G\(_{\alpha_{13}}\) Induces PreproET-1 Gene Expression via JNK


The endothelin B receptor (ET\(_B\)R) has been shown to mediate autoinduction of endothelin-1 (ET-1). We previously reported that the ET\(_B\)R interacts with G\(_{\alpha_{13}}\), a member of the heterotrimeric GTP-binding protein family. In the present study, we examined whether G\(_{\alpha_{13}}\) induces preproET-1 (ppET-1) gene transcription, which could result in ET-1 autoinduction in a renal epithelial cell line. We generated a reporter gene construct under control of the ppET-1 promoter region. The construct was transiently expressed in COS-7 cells. Transient expression of ET\(_B\) R increased the promoter activity of ppET-1 following treatment with 100 nmol/l of ET-1. Expression of G\(_{\alpha_{13}}^{Q226L}\) or G\(_{\alpha_{q}}^{Q209L}\), constitutively active forms of G\(_{\alpha_{13}}\) and G\(_{\alpha_{q}}\), also activated the ppET-1 promoter. ET\(_B\) R-stimulated ppET-1 promoter activity was partially diminished by the expression of dominant negative forms of c-Jun N-terminal kinase (JNK1APF) or MAPK/ERK kinase (MEK K97M). Expression of JNK1APF also inhibited G\(_{\alpha_{13}}^{Q226L}\)-induced ppET-1 promoter activation. These findings indicate that G\(_{\alpha_{13}}\) can induce ppET-1 gene expression through a JNK-mediated pathway. Our results also suggest that this G\(_{\alpha_{13}}\)-coupled signaling pathway may play an important role in a sustained ET-1 autoinduction loop in various pathophysiological conditions. (Hypertens Res 2002; 25: 427–432)

Key Words: G-protein, endothelin, endothelin receptor, transcription

Introduction

G\(_{\alpha_{13}}\), a member of the G\(_{13}\) family of guanine nucleotide-binding proteins (G-proteins), is ubiquitously expressed and regulates multiple complex cellular effects. G\(_{\alpha_{13}}\) is activated by thrombin, thromboxane, thyroid-stimulating hormone, and angiotensin II AT\(_1\)A receptors (1–5). In addition, we previously demonstrated that endothelin B receptor (ET\(_B\)R) activates G\(_{\alpha_{13}}\) (6). G\(_{\alpha_{13}}\) regulates Na/H exchanger activity (7, 8), extracellular signal regulated kinase (ERK), c-Jun NH\(_2\)-terminal kinase (JNK) (9–11), nuclear factor \(\kappa\)B (NF-\(\kappa\)B) (12) and the activity of the low molecular weight GTPase, Rho (13–15), and is also capable of neoplastic transformation, induction of apoptosis, and stimulation of actin re-
protein kinases (ERK, JNK, and p38) (23, 24). Because of its vasoconstrictor and growth-promoting action, numerous studies indicate that ET-1 is involved in the pathogenesis of a broad spectrum of renal and cardiovascular diseases (21, 22, 25–27). Previous studies have demonstrated that ET-1 induced ET-1 production through ETaR and/or ETbR-mediated pathways, suggesting that a sustained ET-1 autocrine loop contributes to the progression of cardiovascular and renal diseases (28–32).

ET and Gaα3 share a number of effects on cells, including activation of JNK, the Na/H exchanger and Rho, and stimulation of cellular proliferation and differentiation (13–15, 21, 23, 24, 33–38). We previously demonstrated that ETaR activates Gaα3 (6). In the present study, we have examined whether Gaα3 could activate the preproET-1 (ppET-1) gene promoter to take part in the ET-1 autinduction loop. We show that the activated Gaα3 is able to induce ppET-1 gene expression via JNK dependent pathway.

Methods

Materials

COS-7 cells were obtained from the American Type Culture Collection (Rockville, USA). Tissue culture medium and fetal bovine serum (FBS) were obtained from GIBCO-BRL (Rockville, USA); ET-1 from Calbiochem (San Diego, USA); restriction and DNA modifying enzymes from GIBCO-BRL or TaKaRa (Kusatsu, Shiga, Japan); and tissue culture plastic wares from Falcon (Becton Dickinson Labware, Bedford, USA).

Cell Culture

COS-7 cells were grown in Dulbecco’s modified Eagle’s medium with 10% FBS in a 10% CO2–90% air incubator. Culture medium was replaced every 3 days. Cells were passed using 0.01% ethlylenediamine tetraacetic acid (EDTA)-trypsin.

Plasmids

A 2.6 kb fragment of human ET-1 promoter region (from -2456 to +168, a gift from G.A. Visner, University of Florida, Gainesville, USA) (39) was subcloned into a luciferase reporter plasmid, pGL3 basic (Promega, Madison, USA). The cDNA encoding the human ETaR was a gift form M Yanagisawa (University of Texas Southwestern Medical Center, Dallas, USA). cDNAs were subcloned into pcDNA3 (Invitrogen, Carlsbad, USA). The sequence of the resulting construct was confirmed using restriction and sequencing analysis.

Transfection and Luciferase Assay

COS-7 cells (4.0 × 10⁵ cells/well) were seeded the day before transfection in 6-well plates. The cells were transfected using the Ca-Po4 co-precipitation method. Each transfection mixture contained 0.25 μg of a reporter construct and 0.5 μg of other cDNA construct(s) as indicated. The total amount of transfected DNA was adjusted to 2 μg by adding an empty vector, pcDNA3.

After incubating the cells with DNA/Ca-Po4 precipitates for 4–6 h at 37°C, the cells were washed three times with phosphate buffered saline, and incubated with Dulbecco’s modified eagle medium (DMEM) containing 0.2% FBS. The transfected cells were harvested 48 h after transfection. Where indicated, ET-1 (100 nmol/l) were added 24 h before the harvest. Luciferase activity was measured using a Luciferase Assay System (Promega) and a TD-20/20 Luminometer (Turner Designs, Sunnyvale, USA) according to the manufacturer’s instruction.

Statistical Analysis

Statistical significance was evaluated using the two-tailed, unpaired Student’s t-test for comparisons between 2 means, or analysis of variance (ANOVA) analysis followed by the Newman-Keuls method for more than 2 means. A value of
Results

The ETbR Activates ppET-1 Promoter Following ET-1 Treatment in COS-7 Cells

Previous studies have demonstrated ETbR-mediated autoinduction of ET-1 in endothelial and mesangial cells (29–31). However, in a study of human keratinocytes, ET-1 treatment decreased ET-1 production presumably through ETbR (44), suggesting that ETbR-mediated induction of ET-1 may be cell type-dependent. In the current study, we examined whether activation of ETbR induces ppET-1 gene expression in COS-7 cells. The ETbR was transiently expressed, and ET-1 (100 nmol/l) was added to the culture medium 24 h after transfection. Transfected cells were harvested 48 h after transfection and luciferase activity was measured. As shown in Fig. 1, treatment with 100 nmol/l ET-1 increased ppET-1 transcription by approximately 2.5-fold in COS-7 cells expressing ETbR, while expression of ETbR itself had no significant effect on ppET-1 promoter activity. These data indicate that ET-1 is able to induce ppET-1 transcription through the activation of ETbR in COS-7 cells.

The Effects of Constitutively Active G-Protein Subunits on ppET-1 Promoter Activity

The ETbR is known to activate G-proteins that are members of the Gaα, Gaβ, Gaγ and Gaε2 families (6). To investigate which G-protein α subunit family is responsible for the activation of ppET-1 gene transcription through the ETbR, we coexpressed constitutively active forms of four α subunits, Gaα1 (Gaα1Q226L), Gaα2 (Gaα2Q209L), Gaα (GaαQ227L) or Gaα2 (Gaα2Q209L), and the ppET-1 promoter construct in COS-7 cells. Luciferase activity was measured 48 h after transfection. Expression of αα1Q226L and αα2Q209L increased ppET-1 promoter activity with similar potency, while αα2Q227L and αα2Q209L had no effect on the ppET-1 transcription (Fig. 2). These results indicate the possibility that Gaα1- and/or Gaα2-coupled signaling systems might be involved in the ETbR-mediated autinduction of ET-1.

Fig. 2. G-protein-mediated activation of ppET-1 promoter. COS-7 cells were transfected with a ppET-1 promoter/luciferase construct and pcDNA3 encoding Gaα1Q226L, Gaα2Q209L, GaαQ227L or GaαQ209L. Forty-eight hours after transfection, luciferase activity was assayed. Values are the fold-increase over the control and expressed as the mean ± SEM (n = 5). * p < 0.05.

Fig. 3. Involvement of JNK- and MEK-dependent pathways in ETbR-mediated ppET-1 promoter activation. COS-7 cells were transfected with a ppET-1 promoter/luciferase construct and ETbR in the presence or absence of either JNK1APF or MEK578M. Twenty-four hours after transfection, cells were treated with 100 nmol/l ET-1 for 24 h. Then cells were harvested and assayed for luciferase activity. Values are the fold-increase over the control and expressed as the mean ± SEM (n = 4). * p < 0.05.

Fig. 4. Involvement of JNK in Gaα13-mediated ppET-1 promoter activation. COS-7 cells were transfected with a ppET-1 promoter/luciferase construct and Gaα13Q226L with or without JNK1APF. Cells were harvested 48 h after transfection and luciferase activity was measured. Values are the fold-increase over the control and expressed as the mean ± SEM (n = 6). * p < 0.05.
Involvement of a JNK-Dependent Signaling Pathway in the Activation of ppET-1 Promoter through ETαR and Gα13

Since the ETαR activates multiple signaling pathways including JNK and MEK, we investigated the involvement of JNK- and MEK-dependent pathways in ETαR-induced ppET-1 promoter activation. COS-7 cells were transiently transfected with ETαR in the presence or absence of either the JNK1APF or MEK (MEK93PM) expression vectors. ET-1 (100 nmol/l) was added to the culture media 24 h before harvest and reporter gene activity was measured 48 h after transfection. Figure 3 shows that expression of JNK1APF or MEK93PM partially inhibited ppET-1 promoter activity following the ETαR stimulation. These findings strongly demonstrate that both JNK- and MEK-dependent signaling pathways are involved in the autoinduction of ET-1 through ETαR. Next, we examined the effect of dominant negative JNK on Gα13-induced activation of the ppET-1 promoter, since Gα13 activates JNK-dependent pathways in a variety of cells. As shown in Fig. 4, expression of JNK1APF partially blocked the Gα13Q202L-induced ppET-1 promoter activity in COS-7 cells. Taken together, our results suggest that autoinduction of ET-1 through ETβ receptor activation involves a Gα13-dependent signaling system that is partially mediated by JNK in COS-7 cells.

Discussion

In the present study, we demonstrated that activated ETαR and constitutively active forms of Gα13 as well as Gα13 activate ppET-1 gene transcription in COS-7 cells. Activation of ppET-1 promoter by Gα13 was partially mediated by a JNK-dependent pathway. ETαR activated Gα13 when they were co-expressed in HEK293 cells (6). Taken together, our previous and current results suggest a role of Gα13 in ETαR-mediated ET-1 autoinduction and other ET-1-mediated pathophysiologic reactions. It is well known that endothelin production is up-regulated by various stimuli such as TGF-β, tumor necrosis factor (TNF)-α, interleukins, insulin, noradrenalin, angiotensin II (via AT1 receptors), thrombin, and other physicochemical factors (22, 23, 27). Since Gα13 interacts with AT1 receptors and thrombin receptors (3, 5), Gα13 could play an important role in induction of endothelin by these peptides. Recently, Offermanns and associates showed that a dominant negative form of Gα13 prevented ET-1-mediated vascular smooth muscle cell (VSMC) contraction in vitro and that intracellular injection of activated Gα13 evoked VSMC contraction (45), which suggests the involvement of Gα13 not only in the regulation of ET-1 production, but also in a broad range of ET-1 bioactivities, including vascular tone regulation.

In our system, Gα13 induces ET-1 transcription presumably through activating protein (AP-1). Because activation of a small GTPase, Rho, is one of the downstream consequences of Gα13 activation (13–15), and because a recent study demonstrated that Rho activates the ppET-1 promoter in bovine aortic endothelial cells (46), Rho might be involved in the autoinduction of ET-1 that is mediated by Gα13. Moreover, since Gα13 has been shown to activate JNK and Rho (47, 48), it is likely that Gα13 induces ET-1 production not only through protein kinase C (PKC)-mediated pathways but also through JNK and/or Rho. Further studies are needed to elucidate the contribution of the Rho-dependent signaling system in the JNK-mediated autoinduction of ET-1 in our system.

We previously demonstrated that ETαR does not activate Gα13 (6). A previous study reported that a blockade of ETαR that was able to activate Gα13 did not inhibit ET-1 production stimulated by ET-1 in rat mesangial cells (29). On the other hand, in a study using a rat model of congestive heart failure, the ETαR antagonist BQ-123 normalized the increased expression level of ppET-1 mRNA (32). In addition, the constitutively active form of Gα13 was not able to increase ppET-1 promoter activity in our system, although Gα13 has been demonstrated to activate JNK and Rho in HEK293 cells (49). These results suggest that the nature of the signaling pathways involved in the autoinduction of ET-1 may vary in different tissues.

In conclusion, we demonstrated that Gα13 induces ppET-1 promoter activation partially through a JNK dependent pathway in COS-7 cells, which suggests a possible role of Gα13 in ET-1 autoinduction. Further investigation is required to complete the picture of the ET-1 autoinduction pathways and G-protein-mediated signaling cascades that are downstream of ET receptors.

References


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