Pure Appl. Chem., Vol. 75, Nos. 11–12, pp. 1743–1756, 2003.

Topic 1.8

Molecular mechanisms of cross-talk between growth factors and nuclear receptor signaling*

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Abstract: Signaling pathways can be linear, but more complex patterns are common. Growth factors and many other extracellular signals cannot directly enter cells and transduce their information via membrane-bound receptors. In contrast, steroid receptors are members of the nuclear receptor superfamily and await their cognate hormones inside the cells. These two types of signaling pathways are extensively intertwined and cross-talk at many different levels. A wide range of extra- and intracellular signals, including a variety of growth factors, can activate the transcriptional activity of steroid receptors in the absence of their cognate hormones. Conversely, steroid receptors lead a double life. By coupling to signaling molecules that mediate signal transduction of extracellular factors, they can elicit very rapid nongenomic responses. The signaling pathways of steroid-independent activation of steroid receptors, on the one hand, and of nongenomic signaling by steroid receptors, on the other, display a remarkable reciprocal relationship suggesting that these two modes of signaling cross-talk may be two faces of the same coin.

DEFINITION AND OVERVIEW

Extracellular signals modify intracellular processes through cognate receptors that elicit a cascade of events. However, a linear view of signal transduction falls short of describing all effects. Instead, branching, feedback, integration, and networking are characteristics of most if not all signal transduction pathways. Signaling cross-talk refers to a situation where one signal affects the output of another, seemingly distinct, signal transduction pathway.

There is signaling cross-talk at all levels of signaling, from affecting availability of the signal to modifying the regulation of expression of target genes. For example, at the level of an organism, steroid hormone concentrations are influenced by signals that affect the levels of their serum binding proteins. Signals can stimulate the biosynthesis of other signals and their receptors. In breast cancer cells, estrogen induces the expression of the progesterone receptor (PR) [1], the EGF receptor [2], and several members of the EGF ligand family [3,4]. With respect to a target cell, signaling cross-talk may take place in the extracellular space or within the cell, inside or outside the nucleus.

In this review, I will focus on the molecular mechanisms by which a variety of extra- and intracellular signals can modulate the activities of steroid receptors, in particular the receptors for the sex steroids, that is, the estrogen receptors (ERs) α and β, PR, and the androgen receptor (AR). These are all members of the nuclear receptor superfamily. Moreover, I will discuss how the very same nuclear receptors appear to elicit very rapid nongenomic effects by feeding into other signaling pathways. There is mounting evidence that these two nuclear receptor activities may be two faces of the same coin

*Report from a SCOPE/IUPAC project: Implication of Endocrine Active Substances for Human and Wildlife (J. Miyamoto and J. Burger, editors). Other reports are published in this issue, *Pure Appl. Chem.* **75**, 1617–2615 (2003).

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(Fig. 1), and that they may be intricately linked in positive feedback loops in some cases. A better understanding of the molecular mechanisms of signaling cross-talk involving steroid receptors will contribute to a framework for assessing the potential dangers of endocrine active substances (EASs).

I will limit my review to posttranslational events, and refer the reader to the extensive literature on cross-talk at the level of the regulation of the biosynthesis of ligands and receptors. Likewise, I will not be able to discuss another platform for extensive signaling cross-talk, the transcriptional regulatory elements themselves. These are typically composite and recruit several transcription factors, which can influence the DNA binding and transcriptional regulatory functions of each other, and be themselves regulated by signaling pathways. The interested reader will easily find both primary and review articles on individual genes and transcription factors.

STEROID-INDEPENDENT ACTIVATION OF STEROID RECEPTORS

Phenomena

Originally, steroid receptors were thought to be exclusively activated as transcription factors by binding cognate ligands. Agonistic ligands induce a conformational change in the hormone binding domain that allows the recruitment of transcriptional coactivators [5]. Assuming that the switch from inactive aporeceptor to active receptor can be described as a chemical equilibrium, it is perhaps not a surprise that the equilibrium can be shifted by altering the concentrations or activities of factors that interact with the active or inactive forms of steroid receptors. Indeed, the overexpression of transcriptional coactivators has often been found to increase the basal activity of steroid receptors. And yet, it came as a surprise when it was discovered in the late 1980s and early 1990s that sex steroid receptors can be activated by a host of extracellular signals in the absence of their cognate ligands [for reviews, see refs. 6–8]. This clearly represents an extreme form of signaling cross-talk. Over the last 10 years, more phenomena have been reported, signaling pathways have begun to be elucidated, but the molecular mechanisms remain poorly understood. Deciphering them should also help to explain the more typical and probably more physiological form of signaling cross-talk: synergy between low levels of steroids and other signals.

Table 1 provides a list of signals that have been reported to activate ER, PR, and AR in the absence of cognate ligand. In addition to a number of growth factors and the neurotransmitter dopamine, it includes several intracellular proteins and drugs. The activation by intracellular factors is of interest for two reasons: (i) It may mimic the stimulation by certain extracellular factors by plugging into their signaling pathways, and (ii) it may be physiologically relevant since intracellular signaling components can be overexpressed or constitutively activated in certain tumors. For example, cyclin D1 overexpression is common in breast tumors [47], and the phosphatidylinositol-3-OH kinase (PI3K)/Akt pathway is activated in many cancers [28,48,49]. In some cases, it is activated indirectly because of the loss of the tumor suppressor PTEN, which is a negative regulator of this pathway [48]. These pathways would be particularly relevant for cancers and diseases that are dependent on ERα.

The phenomenon of steroid-independent activation of steroid receptors may also be relevant to the cell-specific partial agonism of many anti-hormones. Steroid receptors have two main transcriptional activation functions (AFs). AF-1 and AF-2 are associated with the N-terminal domain and the hormone-binding domain, respectively. Antagonistic ligands of steroid receptors bind in the hormone-binding pocket and block AF-2 in an inactive conformation. However, partial antagonists display cell- and promoter-specific agonistic effects that are thought to be due, at least in part, to AF-1 activity. Interestingly, some of the signals that are able to activate unliganded steroid receptors can further increase the agonistic effects of partial antagonists, in some cases by further stimulating AF-1 (see below). In transfection experiments, the ER antagonist tamoxifen is switched to an agonist in the presence of growth factors or their downstream signaling molecules [29,50–53]. Similarly, the PR antagonist RU486 becomes an agonist when the protein kinase A (PKA) pathway is stimulated [44–46].

Table 1 Steroid-independent activators of unliganded sex steroid receptors[a].

Factor[b]	ER[c]	PR[d]	AR
Dopamine	[9]	[9]	
Epidermal growth factor (EGF)	[10,11]	[12]	[13]
Heregulin	[14]		
TGFα	[11]		
Insulin and insulin-like growth factors (IGF)	[15–17]		[13]
Keratinocyte growth factor			[13]
Fibroblast growth factor 2 (FGF-2 = bFGF)	[18]		
Gonadotropin release hormone (GnRH)	[19]	[20]	
Sex hormone binding globulin			[21]
Interleukin-6	[22]		[23,24]
erbB2 = HER-2	[14]		[25]
PI3K	[26]		
Protein kinase B (= Akt)	[26–28]		
v-Src	[29]		
Ras (constitutive mutant)	[30]		
MEKK1 (constitutive mutant)			[31]
MAPKK (constitutive mutant)	[32]		
Cyclin D1	[33,34]		
Cyclin A-Cdk2	[35]		
Ets-1	[36]		
Activators of protein kinase A	[15]	[37]	[38]
Inhibitors of protein phosphatases 1 and 2A	[9]	[9,37]	
Inhibitor of phosphotyrosine phosphatases		[12]	
Activator of protein kinase C	[30,39]		[40]

[a]For a regularly updated table, see <http:/www.picard.ch/>.
[b]This list may be incomplete as the extent of steroid-independent activation varies widely; moreover, several reports have indicated that some of the effects may be cell- and/or promoter-specific [see, e.g., refs. 25,41].
[c]Almost all publications have examined ERα. ERβ has only been shown to be activated by EGF [42,43].
[d]The response of PR displays marked species differences: chicken and rodent PRs can be activated in the absence of cognate hormone, whereas human PR is affected only in the presence of a ligand such as the partial antagonist RU486 [44–46].

Signaling pathways

Efforts have been made to dissect signaling pathways but many gaps remain (Fig. 1). Peptide growth factors signal to ERα through their respective tyrosine kinase receptor and the Ras-MAPK pathway [30,32,54]. Constitutive activation of the MAPK pathway mimics activation of ERα by the extracellular factor, and its inhibition blocks it [30,32]. Activation of ERα by GnRH requires protein kinase C in addition to the MAPK pathway [19]. Interestingly, constitutive activation of the PI3K-Akt pathway is not only able to activate ERα [26–28,55], but this pathway is also required for activation of ERα by IGF-I [27,55]. This suggests that peptide growth factors may signal to steroid receptors through multiple or branched pathways. Further studies are needed to explain why MAPK activity can be sufficient in one case whereas in another activation by growth factors can be blocked by a PI3K inhibitor. Tissue-specific differences in the signaling circuitry may provide part of the explanation [see, e.g., refs. 30,56].

The direct phosphorylation of steroid receptors by some of these pathways is important. MAPK phosphorylates a serine residue in the AF-1 domain of the ERs. This modification is necessary for ac-

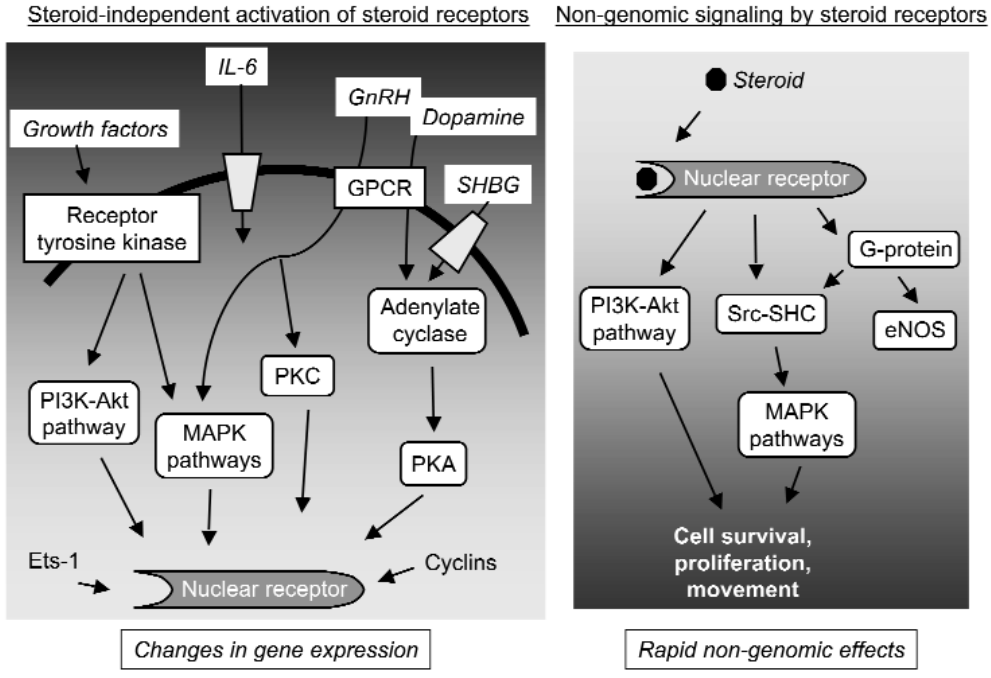

Fig. 1 Schematic comparison of steroid-independent activation of steroid receptors and nongenomic signaling by steroid receptors. Signaling pathways are simplified and do not necessarily apply as shown to all sex steroid receptors (ERα, ERβ, PR, AR). The shading of the two boxes illustrates the reciprocal relationship of the two signaling modes. GPCR, G-protein coupled receptor; SHBG, sex hormone binding globulin.

tivation by peptide growth factors [30,32,42,54]. Since simply putting a negative charge at the AF-1 associated serine 118 of ERα is not sufficient for ligand-independent activation [32], other sites of ERα or other factors may need to be modified as well. Other activating signals and pathways also lead to the hyperphosphorylation of ERα. Heregulin, potentially through the MAPK pathway, leads to ERα hyperphosphorylation [14]. The stimulation of ERα by the PI3K-Akt pathway appears to depend on the direct phosphorylation of yet other sites of the ERα N-terminus, notably S167 [26,27]. The enhancement of ERα activity by the cyclin A-Cdk2 complex, in particular in the presence of estrogen, is linked to the phosphorylation of S104 and S106, but not S118 [57].

There are alternative activation pathways that do not seem to involve the direct phosphorylation of ERα. One and the same signal can target different ERα domains in different cell types: whereas insulin targets S118 in most cell types, in neuronal cells it signals in a S118-independent fashion to the hormone binding domain [58]. Moreover, the same signal can target the same ERα domain through different pathways in different cell types: MAPK and S118 phosphorylation are not involved in the activation of ERα by EGF in vascular cells even though the same domain, AF-1, is the target [56]. Likewise, the stimulatory effect of v-Src on AF-1 activity is only in part due to the phosphorylation of S118 by MAPK; v-Src also stimulates AF-1 activity indirectly through the Rac-MEKK-JNKK-JNK pathway [29]. However, in all of these cases one cannot exclude a role for minor phosphorylation sites of ERα itself.

Dopamine functions through a G-protein coupled membrane receptor. Its activation results in the stimulation of adenylate cyclase, elevation of cAMP, and activation of PKA. Despite the fact that both cAMP and PKA mimic the ability of dopamine to activate steroid receptors in the absence of ligand, there is no evidence that PKA elicits the steroid-independent activity by directly phosphorylating the steroid receptors [59,60]. Rather, the phosphorylation of S236 in the DNA-binding domain of ERα by

PKA is inhibitory for dimerization and DNA binding [61]. The issue is complicated by the fact that cAMP signaling can either inhibit or activate the MAPK pathway depending on cell type [62]. However, cAMP-mediated activation of ERα also works in cells where PKA inhibits the MAPK pathway (our unpublished results). Moreover, EGF and cAMP appear to activate ERα through distinct pathways targeting different domains; cAMP may target AF-2 [63], or possibly both AF-1 and AF-2 (our unpublished results). Further progress towards dissecting steroid-independent activation of steroid receptors by cAMP has been made in whole animal experiments with rodents. Both dopamine and progesterone elicit the lordosis response of females in a PR-dependent fashion [64]. Elegant studies have placed the dopamine- and cAMP-regulated phosphoprotein-32 (DARPP-32) and protein phosphatase 1 (PP1) downstream of PKA. In the presence of either dopamine or progesterone, DARPP-32 is hyperphosphorylated and inhibits PP1 [65,66]. This finding correlates with the original observation that the phosphatase inhibitor okadaic acid activates apo-PR [9].

And yet, the role of direct phosphorylation of PR remains confusing. The mutation of the C-terminal S628 in chicken PR selectively abolishes the response to dopamine while leaving unaffected the response to hormone and okadaic acid [9]. A more recent survey of the PR sites that are hyperphosphorylated in response to either hormone or increased levels of cAMP revealed differences, but failed to provide evidence for a critical requirement of any of the major sites [60]. The role of phosphorylation will have to be reexamined in comparable biological systems, and it will have to be ascertained that the lordosis response to dopamine entails the steroid-independent activation of the transcriptional function of PR.

Molecular mechanisms

The challenge is to explain how the transcriptional activity of aporeceptors is stimulated in the absence of steroid at the molecular level. As alluded to above, the role of phosphorylation of the ERs for transcriptional coactivator recruitment has received particular attention. The EGF-induced phosphorylation of the ERβ AF-1 domain promotes its recruitment of the coactivator SRC1 [42], and transcriptional activity is further increased in the presence of the CREB binding protein CBP [43]. In contrast to the p68 RNA helicase [67], SRC1 recruitment to the ERα AF-1 domain is not stimulated by growth factor signaling and phosphorylation of S118 [42,68]. However, the stimulatory effect of the p68 RNA helicase is weak and not observed in HeLa cells [67] where EGF has been reported to work [32].

The steroid-independent activation of ERα by cyclin D1 overexpression represents an interesting paradigm. Cyclin D1 functions independently of its kinase partner to provide a bridge between p160 coactivators and the ERα, which is unable to bind these coactivators directly in the absence of hormone [69]. Remarkably, cAMP signaling promotes the interaction between ERα and cyclin D1, and this correlates with increased transcriptional activity of apo-ERα [70]. It remains to be determined how cAMP stimulates this interaction and whether the activation of apo-ERα by cAMP (see above) or by other signals requires cyclin D1.

In principle, almost any of the close to 100 ER interacting proteins (for a regularly updated list, see <http://www.picard.ch/>) or proteins interacting with the other nuclear receptors could serve as a platform or conduit for signaling cross-talk and many are [71]. MAPK phosphorylates the p160 coactivators AIB1 [72] and Grip1 [73], and stimulates their intrinsic transcriptional activity and coactivation function. Likewise, MAPK signaling leads to the phosphorylation of SRC1 [74], and thereby stimulates its ability to coactivate the chicken PR in the presence of either cAMP or progesterone [75]. Interestingly, activation of AR by IL-6 is MAPK-dependent, stimulated by MAPK-phosphorylated SRC1, but the IL-6 induced recruitment of SRC1 to AR depends on yet another unidentified signaling pathway [76].

The induced release of a repressor would constitute an alternative mechanism for steroid-independent activation. In the absence of ligand, steroid receptors are associated with molecular chaperones, which are thought to keep them transcriptionally inactive [77,78]. Other targets might be Brca1, which

can function as a repressor of apo-ERα [79], and the transcriptional corepressors NCoR and Smrt. NCoR appears to interact with ERα in the presence of the antagonist tamoxifen, and EGF stimulates the release of NCoR and concomitant activation of ERα [52]. Similarly, cAMP decreases the association of NCoR and Smrt with antagonist-occupied human PR [80].

The association with other transcription factors might contribute to steroid-independent effects. In addition to coactivators (see above), the stimulation of AR by interleukin-6 (IL-6) involves the recruitment of IL-6-activated Stat3 to AR [24]. Even more remarkable is the observation that the transcription factor Ets-1, upon association with nuclear receptors, induces their transcriptional activity in the absence of ligand [36]. Ets-1 appears to induce a conformational change in the unliganded nuclear receptor that allows it to bind coactivators.

Physiological significance

The physiological significance of steroid-independent activation of the ERα is best documented for the growth regulation of the rodent uterus. Both estrogen and peptide growth factors stimulate uterine growth, and both types of signals depend on ERα function [10,81]. In ERα knock-out mice, IGF-I signaling normally activates Akt and MAPK but fails to stimulate uterine growth; moreover, an ERE-luciferase transgene is activated by either IGF-I or estrogen [82]. As discussed above, steroid-independent activation of PR by dopamine may be involved in the lordosis response in female rodents [64]. This behavioral response can also be elicited by EGF, but in this case it depends on ERα rather than PR [83]. But despite the elegance of these in vivo experiments, there is no formal proof yet in this system that ERα and PR are transcriptionally activated by stimulation with EGF and dopamine, respectively. It is still possible that these nuclear receptors must be there as competence factors at some point preceding the stimulation.

Essentially all of the other evidence comes from studies with tissue culture cell lines. For example, androgen- and estrogen-dependent prostate and breast cancer cell lines can lose their steroid- but not their steroid receptor-dependence upon overexpression of the tyrosine kinase receptor HER-2 [14,25].

Considerable circumstantial evidence has led to the speculation that steroid-independent activation of ERα contributes to breast cancer progression, notably to estrogen-independence and tamoxifen resistance of ERα-positive tumors [for discussion, see refs. 6,84]. A large number of growth factors are indeed expressed in breast tumors, and act in both autocrine and paracrine fashions [85]. In addition to extracellular factors, HER-2 and cyclin D1 play a role and are often overexpressed in breast tumors [47,86]. In transfection experiments, both are able to activate the ERα in the absence of estrogen (see Table 1 and above), and overexpression of HER-2 renders breast cancer cell lines less dependent on estrogen and reduces their tamoxifen sensitivity [14,87]. Thus, these pathways may not only stimulate the proliferation of ERα-dependent breast tumor cells by themselves, but they must also be considered for their potential to pervert the action of estrogen antagonists by switching them to agonists [29,50–53]. By stimulating the AF-1 function of ERα, growth factors reduce the requirement for AF-2, and render ERα "resistant" to antagonists such as tamoxifen, which primarily block AF-2. Even worse, tamoxifen may then even facilitate the activation of ERα. Remarkably, this phenomenology in tissue culture seems to correlate with the observation that tamoxifen, in a small percentage of breast cancer patients, ends up stimulating tumor progression. In any case, HER-2 overexpression seems to predict a poor response to endocrine therapy [88,89], but the weakness of the correlation, not surprisingly, emphasizes that signaling cross-talk is but one of several causes of tamoxifen resistance.

NONGENOMIC SIGNALING BY STEROID RECEPTORS

A new role for old receptors

Evidence has accumulated over several decades that steroid hormones also signal through nongenomic pathways with very rapid cellular effects [90–95]. Within seconds or minutes, steroids can induce an increase in several second messengers such as inositol triphosphate, cAMP, Ca^{2+}, and the activation of MAPK and PI3 kinase [e.g., see refs. 96–100]. Biological responses attributed to short-term effects range from vasorelaxation of endothelial cells, neuroprotection, and bone protection to the stimulation of proliferation of carcinoma cells.

The molecular mechanisms and notably the receptor(s) have only recently begun to be unraveled. It now appears that at least some of the reported effects are mediated by the same steroid receptors that are known as nuclear receptors. This alternative lifestyle of the known steroid receptors will be the focus of the following paragraphs. However, it is important to note that they clearly do not account for all nongenomic phenomena elicited by steroids. Several unrelated membrane receptors contribute to a large diversity of rapid responses [90,94,101–103].

The conclusion that the known steroid receptors can themselves be at the top of a signaling cascade for rapid responses is primarily based on the following findings: (i) Rapid nongenomic responses to estrogen, progesterone, and androgen can be reconstituted in unresponsive cell lines by transient expression of ER, PR, and AR, respectively, and these responses seem to be independent of the transcriptional competence of the steroid receptor [97,98,104–115]. (ii) A small fraction of the total cellular (and mostly nuclear) ER complement associates with the cell membrane [110,116–119], extranuclear localization is necessary [109], and cytoplasmic sequestration by a variant of the metastatic tumor antigen 1 promotes nongenomic signaling [120]; in the case of ER signaling to eNOS in endothelial cells, this nongenomic response can even be demonstrated with isolated caveolae, a specialized membrane compartment [110,118,121]. (iii) "Nuclear" steroid receptors can be immunoprecipitated with a whole series of signaling molecules that are typical of signaling pathways triggered by membrane receptors (see below). (iv) Antibodies to "nuclear" ERα added to the culture medium can modulate estradiol responses [119,122].

Signaling pathways

In line with their proposed membrane association, the ERs, AR, and PR have been shown to couple to a large number of signaling molecules (Fig. 1). Signaling of ERα to eNOS takes place in caveolae [110,118,123] and involves the recruitment of a $G_{\alpha i}$ protein to ERα [124]. ERs are also coupled to other types of G proteins [106]. PR may be coupled to G proteins in frog oocytes, in which the rapid response to progesterone leads to the inhibition of adenylate cyclase [125–127]. Many groups have reported that activation of steroid receptors can activate the MAPK pathway. ERα, AR, and PR can elicit this rapid response by binding and activation of c-Src [97,104,109,112,113]. The molecular mechanism of this activation remains unclear. For human PR, it is particularly controversial. One group has provided evidence that suggests PR binds and displaces the inhibitory SH3 domain of c-Src [113], whereas another group has argued that the activation by PR is indirect and depends on the association of PR with ERα [104]. Additional adaptor proteins may also be involved in MAPK activation by steroid hormones since several reports demonstrate a role for SHC and its adaptor proteins Grb2 and Sos [109,128], and the modulator protein MNAR promotes the formation and activity of ternary complexes between ERα and c-Src [129]. The formation of ternary ERα-Src-PI3K complexes [114] may explain why both PI3K and the Src-MAPK pathway have been placed downstream of ERα [28,98,130] and AR [115]. Surprisingly, the kinetics of activation of PI3K by estradiol has been shown to be slower than that of MAPK [98]. As a result of the activation of PI3K by ERα, Akt is activated [28,98,114,119,131]. In adipocytes, MAPK activation by estradiol requires a G protein, Src, PI3K as well as protein kinase C (PKC) [132].

Signaling pathways are likely to differ substantially between cell types and experimental systems. For example, the requirement for c-Src for estradiol signaling mentioned above has not always been observed [128,133]. Moreover, the existence of negative and positive feedback mechanisms may often render a linear view of signaling inappropriate. Indeed, the IGF-I receptor is both upstream and downstream of ERα in a pathway leading to the activation of MAPK [111]. Likewise, Akt is both upstream and downstream of ERα [28, see also ref. 129]. A prediction from this type of positive feedback is that the system should respond to lower levels of stimuli.

Nongenomic signaling: Open questions

Many mechanistic details remain poorly understood. The evidence is confusing regarding the membrane association of steroid receptors. How do they get there? Do they insert into the membrane, and if so on which side? Do they actually expose the ligand-binding pocket of the hormone-binding domain on the outside of the cell? Signaling can be triggered by membrane-impermeable forms of steroids such as BSA conjugates [see, e.g., refs. 108,132], but internalization of the conjugated or deconjugated steroid has not been rigorously excluded [see also ref. 134]. The minimal signaling domain of ERα seems to be the hormone-binding domain [see, e.g., refs. 109,112], but according to another report this domain is not even required [128]. Could different domains couple to different signaling pathways? The findings with PR [113] would seem to support such a view.

Pharmacology has been used and abused extensively to draw conclusions about the type of receptor involved in particular responses. However, there is no reason whatsoever to assume that the ligand specificity and response of a nuclear receptor should be the same once it associates with the membrane or other signaling molecules. Thus, antagonists of the transcriptional function may be either antagonists or agonists for nongenomic signaling by the very same receptor (and even more so of course for unrelated membrane receptors), and vice-versa for antagonists of nongenomic signaling. An intriguing pharmacological study has revealed a correlation between the ability of synthetic estrogens to elicit nongenomic as opposed to genomic signaling in tissue culture and bone protection in whole animals [109,135], but the target cells and molecular mechanisms in vivo remain to be elucidated. Establishing the phenomenology is important, but more caution should be used in interpreting it.

What are the biological consequences of nongenomic signaling? Apart from the stimulation of frog oocyte maturation, little is formally established. And even there, a very recent reassessment demonstrates that it is androgens that signal through the frog AR rather than progesterone through PR; while implicating nongenomic signaling by a nuclear receptor was correct, both the hormone and the receptor had been mistaken [127]. In MCF-7 breast cancer cells, estradiol induces ruffles and pseudopodia in cell peripheral areas where ERα and actin colocalize [128]. A consequence of the activation of MAPK is the activation of their target transcription factors including Elk-1 and the serum response factor [115,128,130,131] leading again to delayed genomic responses. In combination, these responses may contribute to stimulate the motility and proliferation of estrogen-dependent breast cancer cells. In pituitary cells, estradiol modulates the prolactin release from stores [122].

Despite the demonstration that nongenomic signaling by nuclear receptors occurs in a wide variety of different cell types in vitro, the physiological significance beyond the frog oocyte system remains very speculative [for discussion, see ref. 93]. Based on where nongenomic signaling of estrogen has been described, it has been argued that it is neuroprotective, beneficial in the cardiovascular system, antiapoptotic in the bone, and stimulatory for the progression of breast cancer. However, it remains a major challenge for the future to discriminate between genomic and nongenomic effects of steroid hormones in whole organisms and to ascribe them to the correct receptor.

TWO FACES OF THE SAME COIN?

I have mentioned evidence in support of the notion that nongenomic signaling by steroid receptors and steroid-independent activation of steroid receptors could be intricately linked. When ERα stimulates MAPK, MAPK can in turn phosphorylate ERα to influence its subcellular localization and to increase its AF-1 activity. The same can be said for the Akt pathway. Perhaps one of the main functions of nongenomic signaling by steroid receptors may be to provide a rapid boost to their classical function as transcription factors. In turn, by stimulating the expression of signaling molecules, steroid receptors might contribute to more sustained changes in signaling patterns. A comparative analysis of the cross-talk potential of other members of the nuclear receptor family might help to determine which role came first.

SIGNALING CROSS-TALK AND ENDOCRINE ACTIVE SUBSTANCES: RELEVANCE AND RECOMMENDATIONS

Signaling cross-talk modifies and enhances the response to extracellular signals. In this context, two types of interactions with EAS that are steroid receptor ligands must be considered. First, their agonistic or antagonistic genomic effects could be altered by cross-talk with other signals. Second, in addition to their well-documented genomic effects, EAS could elicit nongenomic responses mediated by membrane-associated steroid receptors, a possibility that has not yet received much attention [136].

By acting synergistically, extracellular factors might be expected to lower the EC_{50} and/or to increase the maximal transcriptional response of xenosteroids. Thus, signaling cross-talk must be considered as an additional risk factor because the disruptive potential of xenosteroids may otherwise be underestimated. Remarkably, this has recently been modeled with estrogen-dependent breast cancer cells. They have been shown to display an increased proliferative response to low levels of estrogens upon constitutive activation of the MAPK pathway [137]. Augmented MAPK activity may also contribute to the estrogen hypersensitivity that ERα-dependent breast cancer cells acquire upon long-term estrogen deprivation [138]. Moreover, as in the case of tamoxifen and RU486, signaling cross-talk could convert weakly antagonistic xenosteroids into weak agonists.

RECOMMENDATIONS

The following recommendations primarily call for additional basic research and modifications in the protocols of large-scale screening programs.

- The transcriptional effects of xenosteroids should be examined both in the absence and in the presence of additional stimuli such as growth factors.
- Dose–response curves of xenosteroids should be established both in the absence and in the presence of additional stimuli such as growth factors.
- Screens for xenosteroids should be done in the presence of factors that can synergize with weak agonists.
- Antagonistic xenosteroids should be tested for agonism under conditions of signaling cross-talk.
- Xenosteroids should be systematically assessed for their potential in eliciting nongenomic responses through the classical nuclear receptors as well as unrelated membrane receptors.

ACKNOWLEDGMENTS

I am grateful to members of my group for continuous discussions and critical reading of the manuscript. My laboratory is supported by the Swiss National Science Foundation, Krebsforschung Schweiz, the Fondation Medic, and the Canton de Genève.

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