Biochimica et Biophysica Acta 1820 (2012) 1636–1646

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

PKA and cAMP stimulate proliferation of mouse embryonic stem cells by elevating GLUT1 expression mediated by the NF-κB and CREB/CBP signaling pathways

Mi Ok Kim [a], Yu Jin Lee [a], Jae Hong Park [a], Jung Min Ryu [a], Seung Pil Yun [a], Ho Jae Han [b,*]

[a] Department of Veterinary Physiology, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Republic of Korea
[b] Department of Veterinary Physiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 151-741, Republic of Korea

ARTICLE INFO

Article history:
Received 10 February 2012
Received in revised form 1 May 2012
Accepted 21 May 2012
Available online 29 May 2012

Keywords:
Glucose transporter
Protein kinase A
Nuclear factor kappa B
Embryonic stem cell
Proliferation

ABSTRACT

Background: Regulation of glucose transporter (GLUT) expression and activity plays a vital role in the supply of glucose to embryonic stem (ES) cells.
Methods: To observe the effect of 6-phenyl cyclic monophosphate (cAMP) on glucose uptake and cell proliferation, 2-deoxyglucose (2-DG) uptake, immunohistochemistry, Western blotting, and immunoprecipitation were carried out.
Results: Among GLUT isoforms in mouse ES cells, GLUT1 was predominantly expressed and 6-phenyl cAMP increased GLUT mRNA levels. Among cAMP agonists, 6-phenyl cAMP increased 2-DG uptake more than that of 8-p-chlorophenylthio-2′-O-methyl-cAMP. 6-Phenyl cAMP increased GLUT1 expression and translocation from the cytosol to the plasma membrane. 6-Phenyl cAMP increased 2-DG uptake in a time- and concentration-dependent manner due to an increase in V_{max} but not K_m. 6-Phenyl cAMP increased phosphorylation of nuclear factor-κB (NF-κB) and cAMP response element binding (CREB) and expression of the CREB protein (CBP) and transducer of regulated CREB activity 2 (TORC2) in sequence. 6-Phenyl cAMP induced complex formation of NF-κB/CREB/CBP/TORC2, which are involved in the increase of gluconeogenic enzyme expression. 6-Phenyl cAMP also increased cell cycle regulatory protein expression levels, the proportion of S-phase cells, and proto-oncogene expression via protein kinase A (PKA)-dependent NF-κB signaling. Finally, GLUT1 siRNA blocked the 6-phenyl cAMP-induced increase in ES cell proliferation. We conclude that PKA stimulated the complex formation of CREB/CBP/TORC2 via NF-κB, which induced effective coordination of glucose uptake as well as proliferation in ES cells.
General significance: 6-Phenyl cAMP-induced PKA activation modified the proliferation, which may be beneficial for expanding ES cell use to cell therapy.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Glucose is the major source of energy for pluripotent stem cells and is an important factor for deciding stem cell fate. The movement of glucose in and out of cells is mediated by facilitative glucose transporters (GLUTs), which are differentially expressed as isoforms in most mammalian cells that participate in glucose uptake. Indeed, regulation of GLUT expression and activity plays a vital role in the glucose supply to embryonic stem (ES) cells, as glucose uptake across the plasma membrane is one of the rate-limiting steps in glucose metabolism [1,2]. As a ubiquitous member of the GLUT family, GLUT1 is expressed throughout development and is thought to provide the embryo with its basal glucose requirements. Moreover, GLUT1 expression, which is detectable throughout pre-implantation development from the oocyte through the blastocyst stage [3], increases 11-fold in developing embryos from the two-cell stage to the blastocyst. GLUT expression and localization are regulated in a variety of ways, such as by intracellular and extracellular signals, depending on the cell type and stimulus involved. Cyclic adenosine monophosphate (cAMP) regulates GLUT3 at the transcription level in rat neurons, whereas cAMP increases GLUT1 expression and glucose uptake in a choriocarcinoma cell line [4]. Although protein kinase A (PKA) was thought to be the only direct effector of cAMP, several studies have pointed to mediation by cAMP signals independently of PKA. Tools that allow the specific PKA activation and exchange protein activated by cAMP (Epac) now exist [i.e., N6-phenyladenosine-3′,5′-cyclic monophosphate (6-phenyl cAMP) and 8-p-chlorophenylthio-2′-O-methyl-cAMP (8CPT cAMP)], respectively, but have not been previously used to dissect cAMP-induced signaling cascades in ES cells. Therefore, further investigation is necessary to clarify the molecular mechanisms responsible for the regulation of glucose uptake by cAMP in ES cells, including transcriptional regulation by cAMP, and to provide relevant information of how cAMP regulates ES cell functions.

Abbreviations: PKA, protein kinase A; NF-κB, nuclear factor-κB; CREB, cAMP response element binding; CBP, CREB binding protein; TORC2, transducer of regulated CREB activity 2; GLUT, glucose transporter; 2-DG, 2-deoxyglucose

* Corresponding author. Tel.: +82 2 880 1261; fax: +82 2 880 2732.
E-mail address: hjhan@snu.ac.kr (H.J. Han).

0304-4165/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagen.2012.05.008

Major advances in cell and developmental biology have been made with mouse ES cells as a versatile biological system. These unique cells are characterized by their capacity for prolonged undifferentiated proliferation in culture and maintaining the potential to differentiate into derivatives of all three germ layers. Furthermore, many extracellular factors have been identified that affect ES cell self-renewal [5–7]. GLUT1 responsiveness to extracellular stimuli is extremely important as a mechanism for providing increased substrate to cope with the increased cellular demand arising from self-renewal. Indeed, GLUT1 expression has been described for mouse and human ES cells [8] and the GLUT1 −/− cells were non-viable [9], indicating that GLUT1 is the main glucose transporter isoform in ES cells [10]. However, relatively few studies have examined the signaling systems involved in the regulation of GLUT1-mediated glucose uptake in ES cells, and no previous reports have investigated the role of a signaling pathway as an important regulator of ES cell function including the pluripotent state. Therefore, we determined whether activating the PKA pathway using synthetic analogs would alter glucose uptake. This study examined the effect of 6-phenyl cAMP on glucose uptake and proliferation of mouse ES cells and its related signaling cascades.

2. Experimental procedures

2.1. Materials

Mouse ES cells were obtained (product code: ES E14TG2a) from the American Type Culture Collection (Manassas, VA, USA). Fetal bovine serum (FBS) was purchased from BioWhittaker (Walkersville, MD, USA). Forskolin, 8-Br-cAMP, and fluorescence isothiocyanate (FITC)-conjugated goat-anti mouse IgM and β-actin were acquired from Sigma-Aldrich (St. Louis, MO, USA). The PKA inhibitor 14–22 amide (PKI) was acquired from Merck (Darmstadt, Germany). The Epac agonist (8-[4-chlorophenylthio]-2′-O-methyladenosine-3′,5′-cyclic monophosphate [8-CPT-2′-O-Me-cAMP]) and the PKA agonist (N^6-phenyladenosine-3′,5′-cyclic monophosphate [6-phenyl cAMP]) were purchased from the Biolog Life Science Institute (Bremen, Germany). Phospho-CREB, phospho-NF-κB p65, CREB, NF-κB, TORC2, CBP, CDK2, CDK4, cyclin D1, cyclin E, and GLUT1 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Goat anti-rabbit IgG antibody was acquired from Jackson ImmunoResearch (West Grove, PA, USA). All reagents were of the highest commercial purity available.

2.2. ES cell culture

Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 3.7 g/l sodium bicarbonate, 1% penicillin and streptomycin, 1.7 mM L-glutamine, 0.1 mM β-mercaptoethanol, 5 ng/ml mouse leukemia inhibitory factor (LIF), and 15% FBS without a feeder layer. Cells were cultured for 5 days in standard medium plus LIF, and were grown on 35 mm or 60 mm diameter culture dishes in an incubator maintained at 37 °C in an atmosphere of 5% CO_2. The medium was changed to serum-free LIF containing DMEM prior to the experiments.

2.3. Alkaline phosphatase staining

Cells were washed twice with phosphate-buffered saline (PBS) and fixed for approximately 15 min with 4% formaldehyde (in PBS) at room temperature. After washing with PBS, 2 ml alkaline phosphatase substrate solution (200 mg/ml naphthol AS-MX phosphate, 2% *N*, *N*-dimethylformamide, 0.1 mM Tris and 1 mg/ml Fast Red TR salt) was added and incubated for approximately 10–15 min at room temperature; stained cells were washed with PBS and photographed.

2.4. Immunofluorescence staining of GLUT

Cells were fixed with 3.5% paraformaldehyde in PBS, permeabilized for 5 min with 0.1% Triton X-100, and washed three times for 10 min each with PBS. Cells were preincubated with 10% BSA in PBS for 20 min to decrease nonspecific antibody binding. Cells were incubated with antibody against GLUT1 or pan cadherin (1:50, Santa Cruz Biotechnology, Inc.) for 1 h, and then incubated for 30 min with a 1:100 dilution of FITC or Alexa Fluor 488-conjugated secondary antibody. Cells were stained with antibody pan cadherin as a plasma membrane marker to confirm separation of the cytosolic and membrane fractions. Fluorescent images were visualized using a FluoView 300 fluorescence microscope (Olympus, Tokyo, Japan).

2.5. RNA isolation, real-time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from mouse ES cells using a monophasic solution of phenol and guanidine isothiocyanate (STAT-60™; Tel-Test Inc., Friendswood, TX, USA). RT was performed with 3 μgRNA using AccuPower™ RT PreMix RT System Kits (Bioneer, Seoul, Korea) with oligo-dT18 primers. Real-time quantification of RNA targets was performed in a Rotor-Gene 6500 real-time thermal cycling system (Corbett Research, Sydney, Australia) using QuantiTect SYBR Green RT-PCR Kits (Qiagen, Valencia, CA, USA). The 20 μl reaction mixture contained 200 ng of total RNA, 0.5 μM of each primer (Supplementary Data 1), enzymes, and fluorescent dyes as per the manufacturer's instructions. The Rotor-Gene 3500 cycler was programmed as follows: 50 min at 56 °C for RT, 15 min at 95 °C for DNA polymerase activation, 5 min at 95 °C for denaturation, and 45 cycles of 25 s at 94 °C, 30 s at 57 °C, and 20 s at 72 °C. Data were collected during the extension step (20 s at 72 °C). Melting curve analysis, which distinguishes specific and non-specific PCR products resulting from primer–dimer formation, was performed after PCR to confirm the specificity and identity of the RT-PCR products obtained. The temperature of the PCR products was increased from 65 to 99 °C at a rate of 1 °C every 5 s, and the resulting data were analyzed.

2.6. Small interfering RNA (siRNA) transfection

Cells grown to 50% confluence in each dish were transfected for 24 h with either siRNA specific for NF-κB, GLUT1, CREB1, CBP (100 nmol/l), or a nontargeting siRNA as a negative control (100 nM; Dharmacon, Lafayette, CO, USA) using Hyperfectamine (Qiagen) according to the manufacturer's instructions. The construct targeting NF-κB was comprised of the following 3′ (sense) and 5′ (antisense) pairs: 3′-CUG CAA AGG UUA UCG UUC A and 5′-UGA ACG AUA ACC UUU GCA G; 3′-GAA GAA AAU GGC GGA GUU U and 5′-AAA CUC CGC CAU UUU CUU C; The construct targeting GLUT1 was comprised of the following: 3′-CCA AGA GUG UGC UAA AGA AUU and 5′-CAU CGU GGC UGA ACU CUU CUU. The construct targeting CREB1 was comprised of the following: 3′-CUG UAC AUA UGC UAC UGA U and 5′-AUC AGU AGC AUA UGU ACA G; and 3′-GAG UGU GUG CUA UGG UAC A and 5′-UGU ACC AUA GCA CAC ACU C. The construct targeting CBP was comprised of the following: 3′-UCA UCA CAG CAG CAA CCA A and 5′-UUG GUU GCU GCU GUG AUG A; and 3′-GUG ACA AGC GAA ACC AAC A and 5′-UGU UGG UUU CGC UUG UCA C. The construct targeting CBP was comprised of the following: 3′-UCA UCA CAG CAG CAA CCA A and 5′-UUG GUU GCU GCU GUG AUG A; and 3′-GUG ACA AGC GAA ACC AAC A and 5′-UGU UGG UUU CGC UUG UCA C. The nontargeting siRNA was 5′-UGG UUU ACA UGU CGA CUA A-3′. After 24 h, transfection mixtures were replaced with serum-free DMEM, and the cells were maintained.

2.7. Western blot analysis

Cells were harvested and washed twice with PBS, prior to lysis in buffer (20 mM Tris [pH 7.5], 1 mM EDTA, 1 mM EGTA, 1% Triton

Download English Version:

https://daneshyari.com/en/article/10800729

Download Persian Version:

https://daneshyari.com/article/10800729

Daneshyari.com