Sex Steroids and Stem Cell Function

Rinki Ray,1 Nathan M Novotny,1 Paul R Crisostomo,1 Tim Lahm,2 Aaron Abarbanell,1 and Daniel R Meldrum1,3,4

1Departments of Surgery, 2Pulmonary and Critical Care Medicine, 3Cellular and Integrative Physiology, and the 4Center for Immunobiology, Indiana University School of Medicine, Indianapolis, Indiana, United States of America

Gender dimorphisms exist in the pathogenesis of a variety of cardiovascular, cardiopulmonary, neurodegenerative, and endocrine disorders. Estrogens exert immense influence on myocardial remodeling following ischemic insult, partially through paracrine growth hormone production by bone marrow mesenchymal stem cells (MSCs) and endothelial progenitor cells. Estrogens also facilitate the mobilization of endothelial progenitor cells to the ischemic myocardium and enhance neovascularization at the ischemic border zone. Moreover, estrogens limit pathological myocardial remodeling through the inhibitory effects on the proliferation of the cardiac fibroblasts. Androgens also may stimulate endothelial progenitor cell migration from the bone marrow, yet the larger role of androgens in disease pathogenesis is not well characterized. The beneficial effects of sex steroids include alteration of lipid metabolism in preadipocytes, modulation of bone metabolism and skeletal maturation, and prevention of osteoporosis through their effects on osteogenic precursors. In an example of sex steroid-specific effects, neural stem cells exhibit enhanced proliferation in response to estrogens, whereas androgens mediate inhibitory effects on their proliferation. Although stem cells can offer significant therapeutic benefits in various cardiovascular, neurodegenerative, endocrine disorders, and disorders of bone metabolism, a greater understanding of sex hormones on diverse stem cell populations is required to improve their ultimate clinical efficacy. In this review, we focus on the effects of estrogen and testosterone on various stem and progenitor cell types, and their relevant intracellular mechanisms.

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INTRODUCTION

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Address correspondence and reprint requests to Daniel R Meldrum, 2017 Van Nuys Medical Science Building, 635 Barnhill Drive, Indianapolis, IN 46202. Phone: 317-313-5217; Fax: 317-274-2940; E-mail: dmeldrum@iupui.edu.
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cells” with better therapeutic efficacy. To achieve this goal, the role of sex hormones on stem cell function must be elucidated. It is the purpose of this review to summarize the current knowledge of the effects of estrogens and androgens on various stem cell populations.

**Embryonic Stem Cells**

The expression of estrogen receptors ER-α and ER-β in embryoid bodies takes place as early as d 2, suggesting the influence of estrogen on the differentiation and proliferation of human embryonic stem cells (ESCs) and embryoid bodies (43). The effects of estrogens on ESC proliferation have been demonstrated by Han et al. 17β-estradiol induces an increase of [3H] thymidine incorporation by murine ESCs and enhances DNA synthesis, which is in turn inhibited by anti-estrogen tamoxifen. Estrogens upregulate the expression of ERα and ERβ protein levels and increase mRNA expression of the proto-oncogenes c-fos, c-jun, and c-myc. In addition, 17β-estradiol activates the MAPK cascade as well as cyclin-dependent kinases, with associated increases in cyclins D1 and E, which are important intermediaries in cell cycle progression (42).

Although it is known that estrogens enhance the proliferation of embryonic stem cells, the role of androgens is not well understood. The presence of androgen receptors (AR) in ESCs has been detected as early as 4.5 d in mice and 5 d in humans, and it also was observed that the concentration of AR increases during differentiation of ESCs in a stage-dependent manner (46). Testosterone or dihydrotestosterone treatment is not associated with any significant change in androgen receptor mRNA expression level. But, in contrast that suggests a testosterone depletion effect, nilutamide, a nonsteroidal antiandrogen, causes proliferation of ESCs through an increase of Akt protein expression and decreased cell-cycle inhibitor p27 (Kip1) expression (41). These findings suggest a definite role of both sex steroids in the differentiation and proliferation of embryonic stem cells.

**Mesenchymal Stem Cells**

MSCs are novel therapeutic agents for organ protection, and estrogens may enhance the protective function of MSCs by increasing or decreasing cytokine and growth factor production in these cells (47). Our previous study proved that gender differences exist in activated MSC function. In particular, lipopolysaccharide- (LPS) and hypoxia-induced VEGF production was significantly greater in female MSCs compared with male MSCs. Female MSCs express significantly less pro-inflammatory cytokines, TNF-α and IL-6, compared with male MSCs in response to acute LPS and hypoxia, suggesting their ability to limit inflammatory reactions (18). In males, TNFR1 regulates VEGF, TNF, and IL-6 production, whereas TNFR expression status does not affect cytokine and growth hormone production in females (19). We also observed gender differences in stem cell-mediated protection in a Langendorff preparation. Rat hearts were subjected to 25 min of warm global ischemia followed by 40 min of reperfusion and were assigned randomly to one of three groups: (a) vehicle treated; (b) male MSC treated; and (c) female MSC treated. Female MSC-treated hearts exhibited significantly improved contractility and compliance as compared with hearts treated with male MSC or vehicle (48).

Regarding the role of estrogens in osteogenic differentiation of MSCs, there is evidence that 17β-estradiol supports growth and differentiation mostly through the ERα receptor (49). This receptor bias may be attributed to interindividual variability and gender differences of osteoblast responses of MSCs to estrogen manifested by ERα polymorphism. In terms of male sex hormone effects, testosterone decreases the specific alkaline phosphatase activity in male MSCs but does not affect calcium deposition in either sex (50).

Bone marrow MSCs, when exposed to osteogenic differentiation medium supplemented with 17β-estradiol, increase the expression of bone morphogenetic protein (BMP) and osteocalcin, and significantly increase the deposition of bone matrix protein (BMP) and osteocalcin, and significantly increase the deposition of bone matrix protein.
calcium (51,52). 17β-estradiol also stimulates the expression of osteogenic genes for ALP, collagen I, and TGF-β1 by MSCs (12). These observations suggest the bulk of the heavy lifting in bone metabolism/physiology is handled by the female sex steroids.

Clues to the molecular mechanisms underlying the role of sex hormones in MSC differentiation may be found in the ERK pathway. Resveratrol, a phytoestrogen found in red wine, stimulates the expression of osteoblastic markers such as RUNX2/CBFA1, osteonectin, and osteocalcin in human bone marrow mesenchymal stem cell cultures. This effect is associated with a rapid activation of ERK1 and ERK2, and also can be inhibited by the ERK inhibitor PD98059. Resveratrol enhances osteoblastic maturation and calcium deposition into the extracellular matrix (53) and its effects on osteoblastic differentiation of MSCs (55) and significant E2 linked increases in the lipid stores of differentiated adipocytes (52).

**Hematopoietic Stem Cells**

There are gender differences in hematopoietic progenitor cell concentrations in cord blood samples collected from infants. Specifically, male infants had significantly higher median CD34+ cell concentrations than female infants (31.8/μL compared with 30.2/μL, respectively; P = 0.03). This relative increase is reflected in adult males, who, when compared with age-matched females, have an increased number of colony-forming cells, erythroblast colonies, and granulocyte-macrophage colonies in their peripheral blood (56). This signifies that gender may affect the hematopoietic potential of cord blood transplants (57).

The effect of sex steroids on B lymphopoiesis is the subject of extensive, continuing study. Sex steroids suppress B lymphocyte production in murine bone marrow. Pre-B-lymphocytes produce the heavy chain of IgM (μ chain) (58), under the influence of the IgM constant region gene (cμ) (59), which can act as a marker of pre-B cells. V(D)J recombination confers the ability of the immune system to respond to a vast number of foreign antigens, which occurs particularly in immature lymphocytes and is mediated by the recombination activating gene products Rag1 and Rag2 (60,61). 17β-estradiol treatment reduces cμ+ pre-B cells, associated with a decrease in Ig gene rearrangements and rag1 transcripts (62). It also has been demonstrated that ERα is predominantly responsible for mediating 17β-estradiol induced changes in B-cell precursors (63,64). These findings suggest that 17β-estradiol exerts negative influence on the production of B-lineage cells by modifying the differentiation, proliferation, and survival of early B-cell precursors.

A novel system for expansion of hematopoietic stem cells utilizes “selective amplifier genes” that encode fusion proteins (granulocyte colony-stimulating factor [G-CSF] mutant receptor and delta G-CSF mutant receptor) between the granulocyte colony-stimulating factor receptor (G-CSF-R) and the hormone-binding domain of estrogen receptor (ER). ERs were replaced with a mutant receptor (TmR), which specifically binds to 4-hydroxytamoxifen (Tm). Interleukin-3 (IL-3)-dependent Ba/F3 (mouse peripheral blood pro-B) cells and hematopoietic progenitor cells transduced with the fusion proteins showed IL-3-independent growth in response to Tm, whereas, the cells were insensitive to estrogen at concentrations up to 10^(-7) M to 10^(-6) M. Murine bone marrow cells transduced with G-CSF-TmR and delta G-CSF-TmR formed colonies in methyl-cellulose medium in response to Tm, but no colonies appeared with 10^(-7) M estrogen or without cytokines. These results suggest that the influences of endogenous estrogen can be ablated by using the G-CSF-TmR/Tm or delta G-CSF-TmR/Tm system to expand hematopoietic stem cells with potential therapeutic application (65).

Androgens exert an inhibitory effect on B lymphopoiesis (66,67), but enhance erythropoietic differentiation (68) and...
thrombocytopoiesis (69). Cultures of human erythropoietic precursor cells collected from children’s normal marrow in the presence of erythropoietin demonstrated a significant increase in the number of colony-forming units (CFU-E) and burst-forming units (BFU-E) of derived colonies in the presence of androgens (10^{-5} - 10^{-3} M). These colonies also showed increased uroporphyrinogen I synthase activity, indicating increased heme synthesis (68). Androgens also can rescue mature erythroid colony-forming cells from apoptosis induced by serum and growth factor deprivation (70), thus increasing erythrocyte population. Interestingly, castation of normal male mice leads to splenic enlargement and expansion of the B cell population, which is mediated via androgen receptors present in both immature B cells and marrow stromal cells. These effects can be reversed with androgen replacement (66), further elucidating the role of the male hormone in lymphopoiesis.

**Cardiac Fibroblasts**

Gender may affect the healing of ischemic myocardium through changes in the function of cardiac fibroblasts (CFs). These progenitor cells play a significant role in the remodeling of ischemic myocardium, and the signal transduction pathways controlling the proliferation of CFs under hypoxia-induced stress reveal significant gender differences. These studies found females to be resistant to hypoxia-induced inhibition of DNA synthesis associated with decreased expression of NFκB and increased expressions of p53 and bcl-2 in comparison to males (71,72). Estrogens exert modulatory effects on cardiac fibroblast function. 17β-estradiol inhibits proliferation and collagen synthesis (3H-proline incorporation) in male and female CFs in a similar way, and facilitates beneficial cardiac remodeling following ischemia. Consequently, hormone replacement therapy using 17β-estradiol may exert protective effects on post menopausal women against cardiovascular events (73).

Cardiac fibroblasts express estrogen receptor protein, and stimulation of CFs with 17β-estradiol causes nuclear translocation of these proteins, indicating one effect of estrogen on gene regulation (74). In cardiac fibroblasts, 17β-estradiol plays an inhibitory role on renin-angiotensin system-induced gene expression, signal transduction, and ECM remodeling. Angiotensin II increases fibroblast proliferation and synthesis of collagen types I and III through the upregulation of expression of the angiotensin AT(1) receptor gene and β1 integrins. 17β-estradiol can prevent these increases in proliferation and AT(1) receptor mRNA levels and can attenuate the collagen synthesis in response to angiotensin II. 17β-estradiol inhibits AngII-stimulated expression of β1 integrins significantly and attenuates collagen gel contraction (75).

Estrogens can improve cardiac fibroblast-mediated remodeling of ischemic myocardium through both genomic and nongenomic mechanisms. 17β-estradiol exerts an inhibitory effect on the growth of cardiac fibroblasts through both ERα and ERβ (76). Underlying molecular mechanisms include increased mitogen-activated protein (MAP) kinase p42/44 activation and decreased p38 activation (77). In addition, 17β-estradiol increases the steady-state mRNA level of transforming growth factor-β, and fibronectin in these cells (78). A recent study has demonstrated that the selective estrogen receptor agonists PPT (4,4’4”-[4-propyl-([1]H)-pyrazole-1,3-5-triyl) tris-phenol) for ER-α and DPN (2,3-bis[4-hydroxyphenyl]propionitrile) for ER-β, stimulate the large-conductance Ca^{2+}-activated K’ (BK)[Ca]) channels in cultured human cardiac fibroblasts (HCFs). In whole-cell configuration, depolarizing pulses evoked large outward currents (I_{Ko}) with an outward rectification, the amplitude of which was increased in the presence of DPN or PPT. Paxilline, a selective blocker of BK(Ca) channels, could reverse the DPN- or PPT-induced amplitude of I_{Ko}. However, no change in the transcriptional level of the BK(Ca)-channel α-subunit was observed by RT-PCR analysis in chronic treatment with these two compounds. These findings suggest that estrogen induces a rapid stimulatory effect on human cardiac fibroblasts via a nongenomic mechanism through the activation of BK(Ca)-channels (79). Building on this understanding of the molecular mechanisms in cardiac fibroblasts may enable us to modify the functions of these cells.

**Endothelial Progenitor Cells**

Blood contains endothelial progenitor cells (EPC), which can differentiate into endothelial cells and modulate healing of injured vessels. In one study on a healthy middle-aged population without known cardiovascular risk factors, it has been demonstrated that women exhibited a distinctly higher EPC colony-forming capacity (approximately 150%) and greater migratory activity (40%) compared with men (80). Moreover, human women with a higher plasma estrogen concentration showed a significantly higher level of circulating EPCs. Increase in the number of EPCs by 17β-estradiol is mediated by decreased rate of apoptosis through a caspase-8-dependent pathway (81). The effects of estrogen on EPCs are mediated via ERα receptor (82). However, another similar study failed to demonstrate significant gender-specific differences in the frequency of colony formation (83).

To explore sex hormone specificity in EPCs, ERTα KO mice were treated with 17β-estradiol, which failed to induce migration, tube formation, adhesion, and estrogen-responsive element-dependent gene transcription activities. In bone marrow transplantation models, endogenous EPC migration and capillary density at the border zone of ischemic myocardium was reduced in 17β-estradiol treated ERTα KO mice. Using a murine ischemic heart model, it also was shown that migration of EPCs into the ischemic border zone was impaired in ERTα KO bone marrow transplant mice. Downregulation of VEGF also was noted in EPCs from ERTα KO mice both in vivo and in vitro (44). It was postulated that 17β-estradiol
mobilizes EPCs via endothelial nitric oxide synthase–mediated activation of matrix metalloproteinase-9 (84). Upregulation of MMP-9 results in the release of soluble Kit-ligand (sKitL), which facilitates the transfer of endothelial cells from the quiescent to proliferative pool (85).

Disease models in animals also have been employed to examine the effects of sex hormones on progenitor cell functionality. In spontaneously hypertensive rats, the number of differentiated and adherent EPCs derived from bone marrow was lower compared with age-matched normotensive rats. Treatment with 17β-estradiol significantly increases the number of EPCs. EPCs derived from hypertensive rats show low telomerase activity and early senescence. Estrogen treatment delays senescence and augments telomerase activity through PI3-K/Akt pathway (86,87).

Regarding male sex hormone effects, recent clinical studies suggest that androgens increase the number of circulating EPCs through a possible effect on bone marrow. It has been revealed that hypogonadotrophic hypogonadal men have low circulating EPCs that increase significantly after androgen treatment (88). A direct effect of testosterone also was suggested by expression of androgen receptor (AR) mRNA and protein in human EPCs. Synthetic androgen, methyltrienebolone (R1881), causes AR translocation in the nucleus, suggesting its activation increases proliferation, migration, and colony formation activity of these cells. Proliferation, migration, and colony formation activities of the EPCs could be abolished by pretreatment with flutamide (89). A greater understanding of these molecular mechanisms will yield insights into how gender differences affect the healing process in patients.

**Preadipocytes**

Regulation of lipid metabolism plays a key role in atherosclerosis and ischemic cardiac diseases. Estrogens control the expression of lipogenic genes such as leptin, perilipin, peroxisome proliferation activator receptor-delta, and lipoprotein lipase in adipocytes and estrogen supplementation helps to reduce adipose mass and adipocyte size and prevents development of obesity in postmenopausal state (90–92) by both genomic and nongenomic mechanisms (93). Genomic effects of estrogens are limited to the regulation of leptin and lipoprotein lipase expression whereas nongenomic effects are mediated via the s messenger systems, namely cAMP cascade and the phosphoinositide cascade. Activation of the cAMP cascade by estrogen is followed by activation of hormone-sensitive lipase leading to lipolysis in adipose tissues. Activation of protein kinase C through the PI3K cascade, controls the proliferation and differentiation of preadipocytes (93). Human preadipocytes (PAs) possess ERα protein and express ERα gene, but do not express ERβ receptors, indicating that effect of estrogen on adipogenesis is mediated through the ERα receptor (52,94,95). The effects of estrogens on ERα is site specific (95).

In humans, development of abdominal fat deposition is inversely proportionate to blood testosterone levels (96). Androgen receptors are found on preadipocytes (97,98) and the effects of testosterone on these cells also are site-specific. Castration is associated with increased proliferation and differentiation of epididymal and perirenal preadipocytes in male rats; whereas, peripubertal testosterone supplementation reduces inguinal and retroperitoneal fat deposits of ovariectomized (OVX) rats. Testosterone decreases adipocyte proliferation without affecting adipocyte mean cell size or the size distribution profiles (99). Androgens act directly on fat cells by upregulating α 2-AR expression (100). Androgens also exert their modulatory effects on the transcription factor C/EBP α, which is a key regulator of the expression of adipogenic genes (101), providing molecular context for gender-based effects on adipocyte physiology.

**Osteogenic Progenitors**

Estrogen and testosterone play crucial roles in bone metabolism. Even in males, estrogen is critical for the pubertal growth spurt characterized by skeletal maturation, accrual of peak bone mass, and the maintenance of bone mass in the adult through its effects on remodeling and bone turnover (102).

In OVX rats, estrogen deficiency causes osteopenia and induces bone turnover. Endosteal bone formation in OVX rats is associated with an increased proliferation of both osteoblast precursor cells present in the marrow stroma and along the endosteal bone surface. The osteoblast surface (percent of the bone trabecular surface covered with osteoblasts) also increases in OVX rats following ovariectomy, suggesting that bone formation increases in correlation with bone resorption. Estradiol supplementation reverses both the increase in resorption and formation indices (103).

Interestingly, 17β-estradiol, through the ERα receptor, increases bone matrix protein (BMP)-2, and BMP-4 expression. It also attenuates the self renewal of early osteoblast progenitors (having limited self-renewal capacity) for both osteoblasts and osteoclasts. Both these effects slow down bone remodeling (104,105). Through TGF β, estrogen causes repression of T cell proliferation and differentiation, and inhibits INFγ production. This subsequently decreases TNF production and thus reduces osteoclastic activity (106). Ovariectomy increases IL-12 and IL-18 secretion by macrophages which result in enhanced T cell activation and TNF production causing bone loss (107). IL-6 (108-110) and IL-7 also have been attributed to induced bone loss associated with estrogen deficiency (111), not only through increased osteoclastic activity but also by reducing bone deposition through downregulation of the osteoblast-specific transcription factor, core-binding factor α 1/Runx2 (112). In addition, 17β-estradiol exerts its inhibitory effect on osteoclasts through the regulation of VEGF production; ovariectomized mice were associated
with increased VEGF production and increased osteoclastic activity (113).

Testosterone also has beneficial effects on bone metabolism in adult males mediated by androgen receptors. Different genomic and nongenomic pathways are believed to be involved in mediating the effects of testosterone on bone metabolism. The nongenomic effects are mediated via Akt activation (114) through stimulation of src kinase (115,116). MAP kinase signaling cascade also is activated with testosterone treatment resulting in increased expression of Raf-1 and ERK-2 (115). Insulin like growth factor-I (IGF-I), and insulin like growth factor binding proteins (IGFBP) also play a significant part. Androgen decreases insulin like growth factor binding protein IGFBP-4 which is inhibitory for osteoblasts, and increases IGFBP-2 and IGFBP-3 mRNA and protein levels, which have stimulatory effects on osteoblasts (117).

The genomic effect of testosterone is mediated by the increased osteoprotegerin (OPG) expression. Osteoprotegerin is a receptor activator of NF-kB ligand, which inhibits the differentiation of the osteoclast precursor into a mature osteoclast (118). However, the effect of testosterone on osteoprotegerin expression is controversial. Some authors have demonstrated that 5α-dihydrotestosterone (DHT) reduces OPG in a dose-dependent manner (119). In total, these observations underscore the importance of understanding the differential effects of sex hormones on bone metabolism and physiology.

**Neural Stem Cells**

Estrogens modulate neurogenesis during embryonic development. Estrogens induce the neuronal phenotype in embryonic stem cell culture and enhance proliferation of embryonic neural stem cells, increasing the ratio of neurons to glial cells (120,121). In combination with poly-L-ornithine/fibronectin, estrogens also have been shown to accelerate differentiation and maturation of neurons (121). Furthermore, estrogens also enhance differentiation and survival of dopaminergic neurons harvested from human neural stem cells, suggesting a possible role of estrogens in the transplantation of neural stem cells as a therapeutic approach for Parkinson’s disease (122). Like the previously discussed cell types, beneficial effects of estrogens on neurons are mediated through both genomic and nongenomic pathways (123,124).

Testosterone has a negative influence on neural stem cell proliferation. Nandrolone (19-Nortestosterone) reduces cell proliferation in neural stem cells stimulated with epidermal growth factor, which can be reversed by flutamide, a receptor antagonist. Nandrolone also decreases the BrdU labeling of neural stem cells in the dentate gyrus, indicating reduced cell proliferation in vivo (125). These observations serve to emphasize the differential role of gender specific hormones on neural cell ontogeny.

**CONCLUSION**

Sexual dimorphism clearly influences the function of various stem cell types throughout the body. A better understanding of the effects of estrogen and testosterone on these cells will allow investigators and clinicians to modulate the functions of these cells directly, with the ultimate goal of generating more potent stem cell applications for the treatment of human disease.

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