STEROIDS 73 (2008) 859–863

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17β-Estradiol signaling in skeletal muscle cells and its relationship to apoptosis

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ARTICLE INFO

Published on line 4 January 2008

Keywords:
Skeletal muscle
Estrogen
Apoptosis

ABSTRACT

17β-Estradiol exerts an antiapoptotic action in skeletal muscle cells through extranuclear ERα and β. This protective action, mainly involves a non-genomic mechanism of ERK1/2 and PI3K/Akt activation and BAD phosphorylation. ERβ plays a major role in the inhibition of apoptosis by 17β-estradiol at the level of mitochondria, whereas ERα and ERβ mediate the activation of Akt to the same extent, suggesting differential involvement of ER isoforms depending on the step of the apoptotic/survival pathway involved. The myopathies associated to estrogen deficit states may be related to the mechanisms by which estrogen regulates apoptosis.

1. Estrogens and skeletal muscle function

There is evidence that skeletal muscle is a target tissue for estrogens. Several muscle pathologies are caused, in part, by decreased estrogen levels. It has been shown that muscle performance diminishes during the postmenopausal years leading to sarcopenia [1]. Thus, hormone replacement therapies prevent this myopathy. Congruent with these observations, it has been shown that 17β-estradiol (E2) promotes proliferation and differentiation of skeletal muscle myoblasts which express functional estrogen receptors [2]. Moreover, it has been suggested that apoptosis plays an important role in skeletal muscle development, by controlling the population size of myoblasts which undergo differentiation into mature myotubes [3]. Although, the exact mechanism by which estrogens improve muscle performance remains to be clarified, in the last few years various studies have addressed this issue. Within the scope of this review, we will summarize reports on estrogen signal transduction in skeletal muscle which involve non-genomic mechanisms, in particular pathways and receptors which mediate the antiapoptotic/prosurvival effects of E2 on muscle cells.

2. Non-genomic and genomic actions of 17β-estradiol

17β-Estradiol elicits its actions through nuclear receptor-mediated gene transcription and a non-genomic transmembrane signal transduction mechanism [4].

Most of the effects of the hormone are mediated via ERα and ERβ, by regulating nuclear estrogen responsive genes. These receptors function as transcription factors to increase gene expression by direct binding to specific DNA target sequences.

Non-genomic effects of estrogen refer to fast non-transcriptional responses involving signaling pathways. Among the rapid actions of E2, the activation of PI3K/Akt [5] and mitogen activated protein kinases (MAPKs) [6] have been demonstrated. PI3K activation leads to Akt phosphorylation, which in turn acts on numerous substrates involved

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doi:10.1016/j.steroids.2007.12.027

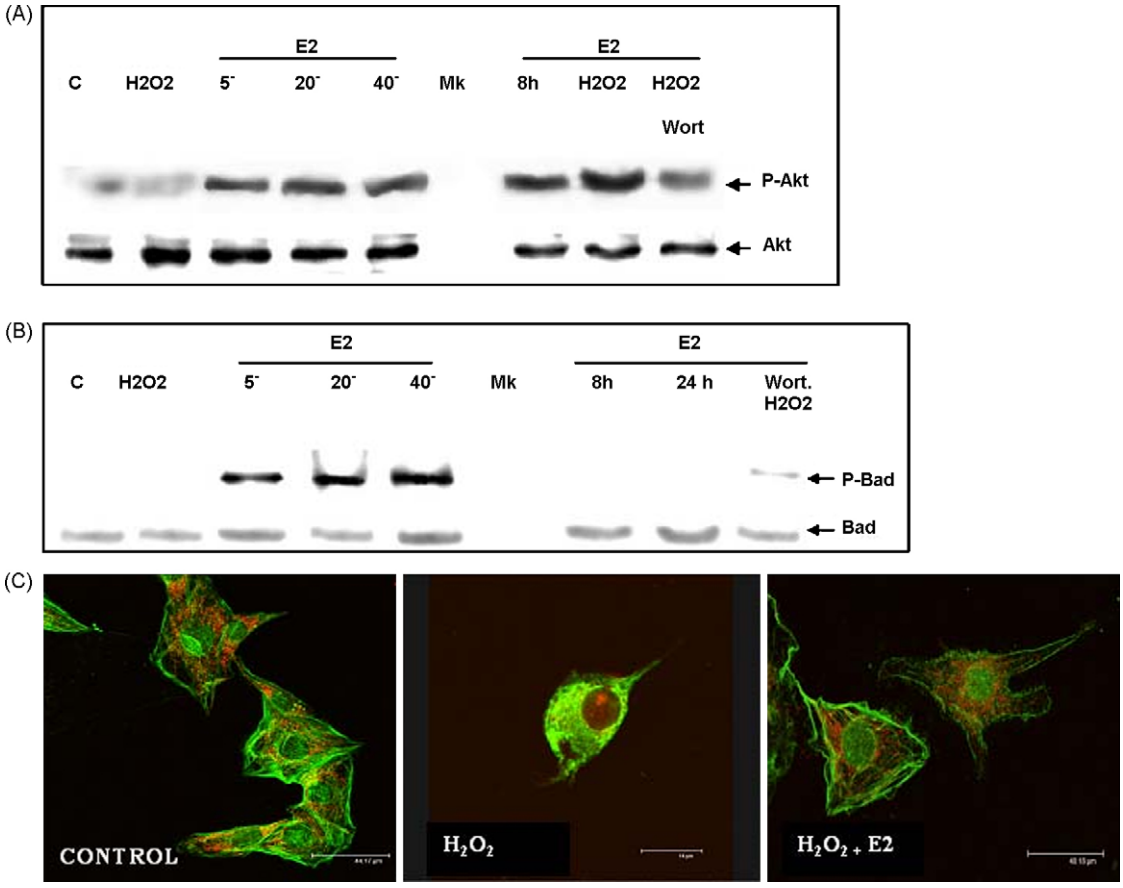

Fig. 1 – E2 induces phosphorylation of Akt (A), and in turn induces phosphorylation of BAD (B). 17β-Estradiol abrogates typical alterations in cytoskeleton structure, mitochondrial morphology and its distribution induced by H_2O_2 (C) [8].

in the regulation of cell functions such as apoptosis [7]. E2 is able to promptly activate the PI3K/Akt pathway by different mechanisms, in an ER-dependent and ER-independent manner, according to the cellular type [5]. We have shown a rapid activation of this cascade by the hormone through ER in the C2C12 skeletal muscle cell line (Fig. 1A and B) [8]. Among MAPKs, ERK1/2, JNK1/2 and p38 MAPK are the most extensively studied. ERK1/2 is considered to respond to growth and differentiation signals, while JNK1/2 and p38 MAPK are activated by cellular stresses [9]. E2 has also been shown to exert in skeletal muscle cells a rapid activation of the ERK1/2 and p38 MAPK pathways leading to the phosphorylation of transcription factors CREB and ELK-1 and c-fos expression [10].

Estrogen regulation of signal transduction pathways may lead to protective actions in skeletal muscle as well as in other tissues [8,11]. Also, as part of the prosurvival mechanism, E2 induces expression of the heat shock protein HSP27 [12]. In agreement with this fact, in skeletal muscle cells, we demonstrated that the steroid increases HSP27 mRNA and protein expression [13]. Immunocytochemistry and co-immunoprecipitation assays demonstrated co-localization and interaction of HSP27 with ERβ in mitochondria, event barely observed when cells are apoptotic [8]. Because of the importance of the antiapoptotic action of mitochondrial ERβ described below, these data highlight an E2-induced extranuclear interaction between ERβ and HSP27 that may be of importance in modulating estrogen signaling.

3. Antiapoptotic effects of 17β-estradiol

E2 can support cell survival or induce apoptosis depending on the cell context [14]. Estrogens exert antiapoptotic effects on various cell types such as vascular endothelial, smooth and skeletal muscles, and breast cancer cells, among others [15,14,8]. As mentioned before, apoptosis exerts a control on the relative proportions of myoblasts and myotubes in muscle [3]. Then, the described effects of the hormone on skeletal muscle development could be regulated, in part, through its effects on apoptosis.

In C2C12 murine skeletal muscle cells, E2 prevents cytochrome c release, caspase-3 and PARP cleavage and DNA fragmentation. Also, estrogen blocked the typical apoptotic morphological changes of the nucleus, mitochondria and cell size, and mitochondrial redistribution (Fig. 1C) [8]. Studies are necessary to elucidate the relative roles of these events and the mechanisms by which estrogens exert their inhibitory effects.

Apoptosis is a complex process in which protein kinases have been implicated both in the upstream induction phase and in the downstream execution stage. The MAPK family, specifically ERK1/2, p38 MAPK and JNK1/2, have been sug-

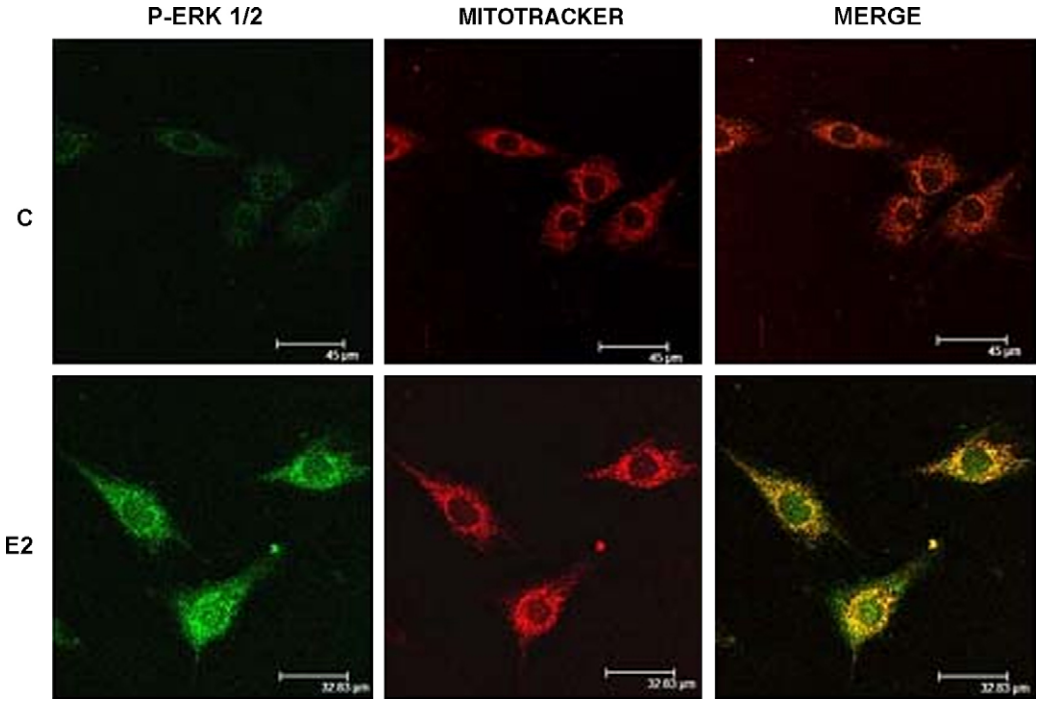

Fig. 2 – E2-activated ERK1/2 localizes in mitochonndria in C2C12 muscle cells [17].

gested to play a role in programmed cell death. The activation of JNK1/2 and p38 MAP kinases is generally associated with promotion of apoptosis, while ERK1/2 activity inhibits it [16]. Accordingly, using U0126 and SB203580, specific inhibitors of ERK1/2 and p38 MAPK, respectively, we have demonstrated that the protective action of E2 on C2C12 muscle cells involves fast activation of these MAPKs. Noteworthy, immunocytochemical assays revealed that ERK1/2 activated by E2 localizes mainly in mitochondria (Fig. 2) [17], further supporting that the organelle is an important target of estrogen action.

Moreover, evidence was obtained suggesting that the PI3K/Akt/Bad pathway also plays a role in the antiapoptotic effects of E2 on skeletal muscle cells. It was shown that after PI3K/Akt activation by E2, Akt phosphorylates serine residues of Bad and blocked its action as mediator of apoptosis. Inhibition of PI3K/Akt by Wortmanin or Ly294002 was unable to totally inhibit E2-dependent Bad phosphorylation, and in view of hormone activation of MAPKs, the possibility that these kinases participate in E2-induced Bad phosphorylation cannot be excluded.

4. Non-classical localization of ERs

The non-genomic events triggered by 17β-estradiol lead to the hypothesis that the hormone is able to activate extranuclear receptors [4]. Accordingly, several investigators have pointed out the possibility that ERα and β could be non-classically associated to the cell surface, intracellular membranes and mitochondrial compartments ([18] and reference therein). Interestingly, the mitochondrial genome also contains ERE-like sequences [19]. In the C2C12 murine skeletal muscle cell line we described a non-classical localization for native ERα and β. Conventional radioligand assays provided information about the presence and subcellular localization of estrogen binding sites. The use of estrogen agonist diethylstilbestrol and antagonist ICI 182780 allowed characterization of these estrogen binding entities as ER. Moreover, radioligand blocking with specific monoclonal antibodies identified the ERs as ERα and ERβ. These ER specific binding sites were concentrated in particulate subcellular fractions, e.g. mitochondria and to lesser extent microsomes. Western and Ligand blot analysis showed higher expression levels and specific estradiol binding of ERβ. In agreement with these findings, immunocytochemical assays with conventional and confocal microscopy confirmed this subcellular localization. ERβ was exclusively detected in mitochondria and ERα in the perinuclear and mitochondrial compartments [20–22]. Unlike other cell lines [23], the subcellular location of the immunoreactive entities was not modified by treatment with E2, reinforcing the hypothesis that one of the two putative ERα localizations detected could be an internal membrane system such as the endoplasmic reticulum or Golgi. Studies employing transiently transfected cells with specific ERα or β siRNA confirmed functional and structural relationships between the classical and the endogenous ERs [21]. Mitochondria are key organelles in the apoptotic process. Localization of ERs in this organelle could be associated to the regulation of apoptosis by the E2.

5. Role of ERs in apoptosis

The antiapoptotic action of E2 on C2C12 muscle cells is exerted through estrogen receptors, involving PI3K/Akt activation and BAD phosphorylation as described before (Fig. 1A and B) [8]. The antagonist ICI182780 blocked the estradiol effect on H_2O_2-induced cytochrome c release and PARP cleavage. Fur-

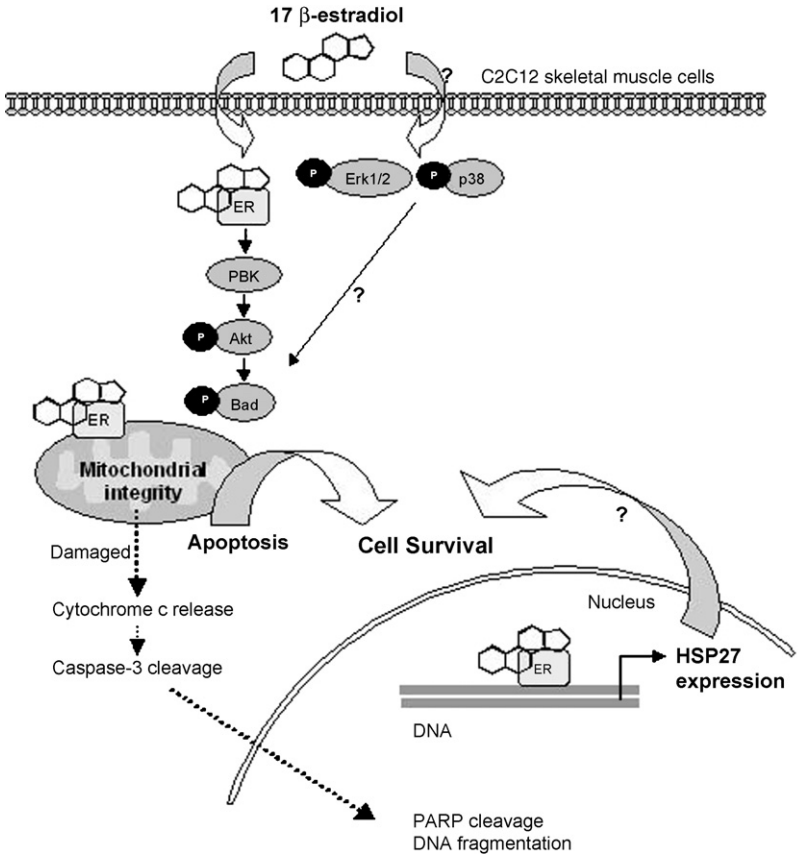

Fig. 3 – Schematic diagram showing events involved in the antiapototic effects of 17β-estradiol in muscle cells.

thermore, the protective effect of the steroid was inhibited both by, antibodies against the ERα or ERβ, or transfecting siRNA specific for each isoform. The inhibition of apoptotic events at the mitochondrial level was more pronounced when ERβ was immunoneutralized or suppressed by mRNA silencing. Whereas under the same experimental conditions, both isoforms participated to the same extent in Akt activation, suggesting differential involvement of ERs depending on the step of the apoptotic/survival pathway evaluated [8].

6. Conclusions

Overall, the data that have been reviewed here indicate that 17β-estradiol plays a non-classical function as anti-apoptotic mediator in skeletal muscle. It is clear that E2 can act through ERs with diverse cellular localizations, but additional investigations are necessary to elucidate the molecular mechanism by which the hormone exerts this role. These studies may sustain an emerging concept of integration between rapid signaling and genomic actions (Fig. 3).

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