxyl-terminal PDZ binding motifs reported to bind to PDZ domain-containing proteins (27, 28). The β₂AR has been...

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The abbreviations used are: βAR, β-adrenergic receptor; β₁AR, β₁-adrenergic receptor; β₂AR, β₂-adrenergic receptor; β₃AR, β₃-adrenergic receptor; CT, carboxyl terminus; ERK, extracellular signal-regulated kinase; NHERF, Na⁺/H⁺-exchanger regulatory factor; PDZ, PSD-95/Dlg/ZO-1 homology domain; PTX, pertussis toxin; ISO, isoproterenol.
shown to interact with Na+/H+ exchanger regulatory factors (NHERF) in an agonist-dependent manner via this PDZ binding domain (27); while the βAR interacts with the posttranscriptional density-enriched proteins, PSD-95 and MAGI-2 (28, 29). Association with PSD-95 regulates agonist stimulated βAR internalization and may also provide a molecular mechanism by which βARs are localized to the synapse, regulating synaptic plasticity (28). Interactions between the βAR and PDZ domain-containing proteins can be regulated by GRK5 (30).

Furthermore, many signaling pathways in cardiac myocytes have recently demonstrated that the PDZ binding motif of the βAR modulates receptor trafficking and signaling (31).

In order to identify proteins that interact with the PDZ binding domain of the βAR, we performed a yeast two-hybrid screen on a human heart cDNA library, using the βAR carboxyl-terminal tail as bait. From this screen we identified GIPC (GaIP-interacting protein, carboxyl (C) terminus), a PDZ-containing protein, as a novel binding partner of the βAR. Here we characterize this interaction and its functional consequences.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Antibodies—Mammalian expression plasmids pCDNA3/FLAG-βAR, pcDNA3/FLAG-β2AR (S475A), and pcDNA3/FLAG-β2AR have been described previously (28, 32). The construction of yeast plasmids pAS2–1/βAR-CT and pAS2–1/β2AR-CT has also been described previously (28). GFP-tagged ERK2 in pEGFP-N1 expression vector was a gift from Dr. Bunnett at UCSF (33). Rat pcACT2/GIPC was a gift from Dr. Farquhar (UCSD) (34). Myc-tagged rat GIPC in pcCMV-Tag2B expression plasmid was constructed by cloning a GIPC fragment from pcACT2/GIPC into BamHI Xhol sites of the pcCMV Tag2B vector (Stratagene). The expression plasmid for the FTX-resistant G protein α1G (C53IS) was purchased from Guthrie cDNA Resource Center. Bait plasmid and library cDNAs were co-transformed into the yeast strain PJ69–4A using a standard yeast transformation protocol. Yeast were plated on selective medium (S.D./Leu/Trp/His, + 10 mM 3AT) and allowed to grow for 4–6 days at 30 °C. Positive colonies were then streaked on selective medium (S.D./Leu/Trp/Ade or S.D./Leu/Trp/His) plates. Plasmid DNA was rescued from positive colonies that grew on selective plates. Plasmid DNA was rescued from positive colonies that grew on selective medium (S.D./Leu/Trp/His, + 10 mM 3AT) and allowed to grow for 4–6 days at 30 °C. Positive colonies were then streaked on selective medium (S.D./Leu/Trp/Ade or S.D./Leu/Trp/His) plates. Plasmid DNA was rescued from positive colonies that grew on selective plates.

**Yeast Two-hybrid Screening—** Plasmid pAS2–1/βAR-CT was used as bait to screen a human heart cDNA library, using the βAR carboxyl-terminal tail as bait. From this screen we identified GIPC (GaIP-interacting protein, carboxyl (C) terminus), a PDZ-containing protein, as a novel binding partner of the βAR. Here we characterize this interaction and its functional consequences.

**Cell Culture and Transfection—** All tissue culture media and related reagents were purchased from Invitrogen. COS-7 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in a 37 °C incubator under 5% CO2. Cells in 100-mm dishes were transfected with LipofectAMINE (at 4:1 ratio with DNA) according to the manufacturer’s protocol. HEK-293 cells were maintained under the same conditions as COS-7 cells except minimal essential medium (MEM) was used. HEK-293 cells were transfected with FuGENE 6 (at 2.5:1 ratio with DNA) according to the manufacturer’s protocol (Roche Applied Science). After transfection, cells were grown 36–48 h before agonist stimulation. The cells were incubated for 30 min at room temperature with continuous slow rocking. The cross-linking reaction was terminated by quickly removing the cross-linking buffer and replacing it with 1 ml of ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 10% glycerol, 0.5% Nonidet P-40, and protease inhibitors). The cells were lysed by incubation on ice for 30 min and then clarified by centrifugation at 21,000 × g for 12 min at 4 °C. The clarified supernatants were then used in cellular coimmunoprecipitation experiments. 50 μl of each supernatant was diluted into an equal amount of 3 × SDS-PAGE sample buffer to serve as whole-cell extract controls.

1 ml of clarified cell extract was then incubated with 25 μl of anti-FLAG M2 affinity gel slurry at 4 °C with gentle rotation for 4 h to overnight. Beads were washed four to five times with ice-cold lysis buffer, and the bound proteins were eluted with 50 μl of 3 × SDS-PAGE sample buffer. Equivalent amounts of sample in 3 × sample buffer were resolved on a precast 4–20% polyacrylamide gel (Invitrogen) and then transferred to nitrocellulose membranes by semi-dry blotting. Nitrocellulose membranes were blocked with 5% fat-free milk in TBST (20 mM Tris, pH 7.4, 500 mM NaCl, and 0.1% Tween 20) and incubated with the appropriate primary antibody at room temperature for 1 h. After washing with TBST, membranes were incubated for 1 h with horseradish peroxidase-conjugated anti-mouse or rabbit IgG secondary antibody (1:2000). Protein bands were visualized by SuperSignal Chemiluminescence Substrate (Pierce). Quantitation of band density was performed with Bio-Rad Fluor-S MultiImager.

**ERK Phosphorylation—** Twenty-four hours after transfection, cells were split into 6-well dishes and then incubated in serum-free media (1% DMEM for COS-7 and MEM for HEK-293, 0.1% bovine serum albumin, 10 mM HEPES, pH 7.4) overnight before agonist stimulation. Agonist stimulation was performed at 37 °C in serum-free media for the times indicated in the figure legends. The medium was removed, and cells were then solubilized in 3 × SDS-PAGE sample buffer. The whole cell lysates were sonicated and resolved by SDS-PAGE. ERK phosphorylation was detected by Western blot using anti-phospho-ERK1/2 antibody described above. The total ERK2 in the cell lysates was detected using anti-ERK2 polyclonal antibody. Protein bands were visualized by SuperSignal Chemiluminescence substrate and quantitated with Bio-Rad Fluor-S MultiImager.

**Receptor Internalization and Cyclic AMP Accumulation Assays—** For receptor internalization assays, HEK-293 cells in 100-mm dishes were transiently transfected with pcDNA3/FLAG-βAR in the presence and absence of Myc-GIPC. One day after transfection, cells were split into poly-n-lysine-coated 6-well plates (Biocoat) and grown overnight at 37 °C. Cells were serum-starved for 1 h before stimulation with 10 μM ISO for 30 min at 37 °C. Cells were then placed on ice and cell surface FLAG-tagged receptors were detected with anti-FLAG M2 antibody followed by FITC-conjugated anti-mouse IgG as described previously (35). Receptor internalization was defined as the percentage of cell surface receptors lost after agonist stimulation, measured by cell flow cytometry.

For cyclic AMP accumulation assays, HEK-293 cells in 100-mm dishes were transiently transfected with pcDNA3/FLAG-βAR in the presence and absence of transfected Myc-GIPC. Twenty-four hours after transfection, cells were split into 12-well collagen-coated plates and then labeled with modified essential medium supplemented with 5% fetal bovine serum and 2 μCi/ml [3H]adenine for 4 h to overnight. Cells were serum-starved for 30 min and then stimulated with βAR selective agonist dobutamine or 10 μM forskolin for 10 min. Cyclic AMP accumulation was quantitated by chromatography and expressed as a percentage of [3H]adenine incorporated into cyclic AMP as described previously (35).

**RESULTS**

**GIPC Binds Specifically to the βAR Carboxyl-terminal Tail—** In our search for potential βAR binding partners, we used the βAR carboxyl-terminal tail (βAR-CT) as bait in a yeast two-hybrid screen of a human heart cDNA library. From a total of 4 million independent colonies screened, 24 positive clones were obtained. Four of the positive clones yielded identical sequence encoding a portion of the gene for human GIPC (hGIPC), with an N-terminal truncation at residue 74. We also isolated rat GIPC from a yeast two-hybrid screen of rat brain. The isolated rat sequence (rGIPC) encoded full-length GIPC plus a portion of the 5′-untranslated region (Fig. 1).

The specificity of the interaction between GIPC and the βAR-CT was confirmed by further yeast two-hybrid analysis. GIPC clones were transformed back into yeast strain PJ69–4A.
GIPC regulates β_{1}AR-mediated ERK activation. HEK-293 cells were transiently transfected with pCDNA3/FLAG-β_{1}AR alone or with Myc-GIPC. Forty-eight hours after transfection, cells were treated with serum-free medium for 30 min and then stimulated with the β_{1}AR-selective agonist dobutamine (at the concentrations indicated) or 50 μM forskolin at 37 °C. It was necessary to use dobutamine instead of ISO for these experiments to avoid activation of endogenous β_{2}-adrenergic receptors in the HEK-293 cells. Whole cell cAMP accumulation was determined by chromatography and presented as percentage conversion of [3H]adenine into [3H]cAMP. Dobutamine-induced cAMP accumulation was normalized to that induced by 10 μM forskolin. These data are representative of three similar experiments.

wild-type β_{1}AR. However, the β_{1}AR variant V477A, which is unable to bind with PSD-95, showed a very strong interaction with GIPC. These studies demonstrate by both yeast two-hybrid screening and cellular co-immunoprecipitation experiments that the GIPC specifically interacts with the β_{1}AR through the PDZ domain. Unlike the association of the β_{1}AR with PSD-95, the Val in the last position (Val-477) of the β_{1}AR association (data not shown). Consist-

ent with the yeast two-hybrid results, GIPC did not co-immunoprecipitate with the full-length FLAG-β_{2}AR when the two proteins were co-expressed in COS-7 cells.

To examine the structural determinants of the GIPC/β_{1}AR interaction, we used several β_{1}AR mutants, in which three out of the last five residues were replaced with Ala individually (28). Similar to that observed with the β_{1}AR/PSD-95 interaction, when the Ser residue at the -2 position of the β_{1}AR was mutated to either Ala (S475A) or Asp (S475D), the association of GIPC with the β_{1}AR could no longer be detected. When GIPC was co-expressed with the β_{1}AR mutant S473A, co-immunoprecipitation of GIPC was reduced only slightly relative to the interaction, when the Ser residue at the -2 position of the β_{1}AR was mutated to either Ala (S475A) or Asp (S475D), the association of GIPC with the β_{1}AR could no longer be detected. When GIPC was co-expressed with the β_{1}AR mutant S473A, co-immunoprecipitation of GIPC was reduced only slightly relative to the

wild-type β_{1}AR. However, the β_{1}AR variant V477A, which is unable to bind with PSD-95, showed a very strong interaction with GIPC. These studies demonstrate by both yeast two-hybrid screening and cellular co-immunoprecipitation experiments that the GIPC specifically interacts with the β_{1}AR through the PDZ domain. Unlike the association of the β_{1}AR with PSD-95, the Val in the last position (Val-477) of the β_{1}AR association (data not shown). Consist-

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**Fig. 4. β₁AR-mediated ERK activation.** COS-7 cells were transiently transfected with the β₁AR and GFP-ERK2. Forty-eight hours after transfection, cells were treated with serum-free medium overnight. ERK activation was performed as described under “Experimental Procedures.”

**A.** β₁AR stimulation enhanced ERK phosphorylation. Serum-starved COS-7 cells were stimulated for 5 min with 10 μM ISO at 37 °C. The cells were

**C.** ELK Activation (Folds Stimulation)

**D.** ELK Activation (% of the ISO control)

**E.** ELK Activation (% maximum)

**B.** ERK activation was measured as described under “Experimental Procedures.”
tamine are shown in Fig. 3. Similar to previous results with PSD-95, GIPC expression had no effect on β1AR-mediated whole cell cAMP accumulation, changing neither the maximal level of cAMP accumulation nor the EC50. Because PDZ domain-containing proteins have previously been shown to regulate receptor internalization (28, 29), we also examined the effect of GIPC on β1AR internalization in HEK-293 cells. GIPC expression had no effect on agonist stimulated β1AR internalization (data not shown).

β1AR-stimulated ERK Activation—In order to examine β1AR-mediated ERK activation, we used a plasmid encoding an ERK2-GFP fusion protein. This allowed for GFP-ERK2 to be easily separated from endogenous ERK1/2 by SDS-PAGE. In COS-7 cells transiently transfected with pcDNA3/FLAG-β1AR and GFP-ERK2, ISO stimulation induced robust ERK phosphorylation/activation compared with non-stimulated cells (Fig. 4A). In control cells transfected with GFP-ERK2 alone, ISO-stimulated ERK phosphorylation was not detected using phospho-ERK antibody.

Similar to previous studies of the β1AR, β1AR-stimulated ERK activation was a relatively fast process, peaking at around 5 min. At maximum, ISO-stimulated ERK activation was 6−8 fold greater than that of non-stimulated control cells (Fig. 4B). After peaking at 5 min, β1AR-stimulated ERK activation steadily decreased to ~50% of maximum at 15 min. β1AR-stimulated ERK activation was observed over a range of agonist concentrations. The dose response curve for ISO-stimulated ERK activation is shown in Fig. 4C.

Next we examined the effect of different agonists on β1AR-mediated ERK activation in COS-7 cells. The β1AR has a high affinity for both norepinephrine and epinephrine (2). As predicted, norepinephrine (NE) stimulation dramatically increased ERK activation (Fig. 4D). The β1AR specific agonist dobutamine (Dobu) also stimulated ERK activation, but was much less active than either NE or ISO. As a control, PMA, the direct activator of PKC, demonstrated robust ERK activation, whereas the direct activator of adenylyl cyclase, forskolin (Forsk), showed no demonstrable ERK activation. As expected, ISO-stimulated ERK activation was completely blocked by the β-adrenergic receptor antagonists propranolol (PRO) and alprenolol (ALP) (Fig. 4E).

Treatment of cells with 10 μM PTX overnight decreased β1AR-mediated ERK activation considerably. To further demonstrate that PTX inhibited the activation of ERK by inhibiting the αi subunit of the G protein, we overexpressed a G protein αi subunit (C351S), which cannot be ADP-ribosylated and is therefore unaffected by PTX treatment. As shown in Fig. 4F, overexpression of Gαi (C351S), while having little effect on the overall ISO-stimulated ERK activity, leads to a marked increase in ISO-stimulated ERK activation observed in the presence of PTX. The residual effect of PTX is presumably due to inhibition of endogenous Gαi. These data further support the idea that the β1AR activates ERK through a Gαi-mediated pathway, similar to that reported for the β2AR (13). In contrast to findings with the β2AR (13), the PKA inhibitor H89 had no significant effect on β1AR-mediated ERK activation.

GIPC regulates β1AR-mediated ERK activation—GIPC is a PDZ domain-containing protein that interacts with a Gi-specific RGS protein called GαiP (Ga-interacting protein) (34); therefore, GIPC could be involved in the regulation of Gi-mediated signaling. Here we tested the effect of GIPC on β1AR-stimulated ERK activation, which may be mediated by the PTX-sensitive G protein, Gαi. As shown in Fig. 5A, expression of GIPC substantially decreased ISO-stimulated β1AR-mediated ERK activation. ISO-stimulated ERK activation in the presence of GIPC was decreased by 45% compared with control cells lacking GIPC (Fig. 5, A and D). Under similar conditions, expression of GIPC had little effect on β2AR-mediated ERK activation (Fig. 5B). The inhibitory effect of GIPC appeared to be specific for the β1AR. GIPC had no significant effect on the ability of the β1AR mutant (S475A), which is unable to interact with GIPC, to activate ERK (Fig. 5C). These data suggest that the ability of GIPC to regulate β1AR-mediated ERK activation is dependent on its ability to interact directly with the β1AR via its PDZ domain.

DISCUSSION

β1ARs are of primary importance in regulating heart rate and contractility. Through a yeast two-hybrid screen of a human heart cDNA library, we identified GIPC as a novel binding partner for the β1AR. GIPC is a PDZ domain-containing protein that was originally identified and named as a protein that interacts with GαiP, an RGS (regulator of G protein signaling) protein (34). In addition to GαiP, GIPC has also been reported to interact with several membrane proteins through its PDZ domain; these include the Glut-1 transporter, transmembrane Semaphorin-F (M-SemF) (36), neurophilin-1 (37), insulin-like growth factor 1 receptor (38), gp75 (tyrosinase-related protein-1), transforming growth factor β (TGF-β) receptor (39), 5T4 transmembrane glycoprotein (40), integrins α5, α6, and 6A (41, 42), and the viral protein TAX (43).

Here we report a specific interaction between the β1AR and GIPC. Despite the fact that both the β1AR and β2AR contain similar PDZ binding motifs, GIPC interacts only with the β1AR, as demonstrated by both yeast two-hybrid screening and co-immunoprecipitation experiments. The GIPC PDZ domain facilitates the interaction with the β1AR. Mutation of Ser-475 to either Ala (S475A) or Asp (S475D) completely eliminated the interaction. However, unlike that reported for PSD-95, changing the last residue Val-477 of the β1AR to Ala had no effect on the β1AR/GIPC association. TAX, a protein containing a similar carboxyl-terminal ETEA-COOH motif has also been shown to interact with GIPC (43). GIPC expression has no significant effect on β1AR-induced cAMP accumulation. Therefore, similar

solubilized in 3 X SDS-PAGE sample buffer. Phosphorylation of the ERK in the whole cell lysates was detected by Western blot analysis using an anti-phospho-ERK1/2 antibody. The data presented in A−D are representative of a minimum of three independent experiments. B, dose-dependent ISO-stimulated β1AR-stimulated ERK activation. Serum-starved COS-7 cells were incubated with different concentration of ISO for 5 min and then phospho-ERK was determined by anti-phospho-ERK1/2 antibody. C, time course of the β1AR-stimulated ERK activation. Serum-starved COS-7 cells were incubated with 10 μM ISO for the indicated times, and then ERK phosphorylation in the whole cell lysates was detected using an anti-phospho-ERK1/2 antibody. D, effect of agonist stimulation on the β1AR-stimulated ERK activation. Serum-starved COS-7 cells were incubated with 10 μM ISO, norepinephrine (NE), dobutamine (Dobu), 1 μM PMA or 10 μM forskolin (Forsk) for 5 min. The ERK phosphorylation in the whole cell lysates was detected using anti-phospho-ERK1/2 antibody. The amount of phosphorylated-ERK from each sample was normalized to that from unstimulated cells. E, β1AR-stimulated ERK activation was inhibited by β antagonist or PTX pretreatment, but not by the PKA inhibitor H89. Serum-starved COS-7 cells were treated with 100 ng/ml PTX overnight, or 10 μM H89, 10 μM propranolol (Pro) or 10 μM alprenolol (ALP) for 30 mins prior to ISO stimulation. The amount of ERK phosphorylation from ISO-stimulated cells was set as control. The amount of phosphorylated-ERK from each sample was expressed as a percentage of control. The data are presented as means ± S.E. from four independent experiments. F, β1AR-stimulated ERK activation was inhibited by PTX. The effects of PTX treatment were reversed by overexpressing a mutant Gαi (C351), which could not be ADP-ribosylated. Serum-starved COS-7 cells were treated with 100 ng/ml of PTX overnight, stimulated with 10 μM ISO, and run on Western blots as described previously. The amount of phosphorylated-ERK from each sample was expressed as a percentage of control in cells expressing β1AR. *, p < 0.05.
to PSD-95, the association of GIPC with the β1AR had no effect on β1AR coupling to Gs. In contrast to PSD-95, which inhibits receptor internalization in HEK-293 cells (31), GIPC had little to no effect on agonist-stimulated receptor internalization.

Recently, many GPCRs have been reported to activate MAP kinase cascades regulating cell growth and/or proliferation (18). In COS-7 cells, it has been clearly demonstrated that stimulation of the β2AR activates the MAP kinase ERK through a Gs-mediated pathway (13, 44). In cardiac myocytes, β2AR stimulation has been shown to activate the MAP kinases ERK, JNK, and p38 (21, 45). However, β1AR-mediated ERK activation or coupling to Gs is still quite controversial. Here, we demonstrate β1AR-stimulated, PTX-sensitive, ERK activation; strongly suggesting that the β1AR, like the β2AR, can signal through both Gs and Gi.

GIPC, similar to many PDZ domain-containing proteins, has been shown to play an important role in organizing signaling cascades (40), anchoring proteins in specific subcellular compartments (40), and regulating cell signaling (39). GIPC regulates the expression of the TGF-β receptor at the cell surface and enhances the cellular response to TGF-β (39). GIPC has also been linked to G protein signaling by its ability to interact with the RGS protein Gaip (46). Overexpression of GIPC in PC12 cells decreases NGF induced MAP kinase (ERK1/2) activation (38). Interestingly, a GIPC-like protein Kermit, which interacts with the Frizzled receptor, has been reported to regulate frizzled 3 signaling in neural crest development (47). Here we provided further evidence that GIPC regulates G protein signaling by directly interacting with the β1AR in a PDZ-dependent manner. Expression of GIPC decreased the ability of the β1AR to stimulate ERK activation, but had no effect on β2AR-mediated cAMP accumulation. This regulatory effect of GIPC on ERK activation is specific for the β1AR and depends on a PDZ-mediated interaction, since expression of GIPC has no effect on the ERK activation stimulated by either the β2AR or the β1AR mutant (S475A). These data suggest that the GIPC/β1AR association affects receptor Gs- but not Gq-mediated signaling.

In mouse cardiac myocytes, stimulation of the β1AR leads to a PKA-dependent increase in the rate of contraction (48). β2AR stimulation shows a biphasic effect on the rate of cardiac myocyte contraction, with an initial PKA-dependent increase followed by a PTX-sensitive decrease, mediated by Gi (48). Disruption of the PDZ binding motif in the β2AR changed the signaling profile of the β1AR in cardiac myocytes to more resemble that of the β2AR (31). These data suggest that the β2AR is capable of coupling to Gi, but that association with PDZ-containing proteins such as PSD-95 could prevent this interaction from occurring. GIPC interacts with the β1AR through its PDZ domain and thereby specifically inhibits β1AR-stimulated Gs-mediated signaling. Thus, the GIPC effect on β1AR-mediated ERK activation might explain the differential effects of β2AR and β1AR stimulation on the rate of cardiac myocyte contraction.

The effect of GIPC on β1AR/Gs-mediated signaling could also explain the differential effects of β1AR and β2AR stimulation on cardiac myocyte apoptosis. β1AR stimulation leads to cardiac myocyte apoptosis, whereas stimulation of the β2AR concurrently activates pro-apoptotic and anti-apoptotic signals, the net effect being increased cell survival (8). Differential coupling to Gs and Gi has been used to explain this differential effect of β1AR versus β2AR stimulation (10). Here we demonstrate that the β1AR can couple to both Gs and Gi, similar to a number of other GPCRs including the histamine, serotonin, and glucagon receptors (20). The continued study of receptor-interacting proteins, such as PDZ-containing proteins, will further our understanding of differential signaling by receptors.

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REFERENCES
1. Brodde, O. E. (1993) Pharmacol. Ther. 60, 405–430
2. Brodde, O. E. (1991) Pharmacol. Rev. 43, 203–242
3. Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002) Nat. Rev. Mol. Cell. Biol. 3, 629–650
4. Lefkowitz, R. J. (2000) Nat. Cell Biol. 2, E133–E136
5. Steinhberg, S. F. (1999) Circ. Res. 85, 1101–1111
6. Xiao, R. P., Cheng, H., Zhou, Y. Y., Kuschel, M., and Lakatta, E. G. (1999) Circ.
GIPC Interacts with the β1-Adrenergic Receptor and Regulates β1-Adrenergic Receptor-mediated ERK Activation
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