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Hormonal modulation of endothelial NO production

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Abstract

Since the discovery of endothelium-derived relaxing factor and the subsequent identification of nitric oxide (NO) as the primary mediator of endothelium-dependent relaxations, research has focused on chemical and physical stimuli that modulate NO levels. Hormones represent a class of soluble, widely circulating chemical factors that impact production of NO both by rapid effects on the activity of endothelial nitric oxide synthase (eNOS) through phosphorylation of the enzyme and longer term modulation through changes in amount of eNOS protein. Hormones that increase NO production including estrogen, progesterone, insulin, and growth hormone do so through both of these common mechanisms. In contrast, some hormones, including glucocorticoids, progesterone, and prolactin, decrease NO bioavailability. Mechanisms involved include binding to repressor response elements on the eNOS gene, competing for co-regulators common to hormones with positive genomic actions, regulating eNOS co-factors, decreasing substrate for eNOS, and increasing production of oxygen-derived free radicals. Feedback regulation by the hormones themselves as well as the ability of NO to regulate hormonal release provides a second level of complexity that can also contribute to changes in NO levels. These effects on eNOS and changes in NO production may contribute to variability in risk factors, presentation of and treatment for cardiovascular disease associated with aging, pregnancy, stress, and metabolic disorders in men and women.

Keywords

Estrogen; Insulin; Glucocorticoids; Growth factor; Progesterone; Testosterone

Introduction

Interest in the possibility that hormones could modulate production of endothelium-derived relaxing factor (EDRF) emerged almost simultaneously with results of large epidemiological studies showing that women who used hormones to manage symptoms of menopause had lower incidence of cardiovascular disease than those who did not [5,6,19]. Indeed, the first studies to demonstrate increases in endothelium-dependent relaxations in estrogen-treated ovariectomized animals were conducted parallel to the identification of EDRF as nitric oxide (NO) [27,59] and the description of the various isoforms of the synthetic enzyme, nitric oxide synthase (NOS). Subsequently, interest in hormonal regulation of endothelial function increased with the concept of “endothelial dysfunction” as a risk factor for cardiovascular disease. The impact of hormones on function of endothelial (e)NOS was found to explain, in

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part, differences in cardiovascular disease incidence between men and women as well as vascular accommodation with pregnancy and increases in cardiovascular risk with aging, diabetes, and infection [2,16,33,40,58,86]. This review focuses on the mechanisms by which eNOS function is modified by hormones. The specific hormones covered in this review are those for which substantial evidence indicates direct modulation of eNOS function. Thus, no attempt has been made to discuss every known hormone.

General considerations

As discussed in great detail in other chapters in this volume, function of eNOS and NO bioavailability can be modified by a plethora of different signaling pathways and factors. Interestingly, despite the diversity of hormones and types of receptors through which hormones act, hormonal modulation of eNOS function appears to converge on two major pathways (Table 1 summarizes key effects on eNOS of the various hormones addressed in this brief review). The first of these major mechanisms for modulation of eNOS function occurs rapidly through phosphorylation of eNOS at serine 1177, resulting in an increase in enzyme activity (Fig. 1). The primary mechanism leading to phosphorylation of eNOS at this site depends on activation of the phosphoinositide 3 kinase/protein kinase B (PI-3 kinase/Akt) pathway. The second major mechanism by which hormones have been shown to modify eNOS depends on increased gene expression involving transcription factors or a change in mRNA stability and translation (Fig. 1). Therefore, this genomic mechanism generally has a longer onset and duration of action. It is intriguing that actions of so many hormones converge mainly on two major mechanisms for control of NO production by eNOS: rapid effects on intracellular signaling and more long-term genomic mechanisms affecting gene transcription. Whether future investigations expand the repertoire by which all the hormones modify eNOS function remains to be seen.

Steroid hormones

Steroid hormone receptors belong to the nuclear receptor superfamily class I [18]. Classically, ligand-bound receptors were thought to initiate cellular changes only through changes in gene transcription. Various co-factors are required for steroid receptors to bind specific nuclear genetic regulatory elements, and these are shared among the various steroid receptors [18]. Therefore, competition for these co-factors among ligand-bound receptors may affect the magnitude and timing of nuclear transcriptional regulation including that of eNOS.

The recognition that membrane-bound steroid receptors can also activate rapid intracellular signaling without requiring nuclear gene transcription (non-genomic) [44] expands the repertoire of actions of steroid hormones. Both of these mechanisms are involved in the actions of steroid hormones on eNOS.

Estrogens—In terms of hormonal influences on eNOS function, perhaps the most studied hormone is 17β-estradiol. Since the vascular actions of estrogen, including effects on eNOS function, have been reviewed in detail [60], only key points will be covered in the present brief review. In humans, flow-mediated vasodilatation (brachial artery reactive hyperemia) and NO production (serum nitrite/nitrates) vary through the menstrual cycle and increase with estradiol supplementation in postmenopausal women, supporting the conclusion that estrogens enhance eNOS dependent vascular reactivity [60,61]. Estrogen supplementation also improves eNOS dependent vascular function in male transsexuals suggesting that effects of this steroid are present in both males and females [69,93].

Mechanistically, two major pathways account for the ability of estrogens to increase function of eNOS: rapid signaling by membrane estrogen receptors through the PI-3 kinase/Akt pathway resulting in eNOS phosphorylation [54,65] and increased eNOS activity and longer term genomically regulated increases in eNOS mRNA and protein [54,89] (Fig. 1). There are two

major forms of the ligand-activated estrogen receptor (ER): termed α and β. In rats and ovariectomized pigs, treatment with estrogen upregulates eNOS in cerebral arteries and aortic endothelial cells, respectively, as demonstrated through isolated arterial responses as well as measurements of plasma NO, eNOS protein and mRNA [23,54,72,89]. Studies of transgenic mice suggest that effects of estrogen on levels of eNOS protein are mediated by ERα [24]. Furthermore, in a man with genetic defects in ERα, acetylcholine-induced increases in forearm blood flow were small and indications of cardiovascular disease were seen early in life [91, 92].

Estrogen also influences eNOS function with a more rapid time course via extra-nuclear ERs associated with the cell membrane. While the identity of the membrane ER has been quite controversial, recent evidence suggests that membrane-associated ERα and/or ERβ mediate eNOS phosphorylation at serine 1177 via activation of the PI-3 kinase/Akt pathway, leading to increased eNOS activity [44,65]. There is evidence that activation of both of these pathways regulates eNOS and NO in humans [22]. Flow-mediated vasodilatation and vascular eNOS expression and activation were measured in estrogen-deficient postmenopausal women as well as healthy premenopausal women during different stages of the menstrual cycle. Flow-mediated vasodilatation and vascular eNOS expression and levels of serine 1177 phosphorylated eNOS were higher in women with higher levels of estrogen. Interestingly, ERα expression was also modulated by estrogen status, with lower levels of ERα when estrogen levels were lower.

It has also been suggested that estrogen may act through a G-protein coupled, membrane-associated receptor, specifically the orphan receptor, GPR30 [60]. Interestingly, however, four different GPR 30 knockout mice have been developed, but they show very little phenotype [44]. Furthermore, in cell models, it does not appear that estrogen binds to GPR30. It remains to be explored whether or not GPR30 collaborates with other ER to influence intracellular signaling [44]. Nothing appears to be known about the possible effect of GPR30 activation on eNOS activity.

In addition to these two mechanisms by which 17-β estradiol increases eNOS activity, given the pleiotropic nature of estrogen effects, a number of other pathways may also affect levels of NO. For example, estrogen has been shown to suppress oxidative stress by a variety of mechanisms [37,61,89,90]. Since free radicals such as superoxide may inactivate NO, the ability of estrogen to suppress superoxide production could also help to increase endothelium-dependent vasodilatation [51]. These studies, and many more like them, emphasize the important impact of estrogen on eNOS function. They also highlight the impact of a variety of factors, such as oxidative stress, estrogen status and age, that all interact to influence function of eNOS and impact cardiovascular health in both men and women.

Androgens—In considering the physiological effects of androgens, it is critical to take into account the local tissue distribution of enzymes that metabolize testosterone: aromatase and 5-α – reductase. As illustrated in Fig. 1, aromatase metabolizes testosterone to estrogen, while 5-α – reductase converts testosterone to dihydrotestosterone. Dihydrotestosterone is a pure androgen, but testosterone, through local conversion to estrogen can activate estrogen receptors as well. Indeed, the localization of aromatase to the blood vessel suggests that vascular effects of circulating testosterone may be mediated by either androgenic or estrogenic pathways or both [29].

Testosterone can affect vascular tone through direct activation of ion channels and thromboxane [15,49,52]. In female to male transsexuals given high concentrations of androgen, flow-mediated vasodilatation is low suggesting a negative effect on eNOS-mediated responses [53]. Furthermore, in male pigs transitioning to puberty, plasma NO decreases with increasing

plasma testosterone, and endothelium-dependent relaxations of isolated coronary arteries are reduced compared to age-matched female [9], also suggesting a negative regulatory effect on eNOS.

However, studies in both humans and animals indicate that, in the male vasculature, aromatase-derived estrogen may have important effects on endothelial function and NO production [56]. In a group of young men administered an aromatase inhibitor for 1 month, endothelial function, as determined by flow-mediated dilatation of the brachial artery, was significantly decreased compared to baseline [46]. Studies in male aromatase-knockout mice also suggest that estrogen, produced by the action of aromatase, modulates NOS activity in the endothelium [39]. Aortic rings from male aromatase-knockout mice show blunted relaxation to acetylcholine compared to wild-type mice. Since endothelium-dependent, acetylcholine-induced relaxation is blocked by a NOS inhibitor, these findings suggest that estrogen as a product of aromatase metabolism can influence vascular eNOS. Thus, it seems that testosterone can influence endothelial NO production indirectly through aromatase-dependent metabolism to estrogen and subsequent stimulation of eNOS and NO production through mechanisms outlined above. Taking into account aromatase-dependent effects of testosterone via estrogenic pathways, is there any evidence that androgens directly influence eNOS function through the androgen receptor? There are reports in the literature exploring effects of testosterone on NO production or eNOS levels [54,74]. However, because of possible conversion of testosterone to estradiol, these studies do not definitively speak to the question posed above. Perhaps future studies using a non-metabolizable AR agonist such as dihydrotestosterone will be able to answer this question.

Dehydroepiandrosterone (DHEA) increases eNOS activity by both genomic and non-genomic mechanisms, perhaps through activation of a specific receptor [85]. However, unlike estrogen, the non-genomic actions of DHEA are mediated by a G-protein-coupled receptor-MAPK cascade [85]. While DHEA has been implicated in cardiovascular disease, more work is needed to further define the mechanism by which DHEA may affect eNOS.

Progesterone—Whether progesterone inhibits or antagonizes the stimulatory effects of 17-β estradiol on eNOS remains controversial [8,11,25,26,45,55,62,98]. This controversy reflects in part the imprecise categorizing of natural and synthetic progestins as a single set of compounds, rather than specific ligands with differential binding properties to progesterone receptors (PR) or other hormone receptors [1]. High concentrations of progesterone, the naturally occurring ligand, and the synthetic progestin, medroxyprogesterone acetate, also bind to glucocorticoid receptors [18,105,106] which inhibit gene transcription of eNOS and its enzymatic activity as described in the next section.

As is characteristic of 17β-estradiol, progesterone affects function of eNOS by both genomic and non-genomic mechanisms, the latter perhaps involving activation of a membrane bound receptor and subsequent activation of PI3K/Akt leading to eNOS activation [18,84,99]. There are two isoforms of the PR (A and B) which when bound to ligand can inhibit each other, as well as other members of the nuclear receptor family. These reciprocal inhibitory effects can occur both directly and indirectly through competition for common nuclear co-activators and co-repressors [18,43]. Thus, in an intact system, varying levels of each hormone, as might be found throughout the menstrual cycle or during pregnancy, may act to reduce fluctuations in endothelial NO production.

Non-genomic actions of progesterone on endothelial production of NO are also mediated through activation of tyrosine kinase, MAPK and PI 3-kinase pathways, but not protein kinase C even though progesterone increases protein kinase C activity [14,57,84]. These rapid effects may influence genomic actions of the hormones by altering phosphorylation of co-activators or repressors required for genomic actions of the hormone [14].

Glucocorticoids—Glucocorticoids are widely used as anti-inflammatory drugs. Cortisol treatment of animals or cortisol excess in humans (i.e., Cushing's disease) results in hypertension, attributed in part to excess sodium retention, but also mediated by inhibitory effects on both inducible NOS as well as eNOS [77,100,101]. As with the other steroids, there are differences in efficacy and mechanism of action of endogenous glucocorticoids compared to synthetic compounds like dexamethasone. These differences reflect not only differences in interactions between the various ligands and the receptor, but also differences in sensitivity of the various compounds to 11β-hydroxysteroid dehydrogenases (11-β HSD) which inactivate the natural ligand [50,104]. For example, inhibition of 11-β HSD potentiates cortisolinduced hypertension [104].

Glucocorticoids decrease endothelial NO production by decreasing eNOS gene transcription as well as by decreasing NO bioavailability through increasing generation of reactive oxygen species (ROS). The ligand-bound glucocorticoid receptor decreases eNOS gene transcription through binding to a suppressive glucocorticoid response element (at −111 to −105 bp) on the eNOS gene [50] and by transrepression with other transcription factors such as NFκB or AP-1 which may inhibit other forms of NOS [3,77]. Indeed, levels of eNOS mRNA and protein are decreased in cultured endothelial cells exposed to dexamethsone, and expression of eNOS protein is decreased in hydrocortisone-treated rats [78,104]. However, acute exposure of rabbit aortic strips or treatment of animals with either dexamethasone or hydrocortisone 12 and 2 h before tissue harvest did not alter endothelium-dependent relaxations to acetylcholine [21]. In contrast, in humans, dexamethasone suppressed reactive hyperemia in forearm blood flow, an effect reversed by administration of the anti-oxidant vitamin C [34]. Endothelium-dependent relaxations also were decreased in the aortae of male rats treated for 2 weeks with hydrocortisone [78]. Differences among studies may reflect differences in type of ligand, dose, and duration of treatment, possible simultaneous release of other endothelium-derived vasoactive factors like hyperpolarizing factor or prostanoids, simultaneous suppression of iNOS and/or tissue specificity of the treatment [78,101].

In addition to inhibition of eNOS protein transcription through receptor binding to repressor response elements on the eNOS gene, these steroids also reduce NO levels by increasing production of ROS by mitochondria, NAD(P)H oxidase as well as xanthine oxidase [34]; reducing synthesis of tetrahydrobiopterin (cofactor required for eNOS enzyme activity); limiting substrate for the enzyme by reducing membrane transport of L-arginine and decreasing agonist-induced intracellular calcium mobilization [83,101,104].

NO inhibits activity of both isoforms of 11-β HSD in a tissue-specific manner, thus representing a feedback mechanism [94]. As this inhibition was identified in placental and chorionic cells, these effects have implications in fetal programming of hypertension especially in male offspring of mothers who are stressed [80]. Other feedback systems of NO on the hypothalamic-pituitary-adrenal axis also have implications for development of cardiovascular disease related to major depressive disorder and stress-induced hypertension [66,76,96] and may contribute to sex differences in presentation and etiology of cardiovascular diseases [12,38].

Insulin

Insulin can bind to two distinct receptors: the insulin receptor (IR) and the related insulin-like growth factor 1 (IGF-1) receptor. Both receptors are ligand-activated tyrosine kinases. When activated, these receptors phosphorylate intracellular substrates that serve as docking proteins for downstream signaling molecules thereby initiating a series of phosphorylation cascades [67,70]. IRs are expressed on the cell surface of human endothelial cells, as are IGF-1 receptors. However, physiological concentrations (<10 nN) of insulin selectively increase autophosphorylation of IR, whereas supraphysiological insulin concentrations (>10 nM) are required to autophosphorylate the IGF-1 receptor [47].

Insulin regulates glucose homeostasis through the IR [67]. As an oversimplification, two major pathways are activated: PI3-kinase and MAPK-kinase, mediating metabolic actions and non-metabolic mitogenic and growth effects, respectively [67]. As discussed above, activation of the PI3-kinase/Akt pathway is known to regulate eNOS activity [32,65]; thus, it is not surprising that insulin increases eNOS activity and NO production. Indeed, insulin resistance is an important risk factor for cardiovascular disease and endothelial dysfunction is often found in patients with metabolic diseases. This coupling of metabolic and cardiovascular diseases through shared insulin-signaling pathways contributes to relationships between insulin resistance and endothelial dysfunction.

Exposure to insulin increases production of eNOS mRNA and protein [20,41]. In bovine aortic endothelial cells treated with insulin concentrations of <100 nM for 4 h, eNOS mRNA levels increased [41]. This effect persisted after addition of inhibitory antibodies to IGF-1 receptors but was blocked by inhibitors of PI-3 kinase. Similar effects of insulin on eNOS mRNA and protein were also shown in porcine aortic endothelial cells [20]; again, these effects were blocked by a PI-3 kinase inhibitor. Furthermore, insulin increased the activity of AP-1, a transcription factor known to bind to the eNOS promoter, and AP-1 decoy oligonucleotides prevented the insulin-induced increase in eNOS [20].

Based on knowledge of the actions of NO released from eNOS, one would predict that insulin would cause vasodilatation, capillary recruitment, increased blood flow, and slower atherosclerotic progression. Indeed, in humans, infusion of insulin causes vasodilatation and increased blood flow, effects dependent on NO [88]. The ability of insulin to cause vasodilatation is not merely a consequence of changes in carbohydrate metabolism [97]. It is thought that these effects of insulin on capillary recruitment and blood flow promote glucose distribution, contributing to metabolic and hemodynamic homeostasis [67].

Given the important role of eNOS in contributing to the physiological effects of insulin, what is the impact of insulin resistance on endothelial function and vice versa? Interestingly, in both humans [13] and in animal models of insulin resistance [36], there is a specific impairment of PI3K-dependent signaling pathways, although other insulin-signaling pathways, including Ras/MAPK appear to remain functional. Thus, insulin resistance would be associated with a decrease in eNOS phosphorylation and decreased endothelial NO production. However, since insulin resistance is usually associated with compensatory hyperinsulinemia, MAPK-dependent pathways will also be over-activated. In the case of the vasculature, this includes a number of pro-hypertensive effects of insulin, including promotion of endothelin-1 secretion, activation of cation pumps and increased expression of VCAM-1 and other adhesion molecules [67].

Additional mechanisms associated with insulin resistance, such as pro-inflammatory signaling due to glucotoxicity and lipotoxicity, may also contribute to endothelial dysfunction [67]. Thus, mechanisms underlying insulin resistance and endothelial dysfunction are multifactorial, with regulation of NO being only one of several cellular autokine/cytokine pathways contributing to a complex linkage between metabolic and cardiovascular disease.

In summary, insulin promotes endothelial function, specifically by activating the PI-3 kinase/Akt pathway leading to eNOS phosphorylation and increased eNOS activity. Insulin also promotes an increase in eNOS mRNA and protein. In contrast, insulin resistance results in a decrease in eNOS function and loss of vascular protective effects of NO. Compensatory hyper-insulinemia may also result in activation of additional mechanisms that contribute to the loss of endothelial function in this condition [67].

Growth hormone

The impact of growth hormone (GH) on eNOS regulation represents another example of the intricate interplay between physiological homeostatic mechanisms. Adult hypo-pituitarism and untreated growth hormone deficiency are associated with endothelial dysfunction, a decrease in NO production, increased peripheral resistance and an increase in risk of cardiovascular morbidity and mortality [71,95]. For example, in adult patients with acquired GH deficiency, administration of recombinant human GH increased indices of NOS activation and decreased total peripheral resistance [4].

The receptor for GH (GHR) belongs to the class I cytokine receptor superfamily [75]. Binding of GH to GHR induces a conformational change that activates downstream signaling, specifically by activating JAK2, a cytoplasmic tyrosine kinase. Activation of JAK2 leads to tyrosine phosphorylation of a number of cytosolic proteins, including STAT, Shc, and IRS-1 and IRS-2, thus activating the PI3 kinase regulatory pathway. Furthermore, GH also induces release of IGF-1, leading to activation of the IGF-1 receptor. As mentioned above, insulin in relatively high concentrations also activates the IGF-1 receptor. Thus, the growth hormone pathway intersects through several mechanisms with those pathways activated by insulin.

A number of the actions of GH are mediated indirectly by induction of IGF-1; administration of human GH to adults with acquired GH deficiency results in a substantial increase in plasma IGF-1 [4]. As mentioned above in the discussion of the actions of insulin, IGF-1 acts on its own membrane receptor. The type 1 IGF-1 R is closely related to the insulin receptor and also has intrinsic tyrosine kinase activity. Indeed, activation of the type 1 IGF R increased eNOS phosphorylation [47]. Thus, one would predict that GH would activate eNOS. Indeed, both in vitro and in vivo studies indicate that eNOS is activated through the GH/IGF-1 pathway. In vivo administration of GH to patients with acquired GH deficiency increased indices of NO production (urinary nitrates and excretion of cyclic GMP) and decreased total peripheral resistance [4]. Furthermore, in patients with childhood-onset GH deficiency who had not received GH treatment, increases in forearm blood flow to infusion of acetylcholine were lower than in control subjects, as were forearm release of nitrites/nitrates and cGMP [7].

IGF-1 administration caused vasodilatation in both humans and experimental animals, and this vasodilatation was blocked by inhibitors of eNOS[81]. In vitro, IGF-1 also caused endothelium-dependent relaxation of isolated arteries, an effect abolished by inhibition of NOS. Furthermore, rapid formation of NO was detected in cultured endothelial cells exposed to IGF-1. Hypophysectomized rats treated with IGF-1 or GH for 7 days showed a significant increase in levels of eNOS protein in the aorta [102]. In contrast, GH treatment for 14 days did not improve the acetylcholine-induced fall in vascular resistance of the skeletal muscle bed of hypophysectomized rats [71]. However, hypophysectomy did produce endothelial dysfunction as determined by response to acetylcholine administration. In cultured human endothelial cells, exposure to GH increased eNOS gene expression within 4 h; eNOS protein expression also increased [95]. These effects were accompanied by an increase in NO production and a fall in intracellular ROS.

In addition to these studies suggesting that GH affects eNOS levels via release of IGF-1, GH may directly increase eNOS activity, independent of actions on IGF-1. In humans, two separate studies demonstrated that infusion of GH increased forearm blood flow and enhanced sensitivity to acetylcholine, effects blocked by NOS inhibition. These effects of GH were not associated with any increase in IGF-1 levels in the forearm circulation [48,68]. Incubation of cultured human aortic endothelial cells with GH showed no change in eNOS protein content, but there was a time-dependent increase in eNOS phosphorylation at Ser 1177 [48]. However, there was no concomitant increase of Akt or AMPK phosphorylation after exposure to GH. Thus it seems clear, from both in vivo and in vitro studies, that activation of the GH/IGF-1

pathway can increase eNOS activity, accounting for some of the cardiovascular effects of GH, as well as the endothelial dysfunction and increased risk of cardiovascular disease in individuals with reduced GH activity. Whether there is also an additional, direct effect of GH, independent of IGF-1, on eNOS levels or activity remains to be proven.

Other considerations

While this short review focuses mainly on mechanisms by which hormones directly regulate function of eNOS, it must be kept in mind that, as described for the glucocorticoids, many hormones may also regulate inducible and neuronal NOS [101]. Understanding possible interactions among these isoforms on the final product, bioavailable NO, still requires investigation. In addition, some hormones may indirectly regulate NO bioavailability through changes in ROS production, NOS substrate availability or the availability of enzyme co-factors.

NO, itself, regulates release of some hormones, for example, NO may decrease renin release by the kidney and release of prolactin from the pituitary (Fig. 2). A decrease in renin would in turn reduce activation of the angiotensin conversion pathways with potential reductions in angiotensin II (AII) related decreases in endothelial NO bioavailability [79,87,96]. Prolactin secretion from the pituitary is enhanced in the presence of estrogen [28,42,64]. However, once in the circulation, the prolactin metabolite, 16K-prolactin, inhibits eNOS [30,63]. These feedback regulatory processes on the pituitary-gonadal and adrenal axes need further consideration in design of physiological experiments evaluating NO in the setting of cardiovascular disease and major depressive disorders and stress-associated hypertension [35,76,82,103].

Whether or not NO affects release of thyroxin from thyroid follicular cells requires further investigation [96]. Even though T3 (L3,5,3′-triiodothryronine) initiates rapid release of NO from cultured endothelial cells, it is unclear if vascular regulation of NO in the setting of hyper- or hypothyroid conditions reflects the direct influence of the hormone on eNOS or indirect effects of altered glucose and lipid metabolism [17,31,96]. Most likely, both direct and indirect effects are involved which may explain the conflicting reports of improved or neutral effects of treatment of hypothyroidism on endothelial function [10,73].

Conclusions

Hormonal regulation of eNOS and bioavailability of NO have implications in maintaining vascular health during natural hormonal transitions with aging, diabetes, and metabolic disorders. Most studies have investigated hormonal effects on eNOS in cultured cells of animals by manipulation of a single hormone (i.e., estrogen or testosterone treatment to cells or castrated animals). However, because hormones regulate eNOS by converging pathways, future studies should address the interactions of hormonal regulation in order to better understand hormonal effects in diseases such as age-associated diabetes, menopause, and stress in which multiple hormones may be involved.

Abbreviations

ACTH	adrenocorticotropic (corticotrophin) hormone
DHEA	dehydroepiandrosterone
E2	17β-estradiol
eNOS	endothelial nitric oxide synthase
FSH	follicle stimulating hormone

GH	growth hormone
Glc	glucocorticoids
LH	luteinizing hormone
NO	nitric oxide
T3	$_{L}$3,5,3′-triiodothryronine
T	testosterone
+	increases in activity
−	decreases in activity

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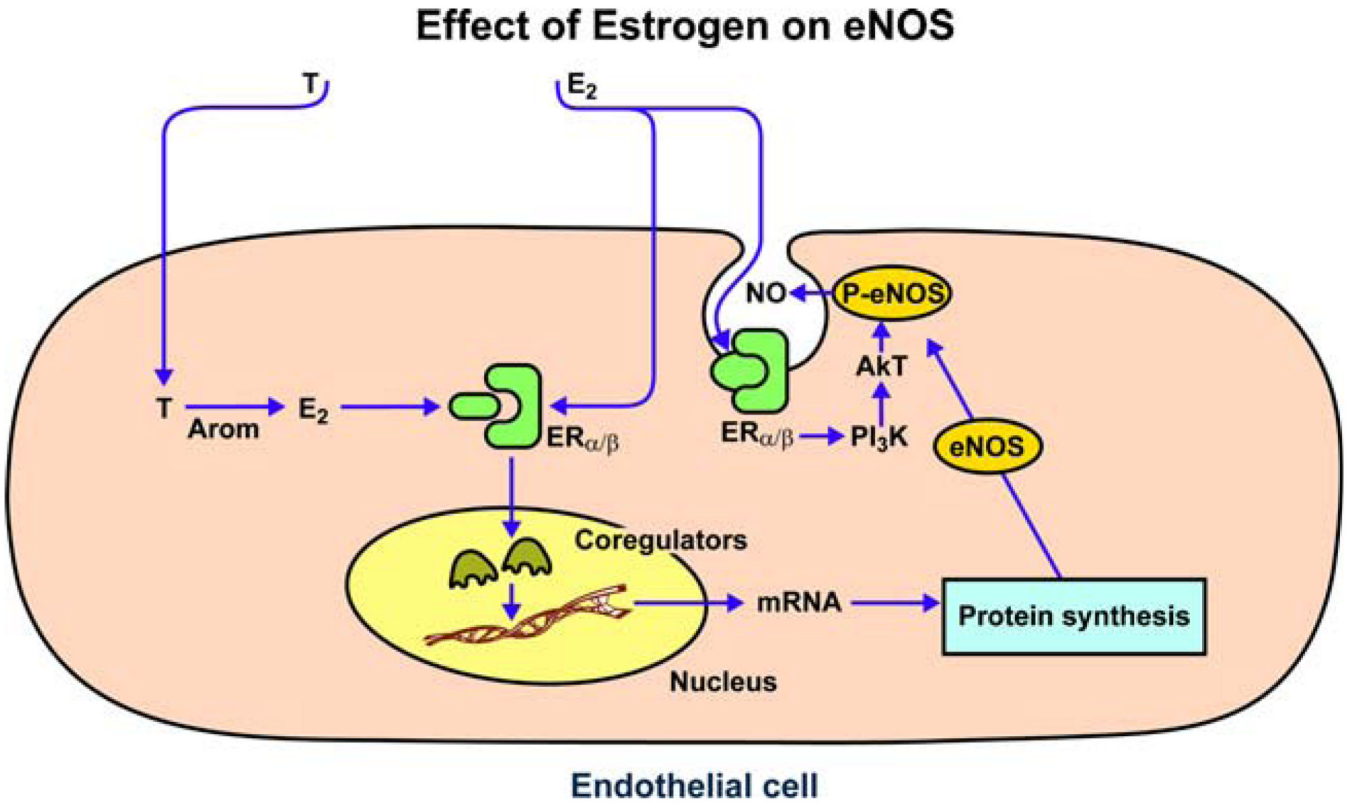

Fig. 1.

Effect of estrogen (E2) on endothelial nitric oxide synthase (eNOS). E2 binds to estrogen receptors α or β (ERα/β) in two locations: membrane-associated or cytosolic. As discussed in the text, binding of E2 to the membrane-associated form activates the phosphoinositide-3-kinase/protein kinase B (PI3 K/AkT) pathway leading to phosphorylation of eNOS at serine 1177. This increases eNOS activity and production of NO. Binding of E2 to cytosolic ERα/β leads to translocation of the bound receptor into the nucleus. There the liganded receptor binds to co-regulators and DNA response elements on the eNOS gene to initiate transcription and increase eNOS mRNA production. mRNA is then translated by the ribosome to increase eNOS protein production

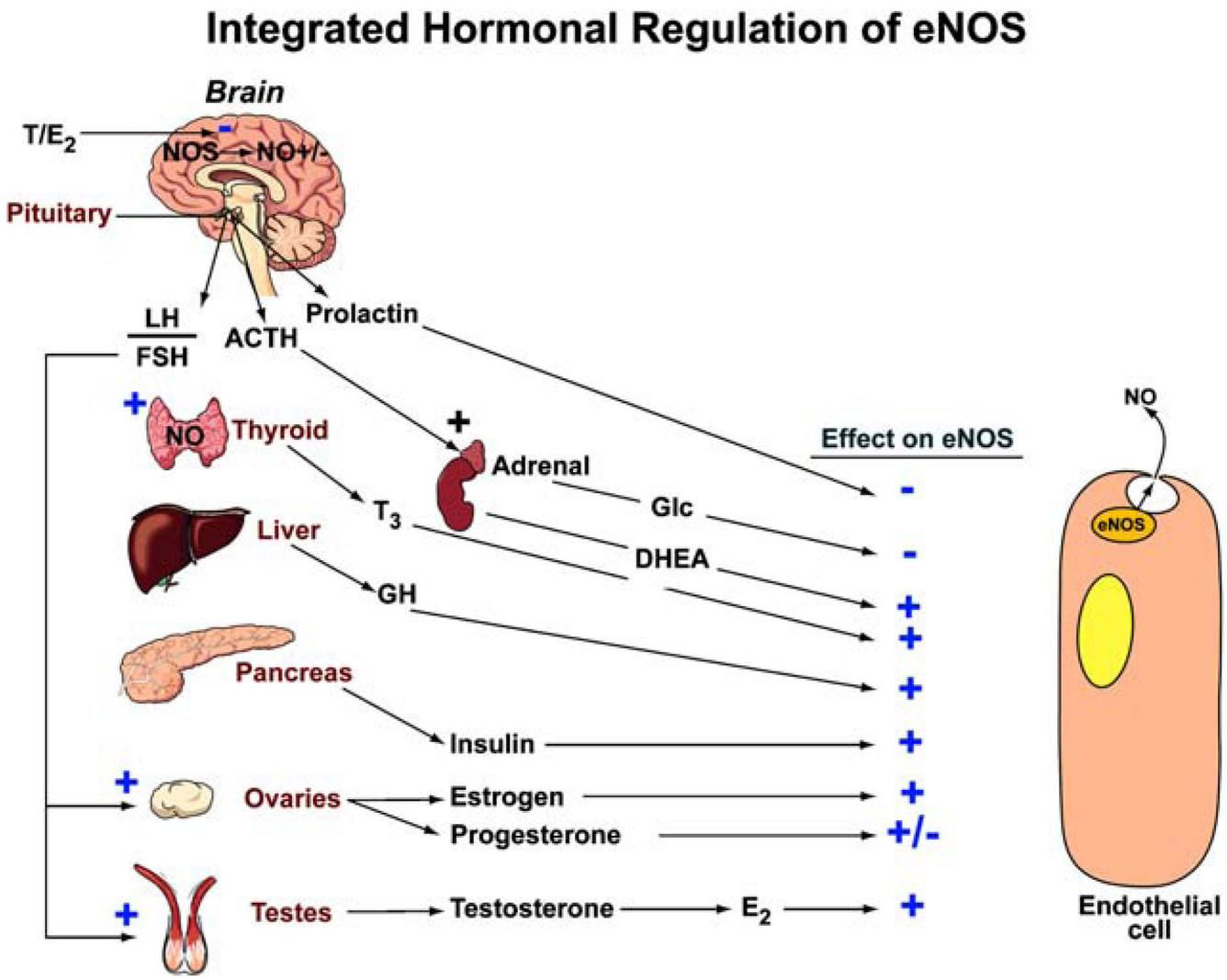

Fig. 2.

Schematic illustrating interactions of hormones in regulation of eNOS. Changes in production of NO will represent the culmination of effects of multiple hormones through both non-genomic and genomic mechanisms. Hormones can also affect levels of NO through indirect mechanisms including alterations in enzyme activity through changes in availability of enzyme co-factors and substrate, or changes in bioavailability of NO through production of oxygen-derived free radicals. In addition, production of NO in specific organs, such as the brain or thyroid, also may affect release of gonadotropins, or thyroid hormone, respectively. Neuronal activity, particularly related to stress, can alter release of ACTH and thus, modify release of glucocorticoids from the adrenal gland. Estrogen and testosterone affect neuronal function and activation of NOS in the brain as well as inhibit release of gonadotropins from the pituitary. These integrated actions of hormones on eNOS will vary across the life span with changes in gonadal function associated with puberty, pregnancy, aging, and stress, thus altering the balance among hormones at the level of the endothelial cell

Table 1

Summary of major hormonal effects on eNOS

Hormone (receptor)	Alter eNOS levels	Alter eNOS activity
A. Steroid hormones (ligand-activated transcription factors)		
Estrogen (ERα and/or β)	Genomic: increase mRNA, protein	Increase (PI-3 kinase/Akt)
Testosterone (AR)	No genomic androgen effect	No androgen effect
Progesterone (PR) (GR)	Genomic: increase mRNA, protein (PR) decrease mRNA, protein (GR)	Increase (PI-3 kinase/Akt)
Glucocorticoids (GR)	Genomic: decrease mRNA, protein	Decrease: reduced bioavailability: Increase ROS, reduce tetrahydrobiopterin, limit L-arginine transport, decrease agonist-induced calcium mobilization
B. Insulin		
IR: ligand-activated tyrosine kinase	Genomic: increase mRNA, protein	Increase (PI-3 kinase/Akt)
C. Growth hormone/IGF-1 pathway		
GHR: cytokine receptor super-family; IGF-1R: ligand-activated tyrosine kinase	Genomic: increase mRNA, protein	Increase (PI-3 kinase/Akt)

ER estrogen receptor, *AR* androgen receptor, *PR* progesterone receptor, *GR* glucorticoid receptor, *IR* insulin receptor, *GHR* growth hormone receptor, *IGF-1R* insulin-like growth factor 1 receptor, *PI-3 kinase* phosphoinositide 3 kinase, *Akt* protein kinase B, *ROS* reactive oxygen species