Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Letters 404 (2006) 87–92
Neuroscience Letters
www.elsevier.com/locate/neulet

Membrane depolarization stimulates the proliferation of SH-SY5Y human neuroblastoma cells by increasing retinoblastoma protein (RB) phosphorylation through the activation of cyclin-dependent kinase 2 (Cdk2)

Miran Seo [a], Yeni Kim [a], Yun-Il Lee [a], So-Young Kim [a], Yong-Min Ahn [b], Ung Gu Kang [b], Myoung-Sun Roh [c], Yong-Sik Kim [b], Yong-Sung Juhnn [a,*]

[a] *Department of Biochemistry and Molecular Biology, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea*
[b] *Department of Psychiatry and Behavioral Science, Institute of Human Behavioral Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea*
[c] *Chookryoung Evangelical Hospital, Kyonggido, Republic of Korea*

Received 12 January 2006; received in revised form 30 May 2006; accepted 31 May 2006

Abstract

Membrane depolarization causes transmembrane ionic influxes that induce various gene expressions, and is involved in the processes of neuronal differentiation and apoptosis. However, the effect of membrane depolarization on neuronal proliferation has not been established. In this study, we aimed to investigate the effect of membrane depolarization on the proliferation of SH-SY5Y human neuroblastoma cells. Membrane depolarization induced by 50 mM KCl for 5 min significantly increased SH-SY5Y cell numbers and thymidine incorporation at 24 h after depolarization, and increased the phosphorylation and expression of retinoblastoma protein (RB), the activity of Cdk2 (without changing the activities of Cdk4 and Cdk6), and the expressions of cyclin A and cyclin E. Single and repeated depolarization (once a day for 6 days) had similar effects on RB, Cdks, and cyclins levels and activities. In summary, our results suggest that membrane depolarization may stimulate cellular proliferation by augmenting the expression of cyclin E leading to increases in Cdk2 activity and RB phosphorylation.

Keywords: Membrane depolarization; Proliferation; Neuroblastoma cells; Retinoblastoma protein; Cyclin-dependent kinases; Cyclins

Changes in the electrical potentials of plasma membranes are known to cause transmembrane ionic fluxes that activate a variety of cellular signaling pathways [14]. Such depolarization-induced signaling pathway activations change cellular metabolisms and the expressions of genes, such as, those of, enzymes required for neurotransmitter metabolism, and receptors required for neurotransmitters [3,15]. Moreover, membrane depolarization has been implicated in neuronal differentiation and in protection against neuronal apoptosis [6], and electrical activities in the nervous system influence more complex intercellular interactions, such as, neural sprouting, long-term potentiation, and map refinement [4,9]. Thus, changes in neuronal metabolism and gene expression induced by membrane depolarization are implicated in the pathogenesis and therapeutic mechanisms of various neuropsychiatric disorders.

Recently, electroconvulsive shock (ECS), which induces massive membrane depolarization and which provides a model for electroconvulsive therapy, was found to dramatically increase neurogenesis in the rat hippocampus [10,16]. In our previous studies, we also found that ECS induces the activations of cell cycle-dependent kinases (Cdks) and the phosphorylations of retinoblastoma proteins (RB) in rat brain frontal cortex [7], suggesting that ECS and membrane depolarization may stimulate neuron proliferation. However, it is not known whether membrane depolarization can stimulate neuronal cell cycle progression and subsequent cellular proliferation. Thus, in this

* Correspondence to: Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, 28 Yongon-dong, Jongno-gu, Seoul 110-799, Republic of Korea. Tel.: +82 2 740 8247; fax: +82 2 744 4534.
E-mail address: juhnn@snu.ac.kr (Y.-S. Juhnn).

0304-3940/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2006.05.061

study, we investigate the effect of membrane depolarization on the proliferation of neuroblastoma cells.

SH-SY5Y human neuroblastoma cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA), and were grown in Dulbecco's modified minimal essential media (DMEM) containing 10% fetal bovine serum (JBI, Korea), 100 IU/ml penicillin, and 50 μg/ml streptomycin in a 5% CO_2 incubator at 37 °C. SH-SY5Y cell line originated from the SK-N-SH neuroblastoma cell line, and exhibits neuronal characteristics, such as, dopamine beta hydroxylase activity and neurite formation, thus reflecting a neural crest cell origin in the sympathetic nervous system [2]. Because of these neuronal characteristics, this cell line is used widely as a model for neuronal functions and differentiation [13], and we also used this cell line to analyze the effect of depolarization on neuronal proliferation.

Membrane depolarization was induced by treating SH-SY5Y cells with 50 mM KCl in complete medium for 5 min, and for repeated depolarization, cells were treated with 50 mM KCl for 5 min every 24 h for 6 days, whereas control mock-treated cells were treated with complete medium. Cells were then washed with phosphate buffered saline (PBS) twice, and maintained in fresh complete medium before harvesting. Analysis was performed 24 h after the last depolarization treatment to examine the delayed effect of depolarization, as was previously described after ECS treatment [7].

SH-SY5Y cells (2×10^6 cells) were plated in 100 mm dishes (55 cm^2) for cell counting, or 1×10^5 cells in 12-well plates (4.5 cm^2) for [^{3}H]thymidine incorporation assays, and 24 h later, depolarized with 50 mM KCl for 5 min. They were then maintained in complete medium for 24 h, detached by trypsin–EDTA treatment, and counted using a hemocytometer. For [^{3}H]thymidine incorporation assays, depolarized cells were incubated in complete medium containing 2 μCi of [^{3}H]thymidine for 24 h, treated with 10% trichloric acid for 15 min, and then with 1 M of NaOH for 5 min. The resulting soluble cell lysates were transferred to scintillation vials for radioactivity measurement.

SH-SY5Y cells were harvested after depolarization with KCl and analyzed by Western blotting [17]. The primary antibodies used were as follows: antibody against phosphorylated RB (Ser795) from Cell Signaling Technology (Beverly, MA), antibody against RB from Lab Vision Corporation (Fremont, CA), and antibodies against cyclin A, cyclin E, cyclin B1, cyclin D3, Cdk2, Cdk4, and Cdk6 from Santa Cruz Biotechnology (Santa Cruz, CA). Proteins were visualized by incubating blots with an enhanced chemiluminescence substrate mixture (Pierce, Rockford, IL) and then by exposure to X-ray film (AGFA Curix RPI, Mortsel, Belgium). Densities of visualized bands were quantified using an image analyzer (Fujifilm, Model Multi Gauge V2.3, Stamford, CT), and band densities were expressed as percentages of the corresponding band densities of control mock-treated cells.

The kinase activities of Cdk2, Cdk4, and Cdk6 were analyzed as described [12]. Briefly, cell lysates from depolarized cells were immunoprecipitated using respective antibodies against

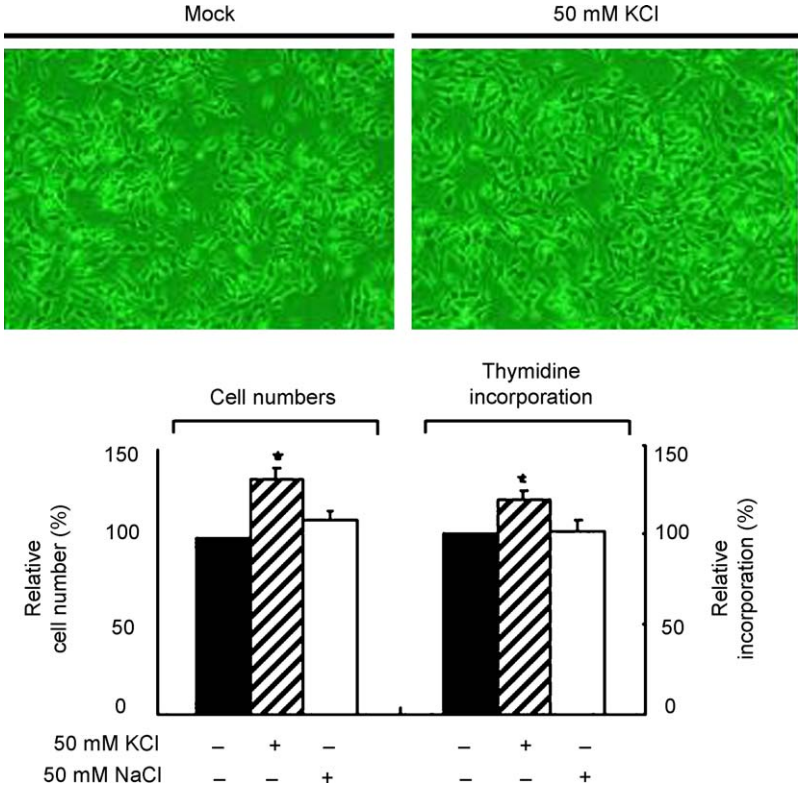

Fig. 1. KCl-induced depolarization increased cellular proliferation and DNA synthesis in SH-SY5Y cells. SH-SY5Y cells were treated with 50 mM KCl or 50 mM NaCl for 5 min. After 24 h, then microscopic view of the cells were photographed, cell numbers were counted using a hemocytometer, and [^{3}H]thymidine incorporation was analyzed. Asterisks (*) mean significantly difference from the respective KCl-mock-treated controls ($p < 0.05$, Mann–Whitney U-test).