A Protein Tyrosine Phosphatase Inhibitor, Pervanadate, Inhibits Angiotensin II-Induced β-Arrestin Cleavage

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β-Arrestins turn off G protein-mediated signals and initiate distinct G protein-independent signaling pathways. We previously demonstrated that angiotensin AT1 receptor-bound β-arrestin 1 is cleaved after Phe388 upon angiotensin II stimulation. The mechanism and signaling pathway of angiotensin II-induced β-arrestin cleavage remain largely unknown. Here, we show that protein Tyr phosphatase activity is involved in the regulation of β-arrestin 1 cleavage. Tagging of green fluorescent protein (GFP) either to the N-terminus or C-terminus of angiotensin AT1 receptor, orthovanadate and pyrovanadate, inhibitors of protein Tyr phosphatase, attenuated the cleavage of C-terminal GFP-tagged β-arrestin 1 in vitro. The inhibitory effects of okadaic acid and pyrophosphate, which are inhibitors of protein Ser/Thr phosphatase, were less than those of protein Tyr phosphatase inhibitors. Cell-permeable pervanadate inhibited angiotensin II-induced cleavage of β-arrestin 1 in COS-1 cells. Our findings suggest that Tyr phosphorylation signaling is involved in the regulation of angiotensin II-induced β-arrestin cleavage.

INTRODUCTION

The angiotensin AT1 receptor belongs to the G protein-coupled receptor family and transduces diverse signals in G protein- and β-arrestin-dependent pathways (Oro et al., 2007). Upon angiotensin II binding to the angiotensin AT1 receptor, a conformational change in the receptor modulates G protein, Gq, and activates phospholipase Cβ, for IP3 and Ca2+-mediated vasoconstriction (Oro et al., 2007). β-Arrestin 1 and 2, ubiquitously expressed in mammalian tissues, are recruited from the cytoplasm to the activated angiotensin AT1 receptor for clathrin-mediated endocytosis (Krupnick et al., 1997). In addition to the role in desensitization and endocytosis, β-arrestins form scaffolds for a number of signaling molecules in mitogen-activated protein kinase (MAPK) cascades and non-receptor Tyr kinases for G protein-independent signals (Lefkowitz and Shenoy, 2005). The β-arrestin 1 and 2 are comprised of N- and C-domains of equal size (Han et al., 2001), with the N-domain serving as a phosphate sensor recognizing the phosphorylated G protein-coupled receptor (Nobles et al., 2007) and the C-domain acting as adaptor binding sites for clathrin and AP-2 (Lin et al., 1997). Previous studies have shown that there are distinct inactive and active conformations in β-arrestin. In the inactive state, the polar core between the N- and C-domain of β-arrestin 1 is kept intact with the C-terminus buried in the structure (Han et al., 2001). However, once β-arrestin 1 is bound to the phosphorylated receptor, a conformational change in β-arrestin occurs and extrudes its C-terminal region towards the outside, thus enabling the binding of clathrin and AP-2 for clathrin-mediated endocytosis (Lefkowitz and Shenoy, 2005). β-Arrestin 1 and 2 have distinct active conformations when bound to the activated receptor (Nobles et al., 2007; Xiao et al., 2004).

In a previous study, we showed that a fraction of β-arrestin 1 bound to the angiotensin AT1 receptor is cleaved upon receptor activation and it requires stable interaction between the angiotensin AT1 receptor and β-arrestin (Lee et al., 2008). Angiotensin II and inverse agonist EXP3174 induced cleavage at distinct sites on β-arrestin 1; after Phe388 upon angiotensin II treatment and after Pro376 upon EXP3174 treatment, respectively. These results suggested ligand-induced selectivity in β-arrestin-mediated signaling. With distinct cleavage sites of β-arrestin 1 and 2 by angiotensin II, our data also demonstrated that the receptor-bound active conformations of β-arrestin 1 and 2 are different. The biological significance and the signaling pathway of ligand-induced β-arrestin cleavage, however, are largely unknown.

In this study, we sought to elucidate the regulatory mechanism of angiotensin II-mediated β-arrestin 1 cleavage in COS-1 cells expressing the angiotensin AT1 receptor. Since protein kinase activation has been implicated in the activation of prote-
ases downstream of angiotensin AT₁ receptor (Eguchi et al., 2001), we hypothesized that β-arrestin proteolysis is regulated by cellular protein kinases or phosphatases. We used inhibitors of protein Tyr or Ser/Thr phosphatase to investigate the effect on β-arrestin proteolysis. Here, we show that cell-permeable pervanadate inhibits angiotensin II-induced cleavage of β-arrestin 1 in COS-1 cells. Our finding suggests that protein Tyr phosphatase activity is involved in the regulation of G protein-coupled receptor-engaged β-arrestin proteolysis.

MATERIALS AND METHODS

Materials
Angiotensin II was purchased from Bachem (USA). Lipofectamine 2000 was purchased from Invitrogen (USA). Monoclonal antibodies to myc and β-arrestin 2 (H-9) were purchased from Santa Cruz Biotechnology, Inc. (USA). Since the anti-β-arrestin 2 antibody (H-9) recognized both β-arrestin 1 and 2 in our previous studies (Lee et al., 2007; 2008), we used this antibody for the detection of transfected β-arrestin 1 in this study. Monoclonal antibody to GFP was purchased from Clontech (USA). Horseradish peroxidase (HRP)-labeled goat anti-mouse secondary antibody was purchased from Upstate (USA). COS-1 cells were purchased from American Type Culture Collection (USA). Western blot stripping buffer was purchased from Pierce (USA). All other reagents, unless stated otherwise, were from Sigma (USA).

Construction of myc-β-arrestin 1, 1-FGP-β-arrestin 1, and β-arrestin 1-FGP plasmids
Three fusion constructs with myc or GFP-tagged β-arrestin 1 were generated by polymerase chain reaction by the following primers using pto DNA polymerase. For Myc-β-arrestin 1, the forward primer was 5'-AACCGGATCCGATGGCCGACAAAGGGAC-3' (BamH1 site underlined, and the N-terminus of β-arrestin 1 in boldface type), and the reverse primer was 5'-AACCCCTCGAGCTATCTGTCGGACCGCCGAG-3' (Xhol site underlined, and the C-terminus of β-arrestin 1 in boldface type). For the N-terminal GFP-tagged GFP-β-arrestin 1, the CDNA for cloning was subconed into pEGFP-C1 vector (Clontech). The forward primer was 5'-AACCCCTCGAGCTATCTGTCGGACCGCCGAG-3' (Xhol site underlined, and the N-terminus of β-arrestin 1 in boldface type), and the reverse primer was 5'-AACCGGATCCGATGGCCGACAAAGGGAC-3' (BamH1 site underlined, and the C-terminus of β-arrestin 1 in boldface type). The C-terminal GFP tag of β-arrestin 1-FGP was generated using pEGFP-N3 vector. The forward primer was 5'-CCCCCCCTCGAGTCTACATGCCCAGAAGGGAC-3' (Xhol site underlined, and the N-terminus of β-arrestin 1 in boldface type), and the reverse primer was 5'-AACGGATCCCTCTGCGGTGCCTG-3' (BamH1 site underlined, and the C-terminus of β-arrestin 1 in boldface type). Accuracy of the fusion constructs in the expression vector was confirmed by DNA sequence analysis.

Cell culture and expression of the angiotensin AT₁ receptor and β-arrestin 1
The synthetic rat angiotensin AT₁ receptor gene, cloned in the shuttle expression vector pM73, was used for expression. To express the angiotensin AT₁ receptor and β-arrestin 1, 60-65% confluent COS-1 cells were grown in 6-well plates and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum. The cells were transfected with 2 μg of purified angiotensin AT₁ receptor and β-arrestin 1 cDNA using Lipofectamine 2000 (Invitrogen), according to the manufac-