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Endothelin-1 induction of Glut1 transcription in 3T3-L1 adipocytes involves distinct PKCε- and p42/p44 MAPK-dependent pathways

Ying-Shiun Kao, Jim C. Fong *

Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan, Republic of China

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Abstract

We have shown previously that chronic exposure to endothelin-1 (ET-1) may stimulate GLUT1-mediated glucose transport in 3T3-L1 adipocytes via both protein kinase C (PKC)- and mitogen-activated protein kinase (p42/p44 MAPK)-dependent pathways. In the present study, by using a luciferase reporter driven by Glut1 promoter and enhancers (pLuc-GT1/E1/E2) and various constitutively active and dominant negative mutants of PKC isoforms, we identified PKCε as the PKC isoform involved. In addition, we provide evidence that there is no direct interaction between ET-1 activated PKCε and MAPK, at least at the kinase activity level. Furthermore, investigations employing deletion mutants of pLuc-GT1/E1/E2 to locate the putative ET-1 responsive sites and inhibitory agents to suppress the activities of putative transcription factors suggested that transcription factors CREB, Sp1 and NF-κB were involved. In summary, the results of this study indicate that ET-1 induction of Glut1 transcription involves distinct PKCε- and MAPK-dependent pathways, as well as downstream transcription factors CREB, Sp1 and NF-κB.

Keywords: 3T3-L1 adipocyte; Glucose transport; PKC; MAPK; GLUT1; Endothelin-1

1. Introduction

Endothelin-1 (ET-1) is a 21 amino acid polypeptide secreted by endothelial cells. Besides being a novel potent vasoconstrictor [1], ET-1 was reported to stimulate glycogenolysis [2] and glucose output in liver [3]. In addition, elevated plasma concentrations of ET-1 has been reported in diabetes mellitus [4], essential hypertension [5], aging [6], uremia [7], sepsis [8] and post-surgery [9], all of which are known to be accompanied by insulin resistance [10–14]. Thus there may be a close relationship between ET-1 and insulin resistance. Indeed, infusion of ET-1 was shown to cause insulin resistance in rat [15] and exposure to ET-1 inhibited insulin-stimulated glucose uptake in isolated rat adipocytes [16]. In addition, whereas ET-1 induced GLUT4 translocation, it decreased insulin-stimulated glucose uptake in 3T3-L1 adipocytes [17,18]. On the other hand, chronic treatment of 3T3-L1 adipocytes with ET-1 potentiated glucose transport [17,19]. Further studies indicated that ET-1-induced glucose transport in 3T3-L1 adipocytes was mainly attributed to GLUT1-mediated sugar uptake and ET-1-stimulated Glut1 transcription seemed to be responsible [19]. Moreover, our previous studies showed that both protein kinase C (PKC)- and mitogen-activated protein kinase (p42/p44 MAPK)-dependent pathways were involved in ET-1 stimulated glucose transport [19,20].

In the present study, we further investigated which PKC isoform is involved, the possible interaction between PKC and MAPK pathways, as well as the downstream transcription factors that may be mediating the effect of ET-1 on Glut1 transcription. Our results indicate that of the two PKC isoforms (ε and δ) that are responsive to ET-1 [20], only PKCε is involved. In addition, there is no interaction between ET-1 activated PKCε and MAPK, at least at the kinase activity level. Finally, we demonstrated that transcription factors CREB, Sp1 and NF-κB may be involved in ET-1 induction of Glut1 transcription.

2. Materials and methods

2.1. Materials

2-deoxy-D-glucose (2-deoxyglucose), manumycin A, GF109203X, dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), 3-isobutyl-1-methylxanthine (IBMX), salicylate, 4β-phorbol 12β-myristate 13α-acetate (PMA), mithramycin A and pertussis toxin (PT) were obtained from Sigma Chemicals (St. Louis, MO). PD98059 was from Calbiochem-EMD Biosciences (La Jolla,

* Corresponding author. Tel.: +886 2 2826 7123; fax: +886 2 2826 4843.
E-mail address: ffong@ym.edu.tw (J.C. Fong).

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CA). ET-1 was purchased from Peninsula Laboratories (San Carlos, CA). Anti-MAPK was from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phospho-MAPK was from New England Biolabs (Ipswich, MA); [^{3}H]2-deoxyglucose (8 mCi/mmol) was obtained from New England Nuclear (Boston, MA). c-Jun-specific phosphorothioated antisense oligonucleotide, 5′-CGTTTCCATCTTTG-CAGT-3′ (AS-cJun)[21], and sense oligonucleotide, 5′-ACTGCAAAGATG-GAAACG-3′, were synthesized commercially.

2.2. Cell culture

3T3-L1 cells, obtained from American Type Culture Collection (Rockville, MD), were grown and differentiated into adipocytes in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum, glutamine, penicillin and streptomycin as described earlier [22]. Briefly, 2 days after confluence (day 0), the medium was removed and fresh medium containing 0.5 mM IBMX and 0.25 mM dexamethasone was added. After another 3 days, the medium was replaced with fresh culture medium and the cultures were then maintained as described above. By day 8, more than 90% of the cells have differentiated into rounded cells with lipid droplets. Days 8–12 differentiated 3T3-L1 adipocytes were used for experiments throughout this study.

2.3. Preparation of cell lysates, membrane and cytosolic fractions

For cell lysate preparation, cells were treated with appropriate reagents, washed with phosphate-buffered saline (PBS) and lyzed in buffer A (20 mM HEPES, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1 mM Na_3VO_4, 10 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM PMSF and 1 μg/ml each of aprotinin, pepstatin and leupeptin, pH 7.5) for 1 h on ice. The cell lysates were centrifuged at 13,000×*g* for 30 min to remove insoluble materials and were collected by a 1 ml syringe through the floating fat layer. For preparation of membrane and cytosolic fractions, cells were treated with tested reagents, washed with PBS and homogenized in buffer B (250 mM sucrose, 20 mM HEPES, 1 mM EDTA, 1 mM PMSF and 1 μg/ml each of aprotinin, pepstatin and leupeptin, pH 7.4) on ice with a motor-driven Teflon-coated pestle. Following centrifugation at 600×*g* for 10 min to remove cell debris and fat cake, subsequent centrifugation at 100,000×*g* for 1 h resulted in cytosolic fraction in the supernatant and membrane fraction in the pellet. The membranes were resuspended to about 5 mg of protein/ml in buffer C (20 mM HEPES, 1 mM EDTA, 1% Triton X-100 and 1 mM PMSF, pH 7.4) and was further incubated for 1 h at 4 °C and the Triton X-100 soluble particulate fraction was collected after centrifugation at 100,000×*g* for 1 h. All centrifugation was performed at 4 °C. With the current experimental protocol, there was little contamination between the Triton X-100 soluble particulate and the cytosolic fractions as justified by immunoblot analyses of the contents of α-tubulin in the Triton X-100 soluble particulate fraction and caveolin in the cytosolic fraction.

2.4. Immunoblot analysis

Cell lysates, membrane or cytosolic samples were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions as described by Laemmli [23], and transferred to a nitrocellulose membrane. The phosphorylated MAPK, MAPK and PKCε were immunodetected by using appropriate primary and secondary antibodies. The blots were developed by the enhanced chemiluminescence method (ECL, Amersham). Quantification of relative band intensity was performed by laser scanning densitometry.

2.5. Plasmids

The expression plasmids for PKCε, δ and α mutants, either constitutively active (CAT) or dominant negative (KR) mutants also carrying N-terminal or C-terminal HA epitope tags, respectively, were kindly provided by Dr. J.W. Soh [24]. The reporter plasmid pLuc-GT1/E1/E2, containing a luciferase reporter construct under the control of the mouse Glut1 promoter along with enhancers 1 & 2 was a kind gift from Dr. C. Montessuit [25], who generated pLuc-GT1/E1/E2 from the chloramphenicol acetyltransferase reporter plasmid pGT1-1.3 [26] by subcloning the promoter and enhancers into the pGL3-basic multicloning site (Promega). The cis-reporter plasmid pAP-1-Luc that is used for determining the transcriptional activity of AP-1 was purchased from Stratagene (La Jolla, CA). The expression plasmid for dominant negative mutant of CREB (dnCREB) was generously given by Dr. Jeffrey J-Y Yen [27].

The deletion mutants (plasmid B–E) of pLuc-GT1/E1/E2 (plasmid A) were constructed by a strategy using various restriction enzymes. Plasmid B was created by inserting *Bgl*II/*Hin*dIII fragment of plasmid A into the Bglll/*Hin*dIII cloning sites of pGL3-basic. Plasmid C was constructed by *Pvu*II digestion of plasmid A, followed by blunt-end ligation. Plasmid D was constructed by

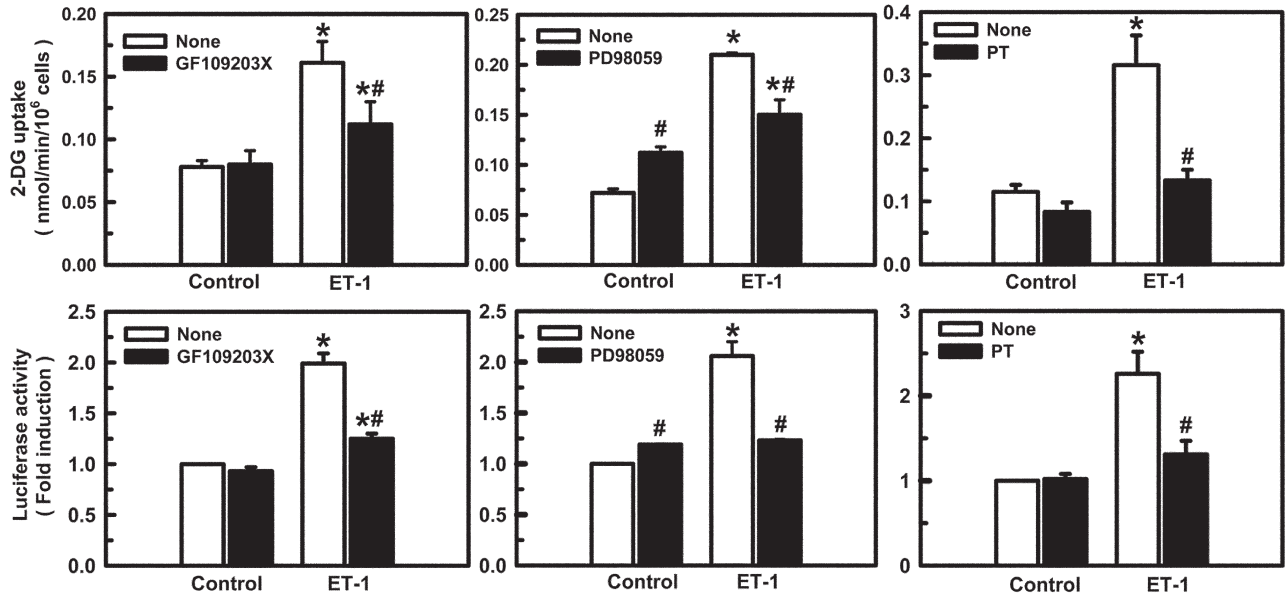

Fig. 1. Effect of PT, GF109203X and PD98059 on ET-1 induced (A) 2-DG uptake and (B) Glut1 promoter activity. (A) After 3T3-L1 adipocytes were pretreated without (none) or with PT (100 μg/ml) for 4 h, GF109203X (1 μM) or PD98059 (50 μM) for 30 min, vehicle (control) or ET-1 (10 nM) was added and incubation was continued for another 8 h. 2-DG uptake was then measured. (B) 3T3-L1 adipocytes were transiently transfected with pLuc-GT1/E1/E2 by electroporation. After recovery for 24 h, cells were treated without (none) or with PT for 4 h, GF109203X or PD98059 for 1 h before the addition of vehicle (control) or ET-1 for another 12 h. The cell lysates were then obtained and measured for the luciferase/β-galactosidase activity ratios that were used for comparison of the induction of Glut1 transcription. Values are means±S.E. from three experiments with duplicate determinations. *Values are significantly different from the corresponding control; #Values are significantly different from the corresponding none.