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Comprehensive Invited Review

Redox Features of the Cell: A Gender Perspective

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Reviewing Editors: Calogero Caruso, Dipak Das, and Tatiana Oberyszyn

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[4]In accordance with the definitions proposed in the Institute of Medicine's Report (290); the term *sex* is used when differences are genetic or phenotypic; the term *gender* is used when referring to social and cultural influences based on sex.

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ABSTRACT

Reactive oxygen and nitrogen species have been implicated in diverse subcellular activities, including cell proliferation, differentiation and, in some instances, cell injury and death. The implications of reactive species in human pathology have also been studied in detail. However, although the role of free radicals in the pathogenesis of human diseases has been extensively analyzed in different systems (*i.e.*, *in vitro, ex vivo*, and *in vivo*), it is still far from elucidated. In particular, the possible role of gender[4] differences in human pathophysiology associated with reactive species is a promising new field of investigation. Although the complex scenario this presents is still incomplete, important gender-associated "redox features" of cells have already been described in the literature. Here we summarize the different aspects of redox-associated molecules and enzymes in regard to gender differences in terms of the intracellular production and biochemical activity of reactive species. These are often associated with the pathogenetic mechanisms underlying several human morbidities (*e.g.*, degenerative diseases) and can represent a specific target for new pharmacologic strategies. Gender differences may thus pose an important challenge for future studies aimed at the clinical management of diseases characterized by a redox imbalance. *Antioxid. Redox Signal*. 9, 1779–1801.

I. INTRODUCTION

BYPRODUCTS OF OXYGEN METABOLISM lead to the production of reactive oxygen species (ROS). This term includes radicals as well as chemicals that, although not having unpaired electrons, can take part in radical-type reactions (*e.g.*, gaining or losing electrons). Examples of nonradical ROS include hydrogen peroxide (H_2O_2), hypochlorous acid, and singlet oxygen. Besides ROS, reactive nitrogen species (RNS) are known, such as nitric oxide (•NO) and nitrogen dioxide, as well as sulfur-based molecules (RSS) such as thinyl and perthinyl, and carbon-centered molecules (110). ROS, reactive nitrogen species, sulfur-based molecules, and carbon-centered molecules are generated by (a) UV light and X and gamma rays; (b) metal-catalyzed reactions; (c) neutrophils, macrophages, and other cells during inflammation; and (d) byproducts of mitochondria-catalyzed electron transport reactions and other mechanisms.These radicals also are present as pollutants in the atmosphere and are produced by xenobiotics (110).

The discovery of •NO, a radical that has a central role in the regulation of vascular tone, nerve function, and immune regulation (77, 195, 258), led to the emergence of the signaling function of RNS, which extends to many molecules including the potentially toxic nitric oxide radical (•NO) and nitric dioxide radical ($•NO_2$–) (3). In this paradigm, all reactive species (RS) are regulators of metabolic processes and are part of the chemistry of life (85, 87). It has also been shown that RS production can be mediated through G protein receptors, as is the case with angiotensin II (Ang II) signaling (99) and platelet-derived growth factor (254). Similarly, it has recently been shown that lipopolysaccharide (LPS) activation of Toll-like receptor 4 and tumor necrosis factor α (TNF-α) increases superoxide anion ($•O_2$–) production through the activation of NAD(P)H oxidase and NF-kB (210).

The production of ROS and RNS is a strongly regulated physiologic process (*e.g.*, by hormones such as estrogens and Ang II (79–81, 99). RNS- and ROS-based signaling involves protein tyrosine phosphatase 1B (153), thioredoxin (TRX) (232), sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) 2 (1), Ras (3), and the activation of numerous transcription factors [NF-κB, activating protein-1 (AP-1), Nfr2, p53, glucocorticoid receptors, *etc*.] (111).

Finally, cells present a complex machinery of antioxidant compounds and enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx), glutaredoxin (GRX), and thioredoxin TRX (110, 242). The activities of these compounds have been shown to be regulated by nutrients (113) and by hormones, including sex hormones (79, 81).

The disruption of redox signaling and control (DRSC) can occur either with or without a modification in the balance between antioxidants and prooxidants (111), and it may be localized in certain cellular compartments (225). Much of the interest in disruption of redox signaling and control stems from its implication in the aging process and in human degenerative diseases (Alzheimer disease, amyotrophic lateral sclerosis, Parkinson disease, diabetes mellitus, atherosclerosis, hypertension, *etc*.) and in some genetic pathologies such as Down syndrome, Fanconi anemia, and Friedreich ataxia (110, 260).

Recently, a different response of males and females to the disruption of redox signaling and control has been hypothesized. In particular, it has been suggested that (a) in comparison with males, female smokers have 50% higher levels of autoantibodies *versus* DNA-derived oxidative products (194); and (b) males and females have a different ability to maintain

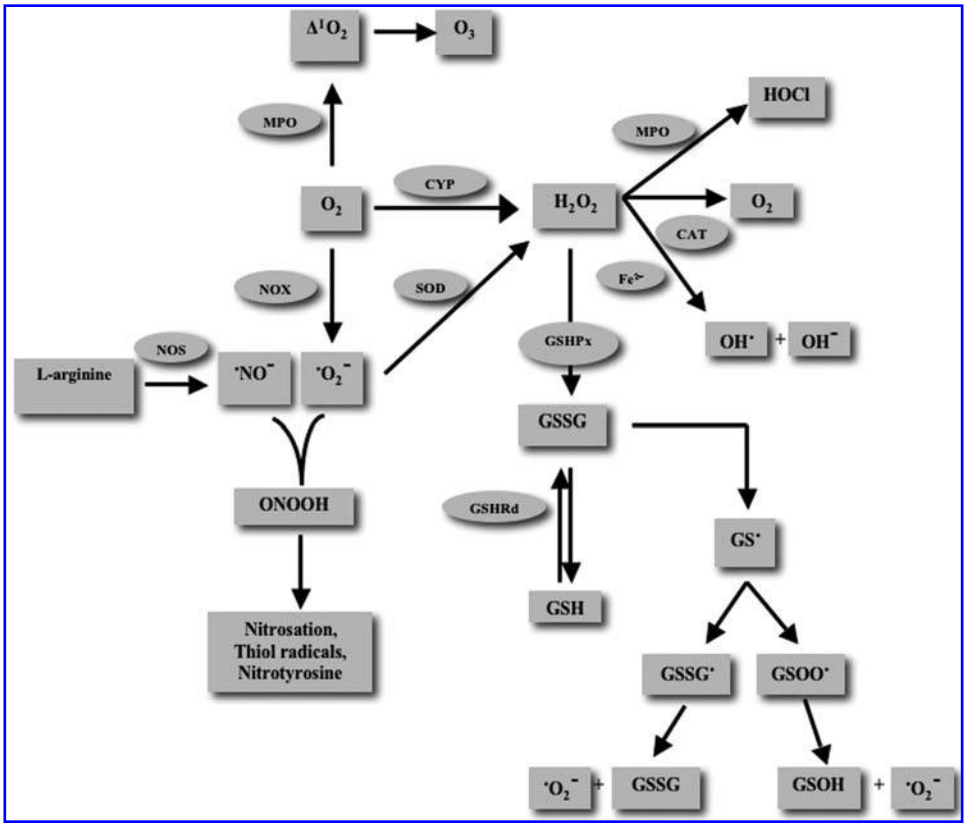

FIG. 1. Schematic representation of different mechanisms leading to the formation of reactive species (RS). CAT, catalase; CYP, cytochrome P-450 enzymes; Δ^1O_2:, singlet oxygen; GSHPx, glutathione peroxidase; GSSG, oxidized glutathione; MPO, myeloperoxidase; NOS, nitric oxide synthase; SOD, superoxide dismutase.

their redox status during aging. This could be of great importance in the development of new therapeutic strategies (154).

The purpose of this review is to provide an overview of the gender-based differences in redox signals and redox alterations. The intention is to provide a conceptual framework of information of relevance for future investigations aimed at understanding the mechanisms underlying these differences and to point to possible implications in the field of gender pharmacology. This analysis is conducted in the context of human pathologic conditions.

A. Endogenous production of oxidants

ROS, reactive nitrogen species, carbon-centered molecules, and sulfur-based molecules are produced endogenously through either enzymatic [NAD(P)H oxidase, xanthine oxidase (XOD), *etc*.] or nonenzymatic reactions (autooxidation of molecules such as glyceraldehydes, FMNH2, FADH2, adrenalin, noradrenalin, dopamine, and thiol-containing molecules such as cysteine in the presence of O_2) (110, 242) (Fig. 1). $\bullet O_2-$ is also produced by numerous enzymes when the substrate is inadequate. For example, nitric oxide synthase (NOS) produces $\bullet O_2-$ when the substrate L-arginine or the cofactor tetrahydropteridines are insufficient (292). Additionally, cytochrome P-450 enzymes (CYP) can produce $\bullet O_2-$ as a side reaction when they break down target molecules (110) (Fig. 2). Radicals can be formed in different cellular organelles, such as mitochondria, endoplasmic reticulum, and peroxisomes (see Fig. 2), as well as in extracellular compartments, and may be tissue spe-

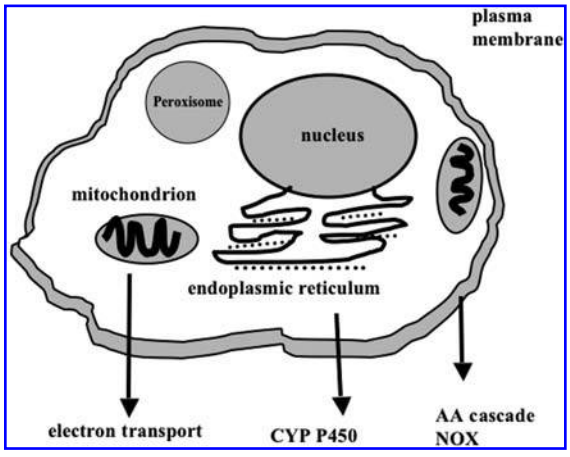

FIG. 2. The major intracellular sites of reactive species production. AA, arachidonic acid; CYP, cytochrome P-450 enzymes; NOX, NAD(P)H oxidase.

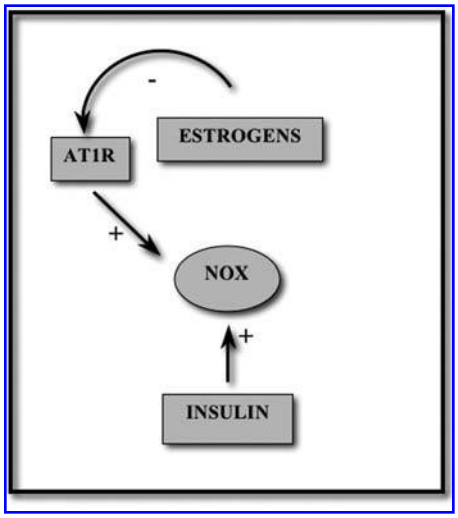

FIG. 3. Schematic representation of NAD(P)H oxidase (NOX) modulators. AT1R, angiotensin receptor 1; (+), activating activity; (-), inhibitory activity.

cific, depending also on the metabolic state and age of the cells (110, 235). Significantly, one metabolic process that contributes to peroxisomal generation of H_2O_2 is the beta oxidation of fatty acids (235).

The role of reduced glutathione as mediators in various vital processes is well established (64, 84, 85). The chemical nature (biologic life, diffusibility through cellular membranes) of individual species as signaling molecules has important implications. Reduced glutathiones can interact with one another, either activating or inactivating different signaling pathways (64). For instance, $\bullet O_2-$ is known to inactivate the vasodilator •NO, leading to endothelial dysfunction and vasoconstriction (35), whereas H_2O_2 has been shown to act as a vasodilator in a number of vascular beds (162, 177).

B. Antioxidants

Cells have developed several lines of defense (which may vary from cell to cell) to nullify the effects of reduced glutathione by using a battery of antioxidant enzymes and molecules such as vitamins E, A, and C; albumin; urate; cysteine; ceruloplasmin; transferring; thyroxine; selenium; *etc*. (242).

The antioxidative enzyme system consists of the family of SOD enzymes, which catalyze dismutation of the $\bullet O_2-$ into H_2O_2, which is removed by GSHPx and catalase in all cells (242). GRX and TRX also participate in reduced glutathione removal (242). The redox state is also controlled through the generation of intracellular reduced glutathione (GSH) and NAD(P)H (110, 242). Thus, glutathione reductase (GSHRd), glucose-6-phosphate dehydrogenase (G6PDH), γ-glutamyl transpeptidase (γ-GT), and glutathione synthetase (GS) play major interactive roles in the replenishment of cellular reducing power (110, 242) (see Fig. 1).

The interplay between prooxidants and antioxidants can also be "dynamic" in nature (84, 110, 242). A single reactive species can reduce the activity of another, as in the case of $\bullet O_2-$ and •NO (201). For example, calcineurin, a calcium-controlled phosphatase that regulates transcription during development, can be blocked by $\bullet O_2-$, and this block can, in turn, be counteracted by •NO (200).

II. THE INTRACELLULAR REDOX MACHINERY AND GENDER

A. NAD(P)H oxidase

1. Features. This enzyme family was first identified in phagocytic cells, where it has an essential role in host defense (110). Today a number of membrane-bound NAD(P)H oxidases have been found in numerous nonphagocytic cells, including wall-vessel cells (30, 151). In many cell types, the NAD(P)H oxidase can be regulated by agents such as Ang II and insulin, which act through G-coupled and tyrosine kinase receptors (125, 220, 224) (Fig. 3).

2. Gender. NAD(P)H oxidase is largely influenced by gender. It has been shown, for example, to interact with estrogens. In particular, hormones seem to reduce the generation of $\bullet O_2-$, decreasing NAD(P)H oxidase in endothelial cells (104, 284) and, through this enzyme, to inhibit endothelin-1 protein secretion and mRNA expression (132). Furthermore, in cultured vascular smooth muscle cells (VSMCs), 17 β-estradiol (10 μM) suppresses Ang II–mediated NAD(P)H oxidase activity, reducing Rac-1 small GTPase translocation that is necessary for enzyme activation (267). In rat cardiac fibroblastic cells, estrogens reduce both angiotensin receptor 1 (AT1R) expression (291) and Ang II–induced cell proliferation and ET-1 gene expression (41) (see Fig. 3). This suggests that ROS have a central role in the estrogen/angiotensin interplay, at least in the cardiovascular system.

NAD(P)H oxidase seems to be influenced by other sexual hormones. In particular, progesterone can increase its activity in VSMCs (283). Nevertheless, microvessels of hypertensive male rats overexpress NAD(P)H oxidase and present higher $\bullet O_2-$ concentrations under basal conditions than those of females (58). In prostatic tissue, NAD(P)H oxidase activity is dramatically increased by castration (202), whereas gene expression of SOD2, GSHPx1, TRX, and peroxiredoxin are decreased. Catalase and GSHRd are unchanged (202). Significantly, these

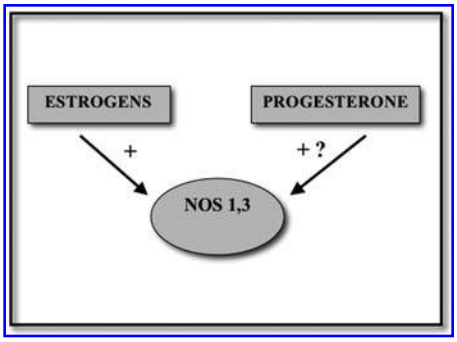

FIG. 4. Schematic representation of the effects of sexual hormones on nitric oxide synthase 1,3 (NOS1,3)(+) activating activity,

changes are partially counteracted by testosterone (202), confirming the importance of this male hormone in controlling the activity of these enzymes. Physiologic variations in hormone levels (menstrual cycle, pregnancy, and menopause) and the interaction between various reactive species could also be of importance. For example, ROS produced from NAD(P)H oxidase have been shown to oxidize the NOS cofactor tetrahydrobiopterin, resulting in the production by NOS of large amounts of $\bullet O_2^-$ in the endothelium (150). However, because sexual hormones may affect many sources of RS and many antioxidant systems, it is difficult to evaluate the real importance of hormonal control on a "single" enzyme.

B. Nitric oxide synthase (NOS)

1. Features. A short-lived free radical, •NO, is produced by a family of enzymes that includes NOS1, NOS2, and NOS3. This family produces •NO from arginine, and all three NOS can also contribute to ROS production, as they are susceptible to the uncoupling that leads to the formation of $\bullet O_2^-$ (rather than •NO) under certain conditions (209) (see Fig. 1).

The activity of •NO depends on both cyclic-GMP–dependent and GMP-independent pathways. The latter involves the stimulation of calcium uptake into intracellular stores by the sarcoplasmic/endoplasmic reticulum calcium ATPase (49). •NO can affect cell function by interacting with cysteine thiol groups, and the modification of proteins by thiols may involve as much as 30–50% of total cellular proteins, including ion channels and pumps, phosphatases, metabolic enzymes, and hemoglobin (2, 49, 66, 86) (see Fig. 1). Finally, $\bullet O_2^-$ and H_2O_2 decrease •NO bioactivity, forming peroxynitrite ($ONOO^-$) and consuming •NO in the presence of myeloperoxidase (MPO) (201).

2. Gender. Several studies indicate that NOS1 and 3 are upregulated by estrogens (7, 95, 97, 143, 168, 187, 285, 299) (Fig. 4). Importantly, physiologic levels of estrogens directly modulate the expression of NOS, particularly the neutrophil NOS1. Thus, changes in circulating levels of oxidized products of •NO during the menstrual cycle, as well as during estrogen replacement therapy, may originate from different cell types, not only endothelial cells (93). In male neutrophils, estrogen induction of NOS1 shows a time and dose dependency (93).

The estrogen activation of NOS1 and 3 seems to be initiated by a membrane receptor, which induces extracellular signal-regulated kinase, cAMP, and Akt/protein kinase B signaling (213, 222). However, the Akt/protein kinase B pathway seems to be more active in females than in males (36), and the increase in •NO production induces vasodilation. This mechanism provides insights into how estrogens may affect vessel function in females (105). Very recently, it also was seen that progesterone increases coronary blood flow in pigs, stimulating endothelial •NO release (55, 105). Thus, female sexual hormones, especially estrogens, seem to play an important role in the regulation of NOS1 and NOS3 activity and expression.

Regarding NOS2, the scenario still appears controversial. Some reports indicate that NOS2 gene is downregulated by estrogens (116, 255), whereas other authors hypothesize that •NO synthesis is enhanced by estrogens (212). It was very recently shown that estrogen inhibits the subarachnoid hemorrhage–induced increase in NOS2 by increasing the association of NF-κB to estrogen receptors (240). NOS2 also appears to be regulated by progesterone (*i.e.*, by exacerbating LPS-induced NOS2 expression) (255).

Gender differences have been hypothesized to be involved in the activity of androgens in modulating NOS2 (*e.g.*, in inflammatory response), a "defensive" response that is gender dependent. Normotensive males display a lower expression of NOS2 in neutrophils (187) and a lower expression/activity of NOS3 than do females (144). Testosterone also promotes a mild increase in postischemic renal NOS2 activity, whereas male castration enhances NOS2 activation (212).

C. Myeloperoxidase (MPO)

1. Features. This lysosomal protein is released from the activated primary azurophilic granules of inflammatory cells (mainly neutrophils, monocytes, and activated macrophages) and is part of the host defense system catalyzing the formation of hypohalous acids from H_2O_2 and chloride (142). In addition to halides, it has been suggested that various organic and inorganic components found in blood plasma may potentially serve as naturally occurring substrates for MPO; these include, but are not limited to, estrogens (141, 142). MPO-derived oxidants have been linked with atherosclerosis, kidney damage, some cancers, multiple sclerosis, and Alzheimer's disease (142). However, they also play an important role in regulating blood vessel tone by acting on •NO levels (73) (Fig. 5).

2. Gender. Relevant gender differences have been described in neutrophils, a cell type rich in MPO. In particular, men have lower systemic neutrophil counts than do women (16). This is probably due to a difference in neutrophil survival, which is significantly higher in women than in men (192). In females, neutrophil counts are particularly high when estradiol and progesterone levels are elevated (16, 280). Hormonal variations influence not only the number of neutrophils, but also the expression of both estrogen receptors alpha (ERα) and beta (ERβ) subtypes, which are increased in the ovulatory phase of the menstrual cycle (191), at least in premenopausal women. However, 17 β-estradiol (10^{-8} *M*) upregulates both receptor subtypes in premenopausal women, whereas in men, it enhances only ERα expression (191, 250).

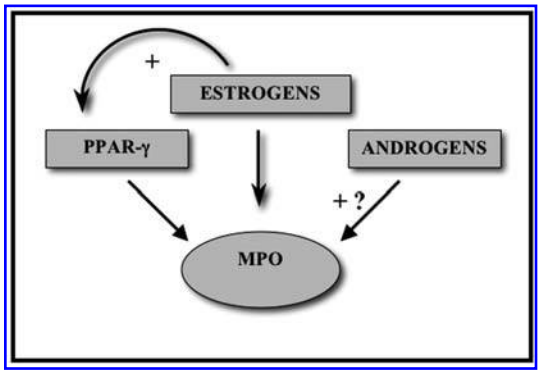

FIG. 5. Schematic representation of myeloperoxidase (MPO) regulation. PPARγ, proliferator-activated receptor γ; (+), activating activity.

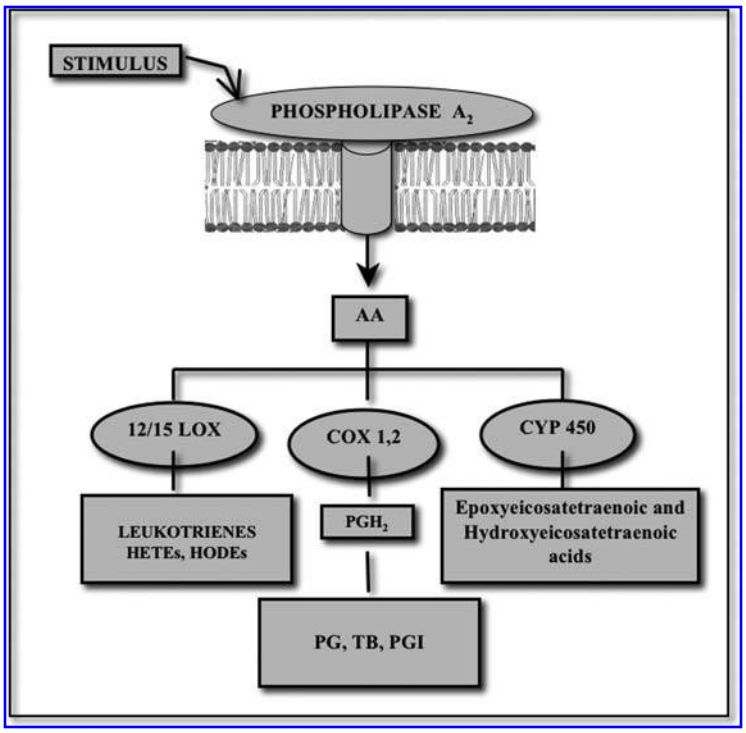

FIG. 6. Schematic representation of arachidonic acid (AA) cascade. COX, cyclooxygenase; CYP450, cytochrome P-450 enzymes; HETEs, hydroxyeicosatetraenoic acids; HODEs, hydroxyoctadecadienoic acids; LOX, lipoxygenases; PGH_2, prostaglandin H_2; PGI, prostacyclin; TB, thromboxane; PG, prostaglandin.

It is of considerable interest that estradiol, at physiologic concentrations, affects the function of neutrophils by enhancing the degranulation (including MPO) of activated neutrophils, and this could lead to an increase in prooxidant products (44). Estradiol promotes leukocyte–endothelial cell interactions that might contribute to the observed predominance of some autoimmune inflammatory diseases in females (47). Estrogen also has "proinflammatory effects" on the uterus, even promoting neutrophil influx, although these effects are "counteracted" by exposure to progesterone (262). Significantly higher levels of MPO activity and of thiobarbituric-reactive substance are present in male rat neutrophils after trauma–hemorrhagic shock than in female rats (266). It has very recently been shown that neutrophil activation induced by trauma–hemorrhagic shock or burn injury seems to be potentiated by testosterone; significantly, naive female neutrophils are more resistant to activation than are naive male neutrophils, and this resistance was found to vary over the estrus cycle (63). Thus, the control of neutrophil function could influence the interplay between sexual hormones. However, in trauma–hemorrhagic shock, gender differences are not limited to neutrophils: they are also detectable in blood plasma (63). The chemotaxis and random migration of neutrophils is enhanced by progesterone (188) and reduced by estradiol (186, 188), whereas testosterone does not have measurable effects on chemotaxis (188). Conversely, sexual hormones do not affect monocyte chemotaxis (188). Finally, neutrophils incubated with 17β-estradiol, progesterone, testosterone, and hydrocortisone show significantly reduced $\bullet O_2-$ production compared with control cells (20). The bulk of these results suggest that neutrophils and sexual hormone interactions may be highly complex. For instance, hormone effects depend partially on their relative concentrations [*e.g.*, during the menstrual cycle (263)], and on their source (different species and different ethnic groups) (241).

A number of studies report gender differences in association with −463GA polymorphism, a genotype with higher MPO

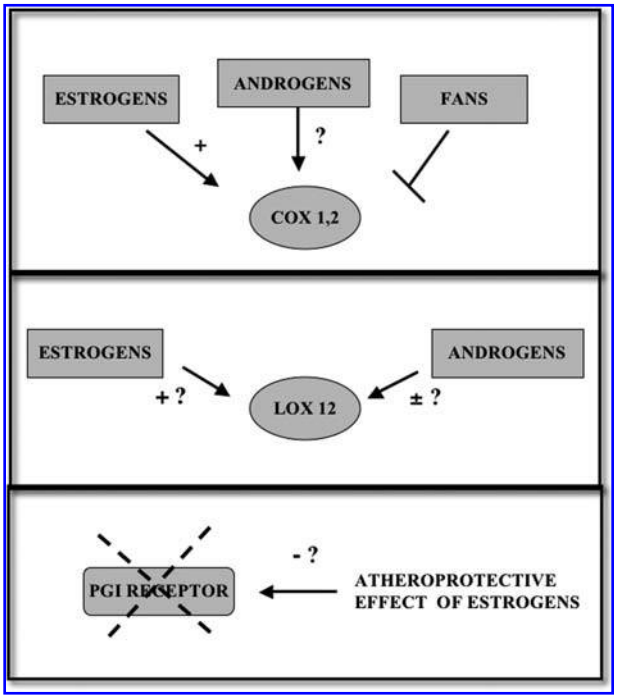

FIG. 7. Gender differences and arachidonic acid (AA) cascade. PGI, prostacyclin receptor; LOX, lipoxygenase; COX, cyclo-oxygenase; (+), activating activity; (-), inhibitory activity.

mRNA (147). Interestingly, this is associated with non–small cell lung carcinoma in women but not in men (161). Recently, it was also shown that the MPO gene is modulated by PPARγ *via* estrogens (147). Hence, in view of the relevance of neutrophils in reactive species generation and inflammatory response, it has been hypothesized that these different features could contribute to the gender differences detected in some inflammatory diseases (20, 53, 54, 231, 241) as well as in the response to infectious agents or in autoimmune diseases (9, 67, 238).

D. Arachidonic acid cascade

1. Features. The three different isoforms of phospholipase A_2 (PLA_2) release arachidonic acid (AA) from membrane phospholipids, whereas the cyclooxygenases (COXs) catalyze the conversion of arachidonic acid to prostaglandin (PGH_2) (197). PGH_2 is a substrate for PGE_2 synthase, which produces PGE_2 and other biologically active prostaglandins through further metabolism. Moreover, arachidonic acid is a substrate for lipoxygenases (LOXs), which catalyze the stereo-specific oxygenation of AA and linoleic acids into a complex series of signaling molecules, including the leukotrienes and hydroperoxides (Fig. 6) (31). Prostaglandins and leukotrienes regulate the inflammatory response and many other physiologic processes through the activation of G protein–coupled receptors (33) and LOX-peroxidized membrane, and the 15-LOX enzyme has been implicated in the oxidation of low-density lipoproteins, a key event in the initiation of atherosclerosis (221).

2. Gender. Some gender differences have been described in the AA cascade (Fig. 7). In particular, the rapid estradiol response in the colon (114) and in embryonic membranes (88), but not in chondrocytes, involves the eicosanoid signaling pathway (115, 257). An interaction between estrogen and AA also was described in vascular tissues. *In vivo*, estrogens inhibit Ang II–induced leukocyte adhesion to endothelial cells, rapidly inducing NOS3 and COX (7), whereas *in vitro*, stimulation of ERα augments PGI_2 production through the increase in COX2 expression (4). Nevertheless, more recent studies show that COX2 is downregulated in the vena cava by estradiol (119). Interestingly, deletion of the PGI_2 receptor removes the atheroprotective effect of estrogens in ovariectomized female mice (4). This mechanism restrains both oxidant stress and platelet activation in females and suggests that treatment of patients with chronic disease with selective inhibitors of COX2 could undermine protection from cardiovascular disease in premenopausal females (72). Finally, aspirin, a well-known inhibitor of COX, initiates the synthesis of 15-epi-lipoxin A4 in a gender-specific manner (45). This could be of interest in studies aimed at developing a gender-specific pharmacology, in particular with regard to antiinflammatory drugs.

The effects of testosterone on the AA cascade are stll to be elucidated. It seems to increase the thromboxane/prostaglandin ratio (281) and platelet aggregation (185). In endothelial cells, dihydrotestosterone decreases the LPS-induced RNA expression of COX2, IL-6, and other inflammatory markers through NF-κB (205). The effects of testosterone on the AA cascade (and on other systems that generate reactive species) have to date been poorly analyzed. In effect, knowledge of male physiology could also provide useful information regarding postmenopausal female physiology (*i.e.*, when testosterone plasma levels are strongly increased when compared with those of estrogen).

Few studies examining sex differences in LOX have been published. Catechol estrogens are more potent inhibitors of leukotriene synthesis (IC_{50} values, 0.044–0.16 μM) than thromboxane (IC_{50} values, 0.99–2.1 μM) and also inhibit PGE_2 synthesis (5). Estradiol significantly increases 12-LOX activity in platelets in a dose-dependent manner. The stimulatory effect of estradiol on platelet LOX is blocked by the antiestrogen nafoxidine hydrochloride (40), but no significant difference in the basal activity or amount of platelet 12-LOX is observed in clinical studies that include men and women (264). However, E-12/15-LOX genes are downstream targets of the progesterone receptor pathway, at least in the uterus (158).

Our knowledge of gender differences detectable in leukotriene synthesis is an important issue in clinical practice. For instance, leukotriene tone is increased in asthmatic patients (207), in whom relevant gender differences have been detected (199, 243). In particular, leukotriene B_4 activates the peroxisome proliferator–activated receptor α (PPARα), a nuclear transcription factor, and participates in the physiologic mechanism that blocks the damaging effects linked to inflammatory response (235), and a sexual dimorphism has been experimentally documented for fibrates, which activate PPARα (131, 259, 295). However, although the details of cellular processes modulated through PPARα are still under investigation in several laboratories, it has been suggested that PPARα could control inflammation, lipid metabolism, carcinogenesis, and some pharmacotoxicologic processes (235). Our knowledge of the sexual dimorphic effects of PPARα should thus be further analyzed and improved.

E. Xanthine oxidase (XOD)

1. Features. Xanthine oxidase (XOD) is a member of a group of enzymes known as molybdenum iron–sulfur flavin hydroxylases and catalyzes the hydroxylation of purines, catalyzing the reaction of hypoxanthine to xanthine and xanthine to uric acid, which also acts as an antioxidant (110). Importantly, the levels of XOD in endothelial cells are modulated by ROS derived from the NAD(P)H oxidase (183).

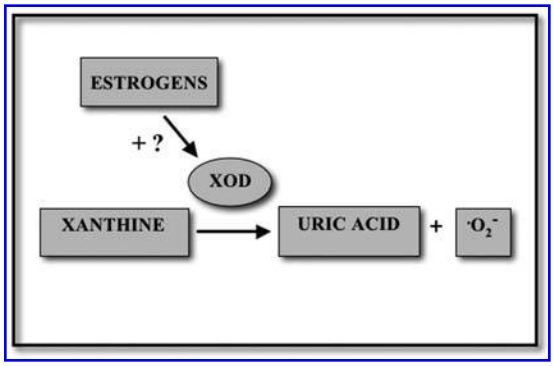

FIG. 8. Schematic representation of the possible effects of estrogens xanthine oxidase (XOD) in endothelial cells. (+), activating activity.

2. Gender. XOD may be induced by low doses of estrogen in endothelial cells (79) (Fig. 8). Studies aimed specifically at evaluating the possible effects of sex on XOD function are lacking. However, some gender differences have been found when uric acid is considered. Men seem to have higher plasma levels of uric acid than women (11), although these differences could also be associated with differences in its elimination (218). Finally, it is not clear whether plasma levels of uric acid should be considered risk factors for cardiovascular diseases in men and women. Most studies have reported that the association of serum uric acid and cardiovascular mortality, hypertensive target organ damage, and cardiovascular events is more pronounced in women than in men (28, 91, 179, 276). In contrast, another study reports an association between serum uric acid and left ventricular hypertrophy in Japanese hypertensive men, but not in women (148).

F. Cytochrome P450 (CYP)

1. Features. The cytochrome P450s (CYPs) are a superfamily of cystein enzymes that are key mediators of the oxidative transformation of endogenous and exogenous molecules (101). The human CYP family contains 57 isoforms. They can generate ROS and have been associated with the pathogenesis of a series of diseases that include cancer, premature aging, heart (arterial) damage, and osteoporosis (in association with estrogen/testosterone imbalance). They also play a role in the adverse reactions of many drugs. In particular, mitochondrial CYP-type enzymes catalyze central steps in steroid biosynthesis (cortisol, aldosterone, pregnenolone, progesterone, androgen, estrogen) and can also act as a NAD(P)H oxidase [*i.e.*, oxidizing NAD(P)H in the absence of substrates]. The degree of uncoupling depends on the CYP and steroid substrate (112) (see Fig. 2).

The CYP enzymes have been reported in both hepatic and extrahepatic tissues. However, tissue-specific metabolism of endogenous substrates, including AA, may be of vital importance for physiologic functions of CYP.

2. Gender. CYP activity is largely modulated by sexual hormones (226). The enzyme is specifically influenced by single sexual hormones, and this happens in a tissue-specific way (149). For example, renal CYP2J5, an enzyme involved in the metabolism of AA to epoxyeicosatrienoic acids, is more expressed in men than in women after puberty, and its expression seems to be upregulated by estrogens (165). Apart from sex-biased regulation, CYP3A levels can also be altered by a number of compounds that are both CYP3A substrates and inducers of CYP3A expression. These include glucocorticoid-receptor agonists and antagonists, phenobarbital, and rifampicin. The increase in CYP3A levels is mediated *via* the pregnane X receptor (PXR) and constitutive androstane receptor (CAR), both members of the nuclear receptor superfamily. On activation by a xenobiotic ligand, PXR and constitutive androstane receptor bind as heterodimers with retinoid X receptor to their respective response elements and thus bring about CYP3A induction (8, 26, 171, 174), which is often sexually dimorphic (296). A gender-specific expression of these nuclear receptors is yet to be reported. Nonetheless, during pregnancy, progesterone mediates the induction of PXR up to 50-fold (176). Conversely, estradiol can activate constitutive androstane receptor by increasing its nuclear translocation (138). Additionally, several clinical studies indicate an increased clearance of many CYP3A drug substrates, such as cyclosporine, erythromycin, diazepam, and prednisolone in women over that in men (126, 300).

Members of the P450 superfamily metabolically oxidize many xenochemicals, thereby forming electrophilic intermediates (46, 102, 145), and it has been proposed that the carcinogenic activity of many chemicals, including estrogens (10), is due to electrophilic intermediates. Radicals may be generated during estrogen metabolism by oxidation of catechol estrogens, which are oxidized to their corresponding semiquinones and quinines (56, 57). Evidence is mounting for a role of CYP metabolites as activators of multiple signaling pathways and their role in inflammation, platelet aggregation, fibrinolysis, cellular injury adaptation, and repair. All in all, additional information on the influence of sex on the regulation of CYP expression and activity in hepatic and extrahepatic localizations is urgently needed.

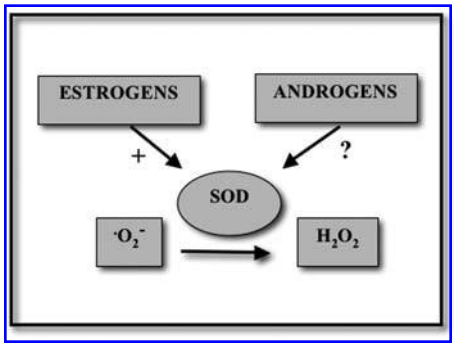

FIG. 9. Schematic representation of the effects of sexual hormones as superoxide dismutase (SOD) modulators. (+), activating activity.

G. Superoxide dismutase (SOD)

1. Features. This important cellular enzyme family catalyzes the dismutation of $\bullet O_2-$ to H_2O_2 and oxygen molecules (242) (Fig. 9). Derangement of the superoxide dismutase (SOD) system and related prooxidant molecules has been described in various pathologic conditions (275, 288).

2. Gender. Clinical and experimental studies suggest that SOD expression and activity are higher in females than in males (24, 234, 283) (Table 1) and are regulated by progesterone and estrogen levels (283). These results support the existence of an intriguing interplay between estrogen and progesterone in controlling SOD activities, suggesting that, to understand the biologic complexity, experimental studies should use both hormones. Furthermore, gender differences also involve Cu,ZnSOD. The difference in myogenic tone, found in control animals, is undetectable in specific knockout animals (272).

H. GSH/GSSG and TRXRed/TRXO cycles, GRX, and NADPH/NADP$^+$

1. Features. The three most relevant redox systems inside cells are NADPH/NADP$^+$, TRXRed/TRXOx, and reduced glutathione/oxidized glutathione (GSH/GSSG) (242). They keep proteins in a reduced state and regulate protein activity, including redox-sensitive transcription factors, *via* GSH transferase (GSHT) and TRX (83, 109). Both oxidized glutathione forms are reduced by GSHRd and TRX/GRX (83), and GSH, under oxidizing conditions, is the source of disulfide-*S*-oxides specifically modulating the redox status of thiols, indicating the existence of specialized cellular oxidative pathways (66, 129) (see Fig. 1).

TRX isoforms modulate many cellular activities (206) and are also specific electron donors for peroxiredoxins as well as directly reducing ROS (83, 133, 204). They cooperate with the GSH/GRX system to maintain the cellular thiol disulfide redox status in living cells (121).

NAD(P)H is a critical factor for the activity of many enzymes (NOS, CYP) and for the Fenton reaction of iron-mediated catalysis of H_2O_2 (237). Thus, a reduction in the supply of NAD(P)H could have a profound effect on ROS production.

2. Gender. The effect of estrogens on GSH may be very complex and tissue specific. For instance, γ-glutamylcysteine synthase (γ-GCS) acts to limit GSH synthesis (193, 269). Nevertheless, Dabrosin and Ollinger (57) suggested that estrogens decrease liver GSH. This decrease may reflect an initial increase in DRCS due to estradiol metabolism, which generates semiquinones and quinines (57) and results in the production of $\bullet O_2^-$ radicals and H_2O_2 (83).

One of the proteins associated with ERα is the protein disulfide isomerase, which has two distinct functions: as a molecular chaperone to maintain properly folded proteins, and as a regulator of the redox state of proteins by catalyzing the thiol-disulfide exchange reaction and influencing the ability of ERα to mediate changes in gene expression (236). The expression of protein thiol/disulfide oxidoreductases such as protein disulfide isomerase, TRX, TRXRd, and GRX in vascular endothelial cells is elevated by micromolar concentrations of estrogens, whereas progesterone and testosterone do not affect these oxidoreductases (74). The effect of estrogen on GRX may be very important in view of the fact that GRX and TRX partially share a redox "sensor" activity (245). Significantly, GRX also regulates the redox state of Akt/protein kinase B (198), which is tightly regulated by estrogens (see later). Concerning GSHPx, some authors (24) have found that its expression and activity are higher in female than in male rats. However, no gender differences have been found in human polymorphonuclear leukocytes (234). The effects of estrogens on antioxidant enzymes are summarized in Table 1.

Regarding NAD(P)H, an important issue could be represented by a human disease: G6PDH deficiency (237). G6PDH deficiency could influence life span in male and female centenarians (62, 90) because G6PDH-deficient subjects produce fewer reduced glutathiones (27, 237). Conversely, the overexpression of this enzyme in adipocytes stimulates oxidative stress and inflammatory responses, thus affecting the neighboring macrophages (211). Significantly, G6PDH also has been implicated in the different levels of ROS production detected in male and female embryos (214).

III. REACTIVE SPECIES AND THE EXTRACELLULAR MILIEU

A. Inflammatory response (cytokine-induced reactive species formation): the paradigmatic role of TNF-α

Several factors indicate that estrogen and androgen are the major causes of the gender differences observed in immune modulation (27, 287). For example, sexual dimorphism has been described in the production of inflammatory cytokines (25, 216), estrogen attenuates immune cell cytokine production after trauma–hemorrhage (256), and significantly, inflammatory cytokines predict type 2 diabetes risk (122, 248). The estrogen effect could be selective for some cytokines; for example, the complete deficiency in aromatase correlates with a decrease in production of the inflammatory cytokine IL-6 but does not affect TNF-α production (217). Female mice produce less TNF-α and macrophage inflammatory protein-1α in response to systemic endotoxin than do male mice and are resistant to endotoxin-induced mortality (166). Pharmacologic inhibition of poly(ADP-ribose) polymerase (PARP) failed to provide further protection in the female animals, whereas in male mice, the inhibition of this enzyme reduces TNF-α and MIP-1β production, reduces mortality, and prevents the development of endothelial dysfunction (166). Fittingly, these gender differences are partially diminished ovariectomized animals. Moreover, in males, the administration of estrogens reduces TNF-α and MIP-1 production, suggesting an important activity of estrogens by a mechanism recently proposed by Mabley *et al*. (166) (Fig. 10). This could be of importance if one considers that RS-induced cell injury, including DNA injury, involves the activation of PARP (130, 279).

Some cytokines are considered inflammatory mediators able *per se* to induce the formation of reduced glutathione (59, 60, 118, 293). For example, the binding of TNF-α to its receptors causes activation of two major transcription factors, AP-1 and NF-κB, which in turn induce genes involved in chronic and acute inflammatory responses such as NOS2 and COX2 (19). The TNF-α promoter itself contains NF-κB and AP-1 binding sites and is subject to positive autoregulation, a property that is important for the amplification of inflammatory responses. It is also worth noting that NF-κB and AP-1 are ROS activated (19).

In this context and in regard to gender differences, several insights come from the study of TNF-α. For instance, in men, androgens may reduce TNF-α levels and inhibit NOS2 activation and Akt/protein kinase B phosphorylation (172, 173). Additionally, in the postischemic kidney, they lead to greater inflammatory responses (212). After the administration of LPS, males develop more severe hypothermia and greater airway hyperresponsiveness than do females, and TNF-α content is greater in the bronchoalveolar lavage fluid of males than in that of females (37). Gonadectomy reduces airway inflammation in

Table 1. Expression and Activity of Antioxidant Enzymes Regulated by Estrogens in Different Cells and Tissues

Tissue/cells	*Proteins*	*Expression*	*Activity*	*References*
Thoracic aorta VSMCs from female Sprague-Dawley rat	MgSOD	+	+	253
Thoracic aorta VSMCs from female Sprague-Dawley rat	ecSOD	+	+	253
VSMCs of thoracic aorta from female Sprague-Dawley rat	CuZnSOD	=		253
VSMCs of thoracic aorta from female Sprague-Dawley rat	GSH	=		253
Thoracic aorta VSMCs from female Sprague-Dawley rat	CAT	=		253
Female mice (C57-BL6) Aortic ring	MgSOD	+	ND	253
Female mice (C57-BL6) Aortic ring	ecSOD	+	ND	253
Monocytes from women from *in vitro* fertilization samples	MgSOD	+	ND	253
Monocytes from women from *in vitro* fertilization samples	ecSOD	+	ND	253
Murine osteoclast	GHSPx	+		152
Cultured normal human breast epithelial cells	CAT		−	56
Cultured normal human breast epithelial cells	GHSPx		+	56
MCF-7 human breast cancer cells	CAT		−	190
MCF-7 human breast cancer cells	SOD I		+	190
MCF-7 human breast cancer cells	SOD II,		+	190
MCF-7 human breast cancer cells	GPx		+	190
MCF-7 human breast cancer cells	GPDH activities		+	190
MCF-7 human breast cancer cells	CAT		=	190
Blood vessel mitochondria	MnSOD	+		251
Blood vessel mitochondria	GHSP	+		251
Blood vessel mitochondria	CAT	+		251

(continued)

TABLE 1. EXPRESSION AND ACTIVITY OF ANTIOXIDANT ENZYMES REGULATED BY ESTROGENS IN DIFFERENT CELLS AND TISSUES (CONT'D)

Tissue/cells	*Proteins*	*Expression*	*Activity*	*References*
Female hepatic tissue	GST		+	184
Female hepatic tissue	GHSPx		=	184
Male aged liver	SOD		−	265
Male aged liver	CAT		−	265
Male aged liver	GSHR		−	265
MCF-7 cells (a mammary gland tumour cell line)	MnSOD	+		23

males but not in females, whereas the administration of exogenous testosterone to intact females increases their inflammatory responses (37). Accordingly, in castrated males, exogenous testosterone decreases the LPS-induced airway hyperresponsiveness, whereas it is increased in sterilized females, suggesting that these gender differences are mediated, at least in part, by androgens. Estrogen-deficient animals and menopausal women have higher serum levels of TNF-α compared with either estrogen-replaced animals or animals treated with etanercept, an antagonist of TNF-α, or premenopausal women (12, 118, 137, 297). Etanercept treatment increases NOS3 expression and decreases NAD(P)H oxidase expression (12). A complex interaction has been described between TNF-α, NOS3, and estrogens (59). Moreover, Imahara *et al*. (127) showed that women produce significantly less LPS-induced TNF-α and IL-1β without reducing IL-6, possibly through an alteration of MAPK phosphorylation. In addition, other inflammatory pathologic conditions, such as wound healing (14), atherosclerosis (120), uveitis (189), and leukodystrophy (178), are shown to be influenced by estrogens, which reduce disease susceptibility, severity, and damage.

Conversely, some reports suggest that the secretion of TNF-α is enhanced by estrogens (230). It has been shown that exposure to estrogen stimulates the production of both TNF-α and IL-1 from human and rat monocytes/macrophages (146). The effects of estrogen appear to be dose dependent, in that physiologic concentrations of estrogen inhibit IL-6 production, whereas high levels of estrogen (during pregnancy) drive IL-6 production and are also suppressive of cell-mediated immunity (146). Several studies of the effects of testosterone on inflammatory states have been conducted. For instance, testosterone elevates monocyte adhesion to endothelial cells (181). Mendonor monocyte-derived macrophages express at least 4 times as many receptors as do those obtained from women (180). Furthermore, they increase foam cell formation in male but not in female donors. This is probably associated with differences detected in atherosclerosis-related genes in males and females (180).

Experimental studies support the *in vivo* findings mentioned earlier. Estrogens downregulate the expression of inflammatory genes, such as those coding for NOS2 or matrix metalloprotease 9, enzymes directly involved in the progression of the inflammatory response, and inhibit the biochemical and morphologic activation of macrophages (34, 116, 273). Recently, it also was shown that estrogen prevents inflammatory gene transcription induced by inflammatory agents by inhibiting NF-κB intracellular transport, an immediate-early event in the inflammatory signaling cascade, which is also activated by ROS (94). Ablation of TNF subtype 1 receptors improves postischemic myocardial function, reducing the activation of p38 mitogen-activated protein kinases (MAPKs) and reducing the expression of IL-1β and IL-6 in males but not in females (282).

At present, many intriguing questions concerning the functions of reactive species are still open, and, considering the central role of inflammation in generating reactive species, it is time to explore further the influence of gender in this crucial event. This must be pursued in a highly integrated manner in view of the fact that reactive species-mediated activation is a critical component in deciding cell fate in response to different stimulations. For example, reduced glutathione generated from

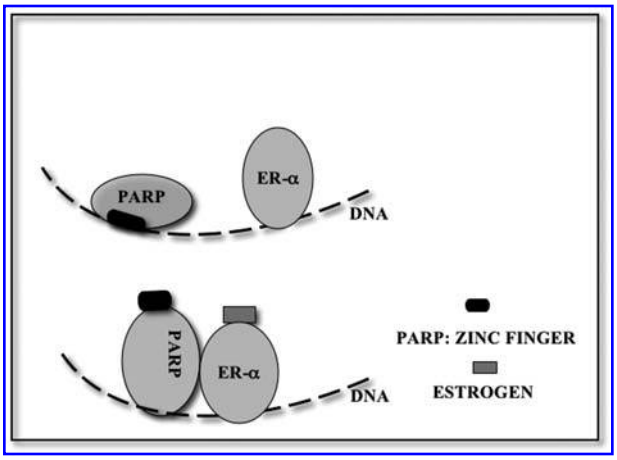

FIG. 10. Schematic model of interplay between estrogen, estrogen receptor alpha (ER-α), and poly(ADP-ribose) polymerase (PARP) with DNA single-strand breakage and PARP activation. *Top:* PARP and ER-α interact weakly in the absence of estrogen, and PARP can recognize DNA strand breaks and become catalytically activated. *Bottom:* Effects of estrogen. Note the interaction between PARP and ERα. PARP may thus anchor to the DNA, reducing its ability to recognize DNA single-strand breaks, thereby preventing its activation.

inflammatory cells is one of the key factors promoting hepatocarcinogenesis (169).

IV. REACTIVE SPECIES, INTRACELLULAR SIGNALS, AND GENDER

A. Molecular targets of reactive species

Until the discovery of •NO as an intra- and intercellular messenger, our knowledge of cellular redox chemistry was confined to the electron-transport chain, the formation of reactive species, and their interaction with cellular macromolecules and antioxidant systems (110, 242). A number of studies revealed the involvement of ROS, reactive nitrogen species, sulfur-based molecules, carbon-centered molecules in cell signaling (108), and the term "oxidative regulation" was coined to indicate the active role of oxide-reductive modifications of biomolecules, mostly proteins. These modifications, formerly known as "oxidative stress," are now referred to as "signals" and contain biologic information that is necessary for the maintenance of cellular homeostasis. Recently, the hypothesis also emerged that cellular oxidant signaling could be mediated by discrete localized redox circuitry (225). Considering the chemical differences in the various reduced glutathiones, it is evident that they have important implications in cellular signaling: different kinds of reduced glutathione are able to activate different signaling pathways, which may then lead to divergent (and potentially opposing) consequences. The generation of ROS, reactive nitrogen species, sulfur-based molecules, and carbon-centered molecules is believed to be a vital component of cellular signaling mechanisms. These species integrate the expression of genes involved in energy production, oxygen transfer, cellular differentiation, and free radical scavenging. For instance, the role of oxidants in insulin action (170), in vascular endothelial growth factor signaling (50), and in signaling of cytokines (TNF-α and IL-1β) (293) has been analyzed. Interestingly, TRAF4, a component of TNF-α signaling, directly binds a component of the NAD(P)H oxidase complex and provides further evidence for the link between ROS generation and signal transduction (293). A further example is represented by the so-called peroxide tone, required for the activity of some enzymes (92). Signaling pathways of reactive species and the influence on them of gender is summarized later.

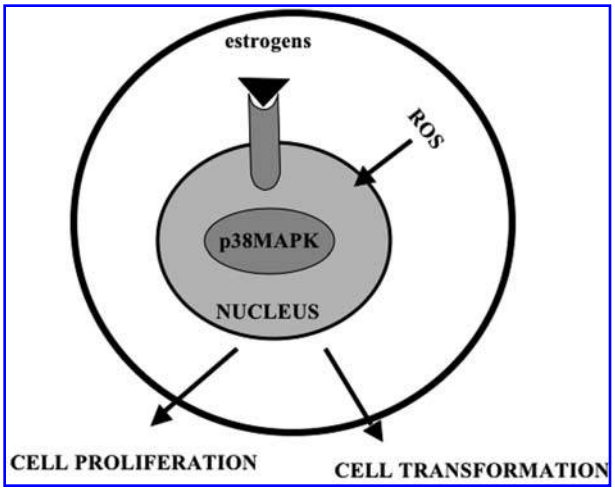

FIG. 11. Schematic representation of p38MAPK activation. Estrogens, through membrane-bound receptors and reactive oxygen species (ROS), activate p38MAPK, promoting cell proliferation and transformation. p38MAPK, mitogen-activated protein kinase.

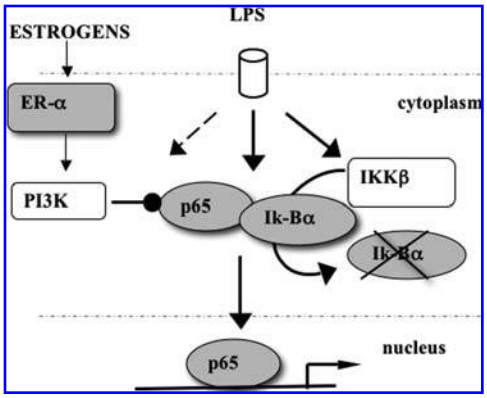

FIG. 12. Schematic representation of estrogen effects on NF-κB activation. Estrogen receptor-α (ER-α) inhibits NF-κB translocation mediated by LPS.

B. Mitogen-activated protein kinases (MAPKs)

These kinases are a family of serine/threonine kinases associated with the signaling cascades that control energy metabolism, cell proliferation, differentiation, and death. Although the ability of exogenously generated reactive species to activate MAP kinases has been known for more than a decade (15), a few studies have investigated MAPK as targets of intracellular ROS (128). For instance, in VSMCs, Ang II–induced p38 MAPK activation is dependent on H_2O_2 (270), most likely derived from NAD(P)H oxidase (277). This could indicate that endogenously generated ROS interact with this cascade. H_2O_2 also activates JNK, and current evidence associates ROS derived from NAD(P)H oxidase, TNF-α and, probably, NOS with JNK stimulation in many tissues (239). However, MAPK is also regulated by estrogens through membrane-bound receptors (229, 246). In rat, hepatic stellate cells, which contain ERβ and are ROS activated, estradiol reduces ROS formation, inhibiting NAD(P)H oxidase, and mitigates the activation of MAPK and, consequently, cell proliferation and transformation. MAPK enzymes are a target for the nongenomic action of progesterone (78), and progesterone-mediated MAPK activation seems to be of relevance in breast cancer (78), where it is largely responsible for cell-cycle progression (219, 244) (Fig. 11). The MAPK cascade also appears to be controlled by androgens, with an activity that seems to be tissue specific. In *osteoblasts,* androgens inhibit the MAPK signaling pathway (289), whereas in other cell types, they induce this enzyme (117). Intracellular protein kinases are emerging as key mediators of steroid hormone receptor action *via* crosstalk with reactive species inside the cells.

C. Akt/protein kinase B

Akt/protein kinase B is a serine/threonine kinase with a critical role in intracellular signaling pathways and exerts its effects on survival and apoptosis (124). Akt/protein kinase B has been found to be responsive to extracellular signaling factors, oxidative and osmotic stress, irradiation, and ischemic stress (32). It is also critical in insulin signaling (48), and the Ser473 phosphorylation of Akt/protein kinase B leads to phosphorylation of a threonine residue on the insulin receptor beta-subunit, resulting in diminished autophosphorylation of the receptor and weakened insulin signaling (261) (see Fig. 3). In neurologic and heart tissue, as well as in ovarian cancer cells, Akt/protein kinase B is controlled by estrogens in a rapid and nongenomic way (36, 164, 167, 213, 298). It is also regulated through ERβ and ERα (89, 252). The former, ERβ, is found to be involved in redox regulation through Akt/protein kinase B *via* the GSH/GRX system (269). Hence, it has been suggested that estrogen-mediated activation of both Akt/protein kinase B and of MAPK could be strongly implicated in the control of the redox state of cells (123) *via* their target activity on NOS, COX, CYP, NAD(P)H oxidase, and SOD (107). Significantly, a gender difference in Akt/protein kinase B has also been observed in *in vivo* studies in humans. Young women possess higher levels of activated Akt/protein kinase B than comparably aged men or postmenopausal woman (36). These findings suggest that the gender differences occurring in Akt/protein kinase B activation could be implicated in the higher "resistance" displayed by females toward cardiovascular diseases (36).

D. Gene expression and transcription factors

Over the years, interest in reduction/oxidation-sensitive transcription factors has gained great importance because redox(y)-sensitive transcription factors are associated with the development and progression of many human diseases. Their ultimate regulation therefore has potential for clinical applications. reactive species and antioxidants are known to influence the expression of a number of genes and signal-transduction pathways; they are thought to act as subcellular messengers for certain growth factors (6). In the last decade, rapid growth has occurred in the identification of genes influenced by redox changes: one example is gene expression governed by the transcription factor NF-κB, which serves as a critical regulator of many genes, including those of proinflammatory cytokines. Cross-talk between estrogen receptors and NF-κB has recently been described (22, 136) (Fig. 12). This cross-talk appears to contribute to the control of inflammatory response. As a general rule, estrogens are antiinflammatory agents, whereas activated NF-κB initiates and maintains cellular inflammation. In this context, recent studies have shown that estrogens inhibit NF-κB activation (22). Estrogens also upregulate the expression of antioxidant genes such as GSHPx and Mn-SOD, although the mechanism through which estrogens upregulate these enzymes remains only partially identified (23).

Regarding *in vivo* studies in human pathology, a striking gender difference in atherosclerotic vascular disease has been hypothesized (17). The mechanism underlying this difference is still unknown, but it could involve the activity of estrogen on the antioxidant enzymes or on NOS3. However, a further point should be considered in this regard: dihydrotestosterone enhances the binding of monocytes to the endothelium, a key early event in atherosclerosis, through a reduction in NF-κB inhibitory protein. This is detected in male-derived endothelial cells only (61), so that androgen has a sexually dimorphic effect.

Concerning the brain, it has been suggested that aging and gender/sex influence the expression of many transcription factors. In the cortex, age increases NF-κB in both male and female adult rats, whereas age decreases AP-1 in the cortex and hippocampus of older females only; these changes seem to be estrogen independent (233), indicating that not all differences are dependent on the hormonal milieu.

A further important question concerns sex chromosome genes, which are present in different amounts in the genomes of the two genders and might be expressed differently, inducing gender-specific patterns of development or functioning or both. Male mammals possess genes on the non-recombining region of the Y chromosome, which are absent in females but which encode 27 different proteins that might cause masculine patterns (38). Alternatively, genes on the non-pseudoautosomal portion of the X chromosome, which are "doubly" present in females but only "singly" in males, could result in sex-specific effects on account of the different dosage. These differences are balanced by X silencing. Nevertheless, a significant percentage of X genes escapes inactivation, at least in humans (38). One example could be that of Ang II type 2–receptor gene. This is located on the X chromosome and is involved in myocardial left ventricular hypertrophy in women, but not in men (223).

V. REACTIVE SPECIES, CELL HOMEOSTASIS, AND GENDER

A. Mitochondria

Mitochondria are an important source of reduced glutathione, and their components are targets of damage associated with disruption of redox signaling and control (268, 278). In eukaryotic cells, mitochondria are organelles devoted to catalyzing the oxidation of organic nutrients by O_2. The mitochondrial respiratory chain is one of the major structural and functional parts of mitochondria and consists of five complexes, known as complexes I–V. Functionally, mitochondria generate the vast majority (>95%) of cellular energy in the form of ATP and ROS from NADH and FADH by using O_2. During normal metabolism, the most important sources of $\bullet O_2^-$ are the respiratory chain complex 1 (NADH dehydrogenase) and complex III (ubiquinone–cytochrome *c* reductase), with the latter being the major source (268). It has been calculated that 1 to 3% of O_2 reduced in mitochondria is in the form of $\bullet O_2^-$ (268). The intramitochondrial steady-state concentrations of $\bullet O_2$ and H_2O_2 are directly related to the production rates and inversely related to the enzymatic activity of SOD and GSHPx, the most relevant mitochondrial antioxidant enzymes. Interestingly, mitochondria also represent sources of ROS in response to external stimuli such as *N*-methyl-D-aspartate, TNF-α, and integrin (70, 96, 118, 286). In summary, the mitochondrial production of reactive species integrates a number of signal-transduction pathways for a wide variety of biologically active molecules such

as inflammatory cytokines (106), thyroid hormone (75), peroxisome proliferators (39), 25H-dihydroxyvitamin D_3 (140), and several steroid hormones, including cortisol and sexual hormones such as estrogen (51, 80, 175).

Remarkably, sexual hormones are also involved in mitochondriogenesis. Testosterone, 17β-estradiol, and progesterone modulate the expression of nuclear factors (PPARγ, PGC1α, NRF1, GABPA, and TFAM) involved in the control of mitochondrial biogenesis and thermogenic function. In particular, 17β-estradiol activates the Akt/protein kinase B pathway by inhibiting PTEN mRNA expression, whereas progesterone positively stimulates mitochondriogenesis and cellular differentiation by increasing the mRNA expression of nuclear factors such as GABPA and TFAM. Finally, testosterone reduces the transcription of peroxisome proliferator–activated receptor coactivator 1α (PGC1α), the master factor involved in uncoupling protein1 (UCP1) expression, an endogenous encoupler, and mitochondrial biogenesis (227).

The activity of estrogens at mitochondrial level has been more extensively studied than that of other sexual hormones. In particular, estrogen exposure triggers the immediate and rapid generation of intracellular ROS ranging from a onefold to severalfold increase in a variety of cells, mainly in perinuclear mitochondria (81). The functional consequences of 17β-estradiol–induced ROS formation includes enhanced cell motility, as shown by the increase in cdc42 and activation of Pyk2 and the elevated phosphorylation of signaling proteins such as c-jun and CREB. Additionally, ROS activate the binding of three oxidant-sensitive transcription factors: AP-1, CREB, and nuclear respiratory factor 1, thus suggesting that the generation of ROS acts as a signal transducer in the estrogen pathway (81). Estrogens also appear to affect mitochondrial DNA *via* NRF, a nuclear transcription regulator responsible for increasing the transcription of nuclear-encoded mitochondrial genes (139, 251). In the cerebral vasculature, estrogen promotes mitochondrial efficiency and decreases oxidative stress, increasing mitochondrial capacity for oxidative phosphorylation while decreasing the production of ROS (69). Although some data suggest that estrogens reduce the formation of ROS at the mitochondrial level, other findings show that low doses of estrogen may increase ROS production, at least in endothelial cells (79). Hence, the possibility of a histotype-specific (*i.e.*, cell-type specific) response to estrogenic stimulation should also be taken into account. Moreover, *in vitro*, estrogens also increase the production of cytochrome *c* (251), which plays a pivotal role in cellular respiration and apoptosis. The presence of estrogen receptors on mitochondria (42, 43, 157, 294) further emphasizes their importance in mitochondrial function (80).

It is not yet known whether other gender-associated mitochondrial differences are linked to estrogens. Significantly, in some tissues such as brown adipose tissue, female mitochondria are larger and have more cristae than those of males (135, 228). Moreover, in the female liver, mitochondria seem to have a higher protein content and higher cardiolipin levels than those in males (134). These variations are associated with a higher capacity and efficiency in inducing substrate oxidation in females (134, 135). Liver and brain mitochondria in females also have more GSH and higher activity and expression of SOD and GSHPx than those in males, and this is associated with a lower (50%) production of H_2O_2 (278).

Several studies have also investigated the relations between calcium ions and mitochondrial energetics with respect to gender differences (21, 29, 52, 65, 82, 156, 160, 208). For example, a gender difference has been implicated in the regulation of mitochondrial calcium, with females showing a lower uptake of calcium, which is critical in certain pathophysiologic conditions (*e.g.*, after ischemia/reperfusion injury) (13).

More in general, females have a higher mitochondrial capacity for oxidative phosphorylation and a lower capacity to generate ROS. This mitochondrial efficiency could also be related to the gender differences detected in the aging process and in the different lifespans observed in the two sexes.

B. Apoptosis

Mitochondria, the major source of reactive species inside the cell, also play a central role in determining cell fate, through the regulation of both cell survival and cell death by apoptosis (98). Depending on the type of stimulus, two different apoptotic pathways have been described. The first involves a receptor-mediated proapoptotic cascade (*e.g.*, induced by ligation of TNF family receptors), whereas the second directly depends on mitochondria (*i.e.*, it is induced by mitochondriotropic drugs). In any case, both pathways lead to the release of apoptogenic factors from mitochondria that result in apoptosis. In light of this, very little has been discovered regarding the possible role of gender differences in determining apoptotic susceptibility. A few significant articles have nonetheless investigated some aspects. Some years ago, it was shown that in monoblastoid cells and in the peripheral blood mononuclear cells of women with normal menstrual cycles, estrogen delays apoptosis through the reduction of oxidative species (274). More recently, it was suggested that estradiol enhances growth and reduces receptor-mediated (TNF-α–induced) apoptosis in endothelial cells (159). Additionally, 17β-estradiol also reduces apoptosis induced by H_2O_2 in endothelial cells through the attenuation of H_2O_2-induced mitochondrial condensation and by decreasing the release of cytochrome *c* from the mitochondria. These findings suggest that 17β-estradiol attenuates H_2O_2-induced apoptosis *via* estrogen-receptor activation (163). It thus seems that estrogen may either interfere with receptor-mediated apoptosis or act directly on mitochondria.

Gender differences are also detectable in the cytotoxic challenge aspect of apoptosis induction. For instance, programmed cell death proceeds mainly *via* an apoptosis-inducing factor-dependent pathway in XY neurons, compared with a cytochrome *c*–dependent pathway in XX neurons. This sex-dependent susceptibility seems to be related to the inability of XY neurons to maintain intracellular levels of GSH (68). This could also be of importance in understanding the gender differences detected in neuroprotection studies that found females to be more resistant to stressing agents (203). Furthermore, it has been shown that female brain neurons display a stronger activation of caspase 3 (301), the effector enzyme leading to apoptosis execution. It has been shown that estrogens delay apoptosis in the female peripheral blood mononuclear cells (76), B cells (100), and endothelial cells (249). Altogether these findings seem to argue in favor of a different susceptibility to apoptosis induction in male and female cells.

Further investigations into apoptotic pathways are still needed in the context of gender differences. In consideration of

the importance of ROS in apoptosis and the relevant role played by the disturbance of apoptosis in a plethora of human diseases, more studies must be carried out in the coming years to ascertain the possible causality of the gender differences–associated occurrence of apoptosis in the pathogenesis of apoptosis-associated diseases characterized by marked gender differences such as immune and neurodegenerative diseases.

VI. CONCLUSIONS AND FUTURE DIRECTIONS

The role played by gender differences in biologic processes is far from being elucidated. We briefly describe here the relations between single actors in intracellular redox balance, taking into account the "gender" data in the literature. However, this approach provides a partial and fragmentary point of view. The whole scenario is obviously more complex. A framework of closely linked subcellular events takes place in male and female cells that still represents a new research field. Some important points should be considered in advance.

1. The widespread distribution of the receptors for steroid sex hormones. The local production of sex hormones and their autocrine/paracrine effects may have important roles in mediating their activity.
2. The importance of aging as a supplementary modulator of the redox state. For instance, markers of oxidative stress in skeletal muscle (oxidative protein adducts, carbonyl groups, 3-nitrotyrosine) are significantly modified only in the elderly postmenopausal women and not in the elderly men, whereas they are lower in young females compared with young males (18). Together with the aging and sex-dependent effect on transcription factors (233), this might imply the presence of a sex-related mechanism in the redox regulation during aging (23, 24).
3. Animal cells clearly possess the biochemical machinery for generating reactive species in a regulated fashion (*i.e.*, they normally undergo a variety of responses to physiologic levels of reactive species). The mechanisms by which the oxidants are synthesized and removed is a strictly regulated process and is also to be considered as cell-type specific.
4. The nature of the oxidative processes that are actually occurring *in vivo* (*e.g.*, during receptor-mediated signaling). For instance, at physiologically relevant concentrations, an ER-mediated pathway is capable of inducing tissue-specific increases in ROS through the regulation of antioxidant genes. Thus, the complex cross-talk between various subcellular targets can result in different outcomes. In addition, the existence of the estrogen receptor–related receptors (ERRs) complicates the pathway that links estrogens to the redox status. The ERRs have some structural homology with the estrogen receptors ERα and ERβ, but do not bind estrogens (55). The ERR's expression is downstream of estrogen signaling *via* ERα in heart and uterus, but not in liver (55). Hence, the regulation mechanisms can be even more complicated in some tissues. A further element of complexity is the "ligand promiscuity" described for estrogen receptors (103, 215).
5. An extraordinary diversity of co-activator and co-repressor protein complexes interacts with nuclear receptors and mediates their transcriptional activities (182, 247). The identification of these proteins, and the understanding of how they interact with nuclear receptors could help to explain the mechanisms of nuclear receptors and the influence of the redox state on their activity and *vice versa*.
6. Next, the importance of gender/sex in regulation of the redox state must be identified in humans. Recent lines of evidence indicate many gender differences in diagnosis, prognosis, and outcome of diseases with significant alterations of redox states, such as diabetes mellitus and coronary artery diseases (155, 196). Actually, it is not clear whether the previous gender differences are linked to variations of redox states.

All these points should be addressed in future studies to provide a clear-cut scenario of the relations between redox state and gender.

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ABBREVIATIONS

AP-1, Activating protein-1; Ang II, angiotensin II; AT1R, angiotensin receptor 1; AA, arachidonic acid; CAR, constitutive androstane receptor; JNK, c-Jun N-terminal kinase; COX, cyclooxygenase; CYP, cytochrome P-450 enzymes; DRSC, disruption of redox signaling and control; Δ^1O_2, singlet oxygen; ER, estrogen receptor; ERRs, estrogen receptor–related receptors; γ-GCS, γ-glutamylcysteine synthetase; γ-GT; GDs, gender differences; G6PDH, glucose-6 phosphate dehydrogenase; GRX, glutaredoxin; GSSG, glutathione disulfide; GSSG$-$, glutathione disulfide radical anion; GSHPx, glutathione peroxidase; GS$-$, glutathiyl radical; GSHRd, glutathione reductase; GST, glutathione S-transferase; GSOH, GS-sulfenic acid; GS, glutathione synthetase; HETEs, hydroxyeicosatetraenoic acids; HODEs, hydroxyoctadecadienoic acids; IL, interleukin; LPS, lipopolysaccharide; LOX, lipoxygenases; MAPK, mitogen-activated protein kinase; MPO, myeloperoxidase; NOX, NAD(P)H-oxidase; NOS, nitric oxide synthase; GSSG, oxidized glutathione; PGC1α, peroxisome proliferators–activated receptor coactivator 1α; PLA_2, phospholipase A_2; PARP, poly(ADP-ribose) polymerase; PXR, pregnane X receptor; PGI, prostacyclin; PG, prostaglandin; PGH_2, prostaglandin H_2; PTP, protein tyrosine phosphatase; PPARα, proliferator-activated receptor alpha; PDI, protein disulfide isomerase; RNS, reactive nitrogen species; ROS, reactive oxygen species; RS, reactive species; GSH, reduced glutathione; GSHT, reduced glutathione transferase; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; RSS, sulfur-based molecules; SOD, superoxide dismutase; GSOO, thiol peroxyl radical; TRXRd, thioredoxin reductase; TRX, thioredoxin; TB, thromboxane; TNF-α, tumor necrosis factor-α; VSMC, vascular smooth muscle cell; XOD, xanthine oxidase.

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