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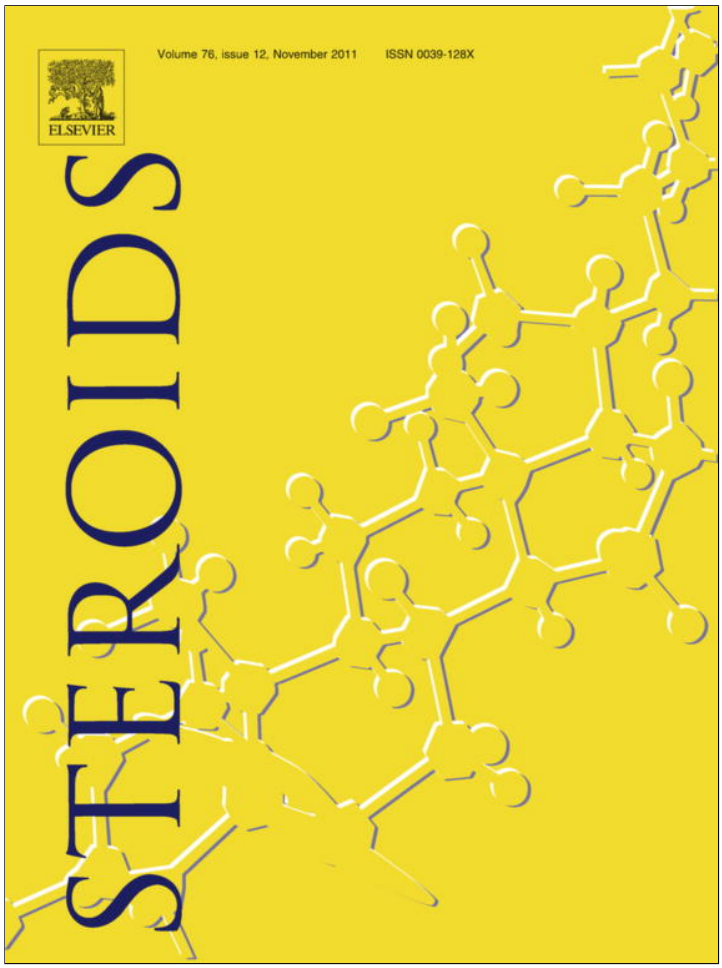

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Review

Role of 17β-estradiol and testosterone in apoptosis

Andrea Vasconsuelo [*,1], Lucía Pronsato, Ana Carolina Ronda, Ricardo Boland, Lorena Milanesi [1]

Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, 8000 Bahía Blanca, Argentina

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ABSTRACT

17β-Estradiol (E2) and Testosterone (T) exert actions in most animal tissues, in addition to the reproductive system. Thus, both sex steroid hormones affect growth and different cell functions in several organs. Accordingly, the nuclear estrogen (ER) and androgen (AR) receptors are ubiquitously expressed. Moreover, ER and AR may have non-classical intracellular localizations, e.g. plasma membrane, mitochondria and endoplasmic reticulum, raising additional complexity to the functional roles of E2 and T. In addition to the modulation of gene transcription by direct interaction with their cognate nuclear receptors, the steroids can rapidly activate signaling pathways by a non-genomic mechanism mediated by receptors identical to or different from known steroid receptors. Among various functions, E2 and T can regulate apoptosis through those pathways. In mitochondria, the presence of ER and AR and actions of estrogen and androgen have been shown, in keeping with the organelle being a control point of apoptosis. The most recurrent action for each steroid hormone is the protection of mitochondria against different insults, resulting in antiapoptosis. This review summarizes the molecular basis of the modulation of programmed cell death by E2 and T in several tissues.

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Contents

1. Introduction

17β-Estradiol (E2) and Testosterone (T) are steroid hormones typically linked to reproductive functions. However, during the last decade it has been discovered that practically every animal cell/tissue/organ system responds to these sexual hormones in some way. Thus, effects by both steroids on differentiation of several tissues and organs, bone metabolism, and modulation of inflammation, brain, liver, nervous system and cardiovascular functions as well have been described ([1–4]; reviewed in [5–7]). One of the reasons of this opening out in the non-conventional actions of E2 and T was the finding of estrogen (ER) and androgen (AR) receptors in non-classical tissues and with non-classical intracellular localizations [8–25]. However, our understanding of the molecular mechanism by which sex steroid hormones regulate these cellular functions is incomplete.

According to the common theory of steroid action, these compounds regulate the expression of various responsive genes and initiate complex events by direct interaction with nuclear receptors. Once activated, the hormone-receptor complex can directly mediate gene transcription or interact with transcription factors to regulate their activity. Changes in gene transcription typically occur after 30–60 min ([26–28]; reviewed in [29,30]). As opposed to this genomic mechanism, steroids can influence cellular functions rapidly activating signaling pathways. Regarding E2, one of the earliest reports about rapid non-genomic effects belongs to Pietras and Szego [31], where they demonstrated a fast cAMP production and calcium flux in the endometrium of ovariectomized rats induced by the estrogen. Afterwards, the interest in the study of these mechanisms increased and fast effects have been

* Corresponding author. Tel.: +54 291 4595100x4337; fax: +54 291 4595130.
E-mail address: avascon@criba.edu.ar (A. Vasconsuelo).
[1] These authors equally contributed to this work.

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reported, which occur from seconds to minutes upon ligand binding, involved in the activation of diverse signal transduction pathways ([32]; reviewed in [29,33]). The activation of this non-genomic mechanism is mediated by receptors identical to or different from known steroid receptors, such as G protein-coupled receptors [34,35]. In connection with the regulation of gene transcription, the sex steroid hormones recruit a complex of coactivators and corepressors to the AR- or ER-ligand-DNA binding site [36,30,37]. Thus, the correct balance between coactivators and corepressors is a key determinant of the capacity of the genomic mechanism to initiate responses. Due to the fact that the relative expression levels of these molecules is cell specific and the vast possibilities of different interactions between genomic and non-genomic actions, E2 and T can exert distinct functions in different cellular types. Besides, the complexity of the mechanism of action of both hormones further increases when the interactions between the two steroid receptors are taken into consideration. Androgen–estrogen interplay may reflect the combined effects of estrogen- and androgen-regulated expression of multiple genes within a target cell. Also, each respective hormone may affect the expression of the other's receptor, i.e. androgens of the estrogen receptor and viceversa [38,39]. Hormone–hormone intercommunication may also result from the contribution of a steroid-receptor coactivator to steroid-binding. For instance, the AR corregulator ARA-70, may confer to the AR an ability to bind estrogen, thus allowing for an estrogen effect on androgen targets [40].

Heterodimerization between members of the steroid receptor superfamily is not unusual [41]. It has been demonstrated that the AR and ERα can interact directly using the yeast and mammalian two-hybrid systems. Consequently, ERα modulates AR transcriptional activity in an E2-dependent fashion [42]. These authors showed inhibitory interactions between AR and ERα but not between AR and ERβ. Further, there was some steroid-dependence of these interactions, since ligand-induced conformational change in at least one receptor type prior to the interaction event is required. These findings support those of Kumar et al. who also found a dose-dependent decrease of AR transcriptional activity when ER was coexpressed in the presence of E2 [43]. A direct interaction between steroid receptors would allow for an additional level of control and adds to the increasing complexity of steroid signaling pathways.

Among the different functions reported for T and E2, both steroids can regulate apoptosis. This review summarizes the available evidence showing the molecular mechanisms activated by E2 and/or T to modulate programmed cell death.

2. Estrogen and androgen actions in mitochondria and their implications on apoptosis

The existence of androgen and estrogen receptors in mitochondria was suggested by early binding experiments using radiolabeled hormone ligands with mitochondrial extracts. The purification of receptor proteins and the availability of the corresponding antibodies allowed the application of immunological techniques for the localization of both receptors in mitochondria [44,22,9]. Thus, mitochondrial DNA (mtDNA) was reported as one of the major targets for the direct actions of steroid hormones and their receptors (reviewed in [45,46]). First, in 1996 the mtDNA was found to contain estrogen-response elements [47]. In addition, the presence of mitochondrial estrogen receptors has been demonstrated in different cell types [48,8,49,9,50]. Even though the localization of ERs in mitochondria is well established, their physiological role at this cellular level remains unclear. Hence, investigations in the last years have been focused to evaluate E2 and ERs actions on mitochondria. The results obtained clearly demonstrate that the organelle is an estrogen target. Indeed, it has been shown that estrogens affect in mitochondria the electron transport chain [51], its morphology [52,53], also gene expression [49] and other multiple effects that E2 exerts, directly on the organelle or indirectly through activation of signaling pathways such as PI3K/Akt or MAPKs, to enhance or preserve mitochondrial function during pathologic/stress circumstances (reviewed in [54]).

Mitochondria are both source and targets of free radicals. Mitochondrial electron transport chain is the main source of reactive oxygen species (ROS) during normal metabolism [55]. ROS affect the mitochondrial machinery. Above all, chronic ROS exposure induces mtDNA mutations that accumulate with age; mitochondrial dysfunction appears to be a major contributor to those age-related diseases of the cardiovascular system or the brain [56–58]. Several studies indicate that estrogen suppresses mitochondrial ROS production [10,59]. Moreover, it has been demonstrated that this antioxidant action of estrogens is due, not to their chemical phenolic structure, but rather to their interaction with ERs which eventually leads to the activation of kinases and nuclear factors [60]. In addition, the effectiveness of estrogen as neuroprotective agent [61,62] or against age-related pathologies as cardiovascular disorders, in part due to their action on mitochondria, is known [63,64]. Questions about the mechanism activated by E2 have arisen, namely, can the estrogens protect mitochondria in a direct fashion, independently of cell membrane, nuclear or cytosolic interactions? The presence of ERs in mitochondria strengthens the hypothesis that estrogens have direct effects on mitochondrial activity but it is also known that estrogens upregulate nuclear gene expression of antioxidant enzymes which are directed to mitochondria [65]. Summarizing, the action of E2 on the mitochondria is mediated by genomic and non-genomic mechanisms involving signals travelling through an intricate net between plasma membrane, mitochondria and nucleus. Evidence suggest that the cytoskeleton could be an important factor in the maintenance of these mitochondrial-membrane and nuclear interactions [66].

Regarding AR, scarce information exists about its mitochondrial localization. AR has been detected in mitochondria of LNCaP cells and of human sperm cells in the midpiece, the region which harbors a high concentration of mitochondria [22]. Nevertheless, testosterone effects on mitochondrial functions have been reported. For instance, the androgen acutely and directly depolarizes and oxidizes cardiac mitochondria in a K^+-dependent, ATP-sensitive, and AR-independent mode [67].

The mitochondria are not only the cell's powerhouses, they integrate a large number of signal transduction pathways for a wide variety of biologically active molecules. Additionally, they could be considered as a cellular arsenal since they enclose a potent cocktail of pro-apoptotic proteins. Really, this organelle represents a control point of apoptosis that is regulated by members of the Bcl-2 family. Then, if mitochondria are a target for sexual hormones these steroids could modulate programmed cell death. The principal mechanism by which Bcl-2 family proteins regulates apoptosis at mitochondrial level is probably by controlling cytochrome *c* release. The Bcl-2 proteins display either antiapoptotic (e.g. Bcl-2, Bcl-xL) or proapoptotic (e.g. Bax, Bad, Bak, Bid) functions that in turn could be regulated by several estrogen or androgen responsive kinases including Akt, MAPKs, PKA, among others ([68]; reviewed in [69,70]).

Cell death has historically been subdivided into regulated and unregulated mechanisms. Apoptosis is an essential cellular response, is a normal event during development and regulation of tissue homeostasis by which unwanted cells are eliminated. It is a form of regulated cell death, reflects a cell's decision to die in response to varied stimuli. Apoptosis is executed by intrinsic cellular machinery, which is activated either by triggering events within the cell or from outside the cell. Multiple molecular components

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such as death receptors, Bcl-2 family proteins, cytochrome *c*, inhibitor of apoptosis proteins (IAP), and many others, are involved in apoptosis signaling that converge on caspase activation, the executers of cell death (reviewed in [71]). This type of cellular death is characterized by distinct biochemical and morphological changes such as DNA fragmentation, plasma membrane blebbing and cellular shrinkage (reviewed in [72]).

Due to the significance of apoptosis in an organism, it is logical to think in more than one (depending on the cell type) form of regulation of this process. Many cytokines, growth factors and hormones control apoptosis in some way (review in [73]). Although it has been demonstrated that E2 as well as T can sustain survival or alternatively induce cell apoptosis according to their biological context [52,74–81], the information reviewed suggests that in general both steroid hormones exert a protective role on the mitochondria at structural and functional levels and thus, they render a survival effect. This steroid hormonal-protective action on mitochondria has been more extensively studied for E2 than for T.

3. 17β-Estradiol and testosterone as modulators of apoptosis: molecular mechanism

Experimental data indicate that androgens and estrogens regulate apoptosis via different cell signaling pathways. In this action the steroids involve or not, specific (with classical or non-classical localizations) and non-specific receptors. Although sometimes androgens and estrogens can trigger apoptosis, protective effects of these steroids, mainly E2, have been widely reported for different tissues. This hormonal regulation of apoptosis depends on factors such as cell type, apoptosis inducer, hormone concentration or cellular environment, as indicated above. Table 1 summarizes relevant data about regulation of apoptosis by sexual hormones, some of which are described in detail below.

It is known that E2 regulates the balance between cell survival, proliferation and apoptosis, processes which are connected in some manner through different mechanisms. However, why the hormone shifts the balance toward cell survival or apoptosis is not totally elucidated. For example, in estrogen receptor (ER)-positive MCF-7 human breast cancer cells, it has been shown that E2 stimulates growth inducing G1- to S-phase transition. This induction is associated with the upregulation of c-myc, affecting cyclin D1, cyclin-dependent kinase (CDK) and retinoblastoma protein [82]. The estrogen also activates cyclin E-CDK2 complexes, accelerating the G1-to-S transition [83], all known events involved in cell proliferation. In addition, through its non-genomic mechanism action, the hormone via the ERα is able to interact with proteins such as c-Src, and activates the MAPK and PI3K/Akt pathways which are classically associated to cell survival [84,85]. Moreover, E2 inhibits apoptosis increasing Bcl-2 and Bcl-X_L without affecting the proapototic Bax and Bak proteins in MCF-7, T47-D, and ZR-75-1 breast cancer cells [86]. However, under some specific conditions E2 could trigger apoptosis in breast cancer cells, opposed to its well studied antiapoptotic role. This peculiar hormone behavior has been observed in cells from breast cancer which have been long-term estrogen-deprived (LTED) or treated exhaustively with anti-estrogens [87]. Curiously, the paradoxical induction of apoptosis by estrogen has been established under several unusual circumstances. For example, in this case, the pre-conditions of prolonged estrogen depletion or exhaustive treatment with anti-estrogens of the breast cancer cells are mandatory requisites to trigger apoptosis by E2 and could explain the dual action of the steroid to stimulate growth or apoptosis. Thus, the development of anti-hormone resistance over years of therapy, reprograms the survival mechanism of the breast cancer cell so that estrogen no longer functions as a survival factor but as a death signal. In this case the author proposes that despite the fact that the ER still regulates the appropriate estrogen target genes, also activates the Fas apoptotic pathway or alternatively has a direct effect on mitochondrial function by downregulation of antiapoptotic members of the Bcl-2 family, leading to apoptosis [87]. Likewise, under other particular conditions such as rat embryo fibroblasts (Rat1 cells) stably transfected with ERα [88] or MCF-7 cells stably transfected with Raf-1 [89], there has been observed that estrogen is also able to induce apoptosis.

In skeletal muscle, there are data demonstrating that apoptosis plays a key role in pathophysiological and physiological conditions that lead to cell loss [90]. Although little is known about the effects of estrogen or androgen on apoptosis and the underlying molecular events in skeletal muscle, the evidence available indicates that the steroids are associated with survival/beneficial effects in this tissue [91,92]. Thus, in the C2C12 murine skeletal muscle cell line, T as well as E2, in a nanomolar dose protect against H_2O_2-induced apoptosis [52,81] (Fig. 1). Typical changes of apoptosis such as nuclear fragmentation, cytoskeleton disorganization, mitochondrial reorganization/dysfunction and cytochrome *c* release induced by H_2O_2, are abolished when cells are previously exposed to androgen or estrogen. Some molecular events that occur during the anti-apoptotic action of T on C2C12 cells have also been identified. At short times of exposure to H_2O_2, cells exhibit a defense response showing ERK2, Akt and Bad phosphorylation and an increase of HSP70 levels. At longer treatment times with the apoptotic agent, dephosphorylation of these proteins, cytochrome *c* release, PARP cleavage and DNA fragmentation occur, but when cells were treated with T prior to H_2O_2, Bad inactivation (phosphorylation), increase in actin levels, translocation of HSP90 to mitochondria and reduction in Bax levels were observed. These findings reveal that, the intrinsic pathway at least, is affected by the steroid hormone. Likewise, E2 inhibits apoptosis in C2C12 skeletal muscle cells through ERs with non-classical localization involving MAPKs, HSP27 and the survival PI3K/Akt pathway which phosphorylates proapoptotic members of the Bcl-2 family inactivating them (Fig. 1) [52,93,94]. Similar to T, the authors observed an important protective effect of estrogen on mitochondria associated to activation of the PI3K/Akt pathway. However, in other cell types under irregular conditions, it has been observed that the hormone can inhibit this classical survival cascade and then induce apoptosis. In tamoxifen-resistant PKC-α-overexpressing cells, E2-induced tumor regression is related to a decrease of Akt activation [95]. In addition, in LTED MCF-7:5C and MCF-7:A 2A cells, the basal level of phosphorylated Akt is markedly upregulated and E2 (in nanomolar range for 72 h) significantly reduces its expression. Also, in MCF-7.beclin-overexpressing cells, E2 treatment significantly decreases Akt activation and then proliferation [96]. As mentioned before, this E2-induced PI3K/Akt inhibition that leads to cellular death requires unusual circumstances as transfected cell lines or tamoxifen-resistant cells. Studies showing the basis by which the hormone is able to activate or inhibit the PI3K/Akt pathway could unravel new therapeutic targets in pathologies associated to deregulation of cellular death. Moreover, the same premise could extend to the MAPK signaling cascade, commonly related to cellular proliferation and, as aforesaid, modulated by E2 too.

Another tissue in which estrogen and androgen clearly have a protective effect against apoptosis is nervous system. In general for both steroids, the dual behavior (apoptotic and antiapoptotic) has not been observed in this system as occur in other tissues. Physiological levels of the female or male sex steroids are neuroprotective both *in vivo* and *in vitro*. With regard to E2, it is well documented that the hormonal protective action is mainly due to its action on mitochondria [97]. Although the mechanism of male steroids in neuroprotection is less clear, the participation of AR is well established. Using an in *vitro*/*ex vivo* model, Ahlbom et al. showed

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Table 1
Regulation of apoptosis by sexual hormones in different cell and tissue types.

Hormone	Tissue/primary culture/cell line	Apoptotic agent	Molecular mechanism activated		Receptor involved	References
			Apoptotic	Antiapoptotic		
Testosterone	C2C12	H_2O_2	–	Bad phosphorylation, downregulation of Bax, inhibition of cytochrome *c* release, PARP cleavage, actin and mitochondrial disorganization	AR	[81]
	LNCaP 104-R1	Overexpression of TNF-α	–	Androgen/AR induces expression of $p21^{Waf1/Cip1}$ that in turn inhibits TNF-α-induced JNK and apoptosis	AR	[108]
	H9c2 embryonic rat heart cell line	Hyperosmotic stress	–	Reduction of DNA fragmentation	Not studied	[109]
	PC12 cells transfected with androgen receptor (AR)	β-Amyloid	–	Phosphorylation of extracellular signal-regulated kinase ERK-1 and ERK-2	AR	[110]
	Cultured hippocampal neurons from rats	β-Amyloid	–	Phosphorylation of extracellular signal-regulated kinases ERK-1 and ERK-2, that induce activation of Rsk and inactivation of Bad	AR	[110]
	Pancreatic β cells from male wistar rats	Streptozotocin (STZ)	–	Decrease in apoptotic pancreatic β cells	AR	[106]
	Pancreatic β cells from male rats	Streptozotocin (STZ)	–	Induction of antioxidant enzyme activities in a sex specific way	Not studied	[107]
	CGC from neonatal rats	H_2O_2	–	Increased catalase activity	AR	[98,99]
	Male Sprague–Dawley (SD) rat germ cells	Heat stress and hormone deprivation	–	Activation of MAPK 1/3 and MAPK14, Bcl-2 phosphorylation and activation of the mitochondria death cell pathway	Not studied	[113]
	Tissue from adult human testes	Serum free condition	–	Reduction in DNA fragmentation	Not studied	[114]
	HK-2	T (nanomolar concentrations)	JNK and c-jun phosphorylation	–	AR	[115]
	LNCaP 104-R1 and 104-R2	R1881 (10 nM)	Androgenic repression of LNCaP 104-R1 and 104-R2 cell proliferation by induction of $p27^{Kip1}$, which in turn inhibits Cdk2, a factor critical for cell cycle progression and proliferation	–	AR	[111]
	Neuroblastoma cell line SHSY5Y	T (micromolar, but not nanomolar, concentrations)	T alters InsP3R type 1-mediated intracellular Ca^2 signaling	–	No studied	[105]
	DPC (cultured dermal papilla cells)	T and 5-alpha-DHT in a dose-dependent and time-related manner	Caspase-8 cleavage and decrease in Bcl-2 protein expression	–	Not studied	[112]
	BMMs Bone marrow derived macrophages	T	Expression of caspase-8, caspase-3, and poly (ADPribose) polymerase (PARP) cleavage via Fas/FasL pathway	–	No studied	[116]
	Caco-2	T-HSA (molar concentration)	Cytoskeleton reorganization and caspase-3 activation	–	mAR	[25]
Estradiol	Heart from Wistar rats	Ischemia	–	Inhibition of cytochrome *c* release, caspase-3 activation and DNA strand breaks	Not studied	[117]
	C2C12	H_2O_2 etoposide	–	PI3K/Akt/BAD, Inhibition of cytochrome c release, PARP cleavage and DNA strand breaks	ERα, ERβ	[52]
	C2C12	H_2O_2	–	Increase in the expression of HSP27, Inhibition of caspase-3 cleavage	ERβ	[94]
	MCF-7:5C	E2 fulvestrant	Increased expression of pro-apoptotic proteins (Bax, Bak, Bim, Noxa, Puma, and p53), decreased ψm, enhanced cytochrome c release, caspase-9 activation and PARP cleavage compared with cells treated with fulvestrant	–	ER	[118]

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	C2C12	H_2O_2	–	ERK2, p38 MAPK and Akt activation. Bad inactivation, inhibition of cytochrome c and Smac/DIABLO release	Not studied [93]
	Adult male Sprague-Dawley rats	Burn injury	–	Increase in the levels of phospho-Akt and inhibition of caspase-3 activation and PARP cleavage	Not studied [119]
	Cultured rat cardiomyocyte	Ischaemia-reperfusion	–	Inhibition of p38α-p53 signalling and downregulation of p53 inhibition on p38β	Not studied [120]
	MCF7	H_2O_2, TNF-α, and serum withdrawal	–	Phosphorylation (S112 and S136)/inactivation of Bad, through ERK and Akt	Not studied [121]
	HEK293 Murine Pancreatic β-cells	E2	Age-dependent action		SUR1 [122]
E2 and T	Chicken osteoblast	–	–	Induction of cell proliferation, ALP activity and stimulation of the osteoblast cell cycle and DNA synthesis	Not studied [123]
E2-BSA and T-BSA	T47D breast cancer cell line	E2-BSA and T-BSA dose-depending	Modulation of Bcl-2 and Bad protein levels		Membrane estrogen and androgen binding sites [20]

that cerebellar granule cells (CGC) from neonatal rats treated with a single dose of T are less vulnerable to damage induced by 50 mM H_2O_2 [98]. They demonstrated a mechanism involving an upregulation of the cellular antioxidant defenses, specifically a two fold T-induced increase in the activity of catalase and superoxide dismutase was detected. The effects of *in vitro* T treatment were also studied showing that CGC treated with the androgen were less susceptible to damage induced by H_2O_2 [99]. The addition of the AR antagonist flutamide abolished the protective effect of T, suggesting an androgen receptor-mediated mechanism. In addition they observed an increase in the antioxidant enzyme catalase in cells treated with T, but not in the cells co-treated with flutamide. The participation of the AR in the protective effects of androgens was further supported by its presence in CGC. In cultured hippocampal neurons from rats and in PC12 cells stably transfected with the AR, androgens protected against apoptosis induced by β-amyloid peptides (Aβ) [100]. In cultured hippocampal neurons, 10 nM of T or dihydrotestosterone rapidly and transiently increased ERK-1 and ERK-2, Rsk-1, and Bad phosphorylation, with discrete but overlapping time courses. This androgen-induced MAPK/ERK signaling occurs only in cells expressing functional AR. Inhibition of the AR-dependent MAPK/ERK–Rsk–Bad signaling pathway at the AR, ERK, or Rsk step, blocks androgen protection against Aβ toxicity. In PC12 cells, androgens were observed to activate MAPK/ERK signaling and provide neuroprotection only in cell lines stably expressing AR. Because of plasma membrane-impermeable testosterone-BSA conjugates failed to induce MAPK/ERK signaling, both in hippocampal neuron and pcDNA3-AR cell cultures, neuroprotective androgen signaling via that pathway does not appear to involve cell surface-associated receptors. With regard to the response of neurons to the female hormone, it generally involves the ER. In various cell types, there has been observed that the final result depends on the subtype of ER. At this point, some contradictions appear, as many reports have established estrogenic protective effects involving both estrogen receptors. However, it has been shown that estrogen-regulated developmental neuronal apoptosis involves ERβ. Indeed, cells which express ERβ undergo apoptosis, whereas cells expressing ERα are protected from apoptosis [101]. Nilsen et al., using *in vitro* approaches, showed that ERβ mediates apoptosis through a mechanism that requires FasL [101]. However, in *in vivo* assays with βERKO mice, it was demonstrated that ERβ is necessary for neuronal survival [102]. Although, *in vivo* assays could be less disputable than *in vitro* assays, it is evident that more evidences are necessary to determine the role of each receptor in apoptosis. Moreover, more in-depth studies are necessary to determine if the effects observed are due to the participation of specific isoforms of each receptor.

Finally, we reviewed singular effects of sexual steroids on apoptosis due to variations in concentrations of hormone or to simultaneous treatment with both steroids. In addition the response to hormone on occasion was sex specific. Thus, opposite effects of E2 and T membrane binding sites on T47D breast cancer cells were observed. Upon activation with BSA-conjugated, non-permeable ligands (E2-BSA and T-BSA), membrane estrogen receptors protected cells from serum-deprivation-induced apoptosis, while androgen receptors induced apoptosis in serum supplemented T47D cells. In addition, co-incubation of cells with a fixed concentration of one steroid and varying concentrations of the other reversed the above mentioned effect (apoptosis for androgen, and anti-apoptosis for E2), suggesting that the fate of the cell depends on the relative concentration of either steroid in the culture medium [20]. It is known that at physiological levels, androgens are involved in neuronal differentiation, neuroprotection, survival and development [103,104]. However, T exerted apoptotic effects in the neuroblastoma cell line SHSY5Y at supraphysiological levels (micromolar range) initiating the apoptotic cascade, and this effect

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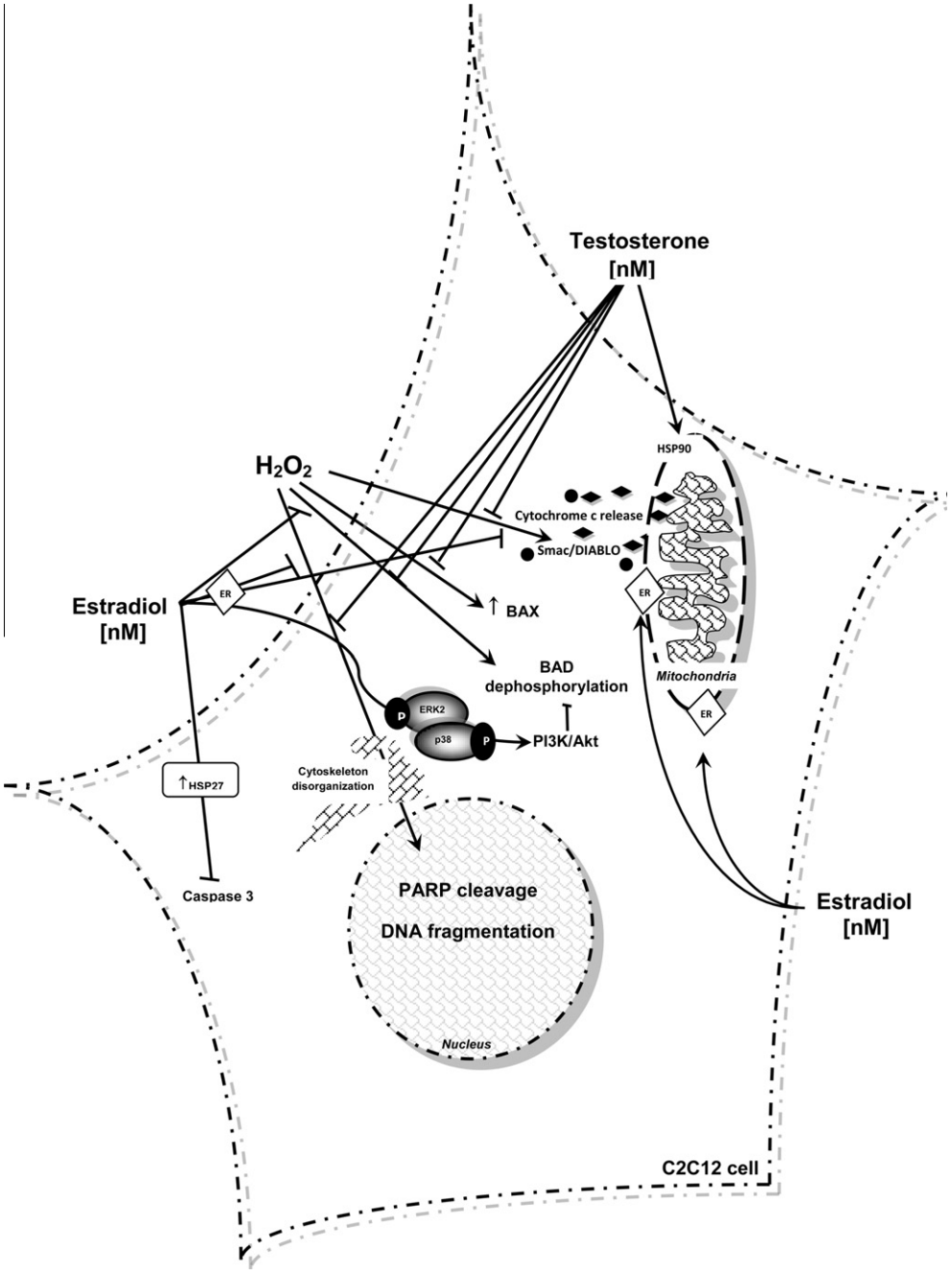

Fig. 1. Schematic diagram showing events involved in the antiapototic effects of Testosterone (T) and 17β-estradiol (E2) in C2C12 muscle cells. The diagram depicts both steroid hormones interacting with apoptotic mediators and activating diverse intracellular signaling pathways to inhibit H_2O_2-induced apoptosis in skeletal muscle cells. E2 (17β-estradiol) can interact with estradiol binding proteins/receptors (ER) localized in cell membrane and mitochondria promoting activation of ERK, p38 MAPK and the PI3K/Akt/p-Bad survival cascade, involving HSP27 and inhibiting caspase-3 activity. Thus, E2 abrogates mitochondrial membrane damage, and consequently Smac/DIABLO and cytochrome c release, induced by hydrogen peroxide. Furthermore, similar to estrogen, T is able to act at the mitochondrial level, probably involving HSP90. T abrogates the H_2O_2-induced Bax expression. Both hormones prevent DNA fragmentation and cytoskeleton disorganization [52,93,81].

was abolished in the presence of either inhibitors of caspases or of the inositol 1,4,5-trisphosphate receptor (InsP3R)-mediated Ca^{2+} release. Futhermore, T induced different concentration-dependent Ca^{2+} signaling patterns: at low levels of T (100 nM), Ca^{2+} oscillations were produced, whereas higher concentrations (1–10 μM) caused a sustained Ca^{2+} increase. Thus, T through a mechanism involving Ca^{2+} signaling may lead to apoptosis [105]. In agreement with these observations, it has been demonstrated that T and 5 alpha-DHT stimulated apoptosis in cultured dermal papilla cells (DPC) in a dose-dependent and time-related manner. The mechanisms involved include a decrease in Bcl-2 protein expression, an increase in the Bax/Bcl-2 ratio and caspase-8 activation.

In pancreatic β cells apoptosis is responsible for the development of insulin-dependent diabetes mellitus in the streptozotocin (STZ) rat model. In this model, Morimoto et al. [106] demonstrated that castrated animals presented higher percentages of apoptotic β cells than intact males and castrated, testosterone-substituted males (castrated rats were substituted with T enanthate). The decrease in apoptotic β cells induced by T was reversed by flutamide, showing a possible involvement of the AR in the steroid protective action. Interestingly, it has been demonstrated that the protective effect of steroid hormones in pancreatic β cells is sex specific. Cytoprotection on STZ-induced apoptosis in rat pancreatic β cells was observed in T but not in progesterone or E2 treated male rats.

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The effect was seen in male but not in female rats. Moreover, the sex specific action of the steroid hormone was related to the induction of antioxidant enzyme activities in pancreatic β cells [107].

Other studies showing the role of androgen in apoptosis and its impact on cancer therapy, have been made in the Caco-2 cell line. In these cells (from human epithelial colorectal adenocarcinoma), using fluorescent non-membrane-permeable AR ligands (T-HAS-FITC), Gu et al. [25] showed not only the presence of membrane ARs but also that the stimulation of these non-classical receptors with T conjugates (T-HAS) induced rapid cytoskeleton reorganization and apoptotic responses via activation of the pro-apoptotic executor caspase-3, even in the presence of anti-androgens. These effects were specific for T and its conjugates, since other steroid hormones such as E2 did not exhibit any pro-apoptotic activity. These data add a clear and significant piece of evidence to the role of membrane ARs in apoptosis. Thus, their activation by steroid albumin conjugates induces potent pro-apoptotic responses involving caspase activation and cytoskeletal rearrangements. Although further experiments are now required for the full identification of the molecular identity of these receptors, they may represent specific targets for the development of novel drugs, since their activation drastically regresses tumor growth and tumor incidence *in vivo* [25]. Anyway one must be cautious to analyze functions of membrane receptors using conjugated ligands. Sometimes these compounds are not stable and the observed effect is not only due to the steroid conjugate that activates a receptor protein membrane, but to fractions of unconjugated compound that act on intracellular receptors. The solution to this problem would be to block intracellular receptors specifically. Then evaluate, under these conditions, the action of hormone conjugates on membrane receptors.

4. Conclusions

17β-Estradiol (E2) and Testosterone (T) regulate, in addition to reproduction, several other functions in a great variety of animal tissues expressing ER and AR receptors mainly with non-classical intracellular localization (extranuclear), or other steroid receptors like G protein-coupled receptors. Apoptosis an important cellular event underlying the actions of both sex steroid hormones on target tissues. The present work reviews the literature available about the effects of 17β-estradiol and Testosterone focusing on apoptosis, with T and E2 acting on mitochondria directly through their mitochondrial receptors or indirectly activating signaling pathways whose target is the organelle, these processes sustaining a predominant anti-apoptotic role for both hormones. E2 and T may also regulate apoptosis through receptors located in the plasma membrane and endoplasmic reticulum. Binding of their cognate ligands activates the MAPK and PI3K/Akt cascades and induces anti-apoptotic members of the Bcl-2 family, altogether leading to survival. Nevertheless, under certain unusual conditions E2 can also inhibit the survival PI3K/Akt pathway and then trigger apoptosis. Likewise, T can also induce apoptotic effects but different signaling pathways may be involved. Therefore, studies elucidating the mechanism by which the hormones upregulate or downregulate these signaling cascades may help to identify new therapeutic targets in pathologies associated to deregulation of cellular death.

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References

[1] Gruber C, Tschugguel W, Schneeberger C, Huber J. Production and action s of estrogens. New Engl J Med 2002;346:340–52.
[2] Pearce ST, Jordan VC. The biological role of estrogen receptors alpha and beta in cancer. Crit Rev Oncol Hematol 2004;50:3–22.
[3] Deroo B, Korach K. Estrogen receptor and human disease. J Clin Invest 2006;116:561–70.
[4] Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med. 1999;340:1801–11.
[5] Katzenellenbogen B. Estrogen receptors: bioactivities and interactions with cell signaling pathways. Biol Reprod 1996;54:287–93.
[6] Yang SH, Liu R, Perez EJ, Wang X, Simpkins JW. Estrogens as protectants of the neurovascular unit against ischemic stroke. Curr Drug Targets CNS Neurol Disord 2005;4:169–77.
[7] Sheffield-Moore M, Urban RJ, Wolf SE, Jiang J, Catlin DH, Ferrando AA. Short-term oxandrolone administration stimulates net muscle protein synthesis in young men. J Clin Endocrinol Metab 1999;84:2705–11.
[8] Chen JQ, Delannoy M, Cooke C, Yager JD. Mitochondrial localization of ERalpha and ERbeta in human MCF7 cells. Am J Physiol Endocrinol Metab 2004;286:E1011–22.
[9] Yang SH, Liu R, Perez EJ, Wen Y, Stevens SM, Valencia T, Brun-Zinkernagel AM, Prokai L, Will Y, Dykens J, Koulen P, Simpkins JW. Mitochondrial localization of estrogen receptor beta. PNAS 2004;101:4130–5.
[10] Pedram A, Razandi M, Wallace DC, Levin ER. Functional estrogen receptors in the mitochondria of breast cancer cells. Mol Biol Cell 2006;17:2125–37.
[11] Matthews J, Gustafsson JA. Estrogen signaling: a subtle balance between ER alpha and ER beta. Mol Interv 2003;3:281–92.
[12] Ruizeveld de Winter JA, Trapman J, Vermey M, Mulder E, Zegers ND, van der Kwast TH. Androgen receptor expression in human tissues: an immunohistochemical study. J Histochem Cytochem 1991;39:927–36.
[13] Kelly MJ, Levin ER. Rapid actions of plasma membrane estrogen receptors. Trends Endocrinol Metab 2001;12:152–6.
[14] Benten WP, Lieberherr M, Giese G, Wrehlke C, Stamm O, Sekeris CE, Mossmann H, Wunderlich F. Functional testosterone receptors in plasma membranes of T cells. FASEB J 1999;13:123–33.
[15] Benten WP, Lieberherr M, Stamm O, Wrehlke C, Guo Z, Wunderlich F. Testosterone signaling through internalizable surface receptors in androgen receptor-free macrophages. Mol Biol Cell 1999;10:3113–23.
[16] Armen TA, Gay CV. Simultaneous detection and functional response of testosterone and estradiol receptors in osteoblast plasma membranes. J Cell Biochem 2000;79:620–7.
[17] Figueroa-Valverde L, Luna H, Castillo-Henkel C, Muñoz-Garcia O, Morato-Cartagena T, Ceballos-Reyes G. Synthesis and evaluation of the cardiovascular effects of two, membrane impermeant, macromolecular complexes of dextran-testosterone. Steroids 2002;67:611–9.
[18] Guo Z, Benten WP, Krucken J, Wunderlich F. Nongenomic testosterone calcium signaling. Genotropic actions in androgen receptor-free macrophages. J Biol Chem 2002;277:29600–7.
[19] Kampa M, Papakonstanti EA, Hatzoglou A, Stathopoulos EN, Stournaras C, Castanas E. The human prostate cancer cell line LNCaP bears functional membrane testosterone receptors that increase PSA secretion and modify actin cytoskeleton. FASEB J 2002;16:1429–31.
[20] Kampa M, Nifli AP, Charalampopoulos I, Alexaki VI, Theodoropoulos PA, Stathopoulos EN, Gravanis A, Castanas E. Opposing effects of estradiol- and testosterone-membrane binding sites on T47D breast cancer cell apoptosis. Exp Cell Res 2005;307:41–51.
[21] Hatzoglou A, Kampa M, Kogia C, Charalampopoulos I, Theodoropoulos PA, Anezinis P, Dambaki C, Papakonstanti EA, Stathopoulos EN, Stournaras C, Gravanis A, Castanas E. Membrane androgen receptor activation induces apoptotic regression of human prostate cancer cells in vitro and in vivo. J Clin Endocrinol Metab 2005;90:893–903.
[22] Solakidi S, Psarra AM, Nikolaropoulos S, Sekeris CE. Estrogen receptors alpha and beta (ERalpha and ERbeta) and androgen receptor (AR) in human sperm: localization of ERbeta and AR in mitochondria of the midpiece. Hum Reprod 2005;20:3481–7.
[23] Vicencio JM, Ibarra C, Estrada M, Chiong M, Soto D, Parra V, Diaz-Araya G, Jaimovich E, Lavandero S. Testosterone induces an intracellular calcium increase by a nongenomic mechanism in cultured rat cardiac myocytes. Endocrinology 2006;147:1386–95.
[24] Cheng J, Watkins SC, Walker WH. Testosterone activates mitogen-activated protein kinase via Src kinase and the epidermal growth factor receptor in sertoli cells. Endocrinology 2007;148:2066–74.
[25] Gu S, Papadopoulou N, Gehring EM, Nasir O, Dimas K, Bhavsar SK, Föller M, Alevizopoulos K, Lang F, Stournaras C. Functional membrane androgen receptors in colon tumors trigger pro-apoptotic responses in vitro and reduce drastically tumor incidence in vivo. Mol Cancer 2009;8:114.
[26] Evans RM. The steroid and thyroid hormone receptor superfamily. Science 1988;240:889–95.
[27] Beato M, Chavez S, Truss M. Transcriptional regulation by steroid hormones. Steroids 1996;61:240–51.
[28] Beato M, Klug J. Steroid hormone receptors: an update. Hum Reprod Update 2000;6:225–36.

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[29] Falkenstein E, Tillmann H, Christ M, Feuring M, Wehling M. Multiple actions of steroid hormones – a focus on rapid, nongenomic effects. Pharmacol Rev 2000;52:513–55.
[30] McKenna NJ, O'Malley BW. Minireview: nuclear receptor coactivators- an update. Endocrinology 2002;143:2461–5.
[31] Pietras RJ, Szego CM. Endometrial cell calcium and oestrogen action. Nature 1975;253:357–9.
[32] Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. Annu Rev Physiol 2005;67:335–76.
[33] Foradori CD, Weiser M, Handa RJ. Non-genomic actions of androgens. Front Neuroendocrinol 2008;29:169–81.
[34] Filardo EJ, Quinn JA, Bland KI, Frackelton Jr AR. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. Mol Endocrinol 2000;14:1649–60.
[35] Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA, Hathaway HJ. Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. Annu Rev Physiol 2008;70:165–90.
[36] Rosenfeld MG, Glass CK. Coregulator codes of transcriptional regulation by nuclear receptors. J Biol Chem 2001;276:36865–8.
[37] Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA. Mechanisms of estrogen action. Physiol Rev 2001;81:1535–65.
[38] Tetsuka M, Hillier SG. Androgen receptor gene expression in rat granulose cells: the role of follicle-stimulating hormone and steroid hormones. Endocrinol 1996;137:4392–7.
[39] Adesanya-Famuyiwa OO, Zhou J, Wu G, Bondy C. Localization and sex steroid regulation of androgen receptor gene expression in rhesus monkey uterus. Obstet Gynecol 1999;93:265–70.
[40] Yeh S, Miyamoto H, Shima H, Chang C. From estrogen to androgen receptor: a new pathway for sex hormones in prostate. Proc. Natl. Acad. Sci. USA 1998;95:5527–32.
[41] Forman BM, Umesono K, Chen J, Evans RM. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. Cell 1995;81:541–50.
[42] Panet-Raymond V, Gottlieb B, Beitel LK, Pinsky L, Trifiro MA. Interactions between androgen and estrogen receptors and the effects on their transactivational properties. Mol Cell Endocrinol 2000;167:139–50.
[43] Kumar MV, Leo ME, Tindall DJ. Modulation of androgen receptor transcriptional activity by estrogen receptor. J Androl 1994;15:534–42.
[44] Solakidi S, Psarra AM, Sekeris CE. Differential subcellular distribution of estrogen receptor isoforms: localization of ERalpha in the nucleoli and ERbeta in the mitochondria of human osteosarcoma SaOS-2 and hepatocarcinoma HepG2 cell lines. Biochim Biophys Acta 2005;1745:382–92.
[45] Psarra A, Sekeris C. Steroid and thyroid hormone receptors in mitochondria. IUBMB Life 2008;60:210–23.
[46] Psarra AM, Solakidi S, Sekeris CE. The mitochondrion as a primary site of action of steroid and thyroid hormones: presence and action of steroid and thyroid hormone receptors in mitochondria of animal cells. Mol Cell Endocrinol 2006;246:21–33.
[47] Demonacos CV, Karayanni N, Hatzoglou E, Tsiriyiotis C, Spandidos DA, Sekeris CE. Mitochondrial genes as sites of primary action of steroid hormones. Steroids 1996;61:226–32.
[48] Monje P, Boland R. Subcellular distribution of native estrogen receptor α and β isoforms in rabbit uterus and ovary. J Cell Biochem 2001;82:467–79.
[49] Chen JQ, Yager JD. Estrogen's effects on mitochondrial gene expression: mechanisms and potential contributions to estrogen carcinogenesis. Ann N Y Acad Sci 2004;1028:258–72.
[50] Milanesi LM, Boland A, Boland RL. Expression and localization of estrogen receptor alpha in the C2C12 murine skeletal muscle cell line. J Cell Biochem 2008;104:1254–73.
[51] Tuquet C, Dupont J, Mesneau A, Roussaux J. Effects of tamoxifen on the electron transport chain of isolated rat liver mitochondria. Cell Biol Toxicol 2000;16:207–19.
[52] Vasconsuelo A, Milanesi LM, Boland RL. 17β-Estradiol abrogates apoptosis in murine skeletal muscle cells through estrogen receptors: role of the phosphatidylinositol 3-kinase/Akt pathway. J Endocrinol 2008;196:385–97.
[53] Vic P, Vignon F, Derocq D, Rochefort H. Effect of estradiol on the ultrastructure of the MCF7 human breast cancer cells in culture. Cancer Res 1982;2:667–73.
[54] Simpkins JW, Yi KD, Yang SH, Dykens JA. Mitochondrial mechanisms of estrogen neuroprotection. Biochim Biophys Acta 2010;10:1113–20.
[55] Lesnefsky EJ, Moghaddas S, Tandler B, Kerner J, Hoppel CL. Mitochondrial dysfunction in cardiac disease: ischemia-reperfusion, aging, and heart failure. J Mol Cell Cardiol 2001;33:1065–89.
[56] Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005;39:359–407.
[57] Madamanchi NR, Runge MS. Mitochondrial dysfunction in atherosclerosis. Circ Res 2007;100:460–73.
[58] Abbott NJ, Ronnback L, Hansson E. Astrocyte–endothelial interactions at the blood–brain barrier. Nat Rev Neurosci 2006;7:41–53.
[59] Razmara A, Duckles SP, Krause DN, Procaccio V. Estrogen suppresses brain mitochondrial oxidative stress in female and male rats. Brain Res 2007;1176:71–81.
[60] Viña J, Sastre J, Pallardó FV, Gambini J, Borrás C. Role of mitochondrial oxidative stress to explain the different longevity between genders: protective effect of estrogens. Free Radic Res 2006;40:1359–65.
[61] Bishop J, Simpkins JW. Estradiol treatment increases viability of glioma and neuroblastoma cells in vitro. Mol Cell Neurosci 1994;5:303–8.
[62] Behl C, Widmann M, Trapp T, Holsboer F. 17-beta estradiol protects neurons from oxidative stress-induced cell death in vitro. Biochem Biophys Res Commun 1995;2:473–82.
[63] Zhai P, Eurell TE, Cooke PS, Lubahn DB, Gross DR. Myocardial ischemia-reperfusion injury in estrogen receptor-alpha knockout and wild-type mice. Am J Physiol Heart Circ Physiol 2000;278:H1640–7.
[64] Zhai D, Huang X, Han X, Yang F. Characterization of tBid-induced cytochrome c release from mitochondria and liposomes. FEBS Lett 2000;472:293–6.
[65] Borrás C, Sastre J, García-Sala D, Lloret A, Pallardó FV, Viña J. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic Biol Med 2003;5:546–52.
[66] Carré M, Carles G, André N, Douillard S, Ciccolini J, Briand C, Braguer D. Involvement of microtubules and mitochondria in the antagonism of arsenic trioxide on paclitaxel-induced apoptosis. Biochem Pharmacol 2002;63:1831–42.
[67] Er F, Michels G, Gassanov N, Rivero F, Hoppe UC. Testosterone induces cytoprotection by activating ATP-sensitive K+ channels in the cardiac mitochondrial inner membrane. Circulation 2004;110:3100–7.
[68] Desagher S, Martinou JC. Mitochondria as the central control point of apoptosis. Trends Cell Biol 2000;1:369–77.
[69] Dimmer KS, Scorrano L. (De)constructing mitochondria: what for? Physiology 2006;21:233–41.
[70] Karbowski M, Norris KL, Cleland MM, Jeong SY, Youle RJ. Role of Bax and Bak in mitochondrial morphogenesis. Nature 2006;443:658–62.
[71] Aslan JE, Thomas G. Death by committee: organellar trafficking and communication in apoptosis. Traffic 2009;10:1390–404.
[72] Degterev A, Yuan J. Expansion and evolution of cell death programmes. Mol Cell Biol 2008;9:378–90.
[73] Kiess W, Gallaher B. Hormonal control of programmed cell death/apoptosis. Eur J Endocrinol 1998;138:482–91.
[74] Choi KC, Kang SK, Tai CJ, Auersperg N, Leung PC. Estradiol up-regulates antiapoptotic Bcl-2 messenger ribonucleic acid and protein in tumorigenic ovarian surface epithelium cells. Endocrinology 2001;142:2351–60.
[75] Okasha SA, Ryu S, Do Y, McKallip RJ, Nagarkatti M, Nagarkatti PS. Evidence for estradiol-induced apoptosis and dysregulated T cell maturation in the thymus. Toxicology 2001;163:49–62.
[76] Florian M, Magder S. Estrogen decreases TNF-alpha and oxidized LDL induced apoptosis in endothelial cells. Steroids 2008;1:47–58.
[77] Seli E, Guzeloglu-Kayisli O, Kayisli UA, Kizilay G, Arici A. Estrogen increases apoptosis in the arterial wall in a murine atherosclerosis model. Fertil Steril 2007;88:1190–6.
[78] Kimura K, Markowski M, Bowen C, Gelmann EP. Androgen blocks apoptosis of hormone-dependent prostate cancer cells. Cancer Res 2001;61:5611–8.
[79] Lin Y, Kokontis J, Tang F, Godfrey B, Liao S, Lin A, Chen Y, Xiang J. Androgen and its receptor promote Bax-mediated apoptosis. Mol Cell Biol 2006;5:1908–16.
[80] Rodríguez-Cuenca S, Monjo M, Gianotti M, Proenza AM, Roca P. Expression of mitochondrial biogenesis signaling factors in brown adipocytes is influenced specifically by 17beta-estradiol, testosterone, and progesterone. Am J Physiol Endocrinol Metab 2007;292:E340–6.
[81] Pronsato L, Ronda AC, Milanesi L, Vasconsuelo A, Boland R. Protective role of 17β-estradiol and testosterone in apoptosis of skeletal muscle. Actual Osteol 2010;2:45–8.
[82] Altucci L, Addeo R, Cicatiello L, Dauvois S, Parker MG, Truss M, Beato M, Sica V, Bresciani F, Weisz A. 17beta-Estradiol induces cyclin D1 gene transcription, p36D1–p34cdk4 complex activation and p105Rb phosphorylation during mitogenic stimulation of G(1)-arrested human breast cancer cells. Oncogene 1996;12:2315–24.
[83] Foster JS, Wimalasena J. Estrogen regulates activity of cyclindependent kinases and retinoblastoma protein phosphorylation in breast cancer cells. Mol Endocrinol 1996;10:488–98.
[84] Pietras RJ, Marquez-Garban DC. Membrane-associated estrogen receptor signaling pathways in human cancers. Clin Cancer Res 2007;13:4672–6.
[85] Moriarty K, Kim KH, Bender JR. Minireview: estrogen receptormediated rapid signaling. Endocrinology 2006;147:5557–63.
[86] Gompel A, Somai S, Chaouat M, Kazem A, Kloosterboer HJ, Beusman I, Forgez P, Mimoun M, Rostene W. Hormonal regulation of apoptosis in breast cells and tissues. Steroids 2000;65:593–8.
[87] Jordan VC. The 38th David A. Karnofsky lecture: the paradoxical actions of estrogen in breast cancer—survival or death? J Clin Oncol 2008;26:3073–82.
[88] Lee Y, Renaud RA, Friedrich TC, Gorski J. Estrogen causes cell death of estrogen receptor stably transfected cells via apoptosis. J Steroid BiochemMol Biol 1998;67:327–32.
[89] EL-Ashry D, Miller DL, Kharbanda S, Lippman ME, Kern FG. Constitutive Raf-1 kinase activity in breast cancer cells induces both estrogen independent growth and apoptosis. Oncogene 1997;15:423–35.
[90] Dirks A, Leeuwenburgh C. Apoptosis in skeletal muscle with aging. AM J Physiol Regul Integr Comp Physiol 2002;282:519–27.
[91] Bhasin S. Testosterone supplementation for aging-associated sarcopenia. J Gerontol A Biol Sci Med Sci 2003;58:1002–8.

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[92] Deasy BM, Lu A, Tebbets JC, Feduska JM, Schugar RC, Pollett JB, Sun B, Urish KL, Gharaibeh BM, Cao B, Rubin RT, Huard J. A role for cell sex in stem cell-mediated skeletal muscle regeneration: female cells have higher muscle regeneration efficiency. J Cell Biol 2007;177:73–86.
[93] Ronda A, Vasconsuelo A, Boland R. Extracellular-regulated kinase and p38 mitogen-activated protein kinases are involved in the antiapoptotic action of 17beta-estradiol in skeletal muscle cells. J Endocrinol 2010;2:235–46.
[94] Vasconsuelo A, Milanesi L, Boland R. Participation of HSP27 in the antiapoptotic action of 17beta-estradiol in skeletal muscle cells. Cell Stress Chaperones 2010;15:183–92.
[95] Zhang Y, Zhao H, Asztalos S, Chisamore M, Sitabkhan Y, Tonetti DA. Estradiol-induced regression in T47D:A18/PKCalpha tumors requires the estrogen receptor and interaction with the extracellular matrix. Mol Cancer Res 2009;7:498–510.
[96] John S, Nayvelt I, Hsu HC, Yang P, Liu W, Das GM, Thomas T, Thomas TJ. Regulation of estrogenic effects by beclin 1 in breast cancer cells. Cancer Res 2008;68:7855–63.
[97] Nilsen J, Chen S, Irwin RW, Iwamoto S, Brinton RD. Estrogen protects neuronal cells from amyloid beta-induced apoptosis via regulation of mitochondrial proteins and function. BMC Neurosci 2006;3:7–74.
[98] Ahlbom E, Grandison L, Bonfoco E, Zhivotovsky B, Ceccatel-li S. Androgen treatment of neonatal rats decreases susceptibility of cerebellar granule neurons to oxidative stress in vitro. Eur J Neurosci 1999;11:1285–91.
[99] Ahlbom E, Prins GS, Ceccatelli S. Testosterone protects cerebellar granule cells from oxidative stress-induced cell death through a receptor mediated mechanism. Brain Res 2001;892:255–62.
[100] Pike CJ, Nguyen TV, Ramsden M, Yao M, Murphy MP, Rosario ER. Androgen cell signaling pathways involved in neuroprotective actions. Horm Behav 2008;53:693–705.
[101] Nilsen J, Mor G, Naftolin F. Estrogen-regulated developmental neuronal apoptosis is determined by estrogen receptor subtype and the Fas/Fas ligand system. J Neurobiol 2000;43:64–78.
[102] Wang L, Andersson S, Warner M, Gustafsson JA. Morphological abnormalities in the brains of estrogen receptor beta knockout mice. Proc Natl Acad Sci USA 2001;98:2792–6.
[103] Hammond J, Le Q, Goodyer C, Gelfand M, Trifiro M, LeBlanc A. Testosterone-mediated neuroprotection through the androgen receptor in human primary neurons. J Neurochem 2001;77:1319–26.
[104] Rubinow DR, Schmidt PJ. Androgens, brain, and behavior. Am J Psychiatry 1996;153:974–84.
[105] Estrada M, Varshney A, Ehrlich B. Elevated testosterone induces apoptosis in neuronal cells. J Biol Chem 2006:25492–501.
[106] Morimoto S, Mendoza-Rodríguez CA, Hiriart M, Larrieta ME, Vital P, Cerbón MA. Protective effect of testosterone on early apoptotic damage induced by streptozotocin in rat pancreas. J Endocrinol 2005;187:217–24.
[107] Palomar-Morales M, Morimoto S, Mendoza-Rodríguez CA, Cerbón MA. The protective effect of testosterone on streptozotocin-induced apoptosis in beta cells is sex specific. Pancreas 2010;2:193–200.
[108] Tang F, Kokontis J, Lin Y, Liao S, Lin A, Xiang J. Androgen via P21 inhibits TNF-α-induced JNK activation and apoptosis. J Biol Chem 2009;47:32353–8.
[109] Sánchez-Más J, Turpín MC, Lax A, Ruipérez JA, Valdés Chávarri M. Pascual-Figal DA: differential actions of eplerenone and spironolactone on the protective effect of testosterone against cardiomyocyte apoptosis in vitro. Rev Esp Cardiol 2010;63:779–87.
[110] Nguyen TV, Yao M, Pike CJ. Androgens activate mitogen-activated protein kinase signaling: role in neuroprotection. J Neurochem 2005;6:1639–51.
[111] Kokontis JM, Hay N, Liao S. Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. Mol Endocrinol 1998;12:941–53.
[112] Winiarska A, Mandt N, Kamp H, Hossini A, Seltmann H, Zouboulis CC, Blume-Peytavi U. Effect of 5 alpha dihydrotestosterone and testosterone on apoptosis in human dermal papilla cells. Skin Pharmacol Physiol 2006;19:311–21.
[113] Jia Y, Castellanos J, Wang C, Sinha-Hikim I, Lue Y, Swerdloff RS, Sinha-Hikim A. Mitogen-activated protein kinase signaling in male germ cell apoptosis in the rat. Biol Reprod 2009;80:771–80.
[114] Erkkilä K, Henriksén K, Hirvonen V, Rannikko S, Salo J, Parvinen M, Dunkel L. Testosterone regulates apoptosis in adult human seminiferous tubules in vitro. J Clin Endocrinol Metab 1997;82:2314–21.
[115] Verzola D, Villaggio B, Procopio V, Gandolfo MT, Gianiorio F, Famà A, Tosetti F, Traverso P, Deferrari G, Garibotto G. Androgen-mediated apoptosis of kidney tubule cells: role of c-Jun amino terminal kinase. Biochem Biophys Res Commun 2009;387:531–6.
[116] Jin L, Ai X, Liu L, Wang Z, Cheng Y, Qiao Z. Testosterone induces apoptosis via Fas/FasL-dependent pathway in bone marrow-derived macrophages. Methods Find Exp Clin Pharmacol 2006;28:283–93.
[117] Morkuniene R, Arandarcikaite O, Borutaite V. Estradiol prevents release of cytochrome *c* from mitochondria and inhibits ischemia-induced apoptosis in perfused heart. Exp Gerontol 2006;41:704–8.
[118] Lewis J, Meeke K, Osipo C, Ross E, Kidawi N, Li T, Bell E, Chandel N, Jordan V. Intrinsic mechanism of estradiol-induced apoptosis in breast cancer cells resistant to estrogen deprivation. J Natl Cancer Inst 2005;97:1746–59.
[119] Gatson JW, Maass DL, Simpkins JW, Idris AH, Minei JP, Wigginton JG. Estrogen treatment following severe burn injury reduces brain inflammation and apoptotic signaling. J Neuroinflammation 2009;6:30.
[120] Liu H, Pedram A, Kim JK. Oestrogen prevents cardiomyocyte apoptosis by suppressing p38α-mediated activation of p53 and by down-regulating p53 inhibition on p38β. Cardiovasc Res 2010 [Epub ahead of print].
[121] Fernando RI, Wimalasena J. Estradiol abrogates apoptosis in breast cancer cells through inactivation of BAD: ras-dependent nongenomic pathways requiring signaling through ERK and Akt. Mol Biol Cell 2004;7:3266–84.
[122] Ackermann S, Hiller S, Osswald H, Lösle M, Grenz A, Hambrock A. 17beta-Estradiol modulates apoptosis in pancreatic beta-cells by specific involvement of the sulfonylurea receptor (SUR) isoform SUR1. J Biol Chem 2009;284:4905–13.
[123] Chen X, Deng Y, Zhou Z, Tao Q, Zhu J, Li X, Chen J, Hou J. 17beta-estradiol combined with testosterone promotes chicken osteoblast proliferation and differentiation by accelerating the cell cycle and inhibiting apoptosis in vitro. Vet Res Commun 2010;34:143–52.