β-Arrestins were initially shown, in conjunction with G protein-coupled receptor kinases, to be involved in the desensitization and internalization of activated seven-transmembrane receptors. Recently, β-arrestin 2 has been shown to act as a signal mediator in mitogen-activated protein kinase cascades and to play a positive regulatory role in chemotaxis. We now show that β-arrestin 1 is required to activate the small GTPase RhoA leading to the re-organization of stress fibers following the activation of the angiotensin II type 1A receptor. This angiotensin II type 1A receptor-directed RhoA activation and stress fiber formation also require the activation of the heterotrimeric G protein Gαq/11. Whereas neither β-arrestin 1 nor Gαq/11 activation alone is sufficient to robustly activate RhoA, the concurrent recruitment of β-arrestin 1 and activation of Gαq/11 leads to full activation of RhoA and to the subsequent formation of stress fibers.

Stress fiber formation and the polymerization of actin structures are fundamental processes required for cell motility, adhesion, and contraction (1–3). The Rho family of small GTPases (Rho, Rac, and Cdc42) is involved in such cytoskeletal rearrangement (4, 5). RhoA in particular is important for stress fiber formation, whereas Rac and Cdc42 lead to lamellipodia and filopodia formation, respectively. Rho is activated by cytosolic receptors, the epidermal growth factor receptor and several 7TMs (6, 7) including AT1R, the angiotensin type II 1 receptor (8, 9). Physiologically, the angiotensin type II 1 receptor plays important roles in the vasculature and renal systems and is implicated in several disease states including hypertension, heart failure, vascular thickening, and cardiac hypertrophy and remodeling (10, 11). Stress fibers in cardiac myocytes, characteristic of cardiac hypertrophy, can be induced by angiotensin II (Ang II)-activated AT1R (12). Furthermore, Rho and its downstream effector of Rho, Rho-associated kinase (ROCK), have been implicated in Ang II-induced effects in vivo (13, 14) and in vitro (15–19).

7TM-mediated Rho activation has been shown to occur primarily through the heterotrimeric G proteins Gαq/11, Gα12, and Gα13 (6). The AT1R signals primarily through the heterotrimeric proteins Gαq/11 to downstream effectors such as phospholipase C and protein kinase C (10). Some controversy exists as to the role of Gαq/11 in Rho activation, as some reports clearly show that Gαq/11 is responsible for Rho activation (20–26), whereas other reports demonstrate no involvement of Gαq/11 in Rho activation (27–30). Furthermore, in studies where Gαq/11 activate Rho, this activation can occur in either a phospholipase C (PLC)- and protein kinase C (PKC)-dependent manner or PLC- and PKC-independent manner (26). Hence, a variety of heterotrimeric G proteins have been shown to link 7TMs to Rho through several distinct pathways that are both 7TM- and cell type-dependent.

Upon the activation of 7TMs, such as the AT1R, scaffolding proteins called β-arrestins are recruited to the cytoplasmic face of the receptor where they desensitize heterotrimeric G protein signaling (31–33) and facilitate receptor endocytosis by recruiting components of the endocytic machinery such as clathrin and AP-2 (34, 35). Furthermore, β-arrestin 2 is required for signaling from 7TMs through the mitogen-activated protein kinase (MAPK) cascade to activate ERK1/2, c-Jun N-terminal kinase, and p38 (36), and in some instances, β-arrestin 2 can activate ERK1/2 in the absence of heterotrimeric G protein activation (37). In these studies, the AT1R proved advantageous for distinguishing between heterotrimeric G protein and β-arrestin 2 signaling because of the availability of mutant receptors (38) and Ang II analogs (39) that fail to couple the AT1R to heterotrimeric G proteins while preserving β-arrestin 2 recruitment and signaling (37).

There is growing evidence for a role of β-arrestins in facilitating small GTPase-mediated events. For example, β-arrestin 2 has been implicated in chemotaxis (40–42) and both β-arrestin 1 and 2 have been shown to directly interact with the small GTPase Arf6 and the guanine nucleotide exchange factors, ARNO (43) and Ras-GDS (44). In light of reports of β-arrestin 2-dependent signaling (37, 45, 46) and β-arrestin involvement in small GTPase pathways (40–44), this study was undertaken to investigate the potential role of β-arrestins in RhoA activation and stress fiber formation. As a model, we utilized human
embryonic kidney 293 (HEK293) cells stably expressing the
AT1AR (AT1AR-HEK293). Here we show that in a concerted
mechanism with G_{q/11}, -arrestin 1 is a critical component of
RhoA activation and stress fiber formation following Ang II
activation of the AT1AR.

EXPERIMENTAL PROCEDURES

Materials—All of the tissue culture reagents, human Ang II, and
G_{q/11} inhibitor pertussis toxin were purchased from Sigma. ROCK in-
hibitor Y-27632, PLC inhibitors ET-18-OCH3 and U-73122, and PKC
inhibitors Go 6976, Go 6983, and RO-31–8425 as well as the tyrosine
kinase inhibitor genistein were all purchased from Calbiochem (Darm-
stadt, Germany). S18I4I8 Ang II (SII) was purchased from the Cleveland
Clinic Core Synthesis Facility (Cleveland, OH). cDNA constructs for
G_{q/11} and G_{q/11}QL were generous gifts from the laboratory of Dr. Patrick
Casey (Duke University, Durham, NC).

Cell Culture—HEK293 cells were cultured in minimum Eagle’s me-
dium supplemented with 10% fetal bovine serum and 1% penicillin/
streptomycin (Sigma). To make HEK293 cells stably expressing the
AT1AR, cells were transfected with rat AT1AR cDNA that contained a
zeocin-selectable marker using FuGENE 6 (Roche Diagnostics, Basel,
Switzerland) according to the manufacturer’s instructions. Stable
clones were selected that contained 1.6 ± 0.2 pmol/mg protein of AT1AR
expression as determined by radioligand binding and were maintained
in zeocin (Invitrogen).

RNAi for -arrestin 1, -arrestin 2, and G_{q/11}—AT 1AR-HEK293 cells
were split to a density of 100,000 cells/well into 6-well dishes at least
24 h prior to the transfection of control siRNA or siRNAs targeted
against -arrestin 1, -arrestin 2, or G_{q/11} using the Gene Silencer
transfection reagent (Gene Therapy Systems, San Diego, CA) as de-
scribed previously (45). The siRNA sequence targeting overlapping
regions of G_{q/11} and G_{q/11} between nucleotides 931 and 951 relative to
their start codons was 5'-AAGATGTCGTCGACCTGAAC-3'. RhoA-
pulldown and stress fiber formation assays were performed 3 days
following the transfection of siRNA(s).

GST-Rhotekin Pulldown Assay—GST-Rhotekin beads were prepared
as described previously (47) with the exception that glutathione beads

FIG. 1. -arrestin 1-dependent RhoA activation by Ang II over time and dose. A, representative blots of RhoA-GTP, Total RhoA,
-arrestin 1, and -arrestin 2 levels over various times. Average time courses for RhoA activation by 10 nM Ang II with normal -arrestin levels
(●) or with -arrestin 2 (■) or -arrestin 1 (▲) depletion. RhoA-GTP levels were normalized to 10 min of Ang II stimulation. Data represent
the mean ± S.E. of 5–8 experiments. B, representative blots of RhoA-GTP, Total RhoA, and -arrestin 1 and -arrestin 2 levels stimulated by
various Ang II concentrations. Average dose response curves for RhoA activation at 5 min with normal -arrestin levels (●) or with -arrestin 1
(▲) depletion. RhoA-GTP levels were normalized to 100 nM Ang II stimulation. *, p < 0.01 and **, p < 0.001 compared with Ang II control
stimulation.
(Amersham Biosciences) with bound GST-Rhotekin were stored at 4 °C for not more than 7 days before being discarded. Following stimulation, cells were lysed in 1× ice-cold Mg2+/HCl lysis buffer (125 mM HEPES, pH 7.5, 750 mM NaCl, 5% Igepal CA-630, 50 mM MgCl2, 5 mM EDTA, 10% glycerol, 10 μg/ml apronin, and 10 μg/ml leupeptin) (Upstate, Waltham, MA). Lysed cells were then incubated on ice for 10 min and then scraped into prechilled 1.5-ml microcentrifuge tubes. Lysates were centrifuged at 15,800 g (13,000 rpm in International Equipment Company Micromax tabletop) for 5 min. A volume of lysate containing 65–80 μg of protein was then pipetted into a fresh prechilled 1.5-ml microcentrifuge tube and mixed with 30 μg of GST-Rhotekin beads in a final volume of 0.5 ml. Lysates with beads were allowed to rotate for 1 h at 4 °C before beads were washed 3× with 0.5 ml of 1× Mg2+ lysis buffer. Following the last wash, the majority of supernatant was removed by pipette and the beads were aspirated to dryness with a flat gel-loading tip (Marsh Biomedical, Rochester, NY).

**Immunoblotting**—Samples were separated by SDS-PAGE under reducing conditions on 12% Tris-glycine polyacrylamide gels (Invitrogen) for the determination of RhoA-GTP or Total RhoA or on 10% gels for the determination of β-arrestin 1, β-arrestin 2, or Gq/11. Bands were detected by immunoblotting with a 1:1000 dilution of rabbit polyclonal anti-RhoA (Santa Cruz Biotechnology, Santa Cruz, CA), 1:10,000 dilution of the rabbit polyclonal anti-β-arrestin 1/2 antibody A1CT (31), or a 1:650 dilution of the rabbit polyclonal anti-Gq/11 antibody (Santa Cruz Biotechnology). Horseradish peroxidase-conjugated polyclonal donkey anti-rabbit IgG was employed as the secondary antibody. Individual bands were then quantified by densitometry with a Fluor-S MultiImager (Bio-Rad).

**F-actin Cytoskeletal Staining and Quantification**—AT1AR-HEK293 cells were treated as indicated in the figure legends before cells were fixed with phosphate-buffered saline (PBS) containing 4% paraformaldehyde without methanol. Cells were then washed and permeabilized with 0.1% Triton X-100 before being stained with phalloidin as per the manufacturer’s protocol (Molecular Probes, Eugene, OR). 25–40 individual cells were then visualized by three-dimensional confocal microscopy (LSM-510META, Carl Zeiss) per treatment group in each experiment. Acquisition parameters were kept constant for all of the experiments. Cell images were then coded and blindly placed into one of four bins: no stress fibers; low stress fibers; medium stress fibers; or high stress fibers. The medium and high stress fiber bins were then combined and shown as the percentage of total cells containing stress fibers.

**Cross-linking Immunoprecipitation of β-Arrestins and HA-AT1AR**—Immunoprecipitation of HA-epitope-tagged AT1,R was performed in

**Fig. 2.** Stress fiber formation was dependent on β-arrestin 1 but not β-arrestin 2. A, representative cells are shown before and after 30 min of 10 nM Ang II stimulation for control, β-arrestin 2, or β-arrestin 1-depleted cells. B, percentage of cells with stress fibers was quantified for each treatment group. Each bar represents the percentage of cells showing stress fiber formation (mean ± S.E.) for three independent experiments in which 25–40 cells were quantified by confocal microscopy. *, p < 0.01 and **, p < 0.001 compared with Ang II control stimulation.
100-mm dishes using AT1R-HEK293 cells. Cells were starved for 2 h in 4 ml of PBS containing 10 mM HEPES and were subsequently stimulated as described in the figure legends. Incubations were terminated by the addition of 1 ml of PBS containing 2.5 mM dithiobis(succinimidylpropionate) (Sigma) in 50% (v/v) dimethyl sulfoxide and 10 mM HEPES. Monolayers were agitated gently for 30 min at room temperature and then neutralized for 15 min in 50 mM Tris-HCl, pH 7.0. Monolayers were then washed with ice-cold PBS, solubilized in 0.6 ml of radiolmmune precipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1.0% (v/v) Nonidet P-40, 0.1% sodium deoxycholate, 100 µM Na3VO4, 1 mM phenylmethlysulfonyl fluoride, 2 µg/ml leupeptin, 2 µg/ml aprotinin, 1 µg/ml pepstatin A, 15 µg/ml benzamidin, and 10 µg/ml soybean trypsin inhibitor), and clarified by centrifugation. 25-µl aliquots of clarified cross-linked detergent lysates were mixed with an equal volume of 2× Laemmli sample buffer. The remainder of each lysate was agitated overnight at 4 °C with 20 µl of 50% slurry of AG1-X8 columns as described previously (48).

**Statistical Analysis**—All of the data were graphed and statistically analyzed using GraphPad Prism (GraphPad Software Inc., San Diego, CA).

**RESULTS**

In AT1R-HEK293 cells, RhoA activation by 10 nM Ang II was quite rapid, reaching a maximal response at the earliest time point measured (1 min) (Fig. 1, A–C). This level of RhoA activation was sustained for 10 min before diminishing, reaching 49% maximal response at 20 min. To determine the role of β-arrestin 1 and β-arrestin 2 in AT1R-mediated RhoA activation, RNAi was utilized, which reduced β-arrestin levels by ~70% in all of the experiments (Fig. 1, A and D). Depletion of β-arrestin 2 by RNAi led to a slight increase in the levels of RhoA activation by Ang II over time (Fig. 1A and 1B, ANOVA p < 0.01) but did not significantly affect RhoA activation by any specific time point measured. In contrast, β-arrestin 1 depletion by RNAi dramatically reduced RhoA activation by 60% across the entire time course (Fig. 1A and C, ANOVA p < 0.0001).

Ang II activated RhoA in a dose-dependent fashion with an EC50 value of 10 nM and maximal RhoA-GTP levels at 100 nM Ang II (Fig. 1, D–E). Following the depletion of β-arrestin 1 by RNAi, maximal RhoA activation was significantly inhibited by 70% (Fig. 1, D and E, ANOVA p < 0.0001) with an unchanged EC50 value of 10 nM. Therefore, β-arrestin 1 is a necessary component of Ang II-activated RhoA-GTP over both time and dose.

The effect of β-arrestin depletion on Ang II-stimulated stress fiber formation, a well established Rho-mediated event, was
next determined. In the absence of Ang II stimulation, control cells, β-arrestin 2-depleted cells, and β-arrestin 1-depleted cells showed little to no stress fiber formation (Fig. 2, A and B). Stimulation with 10 nM Ang II for 30 min provoked robust reorganization of F-actin and stress fiber formation in 73 ± 6.1% control cells. The depletion of β-arrestin 2 had no significant effect on stress fiber formation as 61 ± 10.2% cells showed actin re-organization upon Ang II stimulation. However, upon depletion of β-arrestin 1, only 23 ± 5.2% cells showed stress fiber formation. This 70% reduction in stress fiber formation was consistent with the reduced RhoA activation observed under these conditions. To further elucidate the downstream effector of RhoA involved in the stress fiber formation, cells were pretreated for 15 min with 10 μM ROCK inhibitor, Y-27632. Under these conditions, stress fiber formation was inhibited by 100%, further substantiating RhoA and ROCK as the upstream components of a pathway leading to stress fiber formation following AT1AR activation by Ang II.

Recently, an analog of Ang II, SII, that binds the AT1AR but does not activate heterotrimeric G proteins, has been shown to recruit β-arrestin 2 and activate ERK1/2 (37). However, it is not known whether SII is capable of recruiting β-arrestin 1 and, if it does, whether this recruitment in the absence of heterotrimeric G protein activation would be sufficient to activate RhoA. Therefore, the extent of β-arrestin 1 recruitment to the AT1AR and subsequent RhoA activation was determined using SII. Because the affinities of SII and Ang II are markedly different with $K_d$ values of 310 and 1.6 nM, respectively (37), the AT1AR-HEK293 cells were stimulated with 10 μM SII or 1 or 100 nM Ang II for 1 min, the HA-AT1AR was subsequently immunoprecipitated and subjected to SDS-PAGE, and the ni-trocellulose was blotted for endogenous β-arrestins (Fig. 3, A and B). Whereas 1 nM Ang II weakly recruited β-arrestin 1 and β-arrestin 2 after 1 min, 100 nM Ang II robustly recruited both β-arrestin 1 and β-arrestin 2 with a preference for β-arrestin 2. Upon stimulation with 10 μM SII, β-arrestin 1 and β-arrestin 2 were clearly recruited to the AT1AR in a similar pattern (β-arrestin 2 greater than β-arrestin 1) as that induced by Ang II. To determine whether SII could induce the sequestration of β-arrestin 1 into endocytotic vesicles in a fashion similar to that induced by Ang II, GFP-β-arrestin 1 was transiently transfected into AT1AR-HEK293 cells. Following 20 min of 10 nM Ang II or 10 μM SII stimulation, GFP-β-arrestin 1 had moved from the cytoplasm into internalized vesicles (Fig. 3C), typical of an Ang II-induced pattern of β-arrestin 1 recruitment. Quantification of the percentage of cells showing GFP-β-arrestin 1 or GFP-β-arrestin 2 in vesicles following 100 nM Ang II or 10 μM SII stimulation revealed that SII was less effective than Ang II at inducing β-arrestin endocytosis. Specifically, GFP-β-arrestin 1 was sequestered into vesicles in 94 ± 3.9 and 23.0 ± 3.0% cells for Ang II and SII, respectively, whereas GFP-β-arrestin 2 was sequestered into vesicles in 97.0 ± 1.3 and 72.0 ± 11.0% cells for Ang II and SII, respectively.

To determine whether the SII-mediated recruitment of β-arrestin 1 in the absence of heterotrimeric G protein coupling...
would be sufficient to activate RhoA, AT₁R-HEK293 cells were stimulated with 10 μM SII for 5 min and the levels of activated RhoA were determined. SII was able to reproducibly activate RhoA, but the levels of RhoA-GTP were only 5.7 ± 4.5% compared with a maximal stimulation of RhoA-GTP following 100 nM Ang II (Fig. 4, A and B). Additionally, 10 μM SII was incapable of inducing stress fiber formation after 30 min of stimulation (Fig. 4C). However, in the same experiment, SII stimulated the 40%-meditated MAPK component ERK1/2 by 35 ± 16% Ang II-stimulated ERK1/2, consistent with previous findings (37).

Given that β-arrestin 1 seemed to be necessary but not sufficient to significantly activate RhoA or to induce stress fiber formation in HEK293 cells, the role of the AT₁R-coupled heterotrimeric G proteins Gᵣ/q and Gᵢ was assessed. Gᵣ/q was depleted in AT₁R-HEK293 cells by ~90% using RNAi (Fig. 5A). To show that this level of Gᵣ/q depletion led to a significant loss of AT₁R coupling and signaling through this G protein, a PI hydrolysis assay was performed revealing almost complete inhibition of PI turnover when Gᵣ/q was silenced (Fig. 5B). When Rho activation was determined following the depletion of Gᵣ/q, it was found to be inhibited by 68% following a 5-min stimulation with 10 nM Ang II (Fig. 5C). However, neither PLC (ET-18-OCH₃ and U-73122), PKC (Go−6976, Go−6983, and RO-31-8425) nor tyrosine kinase inhibitor genistein, and Gᵣᵦ, inhibitor pertussis toxin (PTX). Ang II-stimulated controls were normalized to 100%. Data represent the mean ± S.E. for three independent experiments.

The dependence of stress fiber formation upon Gᵣ/q and β-arrestin 1 depletion was determined in AT₁R-HEK293 cells with Gᵣ/q or simultaneous Gᵣ/q and β-arrestin 1 depletion. In the absence of Ang II stimulation, control cells and cells depleted of Gᵣ/q or Gᵣ/q and β-arrestin 1 showed little to no stress fiber formation (Fig. 6, A and B). Stimulation with Ang II showed stress fiber formation in 76 ± 23% control cells. However, only 33 ± 4.2% Gᵣ/q-depleted cells, 23 ± 5.2% β-arrestin 1-depleted cells (shown again from Fig. 2B), or 21 ± 3.5% Gᵣ/q and β-arrestin 1-depleted cells showed significant stress fiber formation upon Ang II stimulation. This 57–72% inhibition of stress fiber formation following Gᵣ/q and β-arrestin 1 depletion is again consistent with the reduced RhoA activation observed under similar conditions. In addition, because silencing both Gᵣ/q and β-arrestin 1 simultaneously did not lead to more RhoA inhibition than silencing either Gᵣ/q or β-arrestin 1 alone,
these data suggest that both molecules act concurrently in the same pathway.

The weak ability of SII to activate Rho, coupled with the apparent involvement of G\(_{q/11}\) in Ang II stimulation, raised the possibility of a concerted mechanism requiring both \(\beta\)-arrestin 1 and G\(_{q/11}\). Such a hypothesis would explain the failure of SII to significantly activate Rho as a consequence of its inability to trigger G protein activation and would further predict that supplementation of SII with G\(_{q/11}\) activity would lead to Rho activation. To test this idea, we transfected cells with either wild type G\(_{q/11}\) or constitutively active G\(_{q/11}\)QL (Fig. 7A). As might be expected, overexpression of G\(_{q/11}\) or G\(_{q/11}\)QL led to modest but significant increases in basal PI turnover (Fig. 7B) but did not cause any significant alteration in sensitivity to Ang II stimulation. Importantly, SII failed to elevate PI turnover even in the presence of overexpressed G\(_{q/11}\) or G\(_{q/11}\)QL, further strengthening the evidence that this Ang II analog is incapable of activating heterotrimeric G proteins.

As shown in Fig. 7, C and D, transfection of G\(_{q/11}\) or G\(_{q/11}\)QL led to weak activation of RhoA similar to that produced by 10 \(\mu\)M SII in the presence of endogenous levels of G\(_{q/11}\). Strikingly, however, the combination of SII stimulation in the presence of transfected G\(_{q/11}\) or G\(_{q/11}\)QL led to synergistic activation of RhoA. In contrast, the activation of RhoA by Ang II was not significantly increased by transfection of G\(_{q/11}\) (\(p = 0.24, n = 8\)) or G\(_{q/11}\)QL (\(p = 0.24, n = 12\)) (Fig. 7A). Thus, these data are consistent with the notion of a concerted \(\beta\)-arrestin 1/G\(_{q/11}\) mechanism for Rho activation in response to Ang II stimulation.

**DISCUSSION**

We show that the activation of the small GTPase RhoA as well as subsequent stress fiber formation following stimulation of the AT\(_{1}\)R requires \(\beta\)-arrestin 1 as well as G\(_{q/11}\). Furthermore, we find that, in HEK293 cells, activated RhoA signals through ROCK to activate stress fiber reorganization, consistent with previous findings (49).

\(\beta\)-Arrestins, which were initially shown to be involved in the desensitization of heterotrimeric G proteins and the internalization of 7TMs, have only recently been recognized as playing roles in cellular events ranging from activation of MAPK cascade components (37, 46) to apoptosis (50, 51) and chemotaxis (41, 42). Most recently, following stimulation of the AT\(_{1}\)R, \(\beta\)-arrestin 2 was shown to signal to the MAPK ERK1/2 in the absence of heterotrimeric G protein activation (37). Additional studies revealed that the \(\beta\)-arrestin 2-dependent activation of ERK1/2 was further enhanced when \(\beta\)-arrestin 1 was silenced, indicating that \(\beta\)-arrestin 1 reciprocally regulates \(\beta\)-arrestin 2-mediated ERK1/2 activation (46). This reciprocal regulation was hypothesized to be the result of competition between \(\beta\)-arrestin 2 and \(\beta\)-arrestin 1 for the same binding sites on the activated AT\(_{1}\)R. Consequently, upon the depletion of \(\beta\)-arrestin 1, more \(\beta\)-arrestin 2 is able to bind the phosphorylated C

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**Fig. 6.** Stress fiber formation is dependent on G\(_{q/11}\) as well as \(\beta\)-arrestin 1. AT\(_{1}\)R-HEK293 cells were transfected with control, G\(_{q/11}\), or both G\(_{q/11}\) and \(\beta\)-arrestin 1 siRNAs. A, representative cells are shown before and after 30 min of 10 nM Ang II stimulation for each condition. B, each bar represents the percentage of cells showing stress fiber formation (mean \(\pm\) S.E.) for three independent experiments in which 25–40 cells were quantified by confocal microscopy. \(\beta\)-Arrestin 1 siRNA data are shown again from Fig. 2B for comparison. *, \(p < 0.01\) compared with Ang II control stimulation.
terminus of the AT1AR, leading to greater ERK1/2 activation.

We find that the recruitment of H9252-arrestin 1 to the AT1AR not only regulates H9252-arrestin 2 activation of ERK1/2 as previously shown (46) but also leads to the activation of the small GTPase RhoA. Furthermore, H9252-arrestin 1 activation of RhoA leads to actin reorganization and stress fiber formation. Not surprisingly, it was also observed that H9252-arrestin 2 reciprocally regulates H9252-arrestin 1 activation of RhoA over time in a manner analogous to the reciprocal regulation of H9252-arrestin 2 by H9252-arrestin 1 with regard to ERK1/2 activation described above.

Hence, it now appears that upon the activation of the AT1AR, H9252-arrestin 1 and H9252-arrestin 2 are competitively recruited to the receptor leading to RhoA activation through a synergistic mechanism requiring β-arrestin 1 and Gq<sub>11</sub> and to ERK1/2 activation through independent β-arrestin 2 and Gq<sub>11</sub> mechanisms (Fig. 8). Furthermore, with respect to RhoA activation, β-arrestin 1 and Gq<sub>11</sub> appeared to be acting coordinately to activate RhoA as silencing β-arrestin 1, Gq<sub>11</sub>, or both together lead to nearly an equivalent (70–80%) loss in RhoA activation and/or stress fiber formation. Importantly, the remaining 20–30% RhoA activation and/or stress fiber formation following silencing of β-arrestin 1 and/or Gq<sub>11</sub> is probably reflective of incomplete silencing of β-arrestin 1 and Gq<sub>11</sub> obtained using RNA interference techniques.

Although the role of the heterotrimeric G proteins Gq<sub>11</sub> in mediating RhoA activation has been somewhat controversial (20), our data support the findings that Gq<sub>11</sub> is involved in the activation of RhoA and stress fiber formation. Importantly, the remaining 20–30% RhoA activation and/or stress fiber formation following silencing of β-arrestin 1 and/or Gq<sub>11</sub> is probably reflective of incomplete silencing of β-arrestin 1 and Gq<sub>11</sub> obtained using RNA interference techniques.

![Fig. 7. β-Arrestin 1 and G<sub>q<sub>11</sub></sub> work coordinately to activate RhoA.](http://www.jbc.org/Downloadedfrom)
ERK1/2. Present data support the hypothesis that mediated RhoA activation, suggesting that PKC and tyrosine heterotrimeric G protein activation is required. Conversely, recent data to that induced by Ang II, thereby suggesting that both arrestin and stress fiber formation via the downstream effector of RhoA, ROCK. Neither arrestin-dependent signaling to RhoA or G\textsubscript{q}\textsubscript{11} recruitment alone by SII nor overexpression of G\textsubscript{q}\textsubscript{11} is sufficient to robustly activate RhoA in a manner analogous to that induced by Ang II, thereby suggesting that both arrestin and heterotrimeric G protein activation is required. Conversely, recent data has been published in which β-arr2 signals to the MAPK component ERK1/2 independently from the heterotrimeric G protein pathway to ERK1/2 via PKC. Therefore, it seems that β-arr1 and β-arr2 are endowed with the capacity to signal in a heterotrimeric G protein-and β-arr1-dependent manner for RhoA activation and stress fiber formation and a heterotrimeric G protein-independent and β-arr2-dependent manner for ERK1/2 activation.

FIG. 8. Model of β-arr-dependent signaling to RhoA or ERK1/2. Present data support the hypothesis that β-arr1 and G\textsubscript{q}\textsubscript{11} work coordinately to activate RhoA. Activated RhoA then leads to stress fiber formation via the downstream effector of RhoA, ROCK. Neither β-arr1 recruitment alone by SII nor overexpression of G\textsubscript{q}\textsubscript{11} or G\textsubscript{q}\textsubscript{QL} is sufficient to robustly activate RhoA in a manner analogous to that induced by Ang II, thereby suggesting that both arrestin and heterotrimeric G protein activation is required. Conversely, recent data has been published in which β-arr2 signals to the MAPK component ERK1/2 independently from the heterotrimeric G protein pathway to ERK1/2 via PKC. Therefore, it seems that β-arr1 and β-arr2 are endowed with the capacity to signal in a heterotrimeric G protein-and β-arr1-dependent manner for RhoA activation and stress fiber formation and a heterotrimeric G protein-independent and β-arr2-dependent manner for ERK1/2 activation.

more robust RhoA activation than does the overexpression of wild type or constitutively active G\textsubscript{q}\textsubscript{11} alone or β-arr1 recruitment alone.

Previously, it has been found that G\textsubscript{q}\textsubscript{11} activates RhoA in either a PLC- and PKC-dependent manner and/or a tyrosine kinase-dependent manner (17, 20). However, we find that PLC and PKC inhibitors as well as the tyrosine kinase inhibitor genistein do not inhibit AT\textsubscript{1}AR-R-mediated RhoA activation. In contrast, PKC inhibitors Gö-6983 and RO-31-8425 and the tyrosine kinase inhibitor genestein actually increased AT\textsubscript{1}AR-mediated RhoA activation, suggesting that PKC and tyrosine kinase might act to inhibit RhoA activation in this system.

The precise mechanism by which β-arr1 and G\textsubscript{q}\textsubscript{11} activate RhoA in a concerted manner remains unknown. How-
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AT1AR-mediated RhoA Activation Is Dependent on β-Arrestin 1 and Gα11
β-Arrestin 1 and \( G_{\alpha q/11} \) Coordinately Activate RhoA and Stress Fiber Formation following Receptor Stimulation

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*J. Biol. Chem.* 2005, 280:8041-8050.
doi: 10.1074/jbc.M412924200 originally published online December 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M412924200

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