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VIEWPOINT

Multiple Mechanisms for Regulation of Steroid Hormone Action

The basic tenets of steroid hormone action developed over the past 25 years have provided us with a unified framework for analyzing hormone effects. The central concept that steroid receptors, activated by cognate steroid ligands, act within the nucleus by binding to specific DNA sequences and modulating target gene transcription has withstood intense scrutiny. Once cDNAs encoding the steroid and thyroid hormone receptors were cloned and sequenced, it became evident that these regulatory proteins share significant structural and organizational motifs. Collectively this large group of proteins, which also includes the vitamin D and retinoid receptors, is termed the steroid/thyroid hormone receptor superfamily [1]. These receptors act through a common mechanism of ligand-dependent transcriptional modulation, which involves: 1) ligand binding to receptor resulting in allosteric activation; 2) binding of hormone-receptor dimers to specific DNA elements; 3) formation of a complex between receptor and the transcriptional machinery and possibly other transcription factors as well; and 4) modulation of the rate of transcription initiation [2]. Recently, a number of findings have added a layer of complexity to our understanding of steroid hormone action. This work reveals that other signal transduction pathways influence steroid receptor function. We will discuss three different mechanisms by which members of the steroid receptor superfamily may functionally interact with or be influenced by other signaling pathways. These mechanisms include 1) activation of receptor function by phosphorylation/dephosphorylation; 2) direct or indirect interaction between steroid receptors and inducible transcription factors; and 3) regulation of steroid receptor levels by factors that mediate other signal transduction pathways.

LIGAND-INDEPENDENT ACTIVATION OF STEROID RECEPTORS

Steroid receptors are phosphoproteins and at least for some members of the steroid receptor family, phosphorylation is induced over basal levels in response to hormone. Virtually every step in steroid receptor function, from hormone binding to transcriptional modulation and receptor recycling, has been postulated to be influenced by changes in receptor phosphorylation state [3]. For example, recently Denner et al. [4] reported that the transcriptional activity of chick progesterone receptors is stimulated, *in the absence of progesterone,* by either 8-bromo-cAMP (activator of protein kinase A) or okadaic acid (inhibitor of phosphatases 1 and 2A). Furthermore, these investigators showed that progesterone activation of progesterone receptors (PR) was repressed by the protein kinase A inhibitor PKI. In line with these findings, preliminary data from Burgos-Trinidad et al. [5] suggest that the unliganded mouse androgen receptor is transactivated by cAMP. These results are consistent with roles for phosphorylation in PR-mediated transcriptional activity and dephosphorylation in decreased transcriptional activity of receptors.

This putative relationship between receptor phosphorylation and receptor transactivation appears to be quite complex and may not be valid for all steroid receptors. Indeed, additional evidence suggests that modulators of phosphorylation may not even have the same effects on PR from different species. Beck et al. [6] reported that the transcriptional activity of human PR is not stimulated by 8-bromo-cAMP or okadaic acid in the absence of progesterone treatment. However, these compounds were found to enhance progesterone-dependent transactivation of gene expression by three- to fourfold over the levels induced by progesterone alone. The reasons for the altered effectiveness of these agents on human compared to chick PR are unclear. One hypothesis is that chick PR are more easily activated in the absence of hormone than their human counterparts [6]. This "intrinsic" difference between the two species types is reflected in the capacity of salt-extracted chick PR to bind specific DNA and to activate transcription in vitro in the absence of progesterone, whereas human PR is dependent on hormone for these

activities [6–9]. Perhaps the unliganded PR complex is less stable due to weaker binding of chick PR by heat shock protein 90 (hsp90) or another stabilizing factor, although simply removing hsp90 does not appear to be sufficient for transactivation (O'Malley, personal communication). Alternatively, there may be differences in specific phosphorylation sites or kinetics of phosphorylation of chick and human PR that are responsible for the variation in sensitivity of these receptors to modulators of phosphorylation.

In another example of ligand-independent activation of steroid receptors, Power et al. [10] reported that the neurotransmitter dopamine caused both chick PR- and human estrogen receptor-mediated gene transcription in the absence of the appropriate receptor agonists. This activation was specific for the D1 subtype dopamine receptor agonists and was not due soley to elevation of intracellular cAMP. These recent findings suggest that alternate pathways for steroid receptor activation exist and that the signal for these pathways may be through alteration of receptor phosphorylation. Of note, the glucocorticoid receptor (GR) was not influenced by dopamine [11]. The reason for the lack of susceptibility of GR to activation by dopamine or other modulators that influence phosphorylation is not known. Perhaps a different mediator promotes ligand-independent GR activation or GR activation may be less promiscuous. Consistent with the failure of unliganded GR to be activated by modulators of phosphorylation, preliminary data reported by Mason and Housley (personal communication) suggest that phosphorylation of GR does not influence receptor transcriptional activity. In this study the seven phosphorylated residues of mouse GR [12] were individually mutated to alanine or aspartic acid. Alterations of these serine residues to either alanine or aspartic acid had no effect on hormone-induced transactivation of a cotransfected reporter plasmid consisting of a glucocorticoid response element (GRE) driving the expression of chloramphenicol acetyltransferase.

The exciting discovery that certain steroid receptors expressed in transfected cells are subject to ligand-independent activation raises numerous questions. Does ligand-independent activation occur in vivo? Before this question can be answered it will be necessary to fully understand the biochemical mechanisms involved in receptor activation. Currently much effort is being directed at identifying the specific residues of steroid receptors that are subject to phosphorylation/dephosphorylation and determining at what point temporally are individual sites phosphorylated/dephosphorylated. How are these events related to receptor functions such as hormone binding, nuclear translocation, dimerization, DNA binding, transactivation (both enhancement and repression of gene transcription), and receptor recycling? Can individual phosphorylation sites be identified that regulate these specific aspects of receptor function? Does the receptor phosphorylation state influence steroid-mediated post-transcriptional effects such as alteration of specific mRNA stability? Understanding the cascade of events that leads to regulation and modulation of steroid receptor phosphorylation will be a herculean task. The pertinent kinases and phosphatases that directly act on the receptors must be identified followed by identification of those kinases/phosphatases that may modulate the phosphorylation state of the "receptor kinases and phosphatases" and so on.

INTERACTION OF STEROID RECEPTORS WITH REGULATORY FACTORS FROM OTHER SIGNAL TRANSDUCTION PATHWAYS

The inhibitory effects of glucocorticoids on various physiologic processes such as lymphocyte and certain tumor cell proliferation and on mediators of inflammation are well established [13]. In order to understand the basis for the glucocorticoid-mediated negative regulation of gene expression that may be involved in cellular differentiation events, the genes for collagenase and proliferin (a placental growth factor) have been studied in great detail [14–18]. Both collagenase and proliferin gene expression are induced by phorbol esters and this induction is blocked by glucocorticoids. This led to the hypothesis that these genes may be regulated via some type of interference between phorbol ester-induced and steroid hormone-induced pathways [19,20]. Although recent studies briefly discussed below indicate that these two genes are regulated by somewhat different mechanisms, both models invoke interaction between GR and the transcription factor AP-1. AP-1 is composed of heterodimers of the protooncogene encoded proteins Fos and Jun and is responsive to stimulation by a number of agents including phorbol esters, certain inflammatory mediators, and growth factors [21].

Regulation of the proliferin gene promoter occurs through a "composite" regulatory sequence that can be bound by both AP-1 and GR [18,19]. Regulation of proliferin gene expression is dependent on which transcription factors (GR, Fos, and Jun) are bound to this "composite" regulatory sequence. In the presence of AP-1 (Fos/Jun heterodimer), glucocorticoids exert a negative effect on proliferin gene transcription. This type of regulatory mechanism is therefore cell specific, i.e., GR action at "composite" DNA elements is dependent on the presence of specific regulators from other families.

Glucocorticoid regulation of the collagenase gene appears to occur through a novel interaction between AP-1 and GR [14–16]. The AP-1 recognition site of the collagenase promoter mediates responsiveness to phorbol esters. Although GR does not bind to this AP-1 recognition sequence, this AP-1 site was found to be essential for glucocorticoid repression of collagenase. Therefore, the interaction between GR and AP-1 is not dependent on cobinding of GR and AP-1 to the collagenase gene promoter. Interestingly, AP-1 can repress GR action on glucocorticoid-inducible genes in a "reciprocal" fashion. Based on mutational analysis and cross-linking studies, it has been postulated that the mutual repression of AP-1 and GR activity involves direct interaction between AP-1 and GR possibly involving the leucine zipper of the Fos/Jun heterodimer and the DNA binding domain of GR [14,15,20]. Alternatively, the interaction between GR and AP-1 may be indirect through a bridging protein.

These findings indicate that GR and AP-1 have the capacity to interact at different levels either "on" DNA (i.e., through a composite regulatory element as occurs at the proliferin promoter) or "off" DNA (i.e., through direct interaction between the two proteins). The mechanism responsible for the functional interaction between these two signal transduction pathways may be gene specific. Since proliferin is regulated differently (either enhanced or repressed by glucocorticoids) depending on cellular context, it is likely that tissue-specific and perhaps stage-specific differences exist that dictate how these transcription factors interact or even whether they interact in all cell types. Regardless of the nature of this interaction, the evidence is strong that two distinct signal transduction pathways contribute to the transcriptional regulation of certain genes. In addition to understanding the precise molecular details of the interaction between these two regulators, another goal is to identify additional genes that are modulated by members of the steroid receptor family and also by members of the AP-1 family and to determine whether steroid receptors and Fos/Jun-related proteins interact (directly or indirectly) to regulate these genes. In other words, how universal is this interaction and under what conditions does it occur? If AP-1 and GR mutually antagonize each other's actions, one might predict that in cells which coexpress AP-1 and GR, phorbol esters would inhibit glucocorticoid responsiveness and glucocorticoids would inhibit phorbol ester-induced responses. Therefore, is there a mechanism to counter the mutual antagonism between these two transcription factors? Do other transcription factors in the leucine zipper family also interact with steroid receptors? The complexities invoked by the interaction between these two transcription factors are enhanced further when one considers that certain members of the steroid receptor superfamily and the AP-1-related proteins have the potential to heterodimerize within their respective families [22].

REGULATION OF STEROID RECEPTORS

Since cellular responsiveness to steroid hormones is dependent on receptor levels, mechanisms that modify receptor concentration can be pivotal controllers of steroid hormone action. One common feature of all steroid receptors and certain other members of the receptor superfamily is their capacity to be regulated by cognate ligand, a phenomenon termed *autoregulation* [23,24]. The highly complex process of steroid receptor autoregulation involves steroid-mediated transcriptional, post-transcriptional (receptor mRNA stability), and post-translational (receptor turnover) mechanisms. In addition, receptor levels may be elevated or depressed in response to agonist depending on the receptor type and even for a given receptor regulation may vary in different tissues. The interesting finding was recently made that the cDNA for the human GR retains sufficient sequence information to confer glucocorticoid-inducible receptor down regulation [25,26]. Although the precise intragenic sequences responsible for down regulation of the human GR cDNA have not yet been identified, these signals may influence both transcription of the receptor cDNA and stability of the receptor mRNA (Burnstein et al., manu-

script submitted). More recent reports also suggest that rabbit and human PR [27–29], human estrogen receptor (Kaneko, Furlow, and Gorski, personal communication), and human androgen receptor [29] also contain hormone-autoregulatory sequences located downstream from the transcription initiation site. Given the structural and functional similarities of the steroid receptor superfamily, perhaps other members of this family also contain intragenic autoregulatory elements. The significance of the intragenic location of these signals is not known; they may provide for more stringent control of receptor mRNA regulation or may be vestiges of the evolution of these genes.

Certain steroid receptors are also subject to regulation by other steroids which do not serve as ligands for these particular receptors (heterologous regulation). One of the best known examples of this type of regulation is the estradiol-mediated stimulation of breast and uterine PR by estrogen receptor [30,31]. Estrogen receptor regulation of vitamin D receptor (VDR) levels has also been reported [32]. Interestingly, in this study, estradiol administration to female rats resulted in increased levels of VDR in liver but decreased levels in kidney. It is not known if this regulation of VDR is a direct effect of estradiol. Further investigation of this process may reveal organ-specific factors that modulate these effects of estradiol.

Regulatory "cross talk" where steroid receptor levels are modulated by non-steroidal hormones and by second messengers has also been reported. GR levels were increased in rat hepatoma cells treated with the cAMP inducing agent forskolin and by the cAMP analog 8-bromo-cAMP [33]. However, unlike the effects of 8-bromo-cAMP on the transcriptional modulating activity of PR, this agent does not promote transactivation of GR as discussed above. Thus this cAMP analog influences steroid receptor function through at least two different mechanisms.

Dopamine appears also to exert differential effects on PR and GR. Although GR is not activated by dopamine as is PR, this neurotransmitter evidently influences GR concentration. GR levels in the intermediate lobe of the pituitary but not in the anterior pituitary are repressed by dopamine [34]. It is not understood how dopamine exerts these tissue-specific effects on GR nor is it clear how these differential dopaminergic effects are achieved on GR and PR.

Agents which activate protein kinase C have been implicated in the down regulation of estrogen receptor mRNA stability [35], VDR gene expression [36], and possibly GR gene expression (Vig and Vedeckis, personal communication). The VDR serves as a substrate for protein kinase C [37], but it is not known if these protein kinase C phosphorylation sites are involved in the down regulation of VDR.

These studies show that various signaling pathways contribute to the regulation of certain steroid receptors and the closely related VDR. How these diverse pathways converge with steroidal ligands to regulate steroid receptor concentration and function will require extensive analysis that encompasses examination of a variety of different cellular control signals. Future approaches must consider the interplay between various signal transduction pathways that emanate from both the cell surface and within the cell.

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