YOUNG INVESTIGATOR PERSPECTIVES

Nuclear Receptor Coactivators in Neuroendocrine Function

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Abstract

Steroid hormones influence a variety of neuroendocrine events, including brain development, sexual differentiation and reproduction. Hormones elicit many of these effects by binding to neuronal steroid receptors, which are members of a nuclear receptor superfamily of transcriptional activators. However, the mechanisms by which activated steroid receptors regulate gene expression in brain are not well understood. Recently, a new class of proteins, known as nuclear receptor coactivators, have been found to dramatically enhance steroid receptor mediated transactivation of genes *in vitro*. Here, the proposed molecular mechanisms of how these coactivators enhance the transcriptional activity of steroid receptors are summarized. While much is known about the mechanisms of these coactivators *in vitro*, it is unclear how these cofactors function in hormone action *in vivo* or in the brain. This paper discusses some of the initial and enticing investigations into the role of these important coregulatory proteins in neuroendocrine events. Finally, some of the critical issues and future directions in nuclear receptor coactivator function in neuroendocrinology are highlighted.

Function of steroid hormones in brain

Steroid hormones act throughout the body to regulate development, differentiation, metabolism and reproduction. During the last half of the 20th Century, there was an explosion in the knowledge of how steroid hormones influence the central nervous system. A variety of studies reveals that the gonadal steroid hormones, oestrogens, progestins and androgens, act in the brain to influence many neuroendocrine events, including those associated with reproduction. These hormones have also been shown in animal and human studies to influence memory and cognition (1). In addition, oestrogens have been linked to depression and delaying the onset of Alzheimer's disease, further implicating a role for steroid hormones in higher order mental processes in humans. However, the mechanisms by which steroid hormones influence gene expression in the brain remain poorly understood. To address these basic cellular and molecular neuroendocrine mechanisms, a variety of model systems have been used to investigate steroid hormone action in brain.

Two widely exploited models for studying steroid hormone action in brain are the hormonal regulation of brain development and hormone-dependent reproduction in rodents. Exposure of the developing brain to gonadal hormones during critical periods results in profound changes in morphology that are sexually differentiated. In adulthood, the ovarian steroid hormones, oestradiol and progesterone, are secreted sequentially during the rat oestrous cycle and act in the brain to regulate female reproductive behaviour (2). Thus, sexual differentiation of the brain and female reproductive behaviour in rats offer excellent models for elucidating how hormones act in the brain to modulate gene expression and behaviour.

Steroid hormones elicit many of their biological effects by binding to their respective intracellular receptors, which belong to a large class of nuclear receptors. Recently, a novel class of proteins, known as nuclear receptor coactivators, has been shown to enhance the activity of nuclear receptors (3, 4). These nuclear receptor coactivators represent an important and critical level of regulation of receptor transcriptional activity. While much is known about how these coactivators influence hormone action *in vitro*, research on the *in vivo* function of nuclear receptor coactivators in neuroendocrine events is in its infancy. Here, the molecular mechanisms

underlying nuclear receptor coactivator modulation of steroid receptor action are briefly described and recent findings on the role of these coactivators in neuroendocrine function are discussed.

Mechanisms of action of steroid receptors

Nuclear receptors represent a superfamily of transcriptional activators that can be divided into subfamilies based on phylogenetic analysis. Receptors for oestrogens (ER), progestins (PR), androgens (AR), glucocorticoids (GR) and mineralocorticoids (MR) represent the type I subfamily known as classic steroid receptors. Receptors for thyroid hormone (TR), vitamin D_3 (VDR), all-*trans* retinoic acid and 9-*cis* retinoic acid comprise the type II receptors. The third subclass includes the orphan nuclear receptors, which have no known ligands (5). These nuclear receptors share a modular structure consisting of a carboxyl-terminal ligand binding domain (LBD), a hinge region, a central highly conserved DNA binding domain and a variable amino terminal domain. In general, nuclear receptors have two transcriptional activation function domains: one in the amino-terminus (AF-1) and one in the carboxyl-terminal LBD (AF-2). A genomic mechanism of action for type I steroid receptors is summarized in Fig. 1. *In vitro* studies reveal that upon binding hormone, steroid receptors undergo a conformational change that causes dissociation from heat shock proteins (hsp), allowing receptor dimerization. These receptor dimers bind to palindromic steroid response elements of steroid-responsive target genes and alter the rate of gene transcription. While hormone action in brain is poorly understood, it is thought that steroid hormones act *via* their respective receptors to alter neuronal gene transcription, resulting in changes in steroid-regulated neuroendocrine events. In contrast to type I receptors, type II receptors bind to DNA in the absence of ligand and often exert a repressive effect on the promoters of target DNA. This repressive effect, known as silencing, is relieved upon receptor binding to ligand. While it is beyond the scope of this review, it should be noted that a variety of studies provide evidence for nongenomic mechanisms of steroid hormone action in brain (6).

Nuclear receptor coregulators

Recent and exciting discoveries have revealed a class of proteins known as nuclear receptor coregulators. These coregulators consist of coactivators and corepressors that
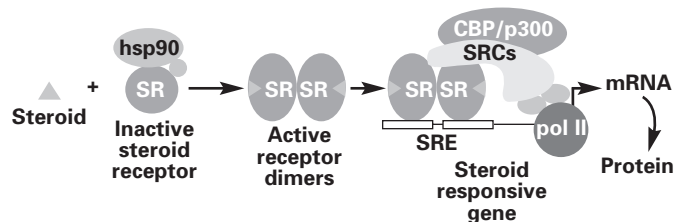

FIG. 1. Model of steroid receptor action and coactivator function. SR, steroid receptor (type I); hsp90, heat shock protein 90; SRE, steroid response element; SRCs, steroid receptor coactivator family (p160s); CBP/p300, CREB-binding protein/p300; Pol II, RNA polymerase II.

are required for efficient transcriptional regulation of nuclear receptors (3, 4). Nuclear receptor coactivators are proteins that interact with nuclear receptors and enhance their transcriptional activity. *In vitro* experiments reveal that these coactivators are often rate-limiting for receptor activation and appear to act as bridging proteins between the receptor and the basal transcriptional machinery (Fig. 1) (3, 4). Corepressors decrease transcriptional activity of nuclear receptors and appear to function more in action of type II receptors (3, 4). Here, I will focus on the behaviourally and physiologically relevant roles of nuclear receptor coactivators in steroid hormone action in brain.

Function of nuclear receptor coactivators in steroid hormone action

Steroid receptor coactivator family

Steroid receptor coactivator-1 (SRC-1) was the first coactivator to be cloned and characterized that dramatically enhances the transcriptional activity of nuclear receptors (7). The following list provides some of the general properties (*in italics*) that define nuclear receptor coactivators as proposed by McKenna *et al*. (8) and the experimental evidence that identifies SRC-1 as a coactivator of steroid receptors. (i) *Receptors physically interact with coactivators in a ligand-dependent manner.* SRC-1, which was identified using the LBD of PR in a yeast two-hybrid screen, interacts with steroid receptors in the presence of an agonist, but not when unbound or in the presence of an antagonist (7). (ii) *Coactivators increase the transcriptional activity of steroid receptors.* In reporter assays, SRC-1 enhances the transactivation of steroid receptors, including PR, ER, GR, and the nuclear receptors thyroid hormone (TR) and 9-*cis* retinoic acid (RXR) (3, 7). (iii) *Coactivators are rate-limiting and required for efficient transcriptional activity of steroid receptors.* Depletion of SRC-1 in cultured cells by microinjection of SRC-1 antibodies prevents steroid receptor-dependent transcription (9). (iv) *Transcriptional interference, or squelching, occurs when one receptor type represses the transcriptional activity of another receptor type by sequestering cofactors required by both receptors. Expression of coactivators can reverse this transcriptional interference.* In cell culture systems, hormone-induced transactivation of PR is reduced by coexpression of ERα. This squelching of PR activity by ERα was relieved by over-expression of SRC-1 (7). (v) *Coactivators contain activation domains that are able to enhance gene transcription when fused to a DNA binding domain.* SRC-1 contains two autonomous activation domains that increase gene transcription when fused to a DNA binding domain in a reporter assay. A recent two-step model by Lui *et al*. (10) proposes that SRC-1 enhances steroid receptor activity by (a) stabilizing interactions between receptor and general transcription factors and (b) remodelling chromatin through its intrinsic histone acetyltransferase activity (11), which unwraps DNA from its condensed histone complex making it more accessible to the transcriptional machinery.

SRC-1 belongs to a novel family of proteins about 160 kDa in size, called the p160s, that also includes SRC-2 and SRC-3 (3, 7). SRC-2 (also known as GRIP1 and related to TIF2) is

similar in sequence and function to SRC-1. As is the case with SRC-1, SRC-2 enhances the transcriptional activity of a variety of nuclear receptors, including ER and PR (12). SRC-3 (also known as RAC3, AIB1 and TRAM-1) coactivates nuclear receptors, as well as other activators such as interferon-α and cAMP regulatory element binding protein (CREB). Interestingly, SRC-3 appears to be selective by enhancing the activity of ERα over ERβ, presumably due to differences between the LBDs of the two ERs (13).

Other coactivators of nuclear receptors

Perhaps one of the more intriguing coactivators described thus far is steroid receptor RNA activator (SRA), which appears to selectively enhance the transcriptional activity of the steroid receptors PR, ER GR and AR, but not other nuclear receptors such as TR or RXR (14). Moreover, SRA functions as an RNA transcript and not as a protein. SRA enhances steroid receptor activity in the presence of cycloheximide, while other coactivators such as SRC-1 do not. Another distinction of this coactivator is that, while most nuclear receptor coactivators enhance the transcriptional potential of the carboxyl-terminal activation function (AF-2) located in the LBD, SRA appears to act through the amino-terminal activation domain (AF-1) of receptors. Lanz et al. (14) propose that SRA functions through the amino-terminal AF-1 of steroid receptors to confer specificity of a multi-protein complex (which includes SRC-1) that is recruited by activated steroid receptors. While SRA is expressed in the brain at low levels (14), its possible function in hormone action in the nervous system has not been explored yet.

CREB binding protein (CBP) is a coactivator for a variety of transcription factors, including CREB, p53 and STAT-2. Recently, CBP has been shown to enhance the transcriptional activity of some nuclear receptors, including ER and PR (3, 15). In addition, CBP acts synergistically with SRC-1 to increase ER and PR function and transcriptional activity in vitro (16, 17). In support of this synergistic effect, CBP interacts with SRC-1 in vitro (15), suggesting that CBP and SRC-1 form a complex with hormone-bound receptor that is integral in mediating steroid receptor-dependent gene transcription. Similar to SRC-1, CBP is thought to remodel chromatin through its intrinsic histone acetyltransferase activity (3). The adenovirus E1A-associated 300 kDa protein (p300) shares many properties with CBP as a coactivator of transcription factors, including nuclear receptors. Because CBP and p300 act with a variety of transcription factors, it has been proposed that they are integrators of convergent signalling pathways, including nuclear receptor pathways (3).

The type II thyroid hormone receptor interacts with a multiprotein complex, termed thyroid hormone receptor-associated proteins (TRAPs) (18), that is distinct from the SRC and CBP/p300 families of coactivators. These TRAPs, which range in size from 70 to 230 kDa, enhance transcriptional activity of TR in an in vitro transcription system (18). Similar to TRAPs, a complex of proteins that associate with VDR, termed VDR-interacting proteins (DRIPs), enhance VDR transcriptional activity in a ligand-dependent manner (19). While some of the TRAPs have been found to associate weakly with type I receptors such as ER (20), most evidence indicates a functional role for TRAPs and DRIPs in type II receptor action. It has been suggested that SRC/CBP and TRAP/DRIP complexes may synergize on specific promoters allowing for regulation of a single gene by different coactivator complexes (4).

Nuclear receptor coactivators in neuroendocrine function

While most research has focused on the biochemical and molecular properties of nuclear receptors in vitro as described above, a few studies have begun to explore coactivator function in vivo. SRC-1 protein and mRNA are expressed throughout the brain, including high levels in the olfactory epithelium and hypothalamus (21–23). While dense expression of SRC-1 mRNA was detected in the mouse suprachiasmatic nucleus, SRC-1 does not appear to be regulated in a circadian fashion (22).

Steroid hormone responsiveness can be regulated at many different levels, predominantly by the availability of the hormone and the regulation of receptor levels. Another level of modulation of hormone responsiveness may be regulation of nuclear receptor coactivators, which are required for full transcriptional activity of steroid receptors. Steroid hormones, which alter the expression of many genes in the brain including steroid receptors, may influence nuclear receptor coactivator expression. The possible regulation of SRC-1 and p300 mRNA by oestradiol and thyroid hormone has been studied in the pituitary (24). While no effect of hormones on p300 mRNA expression was reported, oestradiol decreased SRC-1 mRNA levels in male rat anterior pituitary as detected by Northern blot (24). In this same study, thyroid hormone increased SRC-1 mRNA in the anterior pituitary of euthyroid rats. In further support of hormonal regulation, a sex difference was observed in the anterior pituitary, with females having 40% less SRC-1 mRNA than males (24). However, no sex differences or effects of hormones on coactivator expression were detected in the hypothalamus. It is important to note that this study used Northern blot analysis, which lacks cellular resolution, and may not detect changes in smaller neuroanatomical regions. In future investigations of hormonal regulation of nuclear receptor coactivators, it will be imperative to apply techniques with cellular resolution, such as in situ hybridization and approaches that detect protein expression such as immunocytochemistry. Furthermore, it will be essential to address the possibility that neurotransmitters that elicit profound effects on steroid hormone response, such as dopamine and serotonin, may do so in part by regulating nuclear receptor coactivator expression. Finally, a variety of environmental stimuli (e.g. social contact, stress, food availability, light, etc.) are known to influence steroid-sensitive neuroendocrine events. Future studies of the modulation of steroid-mediated events by environmental stimuli should consider regulation of nuclear receptor coactivators as a potential mechanism. Each of these modes of regulation of receptor coactivator expression provides a possible mechanism by which steroid responsiveness can be further regulated within individual neurones.

In order for coactivators to enhance the activity of steroid receptors, both the coactivator and receptor must be present

in the same cells. Using a double-label immunofluorescent technique, ERα and SRC-1 were observed to be coexpressed in rat mammary gland stroma cells, while these two proteins were segregated in mammary epithelial cells (25). This differential pattern of coexpression suggests that SRC-1 functions in a cell-type specific manner. Preliminary work in brain from our laboratory supports this idea that cells coexpress steroid receptors and coactivators. Oestradiol-induced PR in the hypothalamus is required for the expression of progesterone-facilitated reproductive behaviour (2). Using double label-fluorescent immunocytochemistry, we found that many neurones in the ventromedial hypothalamus (VMH), a brain region critical in the regulation of female reproductive behaviour (2), coexpress steroid receptors and coactivators. Of the oestradiol-induced PR containing neurones in the VMH, most express CBP and more than 50% express SRC-1. Thus, these VMH neurones are potential sites of functional interaction between coactivators and steroid receptors involved in hormone-dependent female reproductive behaviour. The heterogeneity of steroid responsiveness of individual neurones within a brain region is a fundamental issue in steroid hormone action in brain. The presence or absence (as well as the overall ratio) of coactivators within particular neurones may be one mechanism for fine-tuning of steroid responsiveness within individual neurones.

In collaboration with Anthony Auger and Margaret McCarthy (University of Maryland), we have found that SRC-1 is critical in hormone-mediated sexual differentiation of the brain (23). One of the crucial events in steroid mediated sexual differentiation is exposure of male rats to high levels of testicularly derived testosterone around birth. One of the consequences of this hormone surge in the developing brain is the enlargement of the sexually dimorphic nucleus (SDN) of the preoptic area in males. This enlargement of the SDN is mediated by the activation of ER by oestradiol that has been metabolized from testosterone. To test the role of SRC-1 in ER-mediated development of the SDN, female rats were infused with SRC-1 antisense or control oligodeoxynucleotides (ODNs) at birth and on postnatal days 1 and 2. On postnatal day 1, rats were injected with the aromatizable androgen, testosterone propionate, to increase the volume of the SDN. At day 13, hormone-treated females that received SRC-1 antisense ODNs had smaller SDN volumes than hormone-treated females that received control ODNs, suggesting that SRC-1 is required for ER-mediated sexual differentiation of the SDN. The neonatal testosterone surge also results in the behavioural defeminization (decrease in female sexual behaviour due to oestrogen action) and masculinization (increase in male sexual behaviour) in adulthood. Male and androgenized female rats were treated with SRC-1 antisense or control ODNs as described above, castrated in adulthood and tested for male sexual behaviour following treatment with testosterone. Males and androgenized females treated with SRC-1 antisense displayed higher levels of female sexual behaviour compared to animals treated with control ODNs. These data suggest that reducing SRC-1 protein decreases the ability of ER to defeminize behaviour and further implicates an important role for SRC-1 in the developing rat brain (23).

Another approach to studying coactivator function in vivo has been gene targeting to create SRC-1 knockout mice (26). Male and female SRC-1 knockout mice are fertile and have growth rates similar to wild-type controls. However, the steroid target organs of SRC-1 mutant mice, including the uterus, mammary glands, prostate and testis, have decreased growth and development in response to steroid hormones (26). In addition, female SRC-1 null mutant mice have elevated plasma levels of oestradiol and testosterone. The increased levels of these gonadal hormones are similar to those observed in ERα knock-out mice (27), suggesting that the altered regulation of the hypothalamic-pituitary-gonadal axis in SRC-1 knock outs is due to disrupted ER activity. Interestingly, SRC-1 knockouts have a two-fold increase in TIF2 mRNA, which is a member of the SRC family and has similar functions as SRC-1 as discussed above. Thus, this increase in TIF2 may have partially compensated for the loss of SRC-1 in the null mutants (26). These knockout mice have also been used to study SRC-1 function in thyroid hormone receptor action. Compared to their wild-type litter mates, SRC-1 knockouts have elevated thyrotropin (TSH) levels, despite increased thyroid hormone levels (28). The authors suggest that these mice are a model for resistance to thyroid hormone, a syndrome in humans characterized by hyposensitivity to thyroid hormone.

Further evidence that SRC-1 functions in thyroid hormone action is provided by studies of hormone regulation of the preproenkephalin (PPE) gene (29). Oestrogen-induced hypothalamic PPE gene expression is involved in hormone-regulated female sexual behaviour in rats. However, thyroid hormone diminishes oestrogen-induced behaviour, possibly through TR-mediated interference of ER activation of PPE gene expression. Such a mechanism would suggest that TR sequesters cofactors which are also required by ER for efficient activation of the PPE gene. In support of this hypothesis, in a cotransfection assay using a PPE promoter, expression of TR reduced (squelched) the ability of ER to activate a reporter gene under the regulation of a PPE promoter. Overexpression of SRC-1 reversed the transcriptional interference (squelching) by TR of oestradiol-induced expression of the reporter gene (29). These data suggest that the thyroid-mediated decrease of oestrogen-induced female sexual behaviour may be occurring at the transcriptional level through competition of SRC-1 by TR and ER, implying the importance of SRC-1 as a limiting factor in hormone mediated gene expression.

CBP is expressed throughout the brain, including high levels of expression in the hippocampus, dentate gyrus and hypothalamus (30). Alteration of the CBP gene has been used to study CBP function in vivo. While heterozygous CBP deficient mice (CBP+/−) live to adulthood, homozygotes die during mid-embryogenesis, apparently due to neurotube defects (31, 32). In humans, a mutation in the CBP gene causes Rubinstein–Taybi Syndrome, which is characterized by severe mental retardation and craniofacial abnormalities (32). Interestingly, CBP+/− mice have deficits in long-term memory as tested in passive avoidance and fear conditioning tests (32), suggesting these mice are a model for Rubinstein–Taybi Syndrome in humans. While these deficits may be caused by a lack of CBP function in multiple signalling

pathways in the brain, it is possible that a lack of coactivation of steroid receptors by CBP contributes to some of these memory deficiencies due to diminished ER and/or GR action in the CNS (1). These data indicate that CBP is required for normal CNS development and function; however, very little is known about either the mechanisms involved or the role of CBP as a coactivator of steroid receptors in brain development and hormone action. As discussed above, p300 has similar functions as CBP, while other studies have found distinct roles for these coactivators in cellular differentiation (33). In future experiments, it will be important to determine how CBP and p300 function as nuclear receptor coactivators in brain.

Conclusions and future directions

Considerable progress has been made in deciphering the molecular mechanisms governing the enhancement of steroid receptor action by nuclear receptor coactivators from a variety of *in vitro* studies. However, it is important to note that these experiments rely on overexpression systems that do not truly represent the cellular milieu that exists *in vivo*. Steroid hormone action in brain offers an excellent model system for *in vivo* investigation of nuclear receptor coactivator function. Initial studies indicate that these nuclear receptor coactivators are critical for normal hormone-dependent development of the brain. Future studies need to investigate the function of these coactivators in steroid hormone-dependent gene expression in the rodent brain.

It is possible that certain anomalies in hormone action, such as mismatches between receptor levels and steroid responsiveness, could be explained by regulation of coactivators by physiological stimuli. For example, food deprivation in hamsters causes an increase in ER cells in the medial preoptic area (MPOA) (34). It is known that oestradiol priming dramatically increases the concentration of PR in the MPOA and other brain areas. While one would anticipate a corresponding increase (or perhaps no change) in the number of oestradiol-induced PR in the MPOA of food deprived animals, in fact food restriction decreased oestradiol-induced PR. One possible explanation for this mismatch between receptor levels and steroid responsiveness is that food-deprivation, while increasing ER, may decrease nuclear receptor coactivators required for efficient transcriptional activity of ER. In support of a role for nuclear receptor coactivators in ER transcriptional activity in brain, preliminary findings from our laboratory indicate that coactivators (SRC-1 and CBP) are involved in ER-mediated activation of the PR gene in rat brain. Administration of antisense oligonucleotides to both SRC-1 and CBP mRNA decreases the number of oestradiol-induced PR cells in the VMH. While it must be established that antisense treatment decreased expression of coactivators, these data suggest that SRC-1 and CBP function in steroid receptor-mediated transactivation of the behaviourally relevant PR gene in brain. Future studies will need to investigate the role of other coactivators, such as SRC-2 (TIF2) and SRC-3, which may function similarly or have distinct roles in hormone action in brain. Finally, other anomalies in steroid-dependent transcription in the nervous system (35) might be explained by regulation of these coregulatory proteins.

An unresolved question in steroid hormone action has been ligand-independent activation of unoccupied steroid receptors by neurotransmitters *in vitro* and in the brain (36, 37). One explanation for ligand-independent activation of receptor may be that neurotransmitter stimulation causes recruitment of coactivator complexes by the unbound receptor. In support of this hypothesis, SRC-1 interacts with, and enhances the activity of the amino-terminal activation domain (AF-1) of PR (38) and ERα (39) in a ligand-independent manner *in vitro*. Furthermore, phosphorylation of the AF-1 of ERβ results in ligand-independent recruitment of SRC-1 (40). These *in vitro* data suggest an intriguing mechanism in which neurotransmitter stimulation may result in phosphorylation of steroid receptors that allows ligand-independent transcriptional enhancement by coactivators. These hypotheses regarding ligand-independent activation of steroid receptors have yet to be tested in brain.

Many steroid receptors, including ER, are expressed as two distinct forms. Recently there has been a rush to decipher the possible different functions of the two distinct ERs (ERα and ERβ) in oestrogen action in the brain. Behavioural studies on ERα and ERβ knockout mice suggest these two ERs function differently in brain (41, 42). *In vitro* studies indicate that SRC-3 enhances the transcriptional activity of ERα, but not ERβ (13). Taken together, these findings raise the possibility that nuclear receptor coactivators may contribute to the differential function of the two ERs in modulating oestrogen action in brain.

The field of Neuroendocrinology is poised to make dramatic gains in understanding how hormones regulate gene expression in brain. Initial investigations indicate that nuclear receptor coactivators are critical regulatory molecules in hormone-dependent activation of genes in the brain. Future studies of the role of these important cofactors in steroid receptor action in brain will shed more light on how hormones regulate a wide array of neuroendocrine events.

Note added after submission

The reader is directed to two important papers relevant to this review. SRC-1 mRNA has been found expressed throughout rat brain, while SRC-2 mRNA was not detected in brain (Meijer *et al.*, *Endocrinology* 2000; **141:** 2192–2199). A recent analysis of SRC-3 knockout mice reveals that SRC-3 is critical for normal female reproductive physiology (Xu *et al., Proc Natl Acad Sci USA* 2000; **97:** 6379–6384).

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