Nuclear Receptor Coactivators: Essential Players for Steroid Hormone Action in the Brain and in Behaviour

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Steroid hormones act both in the brain and throughout the body to influence behaviour and physiology. Many of these effects of steroid hormones are elicited by transcriptional events mediated by their respective receptors. A variety of cell culture studies reveal that nuclear receptor coactivators are critical for modulating steroid receptor-dependent transcription. Thus, in addition to the availability of the hormone and the expression of its receptor, nuclear receptor coactivators are essential for steroid-dependent transactivation of genes. This review discusses the mounting evidence indicating that nuclear receptor coactivators are critical for modulating steroid hormone action in the brain and in the regulation of behaviour.

Key words: steroid receptor coactivator-1 (SRC-1), oestrogen receptor, progestin receptor, brain development, sex behaviour.

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altering the rate of recruitment of general transcription factors and influencing the recruitment of RNA polymerase II to the initiation site (29, 30). Thus, in the brain, it is thought that steroids can act via their respective receptors to alter neuronal gene transcription, resulting in profound changes in behaviour and physiology (31, 32).

Nuclear receptor coregulators

Nuclear receptor coregulators are required for efficient transcriptional regulation by nuclear receptors (33, 34). The importance of these coregulators in a variety of human diseases, including cancer and some neurological disorders, is becoming more apparent (35). Coregulators consist of coactivators and corepressors that are required for efficient transcriptional regulation by nuclear receptors. Nuclear receptor coactivators dramatically enhance the transcriptional activity of nuclear receptors, including ER and PR (33, 34). Nuclear receptor coactivators influence receptor transcription through a variety of mechanisms, including acetylation, methylation, phosphorylation and chromatin remodelling (33). Studies performed in vitro using antibodies against nuclear receptor coactivators indicate that recruitment of coactivators is rate-limiting in steroid receptor-mediated gene transcription (33, 36). In further support for nuclear receptor coactivator-dependent facilitation of transcription in vitro, squelching, or the repression of the transcriptional activity of one steroid receptor by another, is reversed by the addition of coactivators (37). Thus, a critical component of efficient steroid receptor transcription is the recruitment of nuclear receptor coactivators, which dramatically enhance transcriptional activity. Under most conditions, steroid receptors interact with coactivators in the presence of an agonist, but not in the absence of ligand or in the presence of an antagonist or a selective receptor modulator (37–40); but see also (41–43). Corepressors and their complexes associate with nuclear receptors when unliganded or bound to antagonists and serve to repress nuclear receptor transcription by recruiting corepressor complexes to the cis-active elements in the promoter and enhancers of target genes (33).

Coactivators of steroid receptors

The p160 family

Steroid receptor coactivator-1 (SRC-1/NcoA-1) was one of the first coactivators found to interact with hormone-bound steroid receptors (37). SRC-1 is a member of a larger family of p160 proteins that includes SRC-2 (also known as GRIP1, TIF2 and NcoA-2) (44, 45) and SRC-3 (AIB1, TRAM-1, p/CAF, ACTR, RAC3) (46, 47). The SRC family of coactivators physically interacts with steroid receptors, including ER and PR, in a ligand-dependent manner (33, 34, 37). The SRs physically associate with agonist-bound receptors through multiple LXXLL motifs (L, leucine; X, any amino acid) that make up nuclear receptor boxes (48). Experiments conducted in vitro reveal that depletion of SRC-1 in cultured cells by microinjection of antibodies to SRC-1 prevents receptor-dependent transcription, suggesting that SRC-1 is important for transcriptional activity of steroid receptors (36). In cell culture, hormone induced transactivation of PR is reduced by coexpression of ERa, presumably due to squelching or sequestering of shared coactivators (37). This squelching can be reversed by over-expression of SRC-1, suggesting that coactivators are a limiting factor necessary for full transcriptional activation of receptors (37). In further support, over-expression of SRC-1 relieves thyroid hormone receptor inhibition of ERa-mediated transcription in a neuroendocrine model (49).

The SRC family of coactivators appears to act as a platform for the recruitment of other coactivators, including cAMP-response element binding protein (CREB) binding protein (CBP) and p300/CBP associated factor (p/CAF), that possess histone acetyltransferase activity and aid in chromatin remodelling (50, 51). The p160 coactivators contain two activation domains, AD1 and AD2, in the C-terminal region. AD1 mediates interactions with CBP (52), whereas AD2 allows binding of other proteins, including the protein arginine methyltransferase CARM1 (53).

Studies with knockout mice have revealed much about the in vivo function of these coactivators. SRC-1 knockout mice, although fertile, have decreased responsiveness in some steroid target tissues (54), partial resistance to thyroid hormone (55) and delayed development of cerebellar Purkinje cells (56). In addition, SRC-1 is critical in maintaining energy balance by regulating both energy intake and expenditure (57).

As is the case with SRC-1, SRC-2 enhances the transcriptional activity of a variety of nuclear receptors, including ER and PR (44, 45). The mid-region of the SRC-2 protein, which mediates interactions with steroid receptors, has relatively low homology with SRC-1, suggesting functional differences between these two proteins (44, 45). SRC-2 knockout mice reveal that this coactivator is important in fertility and ducal branching in mammary gland (58–60). Microarray analysis of uteri from SRC-2 null mice reveal that SRC-2 is involved in the ability of progesterone to repress specific genes involved in a variety of functions, including cell cycle and immunity (61).

SRC-3/AIB1, which is amplified in human breast tumors (46), coactivates a variety of nuclear receptors, including ER and PR (36, 46, 62). Female SRC-3 null mice, although fertile, have delayed puberty, longer oestrous cycles, ovulate fewer eggs and have impaired mammary gland development (63, 64). Using chromatin immunoprecipitation assays, gonadotrophin-releasing hormone (GnRH) stimulated more efficient recruitment of SRC-3 by PR, on the progesterin response element of a luciferase reporter gene of the gonadotropin alpha subunit gene promoter, than progesterone (65). These findings suggest that phosphorylation of PR and its interaction with SRC-3 and binding to DNA may play an important role in the possible ligand-independent activation of PR by GnRHs (65).

Other coactivators of steroid receptors

Although CBP was initially discovered to be a transcriptional activator of CREB (66, 67), it is also now known to function as an integrator of nuclear receptors with other cell signalling pathways, including CREB and AP-1 (51, 67, 68). As is the case with the p160 family, CBP is important in ligand-dependent transcriptional activity of nuclear receptors, including ER and PR (69). Interestingly, mutation of the
CBP gene causes Rubinstein–Taybi syndrome, which results in severe mental retardation and a variety of physiological deformities in humans (70). In mice, mutations of CBP lead to similar physical deformities as well as impaired memory (71). A variety of studies performed in vitro indicate that SRC-1 and CBP act synergistically to enhance ER and PR transcriptional activity and function (69, 72–74). In support of this concept, SRC-1 physically interacts with CBP and recruits it to the coactivator complex to form a ternary complex at target gene promoters (51, 69).

Steroid receptor RNA activator (SRA) is a unique coactivator in that it functions as an RNA transcript to enhance transcriptional activity of steroid receptors, including PR, ER, glucocorticoid receptor (GR) and androgen receptor (75, 76). Although liganded ER reduced PR transcriptional activation, addition of SRA reversed this squelching effect of ER (76). Treatment of cells with antisense to both SRC-1 and SRA greatly reduced the activity of ERα or PR (75, 76). Antisense to either SRA or SRC-1 alone had a less dramatic effect on ERα activity, suggesting the association of SRA with SRC-1 (75). In further support, SRA copurified with SRC-1, indicating that SRA exists in a ribonucleoprotein complex containing SRC-1 (76). Expression of SRA is tissue specific, with SRA mRNA being expressed at high levels in the liver, skeletal muscle and heart, and at lower levels in the brain and placenta (76). Overexpression of SRA in transgenic mice reveals a role for SRA in oestrogen-induced expression of PR in mammary gland (77).

Finally, there are a variety of other coactivators, including ERAP140 (78), TRAP220 (79), PGC-1 (80), chromatin high mobility group proteins 1 and 2 (81) and TIP60 (82), that are known to interact with ER and PR. With over 285 coactivators and corepressors identified to date (83), there is much more to be learned about the function of coregulators in nuclear receptor action.

Function of nuclear receptor coactivators in the brain and in behaviour

Although much is known about the molecular mechanisms of nuclear receptor coactivators from a variety of cell culture studies (33, 34), we are just beginning to understand their role in hormone action in the brain. SRC-1 mRNA and protein are expressed at high levels in the cortex, hypothalamus and hippocampus, and at low levels in the lateral septum, of rodents (84–90) and birds (91). For coactivators to function with steroid receptors, they must be expressed in the same cells. Indeed, SRC-1 is expressed in the majority of oestrogen-induced PR cells in reproductively-relevant brain regions, including the ventromedial nucleus (VMN), medial preoptic area and arcuate nucleus (92). Given that virtually all oestadiol-induced PR cells in the hypothalamus contain ERα (93, 94), these findings suggest that these specialised cells represent functional sites of interaction between ovarian steroid receptors and SRC-1 in the brain (92). It is important to note that not all SRC-1 immunoreactive cells expressed PR, suggesting that SRC-1 may function with other nuclear receptors in these cells (92). The expression of the SRC family of coactivators in the brain appears to be regulated by a variety of factors, including hormones (95–101), daylength (102) and stress (97, 103, 104).

The function of nuclear receptor coactivators in hormone action in the brain and in behaviour has been investigated. The role of SRC-1 in hormone-dependent sexual differentiation of the rodent sexually dimorphic nucleus (SDN) of the pre-optic area has been studied (88). On postnatal days (PN) 0–2, the hypothalami of female rat pups were bilaterally infused with antisense oligonucleotides (ODNs) to SRC-1 mRNA or scrambled control ODNs. On PN 1, female pups were treated with the aromatisable androgen, testosterone propionate, to increase SDN volume. On PN 13, antisense to SRC-1 was found to reduce the volume of the SDN of androgenised females by 46% compared to females receiving control ODNs. The testosterone surge in males just after birth suppresses the development of female sexual behaviour in adulthood (105, 106). This suppression is due to oestadiol, aromatised from testosterone, binding to ER (107). To test whether SRC-1 was critical in development of sexual behaviour, androgenised female and male rats were treated with SRC-1 antisense or control ODNs on PN 0–2 (88). Males were castrated in adulthood and following testosterone treatment, were tested for male and female sex behaviour. Males and androgenised females treated with SRC-1 antisense displayed higher levels of female sexual behaviour than did rats treated with control ODNs. Taken together, these findings suggest that reduction of SRC-1 in the brain decreases ER activity, and thus alters brain development and inhibits the defeminising actions of oestrogen during development (88).

CBP is expressed in reproductively-relevant brain areas in a dimorphic manner, and functions in the development of masculine sexual behaviour (108). On the day of birth, males express 53% more CBP-immunoreactive (CBP-IR) cells in the medial pre-optic area, whereas females express 83% more CBP-IR cells in the VMN than males. These findings of differential expression of CBP suggest that gonadal steroid hormones alter levels of CBP in the brain during development, which in turn influence neural steroid responsiveness. In the same study, testosterone-treated females that received CBP antisense in the hypothalamus on PN 0–2 displayed higher levels of lordosis than androgenised females treated with control ODNs (108). Taken together with the findings of the previous study, it appears that both SRC-1 and CBP are necessary for ER action in the developing brain.

Our laboratory and others have investigated the role of nuclear receptor coactivators in hormone-dependent gene expression in the brain and in behaviour in adult rodents (89, 109). Oestadiol-induction of PR gene expression in the VMN is important for hormone-dependent female sexual behaviour (110). Therefore, we tested the hypothesis that SRC-1 and CBP are critical for modulating ER-mediated transactivation of the PR gene in the VMN. Infusions of antisense ODNs to SRC-1 and CBP mRNA into one side of the VMN of adult female rats reduced the expression of ER-mediated activation of PR gene expression compared to the contralateral control ODN-treated VMN (89). These findings are supported by previous in vitro studies indicating that SRC-1 and CBP function together to modulate ER activity (69). In further support of SRC-1 and CBP/p300 functioning together in the brain, neurons in the rat hippocampus and dentate gyrus coexpress SRC-1 and p300 (90). A similar study in the brain supports these findings and extend them to include a
role of SRC-2, but not SRC-3, in ER-mediated induction of PR in the VMN (109). Finally, the p160 coactivators function in GR action in glial cells (111) and in GR-mediated repression of the corticotropin-releasing hormone gene (112). Taken together, these findings indicate that nuclear receptor coactivator action in the brain is essential for full steroid receptor transcriptional activity.

Given that nuclear receptor coactivators are critical for hormone-dependent gene expression in the brain, we tested the hypothesis that these coactivators act to modulate the expression of hormone-dependent behaviours (89). Female rats treated with antisense to both SRC-1 and CBP mRNA into the VMN displayed reduced levels of hormone-dependent female sexual receptivity compared to scrambled treated controls (89). Another study supported these findings with SRC-1 and extended them to include a role for SRC-2 in hormone-dependent behaviour (109). Our laboratory has gone on to isolate the effects of these nuclear receptor coactivators on both ER- and PR-dependent aspects of female sexual behaviour. There are two modes of hormone regulated female reproductive behaviour in rats: oestrogen-mediated (elicited by oestradiol alone) and progesterone-facilitated (requires oestradiol priming followed by progesterone) (32). To test the hypothesis that nuclear receptor coactivators function in the brain to modulate ER-mediated aspects of female reproductive behaviour, animals were injected with oestradiol only (113). Antisense to SRC-1 and CBP infused into the VMN of animals treated with oestradiol alone decreased lordosis intensity and frequency, suggesting that these coactivators modulate ER-mediated aspects of female sexual behaviour. Proceptive behaviours by the female, which serve to solicit interaction by the male, are PR-dependent and include ear-wiggling and hopping and darting (114–119). Infusion of antisense to SRC-1 and CBP mRNA into the VMN around the time of progesterone administration reduced PR-dependent ear wiggling and hopping and darting, but did not alter lordosis (113). Thus, it appears that nuclear receptor coactivators function in the brain to modulate PR and ER action and influence specific aspects of hormone-dependent sexual behaviours in rodents. Interestingly, although SRC-1 and SRC-2 are expressed at high levels in the hypothalamus, SRC-3 is not (101, 109). However, SRC-3 is expressed at high concentrations in the hippocampus (109). In future studies, it will be important to distinguish the functions of these different coactivators in hormone action in the brain.

Recently, we have begun to take a proteomics-based approach to study the interactions of steroid receptors with coactivators from rat brain. To test the hypotheses that SRC-1 from brain physically associates with oestrogen receptor (ER)α and ERβ in a ligand-dependent and receptor isoform-specific manner, pull-down assays with brain tissue from female rats were developed (120). SRC-1 from hypothalamus or hippocampus interacted with ERα and ERβ when bound to oestradiol (Fig. 1a), which was confirmed by mass spectrometry (120). SRC-1 may function with ERα in the hypothalamus to mediate expression of female sexual behaviour (15–17, 121), and with both ER subtypes in the hippocampus to differentially modulate the effects of oestrogen effects on cognition (18, 122) and stress (18, 123). Very little to no association of SRC-1 from brain was detected with ERα or ERβ in the absence of ligand or in the presence of the selective ER modulator tamoxifen.

These findings suggest tamoxifen is functioning as an antagonist to prevent receptor-coactivator interactions, and are consistent with a variety of studies using cell lines demonstrating that oestradiol facilitates, whereas antagonists prevent, SRC-1 association with ER (124–126). By contrast to our findings obtained using brain tissue, cell culture studies suggest that both ERα and ERβ can recruit coactivators to AF-1 in the absence of ligand under certain phosphorylation conditions (127, 128). Although little to no interaction between receptor and SRC-1 from brain in the absence of ligand was detected, it will be important to investigate whether physiologically-relevant events that modulate ligand-independent activation impact on receptor-coactivator interactions in the brain.

In our studies, SRC-1 from the hippocampus appears to interact equally with ERα and ERβ (Fig. 1a). By contrast, SRC-1 obtained
from hypothalamic extracts interacted more with ERα than with ERβ (Fig. 1a). The different functions of the ER subtypes in brain (discussed above) may be explained in part by the lower transcriptional activity of ERβ observed in particular cell lines (19). These differences in transcriptional abilities between ERα and ERβ may be attributed to differential recruitment of coactivators, or differences in the ability of the same coactivator to facilitate transcription of the ER subtypes (129). Although some studies using recombinant SRC-1 (129) are consistent with our findings that SRC-1 from brain interacts more with ERα than with ERβ, other studies suggest that SRC-1 associates equally with each ER subtype (130, 131). Although these later findings are consistent with our results using SRC-1 from hippocampus, we observed that SRC-1 from hypothalamus interacted more with ERα than with ERβ. These data suggest that ERα is a more efficient transcriptional activator of SRC-1-dependent signalling pathways in the hypothalamus than ERβ. In support, previous findings from our laboratory indicate that SRC-1 function in the hypothalamus is important for maximal expression of ERα-mediated female sexual behaviour (113), which appears to be ERα-dependent (15, 132). In addition, SRC-1 from brain interacts more with PR-B than with PR-A (120). These differential interactions of SRC-1 from hypothalamus or hippocampus with the ER and PR subtypes suggest that these brain regions have distinct expression patterns of cofactors involved in these important protein–protein interactions. In addition, it is possible that SRC-1 undergoes differential phosphorylation in these two brain regions, leading to distinct patterns of interaction with receptors. Future experiments will need to apply mass spectrometry analysis to determine whether, in a brain region specific manner, different cofactors are present in the receptor–coactivator complex and/or if SRC-1 undergoes differential phosphorylation. Finally, these findings suggest the importance of using biologically-relevant tissue, in contrast to the use of cell lines alone, in investigating receptor-coactivator interactions. It may be that other cofactors and proteins that are present in tissue (e.g. brain) are important for appropriate SRC-1 interactions with receptor. Understanding how nuclear receptor coactivators function with various steroid receptors, and their subtypes, is critical for understanding how hormones act in different brain regions to profoundly influence physiology and behaviour. Ultimately, the investigation of these receptor-coactivator interactions using brain tissue may allow the identification of novel cofactors involved in the steroid receptor complex in brain.

The function of coregulators has also been studied with respect to hormone action in the bird brain. SRC-1, CBP and L7-SPA are expressed at high levels in steroid-sensitive brain regions of adult quail (91), European starlings (133) and zebra finches (134), respectively. In adult quail, the infusion of antiserum to SRC-1 mRNA reduced testosterone-dependent male copulatory behaviours (135). In addition, SRC-1 was found to function in testosterone-dependent sex differences in brain volume and aromatase expression in the preoptic medial nucleus of the quail (135, 136). These findings indicate that SRC-1 is important in the modulation of hormone-dependent gene expression, brain plasticity and behaviour in birds.

Summary

The mechanisms by which steroids act in a region-specific, and cell type-specific, manner is a fundamental issue with respect to steroid hormone action in the brain. Recent investigations indicate that, in addition to the bioavailability of hormone and receptor levels, nuclear receptor coactivators are critical molecules for modulating steroid receptor-mediated transcription. Studies from cell lines have revealed much about the molecular mechanisms of action of these coactivators. Furthermore, work in the brain, as well as other steroid-sensitive tissues, indicates that nuclear receptor coactivators are critical for the fine-tuning of steroid-responsiveness within individual cells. Understanding the recruitment of different coactivator and corepressor complexes to the promoter, which is likely to be cell and tissue specific, will be critical for understanding how hormones function in the brain to regulate complex behaviours.

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M. J. Tetel


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