Extranuclear Steroid Receptors: Nature and Actions

Stephen R. Hammes and Ellis R. Levin

Division of Endocrinology and the Departments of Medicine and Pharmacology (S.R.H.), University of Texas Southwestern Medical Center, Dallas, Texas 75390; Division of Endocrinology (E.R.L.), Veterans Affairs Medical Center, Long Beach, Long Beach, California 90822; and Departments of Medicine, Biochemistry, and Pharmacology (E.R.L.), University of California, Irvine, Irvine, California 92697

Rapid effects of steroid hormones result from the actions of specific receptors localized most often to the plasma membrane. Fast-acting membrane-initiated steroid signaling (MISS) leads to the modification of existing proteins and cell behaviors. Rapid steroid-triggered signaling through calcium, amine release, and kinase activation also impacts the regulation of gene expression by steroids, sometimes requiring integration with nuclear steroid receptor function. In this and other ways, the integration of all steroid actions in the cell coordinates outcomes such as cell fate, proliferation, differentiation, and migration. The nature of the receptors is of intense interest, and significant data suggest that extranuclear and nuclear steroid receptor pools are the same proteins. Insights regarding the structural determinants for membrane localization and function, as well as the nature of interactions with G proteins and other signaling molecules in confined areas of the membrane, have led to a fuller understanding of how steroid receptors effect rapid actions. Increasingly, the relevance of rapid signaling for the in vivo functions of steroid hormones has been established. Examples include steroid effects on reproductive organ development and function, cardiovascular responsiveness, and cancer biology. However, although great strides have been made, much remains to be understood concerning the integration of extranuclear and nuclear receptor functions to organ biology. In this review, we highlight the significant progress that has been made in these areas. (Endocrine Reviews 28: 726–741, 2007)

I. Historical Background

II. Estrogen Receptors

A. Introduction

B. Nature of membrane estrogen receptors

C. Functions of membrane estrogen receptors

D. Outcomes of extranuclear estrogen signaling

III. Progesterone Receptors

A. Introduction

B. Nature of membrane progesterone receptors

IV. Androgen Receptors

A. Function and importance

V. Glucocorticoid Receptors

A. Function and importance

VI. Mineralocorticoid Receptors

A. Function and importance

VII. Thyroid Hormone Receptors

A. Functions

B. Classical thyroid receptors

VIII. Vitamin D Receptors

IX. Summary

I. Historical Background

HANS SELYE FIRST reported in the early 1950s that glucocorticoids and catecholamines regulate the rapid adaptation to stress (1). In 1967, Clara Szego and June Davis (2) reported that iv administration of physiological amounts of 17-β-estradiol (E2) to ovariectomized rats resulted in a 100% increase in uterine cAMP levels after 15 sec. This observation suggested that steroid hormones rapidly signal, and it was subsequently supported by studies demonstrating rapid calcium responses to E2 in endometrial cells (3). Shortly thereafter, specific, high-affinity binding sites for estrogen were described at the surface of endometrial cells (4). These studies effectively launched the field of rapid steroid responses, eventually extending to many members of the steroid receptor superfamily (5).

II. Estrogen Receptors

A. Introduction

Compelling evidence for the rapid effects of estrogen now exists. E2 rapidly activates several protein kinases [e.g., MAPKs, phosphotyrosinyl-3-kinase (PI3K), and protein kinase C (PKC)] and phosphatases, as well as the release of several cyclic amines (cAMP, cGMP) and calcium, in a variety of cell types (5, 6). These signals subsequently mediate
the posttranslational modification of many proteins, especially by phosphorylation. As a result, enzymes are rapidly induced (6, 7), and cell functions are modulated (8). Rapid signaling by estrogen correlates to the membrane localization of classical estrogen receptors (ERs), but not to their nuclear localization (9).

B. Nature of membrane estrogen receptors

1. Isoforms and sizes. The identity of the cytoplasmic/membrane ER has been an issue of debate. Initial data suggested that the membrane-localized estrogen binding protein shared epitope homology to classical (nuclear) ERα, based upon immunological identification (10, 11). Accordingly, expression of classical ERα or ERβ in ER-null cells results in both nuclear and membrane-localized functional pools of estrogen binding proteins (12). Furthermore, breast cancer MCF-7 cells that lack nuclear ERα (13) or endothelial cells (EC) from combined ERα/ERβ-deleted mice (14) do not demonstrate a functional estrogen binding protein at the cell surface (15). Genetic approaches using antisense oligonucleotides (11), as well as small interfering RNA directed against surface (15). Genetic approaches using antisense oligonucleotides (11), as well as small interfering RNA directed against classical ERα (13) or ERβ (14, 15), also eliminate estrogen binding and membrane-initiated steroid signaling (MISS). Finally, membrane ERs isolated from breast cancer cells are identical to the nuclear ERα by mass spectrometry (15).

Approximately 5–10% of endogenous cellular ERα is present at the membrane, determined by Scatchard analysis of ligand saturation binding (15). Interestingly, receptors expressed by transfection also show approximately the same number of receptors at the membrane in the absence of subcellular targeting (12). This suggests the presence of a saturable transport mechanism for the membrane-bound ER pool (see Section II.B.3). Notably, ER at the membrane and in the nucleus exhibit the same high-picomolar affinity for ligand.

The majority of work in the area of membrane-initiated estrogen signaling identifies a 66-kDa protein as the predominant/exclusive ERα form at the plasma membrane in multiple cell types (16, 17). However, truncated forms of ERα have also been described.

In both MCF-7 cells (13) and immortalized human ECs (18), abundant 46-kDa receptor ERα (lacking the amino terminus) has been demonstrated at the plasma membrane. However, antibodies directed against either the amino or carboxyl terminus of the protein detect ERα in human breast cancer specimens (mainly in the nucleus), suggesting the presence of full length, 66-kDa receptor. Regarding vascular cells, several groups working with freshly isolated EC or aorta from rodents or ECs from human umbilical veins have only described a 66-kDa receptor (15, 16, 19). These ERα exist in the caveolae and noncaveolar rafts of EC membranes and stimulate rapid signaling to endothelial nitric oxide synthase (eNOS) activation and nitric oxide (NO) production (20). Thus, the functional importance of the smaller isoform, described in only a few reports, is unknown.

Additional membrane receptors of 36-kDa size have been isolated mainly from ER-positive breast cancer cell lines and from some human cancers (21). The vast majority of studies in breast cancer do not mention these low-molecular weight isoforms that are apparently low in abundance. ERβ has been much less well characterized, but endogenous membrane-localized receptors have been described as approximately 60 kDa in size (22). A functional 54-kDa ERβ receptor also exists at the membrane of EC (16).

2. G protein-coupled receptor 30 (GPR30) and other putative ERs. A few reports describe the orphan G protein-coupled receptor (GPCR), GPR30, as an ER (23, 24). This protein has been reported to respond to estrogen with high affinity and low capacity binding (25) at both the plasma membrane and endoplasmic reticulum. E2 has been reported to engage this protein to stimulate kinase activation, calcium signaling, and epidermal growth factor (EGF) receptor (EGFR) transactivation in ER-negative breast cancer cells. It has not been demonstrated that this receptor mediates G protein activation in response to E2, as shown for the endogenous or expressed classical ERα (12, 20). Furthermore, many laboratories have found that classical ER-negative breast cancer cells do not respond to E2 with any biological functions.

In contrast, multiple groups have described collaboration between membrane-localized ERs and GPR30, presumably at the membrane of several hormone-sensitive cell lines. In endometrial and ovarian cancer cells, as well as keratinocytes, ERα and GPR30 mediate rapid kinase signaling by E2 to c-fos or cyclin D1 up-regulation and proliferation (26–28). A second GPCR at the membrane, Edg-3, is the binding protein for sphingosine-1 phosphate (29). In MCF-7 cells, membrane ER signaling to EGFR transactivation and ERK may cause sphingosine kinase to release sphingosine, resulting in feed-forward engagement of Edg-3 for further signaling (30). It is unclear from many of these reports whether the GPCR, as opposed to ER, activates the G protein; it may be that the GPR30 or Edg-3 serves as a linker or scaffold protein to help assemble the signal complex that is required for ER signaling (31). Proteins such as the modulator of nongenomic actions of steroid receptors (MNAR) (32), Shc (33), caveolin-1 (9), and striatin (34) have been described to serve these purposes for membrane ER signaling in several cell types. Importantly, most of these studies do not support the idea that GPR30 functions as a stand-alone ER in the absence of classical ER. It is conceptually possible that some cells might lack GPR30 or other downstream signaling proteins, and therefore rapid signaling by E2 is not evident even when membrane ERα is present.

Additional estrogen binding proteins that mediate rapid actions of E2 in the central nervous system have also been functionally implicated in E2 rapid signaling, but the nature of these proteins is currently undefined (35, 36). In both platelets and T-lymphocytes, a body of data implicates undetermined, but nonclassical estrogen binding proteins in the actions of E2. However, recent work implicates ERβ as mediating E2 rapid actions in platelets (37, 38) and ERα or ERβ as supporting E2-induced kinase activation to suppress T-cell cytokine production and transcription factor activity (39, 40).

3. Membrane localization and function. Endogenous ERα and ERβ proteins exist at the plasma membrane of many cell types, but are variable in their expression. Abundant α and β ER are present at the cell surface of ECs and cardiomyo-
cytes as both homo- and heterodimers (15, 16, 20, 22). In contrast, breast cancer cells express much more plasma membrane ERα than ERβ, consistent with much of E2 rapid signaling being mediated through the α isoform in these cells (13, 15).

The endogenous ERα exists as a monomer at the cell surface in the absence of E2 and rapidly dimerizes upon addition of ligand to the cell (14). Dimerization is known to be crucial to nuclear ER function (41), and evidence links this modification of membrane ER to rapid G protein activation and downstream signaling (14). Dimerization may not be required for estrogen-induced signaling to eNOS activation in ECs (42).

At the surface of several cell types, ERα and ERβ are localized to isolated caveolae rafts (13, 42–44). Here, many signaling molecules congregate in a spatially confined area that facilitates rapid signaling (Fig. 1). These signal proteins include G proteins, growth factor receptors (EGFR, IGF-I receptor), non-growth factor tyrosine kinases (Src, Ras), linker proteins (MNAR, striatin), and orphan GPCRs. The signalsome complex provides the necessary protein interactions for membrane ER to transactivate growth factor receptors (30, 33, 45) or engage and directly activate G proteins (14, 20), depending upon cell context. Various domains of ERα contact discrete signal proteins, including the amino-terminal A/B (striatin, Shc) and carboxyl-terminal E (MNAR) domains (32–34). However, A/B domain-deleted ERα still translocates to the membrane and activates ERK comparably to full-length ER (9), emphasizing the point that the precise assembly sequence of multiprotein complexes dictating specific signals to select cell biology events is not known. Undoubtedly, the particular complex assembled in a specific context provides signal specificity. For instance, membrane ERα activates many but not all G proteins in response to E2, and one or two G proteins are often linked to the activation of selective kinase cascades (12, 31).

4. ER translocation. Because the endogenous ER exists as a monomer at the cell surface, it is likely that transport of the protein to the plasma membrane does not require dimerization. The structural requirements for membrane ER localization have been investigated. Expression of the E domain alone into ER-null cells is sufficient for membrane localization and signaling (45), suggesting that this domain contains the key residues that participate in transport to the membrane. Consistent with this idea, serine 522 of the E domain was found to be important for membrane translocation, dictating a physical interaction with caveolin-1 protein that functions to facilitate transport of ERα to caveolae (9). Furthermore, intestinal cancer cells lacking caveolin-1 but containing endogenous ER show only nuclear steroid receptors. Expressing caveolin-1 in these cells results in a small population of ER translocating to the cell surface (9). Similarly, caveolin-1 expression in MCF-7 cells facilitates ERα translocation to the membrane (44). Most epithelial cancer cells have reduced (but not absent) caveolin-1, compared with normal epithelial cells.

Once ER is localized to the membrane, caveolin-1 must be displaced from ER for productive signaling in EC or MCF-7 cells (20, 44). A similar requirement of caveolin-1 displacement from the membrane is known for signaling by membrane growth factor tyrosine kinase receptors. However, the ability of membrane ER to inhibit ERK MAPK in vascular smooth muscle (VSM) cells correlates to a strong interaction with caveolin-1 (44). Also, E2/ER differentially regulate caveolin-1 synthesis, stimulating this scaffold in VSM cells but inhibiting transcription and new caveolin-1 protein production in MCF-7 cells.

Another important determinant of membrane translocation is cysteine 447 in the E domain of ERα. This residue has been determined to be a palmitoylation site (46, 47), and identification of cysteine 447 extends a previous observation that truncated ERα in immortalized human ECs are palmi-
tolytated (48). Both studies indicated that palmitoylation is necessary for membrane localization of ERs. Mechanistically, palmitoylation promotes a physical interaction of ER with caveolin-1, thereby promoting membrane localization. It is not clear where palmitoylation of ER occurs in the cell, but this posttranslational lipidation occurs only on cytoplasmic/membrane ER, and not nuclear ER (49). This is consistent with the importance of this modification to promote membrane transport. Why only a small percentage of ER undergo palmitoylation and membrane localization despite all ER containing the same sequence information is not clear. Perhaps palmitoylation is a saturable process where the relevant palmitoyltransferase protein abundance is limiting. We speculate that palmitoylation occurs on a few ER at any one time. Membrane-localized ER can also translocate to non-clathrin-coated cell endosomes (50); the importance of this for function, recycling, or degradation of membrane ER is currently unknown.

Recently, additional sequences flanking cysteine 447 and comprising a nine-amino acid membrane-localization motif have been identified to promote palmitoylation of ERs (49). Interestingly, this motif is highly conserved in the E domains of ERβ, progesterone, androgen, and glucocorticoid receptors. Mutational analysis of this motif in all the sex steroid receptors indicates that this structure dictates palmitoylation, membrane localization, and all membrane-initiated signaling (49). In contrast, nuclear localization and function of sex steroid receptors is not impaired by mutation of this motif.

C. Functions of membrane estrogen receptors

1. Importance of rapid signaling by E2. Increasingly, the importance of rapid signal transduction to the biological actions of sex steroids has been demonstrated. These signals modify existing proteins (e.g., phosphorylation) and their functions (6) and also modulate gene expression and hence production of proteins (51). As examples, ERK signaling in response to E2 increases c-fos expression in vivo and in vitro in multiple cell types and prolactin gene expression in pituitary cells (52, 53). Hence, the concept that these signals are “nongenomic” is simply inaccurate. The FASEB rapid steroid signaling working group suggested adoption of the terms “membrane-initiated steroid signaling” (MISS) and “nuclear-initiated steroid signaling” (NISS). These distinctions also allow description of signaling originating from additional pools of ER (e.g., mitochondria, see Section II.C.4). Cross-talk between ER pools also occurs, where a signal at one pool modulates the signal from a second discrete pool (54). The full extent of this integration is not well understood.

2. Impact of membrane signaling for transcription. As described from the laboratory of Don Pfaff (54), rapid activation of ERK, calcium, and perhaps other signals from membrane ER amplifies subsequent gene transcription originating from nuclear ER action. Rapid signaling can cause this in numerous ways. First, ERK, PI3K, protein kinase A, or p21-kinase activation phosphorylate key residues of nuclear ERα (54). This augments the transcriptional function of nuclear ER and may contribute to tamoxifen resistance in breast cancer both in vitro and in vivo (55, 56). Interestingly, these signals are known to be activated by growth factor receptors in breast cancer (57), and membrane ERs can transactivate EGF family and IGF-I receptors to stimulate identical signaling (6).

Second, kinase activation by estrogen phosphorylates and thereby recruits coactivators to the nuclear ER transcriptional complex (58). Examples include GRIP-1, SRC family proteins, and CBP (58–60). As a result, rapid signaling by E2 in ECs quickly up-regulates many transcription factor and structural genes, some contributing to the angiogenesis effects of E2 (61).

Third, signaling from membrane ERs may contribute to gene transcription independently of nuclear ER. This may involve posttranslational modification and recruitment of AP-1 family partners to their cognate DNA binding sites, mediating gene modulation (62). Other transcription factors that contribute to gene regulation after rapid signaling by E2 are the Sp-1, nuclear factor κB, and signal transducers and activators of transcription (Stat) 3 and 5 (51, 63, 64). Signaling through PI3K/AKT phosphorylates and inactivates GSK-3B, thereby promoting β-catenin translocation to the nucleus (65). This contributes to cyclin D1 and c-myc induction in breast cancer cells, two targets for estrogen function (66, 67).

Rapid signaling can also suppress transcription. E2 rapidly activates PI3 and AKT kinases in many cell types. AKT phosphorylates and thus retains Forkhead (FOXO) family transcription factors in cytoplasm, preventing their induction of cell death gene transcription (68, 69). PI3K/AKT signaling by membrane ER is known to importantly contribute to cell survival in many target cells (70–72), in part likely to occur through the mechanism described above. Because there are more than 500 kinases coded for in the human genome, it is apparent that kinase activation resulting from membrane ER action is likely to be highly significant in ways currently unappreciated.

Regarding breast cancer, aromatase inhibition has been modeled by long-term culture of MCF-7 cells in estrogen-deprived medium (73). Sustained signaling through ERK, PI3K/AKT, and mTOR results in and mediates low concentration, E2 hypersensitivity in these cells. In another model, continued exposure of MCF-7 cells to tamoxifen simulates tamoxifen resistance. In these cells, a population of ERs moves out of the nucleus to physically associate with EGFR and Src at the membrane. Inhibition of Src reverses tamoxifen resistance (74).

3. Nontranscriptional functions of membrane ER signaling. Rapid signaling by membrane ER modifies existing protein structure, affecting the cell distribution and function of the substrate protein. As examples, kinases and phosphatases can themselves be regulated by calcium or phosphorylation. ER signaling rapidly down-regulates MAPK phosphatase 1, leading to the up-regulation of ERK activity in breast cancer cells (75). PI3K activation in breast cancer cells phosphorylates the proapoptotic Bcl-2 protein BAD, sequestering BAD in the cytoplasm through binding 14-3-3 proteins (76). Bcl-2 and Bcl-xl are subsequently released from complexing to BAD through this mechanism, activating their prosurvival functions. In bone cells, membrane ER signaling modulates...
whether ERK signals predominantly in cytoplasm or nucleus, the latter contributing to osteocyte survival (77).

Membrane ER-initiated signaling through multiple kinases modulates the migration of ECs (8), monocytes (78), breast cancer cells (79), and VSM cells (80). These pathways depend upon the phosphorylation of multiple substrate proteins, altering the activity of these proteins that promote motility.

Extranuclear estrogen signaling also modulates genes whose products regulate phosphatase activity. Rapid signaling by membrane ER through PI3K stimulates the MCIP-1 (modulatory calcineurin-interacting protein 1) gene (22). The MCIP protein clamps calcineurin (protein phosphatase 2B), and down-regulates its activity, preventing the nuclear factor of activated T cells family of transcription factors from moving to the nucleus of the cardiomyocyte. This is an important mechanism through which rapid E2 signaling prevents cardiac hypertrophy (22).

4. ER in the mitochondria. In addition to cytoplasmic ER that are tethered adjacent to the cell membrane, there are discrete cytoplasmic pools of these receptors. A prominent example is mitochondria, where both classical ER isoforms are found (81, 82). In breast cancer cell mitochondria, ERβ is more abundant than ERα. Mitochondrial ER prevent radiation-induced cell death of breast cancer cells by mitigating mitochondrial reactive oxygen species (ROS) formation and death kinase activation. This occurs through the rapid stimulation of mitochondrial MnSOD (manganese superoxide dismutase) activity (83).

Signaling from the membrane to the mitochondria also occurs. In cardiomyocytes exposed to hypoxia/normoxia injury, mitochondrial ROS formation triggers p38α-induced apoptosis. Membrane ER stimulates the rapid induction of p38α activity through PI3K, leading to the inhibition of ROS formation in mitochondria and resulting cell survival (71). Mitochondrial ER also promotes the transcription of mitochondrial-encoded genes (84). It is likely that this ER pool cross-talks with nuclear ER to coordinate the synthesis of proteins that are essential for the normal respiratory function of mitochondria.

D. Outcomes of extranuclear estrogen signaling

Many organ systems are impacted by rapid estrogen signaling to the development, growth, survival, and function of cells. The mechanisms are often consistent from system to system, with some unique aspects conveyed in context. Multiple pathways involving ERK, PI3K, and PKC result from upstream signaling (e.g., G proteins and Src kinase) that triggers effector kinase action. A complete depiction of all signaling studies is beyond the scope of this review, but recent work that highlights the physiological impact of rapid signaling outside the reproductive tract is presented (Table 1).

1. Bone. Rapid signaling by estrogenic compounds impacts many aspects of bone cell biology. The survival (85) and differentiation of osteoblasts (86) depends upon estrogen activation of several kinase systems, including Src, ERK, and PI3K. A purportedly selective agonist for the membrane ER, estren, has been shown to trigger the Wnt/β-catenin transcription system, impacting cell function. Estren also reverses bone loss in ovariec-tomized mice, possibly by signaling through membrane ERα (87). However, estren also engages the androgen receptor (AR) (88), indicating that the mechanisms of this compound’s effects are complex. E2-induced defenses against ROS formation may prevent bone loss and osteoclast formation (89, 90). In part, this may occur from estrogen rapid signaling in mitochondria, as occurs in breast cancer cells (83). Inhibition of osteocyte apoptosis occurs through nuclear-translocated ERK (77), and stimulation of osteoclast death relies on prolonged ERK activation (91).

2. Central nervous system. Several laboratories have shown that estrogens limit neuronal damage and death, possibly by signaling through PI3K, PKC, ERK, or glycogen synthase kinase 3-β (92–95). Models tested include brain injury created by experimental cerebrovascular compromise (stroke) (92, 93) or the administration of glutamate (96), alcohol (94), or MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine, a chemical that creates a rodent model of Parkinson’s disease) (97). Extranuclear ER in neurite extensions may mediate these effects (98, 99).

Rapid signaling by E2 through ERα and ERK phosphorylates cAMP-response element binding protein (CREB) in cortical neurons in vivo (52). Such signaling by E2 has also been reported to modulate the plasticity of sexual behavior in birds and fish (100, 101). Dendritic spine formation in cortical and hippocampal neurons promotes synapse formation and plasticity. Rapid signaling by estrogen has been found to contribute to these sex steroid effects in the brain (102, 103). A more extensive review of the central nervous system effects of rapid signaling by estrogen has recently been published (54).

3. Cardiovascular. Rapid signaling by estrogen modulates EC and VSM migration and proliferation (8, 80). In part this may be related to the ability of estrogen to stimulate NO formation in ECs through Gα and Gβγ proteins (104), activating PI3K and ERK (7, 105). Both ERα and ERβ have been implicated in the rapid signaling to NO production in vivo, resulting in rapid arterial dilation induced by the sex steroid (106). Rapid signaling underlies the E2 activation of gene expression in the heart (107), and PI3K activity has been implicated in the...
molecular program by which E2 prevents cardiomyocyte hypertrophy (22) and the response to arterial injury in vivo (108). E2 also signals through PI3K (109) and p38β (71) to prevent ischemia/reperfusion injury in the heart and in isolated cardiomyocytes.

### III. Progesterone Receptors

#### A. Introduction

Progesterone plays a critical role in regulating multiple reproductive processes, including follicle growth, oocyte maturation, ovulation, implantation, and the maintenance of pregnancy. In addition, progestins are critical mediators of both normal and carcinogenic breast development. Several studies suggest that extranuclear, transcription-independent signaling may be important for many of these progesterone-mediated processes (110).

In general, transcription-independent progesterone effects seem to be mediated via the classical progesterone receptor (PR) B; however, similar to GPR30 and estrogens, membrane progesterone binding proteins that appear to function as GPCRs may also contribute to progestin-mediated signaling outside of the nucleus.

#### B. Nature of membrane progesterone receptors

1. Classical PRs

   a. Breast. As observed with classical ERs, the classical PRB form can mediate progesterone-triggered activation of Src and downstream MAPK signaling in breast cells (111). Unlike ER-mediated Src signaling, human PRB-induced Src activation is regulated by direct binding between a proline-rich domain within the amino terminus of PRB and the SH3 domain of Src (Fig. 2). Mutation of the polyproline motif abolishes both Src binding to and activation by PRB (111). Although no evidence currently demonstrates that direct PRB-mediated activation of Src is membrane initiated, evidence indicates that it is occurring outside of the nucleus (112). In fact, PRB is detected at the cell membrane in some cells, and its sequence contains the palmitoylation site/membrane-localization signal found in multiple classical steroid receptors (49). These findings therefore indicate that PRB is at least in the appropriate location to initiate signaling from the membrane.

   Interestingly, as seen with other classical steroid receptors, rapid PRB-mediated activation of Src leads to transactivation of the EGFR, resulting in sustained MAPK signaling (113) that may have significant biological effects. For example, extranuclear PRB-mediated activation of Src, EGFR, and MAPK appears to be critical for promoting transcription of the cyclin D1 gene (112, 114), as well as sustained progesterone-mediated up-regulation of cyclin D1 protein expression (by increasing translation and/or stability) (113). Because the cyclin D1 promoter does not contain a classical PR response element, these observations suggest that extranuclear PRB-mediated signaling rapidly activates or enhances activity of alternative transcription factors that in turn promote cell cycle progression and possibly proliferation (113, 115).

   In addition to PRB rapidly activating the EGFR, direct activation of the EGFR conversely triggers phosphorylation of PRB and its ligand-independent accumulation in the nucleus (116, 117). This further demonstrates the importance of cross-talk between multiple receptor families (Fig. 2).

   Finally, the amino-terminal proline-rich motif is relatively specific for the human PRB (for example, it is not found in the mouse PRB). This suggests that direct activation of Src by PRB may be specific for both human breast cell development and proliferation, as well as for the stimulation of breast cancer progression.

   b. Oocytes. Because steroid-mediated processes such as breast cell proliferation involve both membrane- and nuclear-initiated steroid signaling, dissecting the relative physiological importance of each is often difficult. One of the oldest models of true nongenomic steroid signaling is steroid-triggered maturation, or meiotic progression, of oocytes (118, 119) (Table 1). Significant evidence accumulated since the 1960s has shown that steroid-mediated oocyte maturation is mediated through the release of membrane-bound EGF-like proteins (e.g., amphiregulin or HB-EGF). Activated EGFR then promotes MAPK signaling, which subsequently enhances the activity of multiple nuclear transcription factors. MAPK signaling also leads to phosphorylation and nuclear accumulation of PRB. In X. laevis oocytes, androgens promote maturation by signaling through the AR and MNAR to suppress constitutive Gβγ and Gαs activity and lower intracellular cAMP (right). In fish, progestins promote oocyte maturation by signaling through a Gαs-coupled mPR. Membrane-bound mPRs may also regulate other Gαs-mediated processes in somatic cells, and endosomal mPRs may regulate calcium flux in response to progestins.
2. Alternative membrane PRs (mPRs)

a. mPRs. A novel class of PRs that might contribute to the regulation of oocyte maturation, as well as to other progestin-mediated reproductive processes, is the membrane PR family, or mPR. The first of these mPRs was cloned from oocytes of the spotted seatrout (125, 126). Similar to frogs, progestins trigger fish oocyte maturation, although the physiological maturation-promoting steroid in sea trout is the progesterone metabolite 21-trihydroxy-4-pregnen-3-one (20β-S).

Interestingly, mPRs bear structural, but virtually no sequence, homology to the GPCR family. Binding studies primarily using bacterially expressed mPR reveal specific, high-affinity interactions between mPRs and progestins. Furthermore, under some conditions, overexpression of mPRs in breast cells mediates a pertussis toxin-sensitive decrease in intracellular Calcium mobilization, presumably from the endoplasmic reticulum. Similarly, stable expression of the human mPRα, -β, and -γ proteins in both HEK293 and MDA-MB-231 breast cells demonstrates expression predominantly in the endoplasmic reticulum. Surprisingly, transfection-dependent progesterone binding or progesterone-mediated signaling was not observed in these transfected cells (132).

Together, these studies indicate that detailed characterization of the mPR family of proteins will be required to elucidate its biological importance as a bona fide membrane PR, including specific mPR knockouts in mouse models.

b. Progesterone membrane receptor component-1 (PGRMC1).

Another rapid transcription-independent progesterone-mediated phenomenon is the acrosomal reaction in sperm (133–135). This response occurs within seconds to minutes after exposure to progesterone, and progesterone binding sites have been identified on the cell surface of sperm. Some evidence suggests that a membrane-localized progesterone-binding protein termed PGRMC1 might be binding to progesterone and in part be regulating this process; however direct evidence supporting its role in the acrosomal response is still forthcoming (135). PGRMC1 and its binding partner, plasminogen activator inhibitor RNA-binding protein-1 (PAIRBP1), are also expressed in primary human granulosa/luteal cell cultures and may play a role in regulating anti-apoptotic actions of progesterone (136). Despite these intriguing observations, however, rigorous demonstration of progesterone-mediated signaling through PGRMC1 or its homolog PGRMC2 has yet to be described. Interestingly, PGRMC1 is also expressed in liver microsomes, and one study suggests that it may in fact serve as an important cofactor for multiple cytochrome P450 enzymes (137, 138).

These results put forth the intriguing possibility that PGRMC1 may not only be a PR, but might also assist in the triggered Xenopus oocyte maturation (128, 129), which is inconsistent with the known signaling properties of the mPRs. Second, substantial evidence suggests that androgens, rather than progesterone, mediate oocyte maturation in frogs (see Section IV.A.1), and evidence from several laboratories shows that mPR do not bind androgens (126, 127, 130). Thus, a generalized role of mPR in regulating oocyte maturation remains to be elucidated.

Interestingly, mPRs have been detected in human myometrial tissue, where progesterone also appears to lower intracellular cAMP levels in a pertussis toxin-sensitive fashion. Progestins also activate multiple kinase cascades in myometrial cells, possibly via mPR, ultimately resulting in the enhanced activation of the classical PRB (130). These progesterone-induced signals may play a role in regulating the quiescent vs. contractile state of the myometrium at the end of pregnancy. Furthermore, this cross-talk suggests that, in some instances, progesterone-mediated biological effects may require complex signaling interactions between multiple PRs.

In contrast to the aforementioned studies, overexpression of an ovine mPR isofrom in CHO cells results in a different phenotype (131). Here, the mPR is present almost exclusively in the endoplasmic reticulum, and progesterone triggers intracellular calcium mobilization, presumably from the endoplasmic reticulum. Similarly, stable expression of the human mPRα, -β, and -γ proteins in both HEK293 and MDA-MB-231 breast cells demonstrates expression predominantly in the endoplasmic reticulum. Surprisingly, transfection-dependent progesterone binding or progesterone-mediated signaling was not observed in these transfected cells (132).

Together, these studies indicate that detailed characterization of the mPR family of proteins will be required to elucidate its biological importance as a bona fide membrane PR, including specific mPR knockouts in mouse models.
IV. Androgen Receptors

A. Function and importance

1. Oocytes. As mentioned, the modest effects of altering expression of classical PR or mPR on progesterone-induced X. laevis oocyte maturation suggest that alternative receptors must be involved. In fact, these differences are not only due to alternative receptors, but also to alternative ligands. Interestingly, Xenopus oocytes express high levels of the cytochrome p450 enzyme CYP17, which converts progestins to androgens. Thus, exposure of oocytes to progesterone results in its rapid metabolism to the androgen androstenedione (139–142). Androgens such as androstenedione and testosterone are at least as potent as progesterone in promoting oocyte maturation in vitro. Therefore, progesterone exposure results in steroid signaling by two agonists, a progestin and an androgen, which likely explains the partial effects of altering PR or mPR levels on progesterone-induced maturation in vitro.

In addition to the androgen production from exogenous progesterone added to isolated oocytes, human chorionic gonadotropin treatment of follicles both in vitro and in vivo induces 10-fold more testosterone production relative to progesterone (140, 143, 144). In vivo, blockade of androgen production significantly reduces human chorionic gonadotropin-induced oocyte maturation as well as ovulation (145). These observations suggest that, contrary to previous assumptions, androgens rather than progesterone mediate oocyte maturation in ovulating X. laevis frogs (Table 1). Evidence suggests that the classical X. laevis AR mediates nongenomic testosterone effects in oocytes, because reduction of classical AR activity abrogates androgen-mediated signaling and maturation (140, 141, 145).

Similar to the ER, androgens (and possibly progesterins) appear to alter G protein signaling. In this situation, however, steroids inhibit constitutive Ga and Gβγ signaling that hold oocytes in meiotic arrest (129, 146–149). Accordingly, electrophysiological studies demonstrate that androgens rapidly attenuate Gβγ signaling in oocytes through interactions with the classical AR (143). This possibly occurs through the scaffolding actions of the MNAR (Fig. 2) (150).

2. Prostate. In addition to mediating androgen-triggered oocyte maturation, MNAR may regulate AR signaling in human somatic cells. Specifically, AR or EGFR activation leads to AR-mediated activation of Src and subsequent downstream signals in prostate cancer LnCAP cells (151–153). This may involve a direct interaction between AR and Src, as well as an MNAR-regulated interaction (154). Furthermore, as seen with the ER, AR signaling appears to originate in caveolae located within the plasma membrane (155, 156). Interestingly, AR and MNAR interact in an androgen-dependent fashion to activate Src in LnCAP cells that require androgen for growth but constitutively interact with and activate Src in LnCAP cells that no longer require androgen for growth (154). This observation suggests that: 1) AR/MNAR/Src interactions may be very important for androgen-mediated prostate cell growth (Fig. 2); and 2) the inevitable androgen-independent growth of prostate cancer may be due in part to constitutive activation of Src by the AR.

3. Testes. In Sertoli cells, testosterone is important for many functions, including tubule development, spermatid growth, and the release of mature spermatozoa (157). Very few transcriptional targets for testosterone have been identified in Sertoli cells, suggesting that extranuclear androgen signaling may be important for these processes. Accordingly, testosterone rapidly activates Src in Sertoli cells by signaling via the AR, leading to activation of the EGFR and subsequent MAPK and CREB signaling (158, 159). These events ultimately promote long-term activation of the CREB, resulting in up-regulation of CREB-responsive genes in Sertoli cells. Because Src and CREB are important for spermatozoa development, we speculate that extranuclear testosterone signaling may promote these processes, although more detailed in vivo work is needed to prove this hypothesis.

V. Glucocorticoid Receptors

A. Function and importance

1. Pituitary. A concern regarding the physiological importance of rapid nongenomic actions of sex steroids is that quick changes in the serum concentrations of these hormones are uncommon in vivo. Thus, the biological need for rapid signaling is questioned. However, one clear example of rapid steroid effects is the immediate suppression of ACTH secretion from the pituitary in patients treated with glucocorticoids (160, 161) (Table 1). This rapid inhibition of ACTH release appears to be mediated by classical glucocorticoid receptors (GRs) signaling via extranuclear pathways. Treatment of human pituitary folliculosectome cells with glucocorticoid leads to rapid phosphorylation of annexin-1, followed by its translocation to the cell membrane where it contributes to the inhibition of ACTH secretion (162). This rapid steroid effect is blocked by the GR antagonist mifepristone, suggesting that classical receptors may be involved. Furthermore, glucocorticoid-induced phosphorylation and translocation of annexin-1 appears to be mediated through activation of the extranuclear signaling molecules PKC, PI3K, and MAPK.

2. Hippocampus. Similar to observations in the pituitary, glucocorticoids trigger rapid signaling in the hippocampus. Introduction of stress-dose concentrations (10–100 nm) of corticosterone to mouse CA1 pyramidal cells of the hippocampus results in a rapid increase in the frequency of excitatory postsynaptic potentials (163). This effect appears to be regulated via the mineralocorticoid receptor (MR) rather than the GR. This action is lost in mice lacking mineralocorticoid receptors in the hippocampus, and synthetic GR antagonists do not inhibit the corticosterone effects. These observations suggest that rapid responses to stress in the hippocampus may be related to extranuclear cortisol signaling, although further studies are needed.

3. Cardiovascular. Administration of glucocorticoids to patients with myocardial infarction or stroke leads to a rapid...
and transient decrease in blood pressure associated with a concomitant increase in coronary and cerebral blood flow (164). In mice, administration of high-dose corticosteroids after a transient ischemic/reperfusion injury decreases vascular inflammation and myocardial infarct size (165). This effect appears to be mediated through classical GRs, because it is blocked both in vitro and in vivo by GR antagonists. Similar to estrogen effects in the vasculature, glucocorticoids rapidly activate PI3K and Akt, which leads to an increase in NO synthase activity. Importantly, the glucocorticoid effects are lost in mice lacking eNOS, demonstrating the importance of this enzyme for glucocorticoid-induced vasorelaxation.

4. Immune system. Glucocorticoids are known to have suppressive effects on immune function. Although the mechanisms regulating these suppressive effects are likely multifactorial, rapid GR-mediated signaling may be playing some role in regulating T cell function. The GR appears to associate with the T cell receptor signaling complex, and stimulation of T cells with dexamethasone inhibits T cell receptor signaling by disrupting the T cell receptor complex (166).

VI. Mineralocorticoid Receptors
A. Function and importance

1. Cardiovascular. Another physiological example of rapid steroid effects is the fast-acting attenuation of baroreflex sensitivity (the change in heart rate in response to increased systemic vascular resistance) by the mineralocorticoid aldosterone (167). Although the underlying mechanisms are not well characterized, rapid aldosterone-mediated signaling has been observed in cardiovascular cells. For example, aldosterone triggers a rapid flux of intracellular calcium in VSM cells (168, 169). This mineralocorticoid-mediated effect may occur independently of the classical MR, because the MR antagonist spironolactone does not block the aldosterone-triggered signals. Similarly, aldosterone activates both a rapid calcium flux and PKC-dependent activation of the Na+/K+ pump in rabbit cardiomyocytes, effects that are also not blocked by spironolactone (170–172). However, the aldosterone responses in cardiomyocytes are abrogated by another MR antagonist, RU28318; suggesting that the pharmacology of rapid classical steroid receptor signaling may differ depending upon the generated signal. More complete studies using genetic knockout models will be needed to delineate the true role of the MR in regulating these extranuclear mineralocorticoid effects in both vascular and kidney cells.

2. Kidney. In contrast to cardiomyocytes, the kidney contains sufficient 11β-hydroxysteroid dehydrogenase 2 to inactivate all local cortisol; thus, MRs in the kidney are extremely sensitive to small changes in aldosterone levels. Accordingly, aldosterone rapidly activates Na+/H+ exchange through PKCα and MAPK signaling in M1 cortical-collecting ducts. Similarly, aldosterone increases Na+/K+ exchange in cultured kidney cells and promotes intracellular calcium flux through cross-activation of the EGFR followed by MAPK activation (174, 175). Finally, aldosterone rapidly inhibits bicarbonate absorption and Na+/H+ exchange in the medullary thick ascending limb in a MAPK-dependent fashion (176). All of these effects are not blocked by spironolactone, bringing into question (but not ruling out) the importance of the classical MR. Interestingly, aldosterone mediates activation of Src and MAPK signaling, as well as calcium flux, when the MR is expressed in CHO and HEK cells (170). Although Src and MAPK activation are blocked by spironolactone in these cells, calcium flux is unaffected by the MR antagonist. This again suggests that the pharmacology of MRs by mineralocorticoids, as well as the receptors mediating these responses, may differ depending upon the generated signal.

VII. Thyroid Hormone Receptors
A. Functions

Rapid signaling by the thyroid hormones T3 and T4 is well established. Fast-acting thyroid-mediated modulation of ion channel function (177), erythrocyte membrane calcium-ATPase activity (178), or protein kinase A activation (179) has been known for 40 yr. Interestingly, the αvβ3 integrin protein is a potential cell surface receptor for T4 (180). Signaling from the engagement of this integrin by T4 promotes ERK activation (181) and other rapid signals (182). Such signaling may contribute to the effects of T3, T4 to promote angiogenesis (183), thyroid cancer and glioma cell proliferation and survival (184, 185), neural cell migration (186), and myocardial function (187).

As seen with steroid hormones, rapid extranuclear T4 actions at the integrin receptor promote the nuclear transcription actions of the classical thyroid hormone receptor. This includes both the acetylation and phosphorylation of thyroid receptor (TR) β (188), perhaps leading to the phosphorylation and displacement of corepressors (SMRT and N-CoR) from the nuclear TR transcriptosome (188). Rapid signaling by T4 has also been reported to modify nuclear ER function, and a cross-interaction by estrogen and thyroid hormone for transcription has been reported (189, 190).

B. Classical thyroid receptors

Evidence also exists that the classical (nuclear) TR mediates rapid signaling by thyroid hormone. TR physically associates with p85α and up-regulates PI3K activity in ECs (108).

A mutant TRβ1 receptor expressed in mice conveys thyroid hormone resistance but also leads to the development of
follicular thyroid cancer (191). The cancerous cells show activation of PI3K, AKT, and the downstream targets integrin-linked kinase and mTOR-S6 kinases. Many of these signaling molecules are thought to contribute to the developmental biology of the thyroid cancer, because human thyroid cancer shows AKT overexpression, and Cowden’s syndrome patients (PTEN mutation) develop thyroid neoplasms (192, 193).

In ECs, TRα1 expression greatly exceeds TRβ1 expression, and only TRα1 interacts with PI3K in thyroid hormone-dependent fashion (108). TRα1 activation results in eNOS phosphorylation and activation, leading to decreased blood pressure due to NO production. Thyroid hormone, probably acting through TRα1, also stimulates cerebral blood flow and decreases the extent and functional consequence of brain damage in mice after focal ischemia (194). These protective effects of T3 are diminished in either eNOS or TRα1/TRβ1 knockout mice.

It is unclear at this point whether and how classical TRα or TRβ translocates to the plasma membrane to promote signaling. Also, it is unknown whether there is cooperation between integrin receptors and classical TR to effect rapid signaling by T3 or T4. In various cell contexts, there may be a range of rapid responses to thyroid hormone linked to activation of one or more TRs.

VIII. Vitamin D Receptors

1,25-Dihydroxyvitamin D3 binds to a protein that is considered part of the steroid receptor superfamily (195). Vitamin D stimulates the rapid absorption of calcium from the intestine in various animal models and modulates insulin secretion from the pancreas, migration of cells, and ion-channel opening in bone cells (osteoblasts) (reviewed in Ref. 196).

Although not yet isolated from the membrane, a caveolae-enriched membrane fraction from various cell types contains a high-affinity 1,25-vitamin D3 binding protein (197). This protein is detected in osteoblast membrane fractions with antibodies to the classical “nuclear” vitamin D3 receptor. Furthermore, several groups have shown that rapid, non-transcriptional effects of 1,25-dihydroxyvitamin D3 fail to occur in mice that either have the DNA-binding domain of the classical vitamin D receptor deleted (198) or lack classical vitamin D receptor altogether (197, 199). This was extended to a patient with vitamin D-resistant rickets, whose cells carried a vitamin D receptor mutation and lacked rapid responses (194).

Rapid extranuclear signals stimulated by vitamin D3 include c-Src, PI3K, and phospholipase C activation (200, 201). These signals lead to VSM migration (202) and the cell survival of osteoblasts and osteocytes (201). In part, the rapid signaling by membrane vitamin D receptors modulates transcription, as shown by rapid phosphorylation of inhibitory kβα, and subsequent nuclear factor kβ translocation to the nucleus of leukemia cells, contributing to cell differentiation (203).

A second putative vitamin D receptor at the membrane of intestinal and other cell types has recently been identified (204). Designated as MARRS (membrane-associated, rapid response steroid binding protein), this protein (also known as Erp57) seems to play a role in phosphate and perhaps calcium uptake/transport in intestinal cells from younger animals. A second function for MARRS may be related to the observation that TGFβ stimulates the transcription of MARRS in intestinal cells (205). Perhaps this receptor provides a means of adapting to stress during inflammation. Detailed studies of these two putative membrane vitamin D3 binding proteins are ongoing.

IX. Summary

The many pathways of signal transduction that result from membrane steroid receptor function influence all cellular processes. However, it is unlikely that MISS is primarily responsible for modulating the fundamental processes that cells undergo in response to steroids. Rather, it is the convergence of signals at the membrane, cytoplasm, and nucleus that results in the integrative effects of steroid hormones. For instance, rapid signaling through kinases modulates mitochondrial ER functions and nuclear receptor-entrained gene transcription. Likewise, nuclear ER-modulated gene transcription promotes the synthesis of functional proteins that constitute essential mitochondrial respiratory complexes, as well as signaling molecules acting at the membrane.

The challenge is to model these integrative functions in vivo and in vitro, affording a better understanding of the kinetics and pathways of steroid function. To define cell surface ER action better, membrane-impermeable estrogen conjugates, such as E2-BSA and E2-horseradish peroxidase, have been used. However, after extended exposure to living cells, E2-BSA may dissociate into BSA and E2 (206) requiring filtration and careful handling. Recently, dendrimeric-conjugated estrogenic compounds have been used to exclude the steroid from the cell interior. These compounds activate kinase signaling and unique gene profiles in breast cancer cells (207, 208). The utility of dendrimeric ER agonists is limited at present to in vitro studies, and ER isoform-specific dendrimeric agonists and antagonists are not yet available.

If these approaches can be applied to in vivo disease, such models might create opportunities for the design of sophisticated new “synthetic” steroids that might selectively avoid undesirable effects but promote desirable actions. In this way, the ability of steroids to prevent inflammation, promote cell differentiation, and augment cell survival and proliferation (when appropriate) can be maximized. Every eukaryotic system is importantly modulated by steroids; therefore developing compounds that selectively target human organs is a primary goal for the near future.

Acknowledgments

Address all correspondence and requests for reprints to: Stephen R. Hammes, M.D., Ph.D., University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-8857. E-mail: stephen.hammes@utsouthwestern.edu; or Ellis R. Levin, M.D., Medical Service (111-I), Long Beach VA Medical Center/University of California-Irvine, 5901 East 7th Street, Long Beach, California 90822. E-mail: ellis.levin@va.gov
This work was supported by grants from the Research Service of the Department of Veterans Affairs, National Institutes of Health Grants CA-100366 and DK59913, and Welch Foundation Grant I-1506.

Disclosure Statement: The authors have nothing to disclose.

References

34. Lu Q, Pallas DC, Surks HK, Baur WE, Mendelsohn ME, Karas RH 2004 Striatum assembles a membrane signaling complex necessary for rapid, nongenomic activation of endothelial NÖ synthase by estrogen receptor α. Proc Natl Acad Sci USA 101:17126–17131

Hammes and Levin • Extraneural Steroid Receptors
cytokine production after trauma-hemorrhage are mediated primarily via estrogen receptor α. Am J Physiol Cell Physiol 292: C2103–C2111.


738 Endocrine Reviews, December 2007, 28(7):726–741


Chen JQ, Yager JD 2004 Estrogen’s effects on mitochondrial gene expression: mechanisms and potential contributions to estrogen carcinogenesis. Ann NY Acad Sci 1028:258–272


Lean JM, Jagger CJ, Kirstein B, Fuller K, Chambers TJ 2005 Hydrogen peroxide is essential for estrogen-deficiency bone loss and osteoblast formation. Endocrinology 146:726–735


Goodenough S, Schlesner D, Pietrzik C, Skutella T, Behl C 2006 Phosphatidylinositol-3 kinase/Akt signaling pathway in the neuroprotective effect of estradiol in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice. Mol Pharmacol 69:1492–1498


Faivre EJ, Lange CA 2007 Progesterone receptors upregulate Wnt-1 to induce epithelial growth factor receptor transactivation and c-Src-dependent sustained activation of Erk 1/2 mitogen-activated protein kinase in breast cancer cells. Mol Cell Biol 27:466–480


Endocrine Reviews, December 2007, 28(7):726–741


180. Bergh JJ, Lin HY, Lansing L, Mohamed SN, Davis FB, Mousa S, Davis PJ 2005 Inegrin αVβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. Endocrinology 146:2864–2871.


Hammes and Levin • Extraneuronal Steroid Receptors


Endocrine Reviews is published by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Course Title: 2nd Annual International Adrenal Cancer Symposium: Clinical and Basic Science
Course Date: March 13–16, 2008

Location: Biomedical Science Research Building
University of Michigan Medical School
Ann Arbor, MI 48109

Websites: http://www.med.umich.edu/intmed/endocrinology/acs.htm
http://cme.med.umich.edu/events/

Contact: Registrar
Office of Continuing Medical Education
University of Michigan Medical School
G1200 Towsley Center
1500 E. Medical Center Drive, SPC 5201
Ann Arbor, MI 48109-5201
Phone: 734-763-1400; Fax: 734-936-1641

Downloaded from https://academic.oup.com/edrv/article-abstract/28/7/726/2354994 by guest on 05 March 2018