Rapid signaling by steroid receptors

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Levin ER. Rapid signaling by steroid receptors. Am J Physiol Regul Integr Comp Physiol 295: R1425–R1430, 2008. First published September 10, 2008; doi:10.1152/ajpregu.90605.2008.—Steroid receptors transcribe genes that lead to important biological processes, including normal organ development and function, tissue differentiation, and promotion of oncogenic transformation. These actions mainly result from nuclear steroid receptor action. However, for 50 years, it has been known that rapid effects of steroid hormones occur and could result from rapid signal transduction. Examples of these effects include stress responses to secreted glucocorticoids, rapid actions of thyroid hormones in the heart, and acute uterine/vaginal responses to injected estrogen. These types of responses have increasingly been attributed to rapid signaling by steroid hormones, upon engaging binding proteins most often at the cell surface of target organs. It is clear that rapid signal transduction serves an integrated role to modify existing proteins, altering their structure and activity, and to modulate gene transcription, often through collaboration with the nuclear pool of steroid receptors. The biological outcomes of steroid hormone actions thus reflect input from various cellular pools, coordinating the necessary events that are restrained in temporal and kinetic fashion. Here I describe the current understanding of rapid steroid signaling that is now appreciated to extend to virtually all members of this family of hormones and their receptors.

steroid hormones; estrogen; progesterone; androgen; glucocorticoids

Background of Rapid Steroid Signaling

The modern era of rapid steroid signaling may have developed from the work on glucocorticoids from Hans Selye (79), but was probably best initially defined by how estrogen rapidly signals (84). Claire Szego and colleagues published a series of papers defining the rapid effects of 17β-estradiol (E2) in animals and cells (68, 69, 84). These investigators identified a high-affinity estrogen binding protein (receptor) at the plasma membrane of several cell types that respond to estrogen administration with cAMP generation and calcium changes (68, 69, 84). These studies encouraged a number of scientists to investigate rapid signaling by glucocorticoids, mineralocorticoids, thyroid hormones, androgens, progestins, and vitamin D (reviewed in Ref. 31). In most situations, the rapid responses result from steroid hormones binding a cell surface receptor. The nature and functions of these plasma membrane-localized steroid binding proteins have been the subject of much investigation and continue to be defined in cell-specific fashion. However, there are great similarities between the actions of all of these hormones and homology of the cell membrane-localized receptors that mediate steroid-induced signal transduction.

The concept of membrane-localized steroid receptors is an ancient one, as plants produce brassinosteroids that are required for flowering and fertility (3). There are no steroid receptors in the nucleus of plants; rather, brassinosteroids bind a cell membrane tyrosine kinase receptor to signal and activate kinase cascades that are essential to plant function (3, 42). Thus membrane steroid receptor signaling has been conserved through phylogeny and has evolved more recently to include nuclear receptor signaling in mammals.

Nature of Membrane Steroid Receptor

Many, but perhaps not all, steroid receptors are the nuclear receptors translocated to the plasma membrane. Nuclear estrogen receptors (ER-α, ER-β), progesterone receptors (PR-A and -B), and the androgen receptor (AR) have been found at the plasma membrane of various cell types (67). A conserved palmitoylation motif in the E domain of sex steroid receptors is required for membrane localization and rapid signal transduction to cell biology (67). Palmitoylation of ER-α enhances the physical association of this receptor with caveolin-1 (1), and this leads to transport to the plasma membrane (43, 71). Palmitoylation of endogenous, monomeric ER-α occurs in cytoplasm. ER-α at the membrane of endothelial cells (ECs) undergoes homodimerization on exposure to E2, and homodimerization is required to generate most rapid signals (74). Interestingly, homodimerization of nuclear ER is also required for transcriptional function (85). Heterodimerization of membrane ER-α and ER-β occurs and importantly contributes to the vasodilation and anti-cardiac hypertrophy effects of the sex steroid in vivo (27, 66). The structural determinants of ER heterodimerization are unknown, and so this area is underdeveloped as to impact for most cell types.

ER-α and ER-β are found mainly, but not exclusively, in caveolae rafts, where a close association with a large number of signaling molecules facilitates signal transduction (11, 38, 72). However, membrane localization varies between cell types, where ER-β is strongly expressed at the membrane of ECs, but not in breast cancer cell lines (65). AR also localizes...
to caveolae, and this is important to rapid signaling (23). Recent work suggests that methylation of ER-α at arginine 260 in the DNA binding domain is initiated by ligand binding the receptor. This methylation promotes cytoplasmic localization of ER-α and association with signaling molecules, such as focal adhesion kinase, Src kinase, and the p85 subunit of phosphatidylinositol 3-kinase (PI3K) (41). Perhaps arginine 260 methylation regulates recycling of membrane-localized ER to cytoplasmic compartments (endosomes), where signaling is known to occur for more typical G protein-coupled receptors (GPCRs) (50).

New work strongly suggests that classical glucocorticoid and mineralocorticoid receptors mediate rapid signaling by their respective ligands when localized to plasma membrane rafts (26, 52). Similarly, nuclear vitamin D receptors have been found in caveolae membrane rafts (35), where rapid signaling commences to downstream biology, including vascular smooth muscle cell migration, osteoblast, and osteocyte survival (9, 76, 89). Exactly how these steroid family receptors traffic to the membrane has not been established. In summary, most members of the steroid receptor superfamily are found localized to the cell membrane and appear to mediate signal transduction by steroid ligands.

**Alternative Steroid Hormone Receptors**

There have also been proposals that alternative binding proteins for steroid hormones mediate signal transduction. For ER, several putative receptors have been described in neural cells, including one that is present in the combined ER-α/ER-β knockout (KO) mouse (70, 86). These proteins have not been isolated, so the nature of these receptors is not clear.

Another recently identified protein that has been claimed as an estrogen binding protein is the orphan receptor, GPCR 30 (GPR30). Initially described as a plasma membrane-localized “ER” (22), this protein has also been found in the endoplasmic reticulum, where it has been reported to mediate PI3K and calcium signaling (77). It must be appreciated that a distinct pool of endogenous ER-α also exists in this cytosolic site and can mediate these same signals in response to E2 (77). Many papers have been published on this protein, with the consensus being that GPR30 collaborates with membrane-localized, classic ER-α and many other signal proteins, to effect signal transduction in some cell types (90, 2). However, there is not good evidence that this protein acts as a stand alone ER (65).

The latter conclusion has been derived from studies in cells from ER-α/ER-β KO mice that show no response to E2, despite the presence of GPR30 (65). Recent work from Otto et al. (61) shows that E2 does not bind GPR30 or activate rapid signals as dependent on this receptor. Also, silencing GPR30 with small interfering RNA has no effect on gene transcription modulated by estrogen dendrimer conjugates (only act at the cell membrane) (49). In addition, whereas the female ER-α KO mouse has a distinctly abnormal reproductive tract and mammary gland phenotype (7, 17, 47), the GPR30 homozygous KO female mouse is viable and fertile and does not show any obvious physical abnormalities, including reproductive, neurological, and immunological deficiencies (91).

Additional receptors that mediate progesterone rapid signaling from the membrane have been proposed and are also controversial. Thomas and colleagues (93, 94) identified a family of membrane-localized progesterone binding proteins [membrane progestin receptor (mPR)] in fish through humans, as distinct products of genes that are separate from the gene encoding classical PR-A/PR-B. In contrast, nuclear PR associates with Src in the vicinity of the plasma membrane and rapidly signals to cell biology (8). KO mouse models for mPR have not yet become available and may be difficult to create because of the potential functional redundancy of mPR family member proteins. Furthermore, convincing biological effects of progesterone in the PR-A or PR-B KO mouse comparable to sex steroid actions in normal mice have not been well established. Thus this area requires additional clarification to know whether receptors at the membrane, in addition to translocated nuclear PR, importantly mediate rapid signaling by this sex steroid. Another membrane-localized progesterone binding protein, progesterone membrane receptor component-1, has been designated PGRMC1. This protein has been proposed to bind progesterone and mediate the acrosomal reaction in sperm (45). PGRMC1 and its binding partner, plasminogen activator inhibitor RNA-binding protein-1, may play a role in regulating antipapoptotic actions of progesterone in granulosa or luteal cells (20). It is unclear whether this receptor extensively mediates progesterone actions in humans.

A second vitamin D receptor, termed MARRS (membrane-associated, rapid-response, steroid-binding; also known as Erp57) has been isolated and may mediate the intestinal absorption of phosphate and calcium, particularly in younger animals (58). Recent work suggests that late chondrocyte differentiation is inhibited by 1,25-dihydroxyvitamin D3 binding to MARRS (16). How classical, membrane-localized vitamin D receptor and MARRS collaborate to mediate many effects on bone and the intestine is under investigation.

The thyroid hormone receptor (TR) that mediates T3 (triiodothyronine) or T4 (thyroxin) rapid signaling is also unclear at present. There is support that classical TR-α1 physically associates with and activates the p85 subunit of PI3K in ECs in response to T3 (80) and may rescue mice from extensive neuronal damage in a stroke model (34). The rapid signaling by TR-α1 results in endothelial nitric oxide (NO) synthase (eNOS) activation, NO generation, and decreased blood pressure. A mutant TR-β1 causes the transformation of thyroid follicular cells into cancer in the mouse, in addition to its recognized role in thyroid hormone resistance (24). The cancer shows activation of PI3K, AKT, mammalian target of rapamycin, and S6 kinases by T3 (10); these kinases appear to be important for the developmental biology of the tumor. Additionally, there is abundant evidence that T3 engages a surface integrin receptor (α5β3), preferentially compared with T3 (5). This interaction causes cAMP/PKA activation, ERK, and other rapid signals to be generated and underlies effects of thyroid hormone on angiogenesis, thyroid cancer and glioma biology, and neuron and cardiomyocyte functions (10, 44, 56). Possible integration of the actions of traditional and nontraditional receptors for thyroid hormone at the surface of target cells is not understood.

**Dynamics of Rapid Signaling by Steroid Hormones**

The assembly of a signalsome, encompassing anywhere from 10–30 signal molecules, occurs in cell or context-specific fashion and determines in part the specificity of signals generated by steroid hormones. A cartoon of signaling by mem-
brane ER-α in breast cancer is shown in Fig. 1. E₂ engaging ER activates some but not all Gα proteins, leading to specific downstream signals (calcium, cAMP, kinase activation) (73). Depalmitoylation of ER-α also occurs at the cell membrane or in endosomes (25, 4) and may be needed for enacting signal transduction, but the details of the results of this process need to be clarified. ER-α also recruits, physically associates with, and activates distinct Gβγ subunits, leading to signal transduction and cell biology (40). One important outcome of this in ECs is the stimulation of eNOS and formation of NO (40, 65). This signaling by ER results in vasodilation in vivo (27). Modulation of small G proteins by other steroid receptors, such as AR, also occurs (19). Thus sex steroid binding proteins qualify as nontraditional, GPCRs, since there is no good evidence that these receptors span the membrane, as seen with traditional GPCRs.

It is clear that the membrane-localized, and not the nuclear steroid receptor, pool initiates rapid signaling (71). The E domain (ligand binding domain) of ER-α is necessary for both membrane localization and rapid signaling by E₂ (71, 75) and may be sufficient for generation of some signals. In part, membrane stability and signaling are mediated through E domain interactions with the MNAR (modulator of non-genomic action of ER) (13) scaffold protein. MNAR mediates the interactions of membrane ER-α with Src, leading to Src, ERK MAPK, and PI3K activation (92). However, other domains, such as the A/B region, interact with additional linking/scaffold proteins, including striatin and Shc (81, 46). These interactions are important to transmit rapid signals to downstream kinase activation in specific cells. As an example, striatin binds both membrane ER-α and the Gαi proteins, leading to eNOS activation in ECs (46).

Consistent with traditional GPCRs, membrane-localized steroid receptors do not contain a catalytic or kinase domain, and thus are not signal molecules themselves. As has been reported for traditional GPCRs, steroid receptors sometimes activate tyrosine kinase growth factor receptors at the membrane in distinct cell types. This includes ER, mineralocorticoid receptors, and PR transactivation of EGF receptors (EGFR) (21, 22, 26, 75). It has also been reported that membrane ER activates the insulin-like growth factor I receptor (82). In breast cancer cells, insulin-like growth factor I receptor then activates the EGFR to transmit downstream signals through a wide variety of kinases that impact breast cancer biology (82). The details of ER growth factor receptor cross talk have been worked out, where rapid G protein activation by membrane-localized ER-α leads to Src activation. Src then activates matrix metalloproteinas that liberate heparin binding-EGF, a ligand for the EGFR (22, 75). Inhibition of any of these steps, or genetic deletion or blockade of EGFR tyrosine kinase functions, prevents steroid hormone signaling to kinase cascades and limits proliferation or survival of breast cancer cells. The interaction of ER-α with Shc may more directly transactivate growth factor receptors (81) as another potential mechanism of receptor cross talk. Under some circumstances, the Edg-3 GPCR (83) or, as mentioned, GPR30 could contribute to overall E₂/ER rapid signaling from the membrane as part of the large signaling complex. Additional cross talk from membrane ER-β to integrin receptors on the surface of platelets has been reported to activate Src kinase and contribute to platelet aggregation (55).

Although membrane-initiated steroid signaling is rapid, it may persist for hours to days (72). It is the persistent signaling that engages protooncogenes such as Ras or Raf, leading to their participation in the development and tumor biology of transformed cells (57). Steroid ligands rapidly activate many signals, sometimes leading to transcription factor activation. Stimulation of calcium, cAMP, and cGMP, PKC isoforms, janus kinase/signal transducer and activator of transcription, and Wnt contribute to many cell functions modulated by steroid hormones, often by posttranslationally modifying existing proteins and thus altering their activities. Protein phosphorylation or new transcription impacts cell growth, migration, and differentiation. Androgen signaling in Xenopus oocytes contributes to maturation, mediated by classical AR (48). In this situation, AR inhibits Gα and Gβγ activity, in part through interactions with the MNAR cell surface protein (28). Androgen rapid signaling in prostate cancer and sertoli cells contributes to gene regulation, modifying tumor biology and sperm development, respectively (88, 12). Cell survival effects of E₂/ER in brain, bone, heart, or breast cancer are mediated in part by engaging membrane or mitochondrial ER, with the latter blocking signal transduction that leads to cell death. As an example, E₂ acting at mitochondrial ER-β blocks radiation-induced death of breast cancer cells (62). This occurs by E₂/ER-β rapidly upregulating MnSOD activity, blocking reactive oxygen species formation and JNK and PKC-δ signaling to Bax phosphorylation. As a result, Bax fails to traffic to the mitochondrial membrane, limiting the release of cytochrome c and apoptotic protease-activating factor-1 that are essential for apoptosome formation and resulting cell death in response to radiation. In another model, E₂ stimulates p38β kinase activation via PI3K activity in cardiomyocytes, blocking reactive oxygen species formation and p38α activation that leads to apoptosis. In this way, E₂/ER protects these cells from simulated ischemia-reperfusion injury (39).

One outcome of rapid signaling is the enhancement of nuclear ER-α action. This can occur when kinases downstream
of membrane ER, including ERK and PI3K, phosphorylate discrete residues of nuclear ER-α, promoting transcription (6). Kinase targets include serines 118, 167, and 305, among others, which impact transcription and the response to tamoxifen (6, 54). Membrane PR signaling may modulate nuclear ER sumoylation, regulating transcription (14). Membrane-initiated steroid signaling also phosphorylates coactivators or corepressors, recruiting them to the promoters of target genes. The resulting impact on transcription may lead to important biological outcomes. For instance, membrane ER-β signaling through PI3K and NF-κB upregulates the myocyte-enriched calcineurin inhibitor protein-1 (MCIP1) gene (63). The MCIP1 protein clamps calcineurin (protein phosphatase 2B) activity in the cytosol, limiting the dephosphorylation of the nuclear factor of activated T cell (NFAT) family of transcription factors. This blocks the ability of NFATs to translocate to the nucleus and enact cardiomyocyte hypertrophy in the setting of hypertrophic stimuli, such as angiotensin II (63). Through this mechanism, E2/ER-β signals to the prevention of cardiac hypertrophy in vivo (66). E2-induced PI3K and NF-κB stimulate the cyclooxygenase 2 gene with subsequent formation of prostacyclins, impacting EC biology (64). This includes EC migration, survival, and primitive tube formation. Another interesting interaction regards the newly described endogenous selective ER modulator, 27-hydroxycholesterol (27HC) (87). This product of cholesterol metabolism is abundant in the arterial wall of diseased blood vessels and competitively inhibits E2 binding to vascular ER, as a selective ER modulator. 27HC prevents both the rapid and transcriptional actions of E2/ER in blood vessels, modulating NOS activity/NO production, the response to vascular injury, and vasorelaxation (87). It can reasonably be hypothesized that competition between this very atherogenic form of cholesterol and E2 for binding to ER prevents the anti-atherogenic properties of E2 (53). Interestingly, 27HC acts as a partial agonist to augment ER signaling to proliferation in breast cancer cells (18).

In vivo requirements of steroid rapid signaling for hormone action have increasingly been described. E2/ER signaling through ERK and PI3K in xenograft models of breast cancer promotes growth and survival (51). E2/ER membrane-initiated signals rescue mice from a variety of central nervous system insults that result in neuronal death. These include protection from alcohol damage and glutamate excitotoxicity (36, 37). E2 also prevents the development of a mouse model of Parkinson’s disease (15), correlating to the signal transduction effects of E2/ER. In Xenopus, progesterins and androgens cause oocyte maturation by decreasing cAMP (30). This is felt to arise from a membrane PR; whether this is classical PR-A or PR-B or mPR is not clear, as discussed earlier. Mineralocorticoid rapid signaling attenuates the baroreflex (78) and modulates ion transport in mammalian colon (32). Glucocorticoids suppress rapid ACTH secretion from the pituitary (33) and cause vasodilation by rapid signaling to eNOS activation (29). As described, thyroid hormone acting at membrane TR provides neuron protection (80).

Perspective and Significance

As we learn more about the rapid signaling pathways enacted by steroid hormones, new models and reagents will be needed to dissect the precise contributions of membrane-localized and nuclear steroid receptor pools. In many situations, integration of steroid receptor pools is required for the overall cellular effects of the steroid ligands. The ER-α KO mouse represents a total cell depletion of this receptor and demonstrates the importance of this E2-binding protein for normal female reproductive tract and mammary gland development. Steroid receptor agonists and antagonists that only act at specific cellular pools will help define the precise contributions of receptors in various locations. In this respect, membrane ER-acting agonists have been synthesized and demonstrate the contributions of membrane-localized steroid receptor signaling to transcription (49). ER modulators and progestins that are more active for nongenomic rather than genomic signaling have also recently been developed and will help address this issue (59, 60). Comparable reagents for other steroid receptors will be useful to test the roles of these binding proteins in vivo. Finally, there may be interventional or therapeutic opportunities using receptor pool-specific reagents to interrupt the pathological effects of steroid hormones while enhancing their desirable effects.

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