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17β-estradiol stimulates MAPK signaling pathway in human lens epithelial cell cultures preventing collapse of mitochondrial membrane potential during acute oxidative stress

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Abstract

17β-estradiol (17β-E_2) protects against H_2O_2-mediated depletion of intracellular ATP and lessens the degree of depolarization of mitochondrial membrane potential ($\Delta\Psi_m$) in cultured lens epithelial cells consequential to oxidative insult. We now report that 17β-E_2 acts as a positive regulator of the survival signal transduction pathway, MAPK which, in turn, acts to stabilize $\Delta\Psi_m$, in effect, attenuating the extent of depolarization of mitochondrial membrane potential in the face of acute oxidative stress. The SV-40 viral transformed human cell line, HLE-B3 was treated with 17β-E_2 over a time course of 60 min and phosphorylation of ERK1/2 was analyzed by Western blot. ERK1/2 was phosphorylated within 5–15 min in the presence of 17β-E_2. Cell cultures were exposed to the MEK1/2 inhibitor, UO126, subsequent to $H_2O_2 \pm$ 17β-E_2 treatment and the $\Delta\Psi_m$ examined using JC-1, a potentiometric dye which serves as an indicator for the state of mitochondrial membrane potential. UO126 treatment attenuated ERK1/2 phosphorylation irrespective of whether estradiol was administered. Mitochondrial membrane depolarization resulting from H_2O_2 stress was substantially greater in the presence of UO126. The greater the extent of depolarization, the less effective 17β-E_2 treatment was in checking mitochondrial membrane depolarization, indicating that the relative degree of ERK phosphorylation influences mitochondrial stability with oxidative insult. The data support a positive correlation between 17β-E_2 stimulation of ERK1/2 phosphorylation and mitochondrial stabilization that would otherwise cause a complete collapse of $\Delta\Psi_m$.

Keywords: ERK; Estrogen; Hydrogen peroxide; Human lens epithelial cell; Mitochondria

1. Introduction

The biological actions of estrogens are mediated by binding to one of two estrogen receptors (ERs), ERα and ERβ, which are members of the nuclear receptor

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superfamily, a family of ligand-related transcription factors (reviewed in Matthews and Gustafsson, 2003). The actions of 17β-estradiol (17β-E_2) occur on binding the ER, and the nuclear pool of these receptors can then transactivate target genes (reviewed in Levin, 2001). In addition to its role in being a prominent transcription factor, studies of the antioxidant activity of 17β-E_2 have demonstrated that estrogens do not necessarily require the classical receptor-dependent mechanism in order to exert their positive effects (Behl et al., 1997; Gridley et al., 1998). It has been recently shown that 17β-E_2 protects against H_2O_2-mediated depletion of intracellular ATP in human lens epithelial cells (HLE-B3) (Wang et al., 2003) and that stabilization of the mitochondrial membrane potential ($\Delta\Psi_m$) by 17β-E_2 plays a crucial role in protecting the lens epithelial cell from oxidative damage (Wang et al., 2003; Moor et al., 2004). The dependency on ER binding for this stabilization process to occur has yet to be definitively demonstrated.

Under conditions of oxidative stress, mitochondria undergo a loss of impermeability of the inner mitochondrial membrane which subsequently causes a complete collapse of mitochondrial membrane potential ($\Delta\Psi_m$) (Murphy et al., 1999). H_2O_2 can collapse $\Delta\Psi_m$ in many cell types including lens epithelial cells (Wang et al., 2003), ultimately eliminating the driving force for ATP production and exacerbating free radical production (Dykens, 1994). The mechanism(s) by which 17β-E_2 stabilizes the inner mitochondrial membrane to maintain $\Delta\Psi_m$ during oxidative stress are unknown. The current study describes the activation of ERK1/2 by 17β-E_2 in both HLE-B3 and secondary cultures of bovine lens epithelial cells (BLECs). We propose that, in spite of acute oxidative stress, the activation of this upstream target may be triggering an anti-apoptotic cascade of events in cultured lens epithelial cells that promotes the downstream stabilization of the inner mitochondrial membrane preventing the complete collapse of $\Delta\Psi_m$.

2. Materials and methods

2.1. Materials

1,3,5(10)-ESTRATRIEN-3, 17β-DIOL (17β-E_2) was purchased from Steraloids, Inc. (Newport, RI). For use in our experiments, the hormone was dissolved in either 100% ethanol or a diluted (0.025%) solution of (2-Hydroxypropyl)-β-cyclodextrin (in water) from Sigma-Aldrich (St Louis, MO). Stock solutions of hormone were prepared fresh for each experiment and diluted in culture medium to a working concentration of 1 μM. 30% hydrogen peroxide (H_2O_2) was purchased from Fisher Scientific (Fair Lawn, NJ) and diluted in water and culture medium to obtain a final working concentration of 100 μM. 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazolylcarbocyanine iodide (JC-1) was purchased from Molecular Probes (Eugene, OR). L-buthionine-[s,r]-sulfoximine (L-BSO), an inhibitor of glutathione biosynthesis was from Sigma-Aldrich (St Louis, MO). The MEK1/2 inhibitor, UO126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio] butadiene), rabbit anti-p44/42 MAP Kinase, mouse anti-phospho-p44/42 MAPK (Thr202/Tyr204), rabbit anti-Akt, rabbit anti-phospho-Akt (Ser473), rabbit anti-phospho-SAPK/JNK (Thr183/Tyr185) antibodies and horseradish peroxidase-conjugated (HRP) goat anti-rabbit and horse anti-mouse IgG were all obtained from Cell Signaling Technology® (Beverly, MA). Rabbit anti-actin and mouse anti-JNK antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). All other chemicals and reagents were of analytical grade and were obtained from commercially available sources.

2.2. Cell culture

HLE-B3 cells, a human lens epithelial cell line immortalized by SV-40 viral transformation (Andley et al., 1994), were obtained from Usha Andley (Washington University School of Medicine, Department of Ophthalmology, St Louis, MO). Cells were maintained in Eagle's minimal essential medium (MEM) supplemented with 20% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT), 2 mM L-glutamine, nonessential amino acids and 0.02 g/L gentamycin solution (Sigma Chemical Co., St Louis, MO) at 37 °C and 5%CO_2/95%O_2. Primary cultures of bovine lens epithelial cells (BLECs) were established from the aseptic dissection of bovine (*Bostaurus*) lenses and cultures were maintained in MEM supplemented with 10% bovine calf serum (BCS) (Hyclone Laboratories, Logan, UT), 2 mM L-glutamine, nonessential amino acids and 0.02 g/L