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Review

Actions of 17β-estradiol and testosterone in the mitochondria and their implications in aging

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ABSTRACT

A decline in the mitochondrial functions and aging are two closely related processes. The presence of estrogen and androgen receptors and hormone-responsive elements in the mitochondria represents the starting point for the investigation of the effects of 17β-estradiol and testosterone on the mitochondrial functions and their relationships with aging. Both steroids trigger a complex molecular mechanism that involves crosstalk between the mitochondria, nucleus, and plasma membrane, and the cytoskeleton plays a key role in these interactions. The result of this signaling is mitochondrial protection. Therefore, the molecular components of the pathways activated by the sexual steroids could represent targets for anti-aging therapies. In this review, we discuss previous studies that describe the estrogen- and testosterone-dependent actions on the mitochondrial processes implicated in aging.

Contents

1. Introduction

1.1. Mitochondria, sexual hormones, and aging

At the molecular level, the aging process implies a gradual and progressive deterioration of biomolecules to yield a variety of pathological outcomes, such as cancer, neurodegenerative diseases, sarcopenia, and liver dysfunction (Chung et al., 2008, 2009; Seo et al., 2006). Of the various hypotheses that have been proposed to explain the age-related decline in physiological functions, the free radical theory proposed by Harman (1956) and later refined by Miquel et al. (1980) has been extensively studied and is the most enduring. This theory suggests that reactive oxygen species (ROS) from the mitochondria are the major contributors to the injury of biological macromolecules and lead to irreversible cell damage (Kowaltowski et al., 2009 and references therein; Harman, 1956). Because the main source of ROS is mitochondrial respiration, the mitochondria are thought to be the primary target of oxidative damage (Sastre et al., 1996; Miquel et al., 1980). As a

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result, the mitochondrial genome, ATP production, structure of the organelle, induction/regulation of apoptosis, and other functions are affected by chronic exposure to mitochondrial ROS during aging (Wallace, 2005). Moreover, because the mitochondrial morphology changes with age, an increased volume and fewer cristae have been observed in this organelle in older cells (Wilson and Franks, 1975; Ozawa, 1997). Although the mechanism responsible for this change is not fully understood, the enlargement of the mitochondria with age is another age-dependent alteration of the organelle. Oxidative stress and subsequent mitochondrial swelling appears to be responsible for this phenomenon (Terman et al., 2003). In fact, H_2O_2 induces changes in the morphology and localization of the mitochondria in C2C12 muscle cells, which become pyknotic and grouped near the nucleus (Vasconsuelo et al., 2008). This displacement of the organelle could facilitate the translocation of mitochondrial proteins, such as apoptosis-inducing factor (AIF), which binds to DNA and triggers its destruction, to the nucleus (Susin et al., 2000). Hence, the decrease in the mitochondrial size could be due to a loss of proteins. Because the percentage of distorted components in the mitochondria is augmented by the action of ROS, the mitochondrial capacity to generate energy decreases, and the ROS production and the cellular propensity for apoptosis increases. As these cells gradually die, the tissue function worsens. Clinical symptoms appear when the number of cells in a tissue decreases below the minimum number required to maintain tissue function (Wallace and Lott, 2002). This deleterious effect is more evident in postmitotic tissues with high-energy requirements, such as the heart, brain, and skeletal muscle (Ojaimi et al., 1999; Trounce et al., 1989; Cooper et al., 1992). For instance, histochemical and molecular assays of the human skeletal muscle of healthy subjects aged 13–90 years have clearly shown phenotypic and genotypic alterations associated with aging. The data in this study demonstrated changes in the mitochondria of human skeletal muscle beginning at 40–50 years of age (Pesce et al., 2001). Coincidentally, changes in the sexual hormonal state of individuals also start at this age interval (Lamberts et al., 1997), which suggests a relationship between hormonal levels and mitochondrial status. In women, the abrupt decrease in estradiol at the beginning of menopause leads to a series of physical and emotional symptoms and an increased risk of cardiovascular diseases, sarcopenia, osteoporosis, and dementia (Burger et al., 2002), and these changes are associated with increased ROS production (Harman, 1956; Miquel et al., 1980). Of importance, it has been shown that estrogen is able to overcome these symptoms through its effects on the mitochondria (Viña et al., 2005). For example, 17β-estradiol (E2) plays a direct role in cardiac myocytes. It has been shown that ovariectomy increases cardiomyocyte apoptosis and induces proapoptotic changes in the Bcl-2 and Bax genes and proteins that directly affect the mitochondria (Fabris et al., 2011). Nevertheless, in addition to the mitochondria, other targets of E2, such as lipids, have been described in association with hormone cardiovascular protection. E2 leads to decreased LDL and increased HDL (Liu et al., 2008).

In contrast, the decrease in testosterone (T) production by the testes is progressive (Van den Beld and Lamberts, 2002). The level of circulating testosterone diminishes not only in men with progressing age but also in women because of the age-dependent decrease in ovarian and adrenal androgen production. As expected, these variations in the hormone levels affect the normal functioning of the body, and one way of achieving this effect may involve the mitochondria. Moreover, in view of the fundamental role of the mitochondria in the aging process, these organelles represent putative targets of anti-aging strategies. Thus, the aging-dependent impairment of many physiological functions that usually trigger diseases could be overcome by specifically protecting the molecular components of the mitochondria that are primarily affected by ROS. However, the relationship between ROS-induced damage, mitochondrial function, the regulators involved in the aging process, and their hormonal regulation are far from being elucidated. In this context, we discuss the effects of estrogen and testosterone on the functions of the mitochondria and their possible implications in the elderly.

2. Mitochondrial localization of estrogen and androgen receptors

The mitochondria, which are widely known for their function as cellular power-generating units, are key regulators of cell survival and death. In fact, the mitochondria manage energy production, free radical formation, and apoptosis (Green and Reed, 1998; Dimmer and Scorrano, 2006). Moreover, this polyfunctional organelle also participates in cellular signaling (reviewed in Brookes et al., 2002). Somehow, these processes are modulated by steroid and thyroid hormones in the course of their actions on metabolism, growth, and development (Green and Reed, 1998).

The mitochondria have their own genome, which is a circular double-stranded molecule of approximately 16 kb. The strands of the mitochondrial DNA (mtDNA) have an asymmetric distribution of purines and pyrimidines, which generates a heavy and a light strand. The mRNA products from the mitochondrial genome encode three subunits of cytochrome oxidase (COX I, COX II, and COX III), seven subunits of NADH-CoQ-reductase (ND 1–6 and ND4 L), one subunit of cytochrome b, two subunits of ATP-synthase (ATP6 and ATP8), rRNAs (12S and 16S), and tRNAs (Montoya et al., 2006). Interestingly, the mitochondrial genome contains sequences that are similar to those of nuclear hormone-responsive elements (HREs) (Sekeris, 1990). The mtDNA was found to contain hormone-responsive elements for Class I and Class II receptors, including estrogen and androgen receptors (ERs and ARs), that are located at various sites of the mtDNA within the ribosomal subunit genes and structural genes (Demonakos et al., 1995, 1996). The fact that a genome as small as the mitochondrial genome, which encodes proteins of great importance to the function of this organelle and thus to the cell, contains hormone-responsive elements demonstrates that the action of steroid hormones is relevant to the organelle. In fact, the appropriate receptors can interact with these sequences to confer the hormone-dependent activation of specific mitochondrial genes. In this context, a direct effect of the steroids hormones on the organelle functions has been proposed (reviewed by Psarra et al., 2006, Klinge, 2008, and Chen and Russo, 2008). In agreement with this proposal, several findings suggest the effects of estrogens and androgens on the transcription of mitochondrial oxidative phosphorylation genes (OXPHOS) (Scheller et al., 2003; Gavrilova-Jordan and Price, 2007; Weitzel et al., 2003; Psarra et al., 2006). Similarly, the effects of E2 on the COXI and COXII mRNA in MCF-7 breast cancer cells have been documented (Felty and Roy, 2005). The stimulation of cells with these hormones through their receptors can induce the OXPHOS genes through the direct activation of the nuclear OXPHOS genes containing HREs or can induce HREs that regulate the expression of transcription factors required for nuclear OXPHOS gene transcription. Moreover, these transcription factors can induce the expression of genes encoding mitochondrial transcription factors that can activate mitochondrial OXPHOS gene expression (reviewed by Psarra et al., 2006).

Despite the controversies between the results obtained by Gustafsson's and Yang's groups on the existence of ERs in the mitochondria, the presence of ERs in the organelle is undisputable. Gustafsson reported that ERβ cannot be positively identified in the mouse liver mitochondria by MALDITOF (Schwend and Gustafsson, 2006). This finding was refuted based on the fact that ERβ expression is low in the mouse liver and that the mitochondrial localization of the ER may be cell-specific because ERβ was

identified in human heart mitochondrial proteins by MALDITOF (Yang et al., 2006). The presence of ERs and ARs in the mitochondria was first suggested based on studies using radiolabeled hormone ligands and binding experiments using mitochondrial extracts. The purification of receptor proteins and the availability of corresponding antibodies permitted the application of techniques, such as Western blotting or immunofluorescence with confocal and electron microscopy, for the identification of ERs and ARs in this subcellular compartment (Hammes and Levin, 2007). Accordingly, ERs were detected in the mitochondria of cells from different tissues and in primary cultures and cell lines, such as rat uterine and ovarian cells (Monje and Boland, 2001), in MCF-7 cells (Chen et al., 2004a; Pedram et al., 2006), human lens epithelial cells (Cammarata et al., 2004), rat hippocampus, neuronal cells (Milner et al., 2005), cardiomyocytes (Yang et al., 2004), endothelia (Duckles et al., 2006), HepG2 hepatocarcinoma, and SaOS-2 osteosarcoma cells (Solakidi et al., 2005a). Similarly, ERs were found in the mitochondria of human sperm cells (Solakidi et al., 2005b), human periodontal ligament cells and tissue (Jönsson et al., 2007), and the C2C12 mouse skeletal muscle cell line (Milanesi et al., 2008, 2009).

2.1. ERα vs. ERβ

The occurrence of individual ER isoforms (α and/or β) in the mitochondria may differ depending on the tissue type. In various types of cells, the presence of both ERα and ERβ in the organelle has been shown (Cammarata et al., 2004; Pedram et al., 2006; Milanesi et al., 2008, 2009). However, in general, the predominant receptor isoform is ERβ (Cammarata et al., 2004; Chen et al., 2004a; Solakidi et al., 2005a; Pedram et al., 2006; Milanesi et al., 2008, 2009). Full structural analysis and knowledge of the amino acid composition and the interactions of both receptor isoforms with other molecules might explain the predominance of ERβ in the mitochondria. For example, it has been shown in C2C12 muscle cells that ERβ interacts with the chaperone HSP27 in the mitochondria (Vasconsuelo et al., 2010). This interaction appears to be specific to ERβ because it was not observed between the chaperone and ERα in C2C12 cells. This finding may explain the fact that E2 mainly involves ERβ in its protective action in skeletal muscle cells (Vasconsuelo et al., 2008). This interplay of the two molecules might confer greater stability to ERβ in the mitochondria such that it can more efficient under stress conditions and/or in the regulation of the estrogen signal. Some researchers have postulated that each receptor regulates the expression of a distinct set of genes (Katzenellenbogen and Katzenellenbogen, 2000; O'Lone et al., 2007). This difference could be due to the different compartmentalization of ERα and ERβ or to the selective estrogen receptor modulators (SERMs). Briefly, the SERMs are ER ligands, and each SERM leads to a different conformational change in the ERs after ligand binding, which causes a differential recruitment of coactivators, corepressors, and other transcriptional factors. Thus, the SERMs can act as antagonists in some tissues and have either partial or full agonist activity in others. In general, most of the genes regulated by ERβ are mitochondrial proteins related to oxidative phosphorylation (O'Lone et al., 2007). Moreover, it is also necessary to take into account that the selectivity of gene expression observed for each receptor could be partly due to the recruitment of different coactivators and adaptor proteins, which play roles in the binding of both ERs and transcriptional activation and whose levels of expression could be cell type-specific. However, how tissue specificity is determined during transcription must be further investigated. Each tissue may have its own coativactors or there might be a small set of proteins that can interact in different combinations to determine where and which ER isoform is recruited in each cell type. For instance, the forkhead protein FoxA1 is a well-characterized factor that helps recruit ERα in breast cancers (Carroll et al., 2005), but this protein is not expressed in bone cells, which are also estrogen targets (Krum et al., 2008). In addition, in osteoblasts, GATA4 helps recruit ERα to osteoblast-specific enhancers (Miranda-Carboni et al., 2011). In addition to mitochondrial factors, there are more than 300 nuclear receptor coactivators and corepressors (O'Malley, 2008), which suggests that the E2 tissue specificity and its related effects during menopause and aging are controlled by an extremely complex mechanism.

In contrast to the ERs, the localization of the AR in the mitochondria is not very well evidenced. Recently, mitochondrial AR was detected in the C2C12 skeletal muscle cell line (Pronsato et al., 2013), in the midpiece of sperm cells, and in the LnCap human prostate cancer cell line (Solakidi et al., 2005b). In the C2C12 cell line, Western blot assays of mitochondrial fractions allowed the immunodetection of a ~100-kDa band, which likely corresponds to the classical AR. Interestingly, additional immunoreactive bands were also detected. The immunoreactive proteins obtained could be the consequence of an alternative usage of different in-frame initiation codons or splice variants of the full-length AR transcript (Pronsato et al., 2013). The identification and characterization of these androgen-binding entities in mitochondrial fractions of the C2C12 skeletal muscle cell line was achieved through conventional competitive radioligand binding assays with [^{3}H] testosterone, which demonstrated specific and saturable binding activity consistent with a single set of affinity binding sites. Solakidi et al. used Western blot analysis with an anti-AR antibody to reveal the presence of a 110-kDa band, which corresponds to the intact AR, and a faint 90-kDa protein, which could represent an AR isoform or an AR degradation product due to the proteases present in sperm. Such cleavage products may represent functionally active receptor species with the same or different properties/functions of the wild-type receptor; however, these aspects have not been evaluated (Solakidi et al., 2005a).

The presence of ERs and ARs in the mitochondria of sperm cells might be related to the regulation of the energy requirements of these cells to maintain flagellar movement. Nevertheless, the findings reported by Diez-Sanchez et al. (2003) demonstrated that sperm cells are not capable of synthesizing proteins because they are unable to modify their capacity for oxidative phosphorylation through the de novo synthesis of proteins. Thus, the presence of ERs and ARs in the mitochondria of sperm may be only indirectly involved in the regulation of their motility. Additional studies silencing these receptors will likely help elucidate the specific roles of ERs and ARs in the mitochondria of sperm cells.

3. Role of estrogens and androgens and their receptors in the mitochondria

Based on the mitochondrial localization of ERs and ARs, the presence of the hormone response elements EREs and AREs in the mitochondrial genome and the fact that the same transcription factors in the nucleus and mitochondria are activated by each receptor, investigations have been performed to evaluate the actions of estrogens and androgens in this organelle.

3.1. Transcription of genes encoding enzymes of oxidative phosphorylation: role of E2, androgens, and their receptors

The mitochondrial respiratory chain (MRC), which is also called the electron transport chain, consists of a series of metalloproteins bound to the mitochondrial inner membrane. There are four large protein complexes (complex I to complex IV) that are associated with the mitochondrial electron transport system and cooperate in electron transfer and proton pumping across the inner membrane. In addition, the coupling of the proton gradient membrane

potential to the synthesis of ATP from ADP + Pi through the mitochondrial F0–F1 ATP synthase generates the major source of cellular energy in the form of ATP. This process generates ROS that play a role in the regulation of gene expression, cell proliferation, and apoptosis. ROS also act as second messengers and in the oxidative damage of cell components, as previously mentioned (Chen et al., 2009).

In the last decade, there has been increased interest in the influence of steroid hormones on the MRC. It is well established that estradiol not only upregulates the transcript levels of several mtDNA genes that encode components of the MRC but also has a direct and indirect effect on the MRC activity, particularly through ERβ (Bettini and Maggi, 1992; Chen et al., 1998, 2004a, 2003; Felty and Roy, 2005). This hypothesis is in agreement with the fact that ERβ appears to be the quantitatively most important isoform in the mitochondria.

In rat cerebrovascular cells, it has been reported that estradiol increases the levels of mitochondrial proteins, such as COXI, cytochrome c, and COX IV of complex IV and manganese superoxide dismutase (MnSOD). MnSOD expression is part of the defense mechanism against the deleterious actions of ROS. Thus, the reduction of E2 levels in the elderly is related to the aging symptoms because it affects MnSOD (Stirone et al., 2005). In mitochondria expressing ERα that were isolated from the cerebral blood vessels of ovariectomized rats with or without estrogen replacement, E2 upregulated various nuclear-encoded proteins, such as ERα, cytochrome c, COX IV, and MnSOD, and the mitochondrial genome-encoded COX I, and this effect is blocked by ER antagonists. Furthermore, an increase in the activity of citrate synthase and COX IV after estrogen treatment has been observed (Duckles et al., 2006; Stirone et al., 2005). Similarly, the brain mitochondria from progesterone and E2-treated rats exhibited increased expression and activity of complex IV coupled to MRC functions, and this increased MRC activity was associated with low reactive oxygen leakage and reduced lipid peroxidation and thus indicating a systematic enhancement of the brain mitochondrial efficiency (Irwin et al., 2008). In male rats that previously experienced severe trauma, E2 or diarylpropionitrile (DPN, a selective agonist of ERβ) treatment increased the expression and activity of mitochondrial ERβ and complex IV. Thus, E2 through mitochondrial ERβ mediates cardioprotection through the upregulation of complex IV and the inhibition of mitochondrial apoptotic signaling pathways (Hsieh et al., 2006). Moreover, in human breast epithelial cells that are negative for ERα but not for ERβ, E2 and DPN treatments induced the synthesis of MRC proteins, which shows that mitochondrial ERβ is directly involved in the expression of mtDNA-encoded MRC proteins in this cell line (Chen et al., 2007). These effects on mitochondrial respiratory functions are not exclusive to estrogen. In fact, the androgenic effects on MRC at the levels of RNA and protein synthesis have been documented in various tissues (Pegg and Williams-Ashman, 1968). Using a mammalian two-hybrid system, Beauchemin et al. (2001) showed that COX Vb physically interacts with the AR. Additionally, in the adrenal cortex and in the liver of rats treated with androgens, an increase in the cytochrome c oxidase activity and an increase in the levels of the COX subunits II, III, and IV were observed. These effects involved the ARs because these were reversed by treatment with the antiandrogen flutamide, which binds specifically to the AR (El-Migdadi et al., 1996). In the LNCaP cell line, androgens induce the expression of various genes, some of which are involved in mitogenesis and bioenergetics (Xu et al., 2001; Koenig et al., 1980).

The stimulation of MRC biogenesis/functions by sexual hormones is highly beneficial because it provides the cells in different tissues sufficient MRC-derived energy for their proper functions. The maintenance of cell survival by these hormones through their receptors, which mediate MRC biogenesis, confers significant protection against aging-dependent diseases.

3.2. Activation of nuclear respiratory factors (NRFs) and mitochondrial transcription factor A (Tfam) by E2/ERs

Although the molecular mechanisms underlying the above enumerated effects of estrogens and androgens are mediated through their respective receptors, the hormonal regulation of mtDNA-encoded MRC proteins is not completely understood. Several studies have highlighted the nuclear respiratory factors 1 and 2 (NRF1 and NRF2) as mediators of these effects.

NRF1 and NRF2 are primary transcription factors of nDNA-encoded mitochondrial proteins, such as the majority of MRC complex proteins, mitochondrial transcription factor A (Tfam), and protein factors that play a crucial role in the replication, transcription, and translation of mtDNA (Kang et al., 2007; Chen et al., 2009 and references therein).

Estrogens can regulate the expression of NRFs in the cerebral blood vessels and heart cells of female and male rats, respectively, and in the MCF7 breast cancer and H1793 lung adenocarcinoma cell lines (Stirone et al., 2005; Hsieh et al., 2005; Mattingly et al., 2008). In MCF7 cells, the effect was mediated by ERs at the transcriptional level. Moreover, the NRF1 promoter contains an ERE that specifically binds both ER isoforms in an estrogen-dependent manner. Similar results were obtained for the mitochondrial transcription factor A Tfam. This nDNA-encoded gene is regulated by E2 via ERs (Nilsen et al., 2007). In male rats, E2 increased the expression of cardiac Tfam, and this effect was associated with increases in COX IV, mtDNA-encoded COXI, ATP synthase β-subunit, mitochondrial ATP, and COX activity (Hsieh et al., 2005). Because the promoter of human Tfam contains functional NRF1 and NRF2 binding sites and E2 upregulates these transcriptional factors, as shown in MCF7 cells, this upregulation of Tfam is most likely an indirect effect mediated through both NRFs (Virbasius and Scarpulla, 1994). Clearly, the localization of ERs and EREs in both the nuclear and the mitochondrial compartments suggests a coordinated regulation of the nuclear and mitochondrial gene expression in response to estradiol. However, there is less evidence available for testosterone and AR. Thus, additional studies are necessary to firmly establish the T/AR effects on the mitochondrial–nuclear crosstalk that result in the regulation of gene expression.

3.3. Role of estrogens, androgens, and their receptors in oxidative phosphorylation and cytoskeletal structure: implications in lifespan

The energy production by oxidative phosphorylation requires both nuclear- and mitochondrial-encoded enzymes (Attardi and Schatz, 1988). Because the majority of mtDNA-encoded genes are related to oxidative phosphorylation, this may be the main area of steroid influence. ERs and ARs can regulate energy production by inducing nuclear and mitochondrial OXPHOS genes. The presence of steroid hormone receptors in the mitochondria and of HRE-like sequences in the mitochondrial genome (Sekeris, 1990; Wrutniak et al., 1998; Chen et al., 2004b; Morrish et al., 2006; Ioannou et al., 1988; Demonakos et al., 1995; Tsiriyotis et al., 1997) suggests an additional mode of coordination through a direct effect of the mitochondrial receptor on the transcription process in the organelle (Scheller et al., 2000, 2003; Sekeris, 1990; Wrutniak et al., 1998; Chen et al., 2004b; Casas et al., 2003; Psarra et al., 2006). In addition, the presence of similar transcription in the nucleus and mitochondrion further ensure the coordination of a process requiring the parallel transcription of genes residing in these two cellular compartments. The fact that the genes responsible for the energy production by oxidative phosphorylation are found in separate

compartments may represent a defense mechanism against ROS in itself. In fact, it has been traditionally understood that nuclear DNA is relatively insensitive to damage by ROS compared with the mitochondrial genome. Studies have shown that the overexpression of mitochondrial-targeted DNA repair enzymes protects cells against death. Thus, mitochondrial DNA damage may play a protective role by triggering death pathways before the oxidant burden increases to a point that threatens the nuclear genomic integrity (Dobson et al., 2000, 2002).

The mitochondrial dysfunction induced by ROS represents the major contributor to the age-related diseases of the cardiovascular system and the brain (Wallace, 2005; Madamanchi and Runge, 2007; Abbott et al., 2006). There is evidence that the vasoprotective action of 17β-estradiol is exerted on the mitochondria (Duckles et al., 2006; Stirone et al., 2005). The effectiveness of estrogen against age-related vascular disorders may be in part mediated by the modulation of the mitochondrial functions, which would result in a greater energy-producing capacity, a decreased ROS production, and therefore a longer lifespan in women. Accordingly, sex differences were also observed in the rat brain mitochondrial oxidative status. The aging effect of oxidative damage was less marked in females than in males. This damage, which gradually accumulates in the rat brain due to the increasing activity of the mitochondrial respiratory chain and the failure of antioxidant defenses, is more evident in males because females exhibit greater antioxidant capacity, such as higher glutathione peroxidase activity and higher ion carrier protein 5 (UCP5) level. This sexual dimorphism gradually increased during aging (Guevara et al., 2011).

Furthermore, it has been demonstrated that sexual hormones are able to protect the mitochondria by acting on the adapter protein p66shc. ROS production is amplified by p66shc, which is released from an inhibitor complex in the inner mitochondrial membrane in response to a variety of proapoptotic stimuli and catalyzes the reduction of O_2 to H_2O_2 through an electron transfer from cytochrome c (Camici et al., 2007; Migliaccio et al., 1999; Giorgio et al., 2005). Thus, the p66Shc protein can control the mammalian lifespan by regulating the cellular response to oxidative stress (Migliaccio et al., 1999). The p66Shc protein contains a serine phosphorylation site, Ser36. It was recently shown that the phosphorylation of this residue plays an important role in the cellular response to oxidative stress and in aging. This phosphorylation is necessary for p66Shc translocation to the mitochondria during aging and can be mediated by PKCβ (Lebiedzinska et al., 2009; Pinton et al., 2007). The p66shc protein in the mitochondria perturbs its structure and functions to result in accelerated ROS production (Pinton et al., 2007). Estrogens attenuate the phosphorylation of p66shc in osteoblastic cells through a process involving ERKs. Thus, the loss of estrogens accelerates the effects of aging in bone (Almeida et al., 2010a). Moreover, E2 and T prevent the H_2O_2-induced apoptosis by suppressing the PKCβ-mediated p66shc phosphorylation and stimulating antioxidant defenses (Almeida et al., 2010b). In connection with p66shc, the phosphorylation of the p53 tumor suppressor is also associated with aging (Tyner et al., 2002). In fact, it has been reported that p66shc acts as a downstream target of p53 and is indispensable for the ability of stress-activated p53 to induce an elevation of intracellular oxidants, cytochrome c release, and apoptosis (Trinei et al., 2002). Therefore, p53 represents an additional aspect of hormonal regulation. For example, E2 protects cardiomyocytes by inhibiting p38α-p53 signaling in apoptosis (Liu et al., 2011).

All of the data reviewed here indicate that the antioxidant/survival action of estrogens is due not to their chemical phenolic structure but rather to their interactions with ERs in cells, which eventually lead to the activation of signaling pathways involving multiples organelles and components, such as kinases and nuclear/mitochondrial factors (Fu and Simoncini, 2008; Hsieh et al., 2005; Attardi and Schatz, 1988; Viña et al., 2006). However, although most evidence suggests a beneficial role of this hormone, other studies have shown that this steroid exerts a prooxidant action. In fact, it has been reported that estrogen is able to generate reactive oxygen species that are linked to cancer progression (reviewed by Okoh et al., 2011). It is hypothesized that cellular, tissue, receptor, and context specificity are all involved in the regulation of the estrogen hormone responses (reviewed by Vasconsuelo et al., 2011). More extensive research is necessary to corroborate and understand the dual action of E2.

In contrast to estrogen, androgens have been less frequently associated with prolonged lifespan (Liu et al., 2003). However, functional ARs are present in the heart, and there is increasing evidence to suggest that testosterone confers cardioprotection through its direct action on the myocardium (Tsang et al., 2007). Moreover, it has been demonstrated that testosterone may confer protection via various mechanisms, which may involve both genomic and non-genomic effects (Tsang et al., 2008).

In the human coronary artery, testosterone enhances the endothelial cell (HCAEC) proliferation. Testosterone and dihydrotestosterone (DHT) have been found to attenuate cell adhesion and the expression of VCAM-1 and ICAM-1 in a dose- and time-dependent manner. Furthermore, long-term treatment with androgen inhibits cell migration and promotes tube formation. A Western blot analysis demonstrated that the expression of phosphorylated endothelial nitric oxide synthase (eNOS) increase and that of inducible nitric oxide synthase (iNOS) decreased after steroid treatment of TNF-α-stimulated HCAECs, which suggests that androgens modulate endothelial cell functions by suppressing the inflammatory process and enhancing wound-healing and regenerative angiogenesis, likely through an AR-dependent mechanism (Liao et al., 2012).

Clearly, the data described show that estrogens and androgens affect mitochondrial function. The presence of HREs, ERs, and ARs in mitochondria strengthens the hypothesis that these hormones exert a direct action, as well as various indirect effects, on the organelle activity that are mediated by their respective receptors in a genomic and non-genomic fashion.

3.3.1. *Sexual hormones and cytoskeleton-ROS protection*

Based on the discussions presented above, we can deduce that the action of the steroid hormones on the mitochondria involves an intricate signaling network between the plasma membrane, mitochondria, and nucleus. There is evidence suggesting that the cytoskeleton might play an important role in the maintenance of these cell membrane and subcellular organelle interactions (Carre et al., 2002). In fact, one emerging area of research is the role of the actin cytoskeleton in the mitochondria-dependent cell survival. Of importance, it has been recently found that estrogens regulate several cytoskeletal components, particularly actin fibers (Fu and Simoncini, 2008; Simoncini et al., 2006; Giretti et al., 2008). In agreement with this observation, E2 and T abrogate the typical alterations of the cytoskeleton during H_2O_2-induced apoptosis in C2C12 and primary mouse skeletal muscle cells (Vasconsuelo et al., 2008; Pronsato et al., 2010a; Ronda et al., 2010). In agreement, in the brain, E2 regulates the flexibility of the cytoskeleton through calpain and the small G protein ADP-ribosylation factor-like 3 (Arl3) (Szego et al., 2010), which is important in tubulin deacetylation. The majority of the α-tubulin content is acetylated in neuronal cells, and the polymer becomes destabilized after losing its acetyl group (Black et al., 1989). However, the molecular mechanism responsible for the protection of the cytoskeleton by E2 needs to be further studied in the above-mentioned and other cell types. During menopause, when the estrogen levels are low, it is conceivable that the cytoskeleton is more prone to be injured by ROS and that all of its cellular interactions are therefore affected.

4. Role of estrogens, androgens, and their receptors in apoptosis

Cell death has been traditionally subdivided into regulated and unregulated mechanisms. Apoptosis is a highly organized mode of cell death that plays an essential role in growth, development, and the elimination of unwanted cells. At the end stage of the cell cycle, the cellular structures are degraded by proteases, such as caspases and nucleases that orchestrate the efficient and non-inflammatory destruction of cells (Jacobson et al., 1997; reviewed in Zhang et al., 2004). The multiple molecular cascades that are triggered during apoptosis to activate these proteases may be part of the intrinsic or the extrinsic apoptotic pathways. The former can be stimulated by various factors, such as chemotherapeutics, UV radiation, and viral infection. The other pathway involves the death receptors (Putcha et al., 2002). The proper regulation of both cascades is important for correct tissue function. The mitochondria play a key role in the connection of the components of both pathways and as a control point of programmed cell death because this organelle is a target of Bcl-2 family members (Wang and Youle, 2009; Jeong and Seol, 2008). Thus, all stimuli that act on the mitochondria may affect the apoptosis process in some way. Of the numerous signals that are capable of modulating apoptosis, several cytokines, growth factors, and hormones should be considered (reviewed by Kiess and Gallaher, 1998). In fact, the hormonal action on apoptosis has been well documented (reviewed by Evans-Storms and Cidlowski, 1995, Gompel et al., 2000, and Amsterdam et al., 2002). With regard to the sexual hormones E2 and T, the presence of ER and AR and the effects of both steroids in the mitochondria are associated with the maintenance of the organelle as a control point of apoptosis (reviewed in Vasconsuelo et al., 2011). Although it has been shown that E2 and T can sustain survival or induce apoptosis depending on their biological context (Choi et al., 2001; Okasha et al., 2001; Florian and Magder, 2008; Seli et al., 2007; Vasconsuelo et al., 2008; Kimura et al., 2001; Lin et al., 2006; Rodríguez-Cuenca et al., 2007; Pronsato et al., 2012), the prevalent action for each sexual hormone is the protection of the mitochondria, which results in antiapoptosis (reviewed in Vasconsuelo et al., 2011).

The Bcl-2 proteins display either antiapoptotic (e.g., Bcl-2 and Bcl-xL) or proapoptotic (e.g., Bax, Bad, Bak, and Bid) functions that in turn may be regulated by estrogen- or androgen-responsive kinases, including Akt, MAPKs, and PKA (Desagher and Martinou, 2000; reviewed by Dimmer and Scorrano (2006); Karbowski et al., 2006). For example, in C2C12 muscle cells, it has been shown that E2 can interact with ERs localized in the cell membrane and mitochondria to promote the activation of ERK, p38 MAPK, and the PI3K/Akt/p-Bad survival cascade, which involves HSP27 and the inhibition of caspase-3 activity. Thus, E2 abrogates the mitochondrial membrane damage and consequently the Smac/DIABLO and cytochrome c release induced by hydrogen peroxide. Furthermore, similar to estrogen, T exerts protective effects on the mitochondria that involve HSP90 and Bax expression (Pronsato et al., 2010b, 2012). Both hormones prevent DNA fragmentation and cytoskeleton disorganization (Vasconsuelo et al., 2008, 2010; Ronda et al., 2010; Pronsato et al., 2012). Then, the question of whether a decrease in these hormone levels can induce the deregulation of apoptosis in old age arises. Because both steroids abrogate apoptosis in most cases, the rate of apoptosis will increase in the presence of decreased hormonal levels due to aging, and this could represent a mechanism to eliminate aged/damaged cells. However, this inverse relationship between apoptosis and hormonal levels represents an additional complexity in pathologies such as sex hormone-dependent cancer. In contrast, there is evidence that apoptotic mechanisms may become less efficient with age, at least in some tissues and under defined stimuli, most likely due to the alterations observed in elderly mitochondria (Gershon, 1999; Camplejohn et al., 2003). However, in mitochondrial failures, apoptosis may also increase with age due to the increased level of oxidative stress and the deterioration of the antioxidants systems (Schindowski et al., 2000). Through an analysis of these processes as a whole, it may be concluded that apoptosis fulfills its function in old age, even though each event separately (hormone levels, molecules damaged by ROS, and increased production of ROS) appears to indicate the contrary. Further studies are necessary to evaluate the extent to which apoptosis can continue to function during aging.

5. Risks and benefits of replacement therapy with sex hormones: clinical trials

Based on the information discussed, estrogen and testosterone elicit mitochondrial protection. Thus, due to the role of the mitochondria in aging, both steroids represent a potential therapeutic option against the general weakening associated with aging. However, the use of testosterone or estrogen replacement remains controversial due to a shortage of clinical trials demonstrating the benefits and adverse effects of these steroids. The ability of both hormones to act in almost all tissues could explain the multiple effects of hormonal replacement therapies (HRT) (Wend et al., 2012; Omwancha and Brown, 2006). Moreover, the few clinical trials that are available do not examine whether the observed effects are due to hormonal action in the mitochondria (Viña et al., 2006).

In the past, estrogenic therapy was often prescribed for the prevention of coronary heart disease and osteoporosis based on epidemiologic data demonstrating the protective effect of estrogen on the heart and bone. However, data from the Women's Health Initiative, which consists of a set of two hormone therapy trials (unopposed estrogen and continuous, combined estrogen-progestin therapy versus placebo) in healthy postmenopausal women showed a number of adverse outcomes, including an excess risk of coronary heart disease, stroke, venous thromboembolism, and breast cancer (Rossouw et al., 2002). Thus, the use of HRT changed abruptly when a large clinical trial found that the treatment actually posed more health risks than benefits associated with one type of hormone therapy, particularly when it was administered to older postmenopausal women (reviewed by Manson and Martin, 2001; Anderson et al., 2004). The Heart and Estrogen/Progestin Replacement Study (HERS), HERS-II, and Women's Health Initiative clinical trials demonstrated that the HRT adverse outcomes could be due to the type of hormone used. For example, oral estrogens are biologically transformed in the liver. In contrast, transdermal preparations avoid this first-pass metabolism. This difference could affect the hormonal behavior in the body. In addition, phytoestrogens may provide alternatives to synthetic estrogens (Viña et al., 2005). Phytoestrogens increase the expression of antioxidant enzymes without the feminizing effects of estradiol. Nevertheless, not all phytoestrogens act in the same manner. These compounds may behave as estrogens or may elicit different effects than estrogens, and this dissimilar behavior depends on their structure, doses, and/or cellular environment. If they exhibit estrogen-like activity, their administration may be beneficial for neuronal diseases or osteoporosis but could also result in a potential risk for breast cancer development. It is currently unclear whether phytoestrogens from soy foods, which act as estrogens, affect breast cancer risk (Murkies et al., 2000). Studies that directly analyze the breast cancer progression and soy in the diet do not agree. Almost half of the investigations have reported that soy has no effect on breast cancer risk. Animal studies have shown that soy phytoestrogens can decrease breast cancer formation in rats. However, several clinical works have suggested that women should be cautious in using large amounts of the soy products that can

behave similar to estrogen because these products may increase breast cancer risk. A possible molecular mechanism would involve the abovementioned estrogen-antiapoptotic actions, which would inhibit the death of cancer cells (Schardt, 2000; Murkies et al., 2000; Wiseman, 2000; Ginsberg and Prelevic, 2000). Another therapeutic approach may involve the use of specific ER modulators (SERMs), which minimize the adverse effects of HRT, including breast cancer. Thus, the beneficial effects of HRT in postmenopausal vascular disease can be enhanced by customizing the HRT type, dose, route of administration, and timing depending on the subject's age (Koledova and Khalil, 2007). Therefore, the use of specific phytoestrogens or SERMS as an alternative therapy could be employed to correct alterations of estrogen deficiency or to decrease malignancy outcomes if these compounds are prescribed correctly.

Cunningham and Toma (2011) provide a critical review of the evidence that supports testosterone replacement treatment. These researchers describe the potential risks of this procedure and propose methods that can be used to reduce these risks. Additionally, Bhasin et al. (2010) recommend testosterone therapy only for men with consistent symptoms of androgen deficiency and unequivocally low serum testosterone levels. These researchers author found controversial results from androgenic therapies depending of the age of the patients (Spitzer et al., 2013), and this result is in agreement with the observations reported by Koledova and

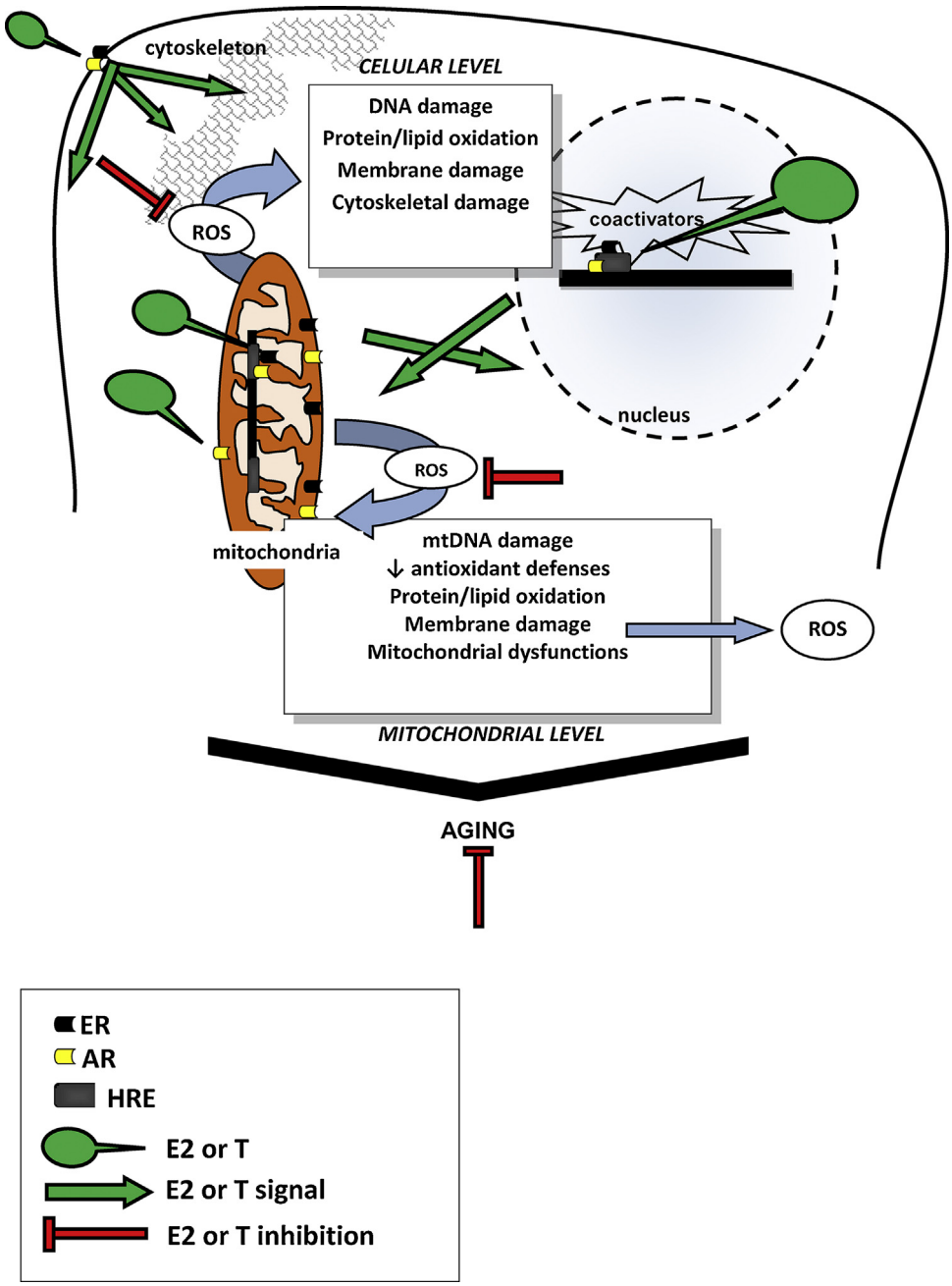

Fig. 1. Schematic diagram showing the effects of steroid hormones 17β-estradiol or testosterone on mitochondria, which in turn, regulate aging. Mitochondria are source and target of reactive oxygen species (ROS). ROS induce mitochondrial alterations and consequently to a general decline in mitochondrial function that, in a vicious cycle-like manner, results in more oxidative stress. The increased production of ROS in aging affects the entire cell, leading to mtDNA and nDNA damage, decrease of antioxidant defenses, cytoskeletal injury, protein/lipid oxidation as well as membrane damage. In this context, estradiol and testosterone could act through plasma membrane, mitochondrial and nuclear ERs, ARs and HREs; establishing an intricate signaling network between plasma membrane, mitochondria and nucleus, which results in protective action on mitochondria and in turn anti-aging effect. Probably the cytoskeleton plays a central role integrating all these mechanisms.

Khalil (2007). However, these researchers found that the administration of T with low-intensity physical training improves grip strength, spontaneous movements, and respiratory activity. These functional improvements were associated with increased muscle mitochondrial biogenesis and improved mitochondrial quality control in mice (Guo et al., 2012). In addition, these improvements could be associated with the role of T and AR in the mitochondria that was previously discussed, which is important for the management of sarcopenia with HRT because the age-related hormonal decline is one of its possible etiologic factors (reviewed by Kamel et al., 2002). Therefore, although the evidence linking age-related hormonal changes to the development of sarcopenia is rapidly growing, it is still too early to determine the clinical utility of hormonal supplementation in the management of this muscular disease. Further research is necessary to establish the proper roles, efficacies, and safe applications of hormones, SERMs, natural compounds, and exercise training therapies for the mitigation of the sharp and rapid decreases in androgen and estrogen concentrations with advancing age and to establish the contribution of these hormones to the overall human longevity.

6. Conclusions

It is clear that 17β-estradiol and testosterone exert actions on the mitochondria and that these organelles play an important role in age-related processes and are thus putative targets for anti-aging strategies. Both steroids affect the mitochondrial function directly and/or indirectly: E2 and T directly act through ERs, ARs, and HREs localized in the organelle and indirectly regulate the nDNA-encoded mitochondrial proteins and nuclear transcription factors that affect mtDNA-encoded proteins. Similarly, these sexual hormones indirectly control various mitochondrial functions, such as ROS production and apoptosis, through kinase signaling pathways induced by plasma membrane receptors that regulate the Bcl-2 family or through cytosolic signaling peptides. The cytoskeleton likely plays a central role in the articulation of all of these mechanisms (Fig. 1).

A full understanding of the molecular mechanism triggered by both steroids at the mitochondrial level and their effects in aging populations carry profound and exciting possibilities for the future treatment of age-dependent diseases associated with the deregulation of sexual hormones levels.

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