G PROTEIN-COUPLED RECEPTOR SIGNALLING IN NEUROENDOCRINE SYSTEMS

Signalling mechanisms in progesterone–neurotransmitter interactions

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Abstract

The current and expanded view of transcriptional regulation by the steroid/thyroid superfamily of nuclear transcription factors integrates not only the hormone-dependent but also the hormone-independent cellular signaling mechanisms in physiology and reproduction. This effort has vastly been aided by the identification of steroid hormone receptors as transcriptional mediators of a variety of ligands, whose transcriptional response is dependent upon cross-talk with distinct signal transduction pathways, their recruitment of coregulators, alteration of chromatin structure and identification of specific interactive motifs within the receptors themselves. This review will provide a framework for the current concepts in the field of steroid hormone action as exemplified by our studies on progesterone receptor in female sexual behavior.

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Introduction

Steroid hormones, estrogen and progesterone, play an important role in the development, differentiation, metabolism and reproduction of various species. The physiological effects of these molecules are primarily mediated by their binding to specific intracellular receptors that act as ligand-inducible transcription factors, inducing structural and functional changes to facilitate target gene expression and regulation. However, not all the biological effects of steroid hormones are ‘genomic’ mediated by direct receptor modulation and target gene expression. There are several reports of their rapid ‘non-genomic’ effects on cell membrane/cytoplasmic signal transduction pathways that are independent of protein synthesis and transcriptional regulation at the genomic level. In addition to these, our laboratory has described a ‘ligand-independent’ mechanism of activation, a phenomenon characterized by the activation of the

progesterone receptors (PRs) by factors other than their cognate ligands. These studies have led to the current understanding of the interactions between distinct signaling mechanisms in the mediation of progesterone action *in vivo*. In this review, the distinct cellular and molecular mechanisms of steroid hormone action will be discussed with specific reference to progesterone effects in brain and behavior.

Structure and function of PRs

Progestins, including progesterone, exert their physiological effects primarily by binding to cognate intracellular PRs (Tsai & O’Malley 1994, O’Malley *et al.* 1995). PRs are ligand (hormone)-inducible members of a superfamily of transcription factors that undergo conformational changes upon hormone binding, leading to their nuclear translocation, dimerization (Guichon-Mantel *et al.* 1989),

Figure 1 Schematic representation of steroid hormone receptor activation. Unliganded receptor (Rc) is present as an inactive complex associated with heat-shock proteins (HSP). The classic ligand-dependent activation by steroid hormone (►) results in conformational change, dissociation of HSP proteins, dimerization of the receptor and binding to target DNA sequences (HRE). The hormone-induced conformational change facilitates recruitment of cofactors and other basal transcription factors to the promoter, producing a transcriptionally active complex that can direct target gene transcription. The activity of protein kinases and phosphatases in the cell contributes to Rc activation by altering the phosphorylation of both Rc and coregulators (SRC-1, CBP).

increased receptor phosphorylation (Weigel 1996, Weigel & Zhang 1998), DNA binding and their subsequent interaction with specific coregulator proteins and general transcription factors (GTFs) leading to the modulation of target gene expression (Horwitz *et al.* 1996, McKenna *et al.* 1999). Coregulator proteins consisting of coactivators and corepressors enhance or inhibit receptor-dependent target gene transcription (McKenna *et al.* 1999, McKenna & O'Malley 2002) through multiple mechanisms, including stabilization of nuclear receptors and the basal transcriptional machinery at the promoter; covalent modification of histones, activators and other coregulators; turnover of activators and other proteins; and also phosphorylation of the coactivators (Rowan & O'Malley 2000, Rowan *et al.* 2000a,b) (Fig. 1).

Structural organization

Similar to the other members of the nuclear receptor superfamily, PRs have a modular protein structure consisting of distinct functional domains capable of binding steroidal ligand (the ligand-binding domain (LBD)), dimerization of liganded receptors, interaction with progesterone-responsive DNA elements (PREs) and interaction with coregulator proteins required for bridging PRs with the transcriptional machinery (Fig. 2). The N-terminal region contains a transactivation function (AF1) that modulates the level and promoter specificity of target gene activation, by interacting with components of the core transcriptional complex and coactivator proteins. The LBD region contains a second activation function (AF2), which

Figure 2 Structural domains of PR. The N-terminal region interacts with the transcriptional machinery, coactivators and other transactivating proteins. This region also contains the two alternate transcription initiation sites that give rise to the two receptor isoforms PR-A and PR-B. The DNA-binding domain (DBD) is associated with the dimerization function. The LBD and the DBD domain are connected by a variable hinge domain, which contains the nuclear localization signal sequence. The AFs AF1 and AF2 are localized to the N-terminal of DBD and LBD respectively. The third AF, AF3, is located at the N-terminal segment of PR-B.

in addition to progesterone-binding function contains sequences for dimerization, heat-shock protein association, intermolecular silencing and intramolecular repression. A unique third activation function (AF3) is present in the N-terminal segment of human PR (B-isoform), which can function autonomously or synergize with downstream AFs (AF1 and AF2) to enhance their activity (Horwitz *et al.* 1995).

PRs exists in two isoforms, PR-A and PR-B, which are structurally related but functionally distinct. The two isoforms differ at the N-terminus, with PR-B containing an additional stretch of amino acids (128–165) located at the N-terminus of the receptor.

Gene activation

In the absence of ligand, the inactive PRs are associated with a large complex of heat-shock proteins in the nuclei of target cells (Smith *et al.* 1990). Upon hormone binding, the receptors dissociate from the heat-shock proteins, dimerize and bind to PREs in the regulatory regions of target gene DNA. Activated, DNA-bound receptors stimulate the rate of formation and/or stabilization of a preinitiation complex, consisting of general transcription factors (GTFs), at enhancer-

controlled promoters. The action of PRs to stimulate preinitiation complex formation occurs primarily via direct protein-protein interactions with ancillary factors such as coregulators (see below).

Formation of the preinitiation complex is initiated by the binding of transcription factor-IID (TFIID) to the TATA box of the promoter, a short distance from the transcriptional start site. TFIID functions as a multiprotein complex composed of TATA-binding protein (TBP) and highly conserved TBP-associated factors. The change in DNA topology induced by TBP facilitates the binding of bridging factor TFIIB, a GTF with affinity for single-stranded DNA, to the sequences adjacent to the TATA box (Lee & Hahn 1995). Binding of RNA polymerase II (Pol II) follows recruitment by TFIIB of TFIIF- α . The remaining GTFs then assemble to form the complete preinitiation complex (Roeder 1996). While this implies a sequential recruitment of GTFs, recent evidence indicates the existence of preformed, stable basal transcription complexes, which contain Pol II and other GTFs. The rate of assembly of the complexes induced by PRs, in association with their coregulators, ultimately defines the transcriptionally permissive or non-permissive environment at the hormone-regulated promoters.

Coactivators and repressors

In the past decade, several biochemical and genetic screens have identified a number of proteins, termed the ‘coregulators’, that form a functional link between the activated receptors and transcription complex to effect an efficient transcriptional regulation. These proteins consist of coactivators and corepressors that enhance or inhibit receptor-dependent target gene transcription. Coregulators are organized into stable, preformed subcomplexes, the modular arrangement of which facilitates the efficient assembly of functionally diverse complexes by a single receptor dimer. The modular arrangement of these complexes appears to provide a potential for different activators to assemble diverse configurations of regulatory complexes at the promoters (McKenna *et al.* 1999). The relative expression level of the coactivators and corepressors determines cell-specific, appropriate and graded responses to ligand.

Coactivators

Coactivators are often rate-limiting for receptor activation and achieve their effects through multiple mechanisms, including stabilization of nuclear receptors and the basal transcriptional machinery at the promoter; covalent modification of histones, activators, other coregulators; and possibly turnover of activators and other proteins. Coactivators have histone acetyl transferase activity that promotes acetylation of core histones to promote chromatin remodeling. Several coactivators for PRs have been identified, the principal ones belonging to the steroid receptor coactivator (SRC) family of coactivators (SRC-1, -2 and -3). These family members interact with co-integrator, CREB-binding protein (CBP), to enhance PR transactivation in various tissues. In addition, E3 ubiquitin protein ligases, leucine zipper-containing proteins, high mobility group proteins 1/2 and steroid receptor activator protein have also been identified to have the coactivator functions.

Corepressors

Several proteins, i.e. nuclear corepressor/receptor interacting protein and silencing mediator for retinoid and thyroid hormone receptor, have been identified as potential mediators of repression. These proteins have the histone deacetylase activity and repress target gene activation. Unliganded receptors maintain a transcriptionally inactive steady state at the promoter by recruitment of corepressors and their associated histone deacetylase activities. Ligand binding is thought to induce release of corepressors enabling the receptor to recruit p300/CBP associated factor, p300/CBP and SRC family members to effect histone acetylation and the creation of a transcriptionally permissive environment at the promoter. For a detailed description of coregulators, the reader is referred to several recent reviews and the references therein (McKenna *et al.* 1999, McKenna & O'Malley 2002, Xu & O'Malley 2002).

PR activation in the brain: relationship to reproductive behavior

Ovarian steroid hormones, estradiol and progesterone, regulate cellular functions in the central

nervous system (CNS) resulting in alterations in reproductive physiology and behaviors. In gonadally intact female rodents, the sequential release of ovarian estradiol and progesterone integrates the appearance of sexual behavior (heat, behavioral estrus) with ovulation. Sequential treatment of an ovariectomized female rodent with estradiol and progesterone maximizes the probability that the female will assume the lordosis posture, a behavioral component of female sexual behavior, when mounted by a conspecific male (Pfaff *et al.* 1994, Blaustein & Erskine 2002).

The regulatory action of estradiol on sexual behavior is believed to involve the activation of estrogen receptors (ERs), altering the expression of a number of genes, including the PR gene. The time-course for estradiol-induced increase in PR mRNA levels parallels the increase in the protein levels indicating the involvement of ERs in the action of estradiol (Romano *et al.* 1989). Recent studies using the mutant mouse strains with targeted deletion of the ER genes, α and/or β , indicate that ER α receptors influence sexual receptivity in female mice (Rissman *et al.* 1997, Ogawa *et al.* 1998, 1999).

Spatial, temporal and functional correlations suggest that estradiol-induced PRs, upon occupation by their cognate ligand, progesterone, function as transcriptional mediators and regulate transcription of target genes to affect the neural networks in the control of reproductive behavior (Pfaff *et al.* 1994). The time course of activation and termination of reproductive behavior parallels estradiol-induced increase and decline in PRs in the hypothalamus of the brain. A wide body of literature has identified different neuroanatomical sites in the regulation of female sexual behavior by steroid hormones, the details of which can be found in several excellent reviews (Blaustein 1992, Blaustein & Erskine 2002).

Several distinct signaling mechanisms for PRs activation in the brain have been identified to date. They are: (i) classic or genomic, (ii) non-classic or non-genomic and (iii) ligand-independent. Genomic and non-genomic mechanisms are both 'ligand (progesterone)-dependent' while ligand-independent mechanisms occur by the activation of the PRs by factors other than their cognate ligands. Recent studies suggest that these mechanisms are not mutually exclusive, but interact with each other to achieve the physiological end-point.

Genomic mechanisms

In female mammals, the circulating estradiol induces PRs and increases behavioral response to progesterone, and progesterone facilitates the expression of reproductive behavior acting primarily via intracellular PRs, which modulate target gene expression in neuronal networks mediating behavior. A temporal relationship between PR expression and display of sexual behavior has been demonstrated in guinea pigs and rats (Blaustein & Feder 1979^{a,b}, McGinnis *et al.* 1981). Studies using PR antagonists, protein and RNA synthesis inhibitors indicate a requirement for PRs in progesterone-mediated facilitation of sexual behavior (Whalen 1974, Rainbow *et al.* 1982, Brown & Blaustein 1984, Meisel & Pfaff 1984, 1985).

Studies using antisense oligonucleotides to PR mRNA, administered intracerebrally into the brain, provide definitive proof for an obligatory requirement for intracellular PRs in female sexual behavior. Three independent groups demonstrated the inhibition of both PR synthesis and progesterone-facilitated sexual behavior by infusing the antisense oligonucleotides to PR mRNA into the third cerebral ventricle (Mani *et al.* 1994^a) or ventromedial hypothalamus (Pollio *et al.* 1993, Ogawa *et al.* 1994). Studies from our laboratory using mutant mice with targeted deletion of the PR gene provided additional evidence for the involvement of intracellular PRs in progesterone-facilitated rodent female sexual behavior (Mani *et al.* 1996). Female mice carrying a null mutation for the intracellular PRs gene do not display the progesterone-facilitated lordosis response. The aggregate results of these studies support the requirement of a classic genomic mode of activation for neural PRs in progesterone-facilitated sexual behavior of rats and mice.

Thus, as described in the earlier section, progesterone binding to the intracellular PRs in the ventromedial nucleus of the hypothalamus (VMH) and pre-optic area (POA) of the hypothalamus induces their conformational change, leading to their nuclear translocation, dimerization, increased PR phosphorylation, DNA binding and recruitment of coactivators. The recruited coactivators facilitate their interactions with the general transcriptional machinery to modulate downstream target gene expression and behavior (Fig. 1). In addition, other signaling mechanisms, as described below, could converge with this

pathway to regulate the complex physiological response

Non-genomic mechanisms

While genomic effects have been assumed to be the primary pathway for hormone action in the brain, there are numerous studies indicating short-latency effects of progesterone which suggest the involvement of 'non-classic' effects via putative cell surface receptors and other mechanisms coupled to second messenger signaling cascades. Rapid effects of progesterone have been reported in the release of luteinizing hormone-releasing hormone (Ramirez *et al.* 1990), dopamine (DA) and acetylcholine (Meiri 1986) and changes in neuronal activity (Havens & Rose 1988). These rapid effects by progesterone are not blocked by protein synthesis inhibitors (Schumacher *et al.* 1999) and are believed to be mediated by binding to putative cell surface membrane receptors (Ramirez *et al.* 1996) or gated ion channels (McEwen 1994), and may be coupled to second messenger pathways (Kelly *et al.* 1999). Furthermore, metabolites of progesterone having low affinity for intracellular PRs have been demonstrated to be effective in facilitating the lordosis response in estrogen-primed rats and mice (Glaser *et al.* 1985, Frye & Vongher 1999^a). Functional interactions between membrane-mediated progesterone-regulated pathways and intracellular PRs have been observed in the facilitation of sexual behavior in female hamsters (DeBold & Frye 1994^{a,b}) suggesting that both classic and non-classic mechanisms act in concert rather than independently. Recent *in vitro* studies also demonstrate the ability of certain second messenger signaling cascades, initiated at the membrane, to interact with distinct proline-rich motifs of the nuclear PRs for their transactivation (Boonyaratanakornkit *et al.* 2001). These findings suggest the possibility that progesterone-induced membrane effects may influence PR gene expression via specific intracellular signaling via cross-talk and convergence with other pathways in the brain and behavior.

Ligand-independent mechanism: DA-PR interaction in the CNS

In vitro studies using cell culture systems have shown that compounds other than steroid hormones can

Figure 3 (A)

activate a significant number of steroid receptors, including PR, in a 'ligand-independent manner'. These include cyclic nucleotides that increase intracellular kinase activity (Denner *et al.* 1990), as well as extracellular compounds that interact with cell membrane receptors and stimulate intracellular phosphorylation pathways, including growth factors and the neurotransmitter DA (Aronica & Katzenellenbogen 1991, 1993, Power *et al.* 1991*a,b*). The earliest observation that progesterone-dependent, PR-mediated transcription could be activated in a ligand-independent manner by 8-bromo-cAMP in the absence of progesterone (Denner *et al.* 1990), was followed by studies demonstrating that PR could be activated by other pathways as well. Power *et al.* (1991*a*) showed that DA could activate the chicken ovalbumin upstream promoter transcription factor, a member of the steroid receptor superfamily. DA was also found to be capable of translocation of other transcription factors, PRs and ERs, from the cytoplasm to the nucleus in an *in vitro* cell transfection system (Power *et al.* 1991*b*).

The physiological relevance of ligand-independent activation of PRs by DA in female sexual behavior was demonstrated in rats and mice.

Intracerebroventricular (i.c.v.) administration of apomorphine, a DA receptor stimulant, or the DA receptor subtype-1 (D_1) agonist, SKF 38393, facilitated sexual receptive behavior in female rats mimicking the effects of progesterone (Mani *et al.* 1994*b*). The facilitatory effect of DA was determined to be specific to the D_1 receptor subtype confirming and extending an earlier report in which DA agonists infused into the hypothalamus and POA facilitated receptive behavior (Foreman & Moss 1979). Interestingly, similar to the facilitation of sexual behavior by progesterone, the facilitatory effect of DA agonist was also blocked by i.c.v. administration of PR antagonists, D_1 receptor antagonist or antisense oligonucleotides to PR mRNA (Fig. 3A), suggesting that neural PRs were required for the DA activation of female sexual behavior (Mani *et al.* 1994*b*). Importantly, the inability of PR knockout mice to exhibit D_1 agonist-facilitated sexual behavior, while their wild-type littermates responded to the agonist with good receptive response (Mani *et al.* 1996) (Fig. 3B), provides definitive evidence for the obligatory role of PRs as transcriptional mediators for a membrane receptor-dependent pathway initiated by DA,

Figure 3 DA agonist–PR interactions in sexual receptivity of female rats and mice via ligand-independent mechanism. Sexual receptivity was quantitated and represented as lordosis quotient (LQ) defined as a percent of the number of times a female rodent exhibits lordosis response divided by the number of mounts attempted by the male. (A) Antisense oligonucleotides to PR (AsPR) significantly ($*P<0.05$) inhibit DA agonist, SKF 38393 (SKF)-facilitated lordosis response (measured in terms of LQ) in estradiol benzoate (EB)-primed female rats. Sense oligonucleotides to PR (SPR) do not have any significant effects ($P>0.05$). (B) PR null mutant mice ($-/-$) do not exhibit progesterone (P) or DA agonist (SKF)-facilitated sexual receptive behavior compared with their wild-type littermates ($+/+$). Statistically significant ($*P<0.05$) differences were observed in ovariectomized, EB-primed, progesterone and SKF 38393-treated homozygous null mutants ($-/-$) compared with their wild-type littermates ($+/+$) that received the same treatments. Values are presented as mean $LQ \pm S.E.M.$ (Adapted from Mani *et al.* 1994b (A) and Mani *et al.* 1996 (B).)

converging on PR, and resulting in sexual behavior.

Studies using antisense oligonucleotides to DA receptor subtypes indicate that DA-facilitated, PR-mediated behavioral effects occur via the D_1B (D_5) subtype and not the D_1A subtype (Mani *et al.* 2001). Ligand-independent activation of PRs has also been observed in other studies. Behaviorally relevant stimuli such as vaginal–cervical stimulation (VCS) activate neural PRs in the absence of progesterone (Auger *et al.* 1997). Administration of the progesterone antagonist RU38486 to estradiol-primed female rats blocked sexual receptive responses to mating stimuli by VCS or mounting by a male rat, suggesting that the somatosensory information provided by either of the stimuli

could be due to ligand-independent activation of PRs. Induction of the immediate early gene ‘Fos’ was reduced in PR-rich areas like the medial POA and ventromedial nucleus of the hypothalamus upon RU38486 treatment (Auger *et al.* 2000a). Based on PR immunostaining studies, Auger *et al.* (2000b) suggest that PRs could be activated differentially by progesterone-dependent or progesterone-independent mechanisms, possibly leading to different neuronal consequences.

Convergence of signaling mechanisms

Protein phosphorylation is common to the pathways and molecular mechanisms through which

neurotransmitters and steroid hormones produce their biological effects. The regulatory mechanisms governing a variety of cellular processes in target cells are not only dependent upon the state of intracellular phosphorylation of the receptor, but are also dependent on the dynamic balance between cellular protein kinases and phosphatases. This has been found to be the case in PR, wherein the equilibrium between transcriptionally active and inactive forms of the receptor is under the regulation of kinases and phosphatases (Denner *et al.* 1990).

Modulation of protein kinases and protein phosphatases in phosphorylation and signal transduction mechanisms occurs in the mammalian brain, a tissue having an abundance of kinases and phosphatases (Shenolikar & Nairn 1991). Neuronal phosphoproteins, like neurotransmitters and cyclic nucleotides, are components of the signal transduction pathway in the nervous system (Greengard 2001) and can be phosphorylated/dephosphorylated in response to extracellular stimuli; such dynamic covalent modification is evident in modulation of the activity of protein phosphatases 1 and 2 (PP-1 and PP-2). Several studies have documented that DA signaling through the D₁ subclass of receptors induces increases in the levels of second messenger cAMP and activates cAMP-dependent protein kinase (PKA) in the neostriatum. Increased PKA activity leads to the phosphorylation of the neuronal phosphoproteins, DA and cAMP-regulated phosphoprotein-32 (DARPP-32), and/or inhibitor-1 (I-1). The phosphoprotein I-1 is closely related structurally, enzymologically and functionally to DARPP-32 (Shenolikar & Nairn 1991). In its phosphorylated state, DARPP-32 and/or I-1, by inhibiting the activity of PP-1, increases the state of phosphorylation of many substrate proteins, leading to induction of physiological responses. Thus, PR is one of the potential substrate proteins phosphorylated by DARPP-32. To determine whether DARPP-32 and/or Inh-1 could be the downstream mediators in the DA-PR interactions in the facilitation of sexual behavior, female rats and mice were investigated (Mani *et al.* 2000).

Antisense oligonucleotides to DARPP-32 administered i.c.v. into the third cerebral ventricle inhibited D₁ agonist- and progesterone-facilitated sexual receptivity in estradiol-primed female rats (Mani *et al.* 2000). D₁ agonist- and progesterone-

Figure 4 Progesterone (P) and DA agonist, SKF 38393 (SKF)-facilitated lordosis in ovariectomized, estradiol benzoate (EB)-treated null mutant mice (-/-) for DARPP-32. The response was significantly reduced ($*P < 0.05$) in null mutant mice (-/-) compared with their wild-type littermates (+/+). All values are presented as mean LQ $\pm$ S.E.M. (Adapted from Mani *et al.* 2000.)

facilitated sexual receptivity also was inhibited in estradiol-primed female mice carrying a null mutation for DARPP-32 gene (Fig. 4). However, I-1 null mutant mice exhibited no deleterious defects in D₁ agonist- and progesterone-facilitated lordosis response compared with their wild-type littermates, revealing that involvement of the DARPP-32 pathway is specific. Similar to DA effects in the neostriatum, D₁ agonist, as well as progesterone, significantly increased hypothalamic cAMP levels and PKA activities, and enhanced phosphorylation of DARPP-32 on threonine-34. D₁ agonist-induced increases were inhibited by the D₁ subclass DA antagonist, SCH 23390, indicating that the increases were due to the effects of DA initiated at its membrane receptor. Progesterone-induced increases, however, were not inhibited by SCH 23390, suggesting that the observed increases were due to the direct effects of progesterone and not secondary to modulation of DA receptors by progesterone (Mani *et al.* 2000). Rp-cAMPS, a compound that blocks the cAMP signal transduction cascade by inhibiting PKA, inhibited D₁ agonist- and progesterone-facilitated sexual receptivity in estradiol-primed female rats. While the studies identify DARPP-32 activation as an obligatory step in PR regulation of sexual receptivity, the sequence of events leading to the activation of PRs are under investigation. These mechanisms possibly include not only a direct,

decreased dephosphorylation of PR, but also enhanced phosphorylation of PR-associated coactivators leading to rapid efficient transcriptional activation (Rowan *et al.* 2000).

Studies (Mani *et al.* 2000) demonstrating the involvement of the cAMP/PKA cascade in sexual behavior is consistent with published reports. These reports demonstrated increased hypothalamic cAMP levels on the evening of proestrus, concomitant with the exhibition of sexual behavior and the facilitatory effects of cAMP analogs and phosphodiesterase inhibitors on sexual behavior (Beyer & Gonzalez-Mariscal 1986, Whalen & Lauber 1986, Kow *et al.* 1994). Studies have also reported increased immunoreactive phospho-DARPP-32 cells in the PR-containing areas of the rat hypothalamus, the areas known to be involved in the expression of sexual behavior (Meredith *et al.* 1998). Taken together, the results suggest that both progesterone and DA increase DARPP-32 phosphorylation by activation of PKA in the neurons of the hypothalamus, resulting in an enhanced lordosis response. While the observations indicate that DARPP-32 activation is an obligatory step in PR regulation of sexual receptivity, the sequence of events leading to the activation of PR remain to be defined. Potential mechanisms could include not only a direct, decreased dephosphorylation of PR, but also enhanced phosphorylation of its associated coactivators leading to efficient transcriptional regulation (Rowan *et al.* 2000a,b, Mani & O'Malley 2002).

SRCs and female sexual behavior

Steroid hormone effects on female sexual behavior are mediated by estradiol-induced PRs in the brain. Local regulation of SRCs present in the brain could enhance these steroid receptor-mediated hormone effects, leading to region-specific changes in steroid sensitivity. Meijer *et al.* (2000) demonstrated a differential expression profile and distribution of coactivators, SRC-1 and SRC-2, in the rat brain. Immunocytochemical analyses have also revealed high levels of expression of yet another coactivator protein, CBP, in the hypothalamus, cortex and cerebellum (Stromberg *et al.* 1999) and its potential role in brain development (Oike *et al.* 1999). CBP and SRC-1 proteins are expressed in estradiol-induced PR-containing neurons in the VMH and medial POA (Stromberg *et al.* 1999).

The functional importance of both coactivators, SRC-1 and CBP, in estrogen-induced PR expression and hormone-dependent regulation of female reproductive behavior has been demonstrated by Molenda *et al.* (2002). Antisense oligonucleotides to SRC-1 or CBP independently infused into the VMH of ovariectomized, estrogen-primed female rats showed no differences in PR expression in the VMH, compared with their scrambled oligonucleotide-treated controls. In contrast, antisense oligonucleotides to both SRC-1 and CBP administered together into the VMH decreased estradiol-induced PR expression, compared with the control-treated contralateral VMH. The studies suggest that functional interactions between the coactivators, SRC-1 and CBP, and ovarian steroid receptors could potentiate the hormone-dependent effects on gene expression in the VMH of the brain (Tetel 2000, Molenda *et al.* 2002). Interestingly, while the antisense oligonucleotides to SRC-1 and CBP decreased the PR expression in the VMH, their effects on hormone-dependent female reproductive behavior were very distinct. While the overall lordosis intensity of progesterone-facilitated lordosis was altered by the antisense oligonucleotides there was no difference in the lordosis frequency (represented by lordosis quotient) between the two treatment groups. Since coactivators like SRC-1 are also targets for multiple signaling pathways, it is plausible that they could play a role in ligand-independent activation of PRs in the brain and behavior.

It is becoming abundantly clear that steroid hormone modulation of the brain and behavior is more complex than previously envisioned. Mutual interdependence and convergence of signaling pathways appear to be a 'reinforcing' mechanism to achieve the neuroendocrine integration required for the complex reproductive behavior. While the molecular and cellular findings discussed in this review have allowed us a greater appreciation of the complex signaling mechanisms involved in steroid hormone action, future studies will reveal the nuances in such an integrative model of steroid hormone action.

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