Review

MOLECULAR METABOLISM

Estrogen receptors subcellular localization and cardiometabolism

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ABSTRACT

Background: In addition to their crucial role in reproduction, estrogens are key regulators of energy and glucose homeostasis and they also exert several cardiovascular protective effects. These beneficial actions are mainly mediated by estrogen receptor alpha (ERα), which is widely expressed in metabolic and vascular tissues. As a member of the nuclear receptor superfamily, ERα was primarily considered as a transcription factor that controls gene expression through the activation of its two activation functions (ERαAF-1 and ERαAF-2). However, besides these nuclear actions, a pool of ERα is localized in the vicinity of the plasma membrane, where it mediates rapid signaling effects called membrane-initiated steroid signals (MISS) that have been well described *in vitro*, especially in endothelial cells.

Scope of the review: This review aims to summarize our current knowledge of the mechanisms of nuclear vs membrane ERα activation that contribute to the cardiometabolic protection conferred by estrogens. Indeed, new transgenic mouse models (affecting either DNA binding, activation functions or membrane localization), together with the use of novel pharmacological tools that electively activate membrane ERα effects recently allowed to begin to unravel the different modes of ERα signaling *in vivo*.

Conclusion: Altogether, available data demonstrate the prominent role of ERα nuclear effects, and, more specifically, of ERαAF-2, in the preventive effects of estrogens against obesity, diabetes, and atheroma. However, membrane ERα signaling selectively mediates some of the estrogen endothelial/vascular effects (NO release, reendothelialization) and could also contribute to the regulation of energy balance, insulin sensitivity, and glucose metabolism. Such a dissection of ERα biological functions related to its subcellular localization will help to understand the mechanism of action of "old" ER modulators and to design new ones with an optimized benefit/risk profile.

Keywords Estrogen receptors; Genomic effects; Membrane-initiated steroid signals; Energy balance; Glucose homeostasis; Cardiovascular system

1. STEROID HORMONE RECEPTORS: FROM SUBCELLULAR LOCALIZATION TO SIGNALING PATHWAYS

In a classical view of steroid receptor activation, the hormone binds to its cognate receptor in the cytoplasm, leading to dimerization and nuclear translocation. Then, this complex interacts with specific DNA sequences in target genes, providing the basis of the initial "two-step mechanism" of hormone action [1]. Accordingly, steroid receptors were initially viewed as primarily localized in the cytoplasm as monomers bound to heat shock proteins (HSPs). Steroid binding alters receptor conformation and triggers release from the HSPs, thereby allowing receptor dimerization and translocation in the nucleus where these dimers bind to specific DNA sequences and recruit numerous co-factors to regulate gene transcription [2,3]. This scheme perfectly applies to glucocorticoid and androgen receptors, as well as to estrogen receptors (ER) [4], although in many cells and tissues, unliganted ER have been mainly characterized as monomers primarily located in the nucleus [5,6].

Importantly, numerous steroid and non-steroid nuclear receptors have now been reported to be also present at the plasma membrane, even though their physiological functions *in vivo* remained completely unknown until recently. In addition to nucleus and plasma membrane, steroid receptors have been also visualized at the level of the mitochondria, endoplasmic reticulum and Golgi [6,7]. Interestingly, extranuclear actions of steroid receptor pools have been also described in plants, contributing to flowering and fertility regulation through the activation of tyrosine kinase receptors expressed at the cell plasma membrane [8]. The existence of a pool of steroid receptors at the plasma membrane suggests that these receptors have evolved to mediate extranuclear membrane-initiated signaling in addition to their role of transcription factor in the nucleus. Over the past few decades, more and more proteins have been identified for their ability to perform two or more distinct and relevant biochemical or biophysical functions that cannot be explained by gene fusions, multiple RNA splice variants, or pleiotropic effects [9]. We will see that ER should be considered as

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moonlighting proteins that switch between nuclear and membrane functions after undergoing post-translational modifications. According to the tissue-specific expression and subcellular localization of these receptors, the membrane and nuclear pools play their specific roles in rapid signaling and regulation of transcription, respectively, but can also probably interact in a still poorly recognized way.

Estrogen actions essentially result from the activation of two molecular targets, the estrogen receptors alpha (ERα) and beta (ERβ), which are encoded by two distinct genes (*ESR1* and *ESR2*, respectively) [10,11]. However, beside these two well recognized ER, two other G-protein-coupled membrane receptors (GPCR) have been reported to be activated by 17β-estradiol (E2), namely the G-protein-coupled receptor 30 (GPR30) and Gq-mER, this latter being identified mainly thanks to a pharmacological compound [12]. A paragraph will be devoted to ERβ and these two GPCRs, but the present review will be mostly focused on ERα, as a consequence of its prominent role in vascular and metabolic physiology and pathophysiology. We will attempt to summarize our current knowledge on the role of ERα subcellular localization, eliciting either nuclear or MISS effects, with a focus on their respective involvements in the vascular and metabolic actions of estrogens. Indeed, as described below, recent transgenic mouse models (targeting ERα activation functions or membrane addressing elements), together with the availability of new pharmacological compounds that selectively induce ERα MISS effects, recently provided significant new insights into the understanding of ERα signaling *in vivo*.

2. ERα IS THE MAIN MEDIATOR OF METABOLIC AND VASCULAR EFFECTS OF ESTROGENS

2.1. Evidence for the metabolic and vascular protection conferred by estrogens

Classically considered as reproductive hormones, estrogens have been recognized to also influence numerous physiological or pathophysiological processes. Among them, both clinical and experimental data demonstrate that estrogens elicit numerous beneficial actions on energy and glucose homeostasis. After menopause, estrogen deficiency favors visceral fat deposition, insulin resistance, and beta-cell dysfunction, leading to significantly increased risk of type 2 diabetes [13,14]. Conversely, in the main randomized trials, hormonal replacement therapy has been shown to reduce the incidence of type 2 diabetes in post-menopausal women [15–17]. Accordingly, in animal models from rodents to monkeys, bilateral ovariectomy impairs energy balance, insulin sensitivity, and glucose tolerance, while exogenous estrogen administration restores this protection [18]. Finally, the abolition of estrogen synthesis in subjects bearing inactivating genetic mutations of aromatase leads to obesity, visceral adiposity, insulin resistance and impaired glucose tolerance [19,20]. Genetically engineered mice models allowed to phenocopy these clinical observations, since aromatase gene invalidation similarly favors several features of the metabolic syndrome in both males and females [21].

Clinical and preclinical studies also provided considerable evidence that estrogens modulate cardiovascular physiology and responses to various situations [22]. The first decade after menopause is accompanied by an increase in blood pressure and has been associated with a higher risk of cardiovascular events such as myocardial infarction and stroke [23]. Even if the Women's Health Initiative (WHI) [24,25] did not initially confirm the expected protective action of estrogens against coronary heart disease and questioned their overall benefits, posthoc analysis suggested that the incidence of coronary artery events was reduced in women who initiated estrogen therapy soon after the onset of menopause, in contrast to the neutral or even increased risk observed in more aged women [26,27]. Experimental data demonstrate the major protective actions of estrogens on arteries. In particular, chronic E2 administration strongly prevents lipid deposition in mouse models of atherosclerosis, namely apolipoprotein E–deficient (*ApoE*$^{-/-}$) [28,29] and low-density lipoprotein receptor-deficient (LDLr$^{-/-}$) [30] mice. In addition, in several experimental models, ovariectomy exacerbates, whereas estrogen replacement attenuates, the course of hypertension [31,32]. E2 also increases basal nitric oxide (NO) production [33], accelerates reendothelialization [30,34] and prevents medial as well as neointimal hyperplasia after vessel injury [35]. Finally, E2 plays a crucial role in the ability of resistance arteries to remodel in response to a chronic increase in blood flow, which is necessary to optimize tissue perfusion [36,37].

Estrogens also exert protective effects in various animal models of myocardial and brain ischemia [38,39]. Enhancement of post-ischemic cerebral reperfusion, limitation of endothelial dysfunction, anti-inflammatory, and anti-apoptotic effects could participate to the neuroprotective action. Chronic and acute E2 treatment improves functional recovery after cardiac ischemia/reperfusion injury [40,41] and reduces cardiac necrosis [42]. E2 is also beneficial in a model of ischemic rabbit hindlimbs by favoring angiogenesis and perfusion [43]. However, the most impressive prevention of tissue necrosis elicited by E2 probably occurs at the level of the skin. Indeed, in a mouse model of skin ischemia, mimicking the surgery of skin flaps, we reported that E2 reduced up to 10-fold the necrosis as compared to untreated ovariectomized mice [44]. Altogether, it is now well accepted that estrogens elicit numerous beneficial actions on whole-body metabolism as well as on the vascular system.

2.2. Key role of ERα in vascular and metabolic protection

The crucial involvement of ERα in the metabolic and vascular protection conferred by endogenous estrogens was first suggested by a case report describing a man with a mutation in *ESR1* gene (coding for ERα) that led to a premature and severe metabolic syndrome associated to arterial dysfunctions [45]. A few years later, the first mouse model of ERα gene invalidation resulted in a similar phenotype characterized by accelerated weight gain, visceral adiposity, insulin resistance and glucose intolerance in both males and females [46,47]. However, these mice, generated by inserting a neomycin resistance cassette into *ESR1* exon 2 and thus named *ERα-Neo-KO* mice, were later demonstrated to still express a truncated 55 kDa ERα mutant form, resulting from a non-natural alternative splicing [48]. This ERα isoform, lacking a major part of the B domain and thus probably the functional AF-1 (see below for detailed ERα molecular structure), was sufficient to mediate the effects of E2 on endothelial NO production [48], on post-injury medial hyperplasia [49], as well as on endothelial healing [50]. Nevertheless, using a second mouse model characterized by a complete deletion of ERα (*ERα*$^{-/-}$) [51], our group demonstrated that ERα, but not ERβ, is absolutely necessary for E2 effect on reendothelialization [34] and on endothelial NO production [33]. Then, Pare et al. reported the same essential role of ERα for the protective action of E2 against medial hyperplasia in response to vascular injury [52].

Along with pharmacological approaches using selective ERα or ERβ agonists [53], studies in transgenic mouse models indicate that ERα is absolutely required for almost all the beneficial vascular actions of E2 [54]. The same conclusion applies to the metabolic influence of estrogens since ERα-deficient male and female mice (both *ERα-Neo-KO* and *ERα*$^{-/-}$ models) spontaneously develop a severe dysmetabolic phenotype associated with impaired energy expenditure and locomotion [46,47,55]. Moreover, we observed that ERα deletion (in *ERα*$^{-/-}$ males and females) exacerbates all the metabolic disorders (obesity, insulin

