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Cells in Behaviourally Relevant Brain Regions Coexpress Nuclear Receptor Coactivators and Ovarian Steroid Receptors

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Abstract

Oestradiol and progesterone act in the brain to elicit profound effects on behaviour and physiology. One physiological function of oestradiol is the induction of progesterone receptor (PR) expression in a variety of behaviourally relevant brain regions, including the ventromedial nucleus of the hypothalamus (VMN), the medial preoptic nucleus of the preoptic area (MPOA), the arcuate nucleus (ARC) and the medial central grey (MCG). Ligand-dependent transcriptional activity of steroid receptors, including oestrogen receptors (ER) and PR, is dramatically influenced by nuclear receptor coactivators. In previous studies, we have found that two of these nuclear receptor coactivators, steroid receptor coactivator-1 (SRC-1) and CREB-binding protein (CBP), are important in ER-mediated induction of PR in the VMN and in steroid-dependent behaviours. For nuclear receptor coactivators to function in hormone-dependent transcription in the brain and regulate behaviour, both receptor and coactivator must be expressed in the same cell. In the present study, we used a dual-label immunohistochemical technique to investigate if individual cells in behaviourally relevant brain regions coexpress nuclear receptor coactivators and steroid receptors. Confocal analysis revealed that in oestrogen-primed rats, most of the E-induced PR cells in the VMN (89.6%), MPOA (63%), ARC (82.6%), and many in the MCG (39%), also express SRC-1. In addition, the majority of the cells containing E-induced PR in the VMN (78.3%), MPOA (83.1%), ARC (83.6%), and MCG (60%) also express CBP. These results, taken together with the findings that virtually all oestradiol-induced PR containing cells in the brain express ER, suggest that these neurones represent sites of functional interaction of nuclear receptor coactivators with ovarian steroid receptors in the brain. The present findings provide neuroanatomical evidence that nuclear receptor coactivators are integral in mediating steroid hormone action in behaviourally relevant brain regions.

Keywords

oestrogen receptors; progestin receptors; SRC-1; CBP; reproductive behaviour

The ovarian steroids, oestradiol and progesterone, act in the brain to regulate a variety of behavioural and physiological processes (1,2). These steroids act in the hypothalamus and other brain regions to regulate female reproductive behaviour and physiology in rodents (1-3). In addition, animal and human studies reveal that these hormones act in the hippocampus to influence memory and cognition (4-6).

Many of the effects of oestradiol and progesterone are mediated by their respective intracellular receptors, oestrogen (ER) and progestin receptors (PR), which are members of a large superfamily of nuclear transcription factors (7-9). Upon binding hormone, these receptors undergo a conformational change that causes the dissociation of heat shock proteins and immunophilins, transforming the receptor into the active form (10). The activated receptors dimerise and preferentially bind to steroid response elements of steroid-target genes to influence gene transcription (7-9). A classic example of a steroid-responsive gene is the induction of the PR gene by oestradiol in a variety of hormone-responsive tissues, including brain (11-14). This ER-dependent induction of PR gene expression is thought to occur via an oestrogen response element in the promoter of the PR gene (15-17). Oestradiol induces PR expression in behaviourally relevant brain regions, including the ventromedial nucleus of the hypothalamus (VMN), the medial preoptic area (MPOA), the arcuate nucleus (ARC) and the midbrain central grey (MCG) (13,14,18-26). In addition, virtually all hypothalamic oestradiol-induced PR cells also express ER α (27,28). Finally, this ER-mediated induction of PR gene expression in the VMN is important for progesterone-facilitated reproductive behaviour (18).

Nuclear receptor coactivators dramatically enhance the transcriptional activity of nuclear receptors, including ER and PR (29-31). It has been proposed that nuclear receptor coactivators influence nuclear receptor transcription through a variety of mechanisms, including acetylation, methylation, phosphorylation and chromatin remodelling (30). Steroid receptor coactivator-1 (SRC-1, also known as NCoA-1), was the first coactivator of steroid receptors to be identified and cloned, and belongs to a larger p160 family of nuclear receptor coactivators (32). The p160 family also includes SRC-2 (also known as NCoA-2, TIF-2, GRIP-1; 33,34), and SRC-3 (AIB1, TRAM-1, p/CIP, ACTR, RAC3; 35-37). These p160 coactivators enhance the transcriptional activity of steroid, retinoid and thyroid hormone receptors through direct ligand-dependent interactions (29-31). CREB binding protein (CBP), and its homolog p300, are coactivators for a variety of transcription factors and have been proposed to be integrators of nuclear receptors with other signalling pathways, including CREB and AP-1 (38,39). CBP enhances the activity of nuclear receptors (39-41) and is thought to be recruited to the coactivator complex by the SRC family (42). In support, SRC-1 and CBP physically associate with each other (43) and act synergistically to enhance the activity and function of ER and PR *in vitro* (44-47).

Although much is known about the molecular mechanisms of nuclear receptor coactivators from a variety of studies performed *in vitro* (29-31), we are just beginning to understand their role in hormone action *in vivo*. SRC-1 knockout mice, although fertile, have decreased hormone-dependent growth and development in a variety of steroid target organs, including the uterus, mammary gland, prostate and testis (48). However, it should be noted that SRC-1 knockouts have a two-fold increase in TIF-2 mRNA, which may partially compensate for the loss of SRC-1 (48). SRC-1 is expressed in a variety of hormone-responsive tissues, including the brain (49). SRC-1 mRNA and protein are expressed at high levels in the cortex, hypothalamus, and hippocampus of rodents (50-55) and birds (56,57). SRC-1 expression in the brain appears to be regulated by a variety of factors, including hormones (58-61), day length (62) and stress (60,63; for a review, see 64). Immunohistochemical studies reveal that CBP is expressed throughout the brain, including high levels in the hypothalamus, preoptic area, thalamus, amygdala, hippocampus and cortex (55,65-67).

More recently, the function of these nuclear receptor coactivators with respect to hormone action in the brain and behaviour has been investigated (49,68,69). Our laboratory has found that SRC-1 and CBP function in the VMN to modulate ER-mediated transactivation of the behaviourally relevant PR gene (55). Others studies provide support for these findings, and extend them to include a role for SRC-2 in this hormone-dependent event (70). In addition, SRC-1 and CBP function in the VMN to regulate both ER and PR-dependent aspects of female

sexual behaviour (71), providing further support for these nuclear receptor coactivators being involved in hormone action in the brain. In the developing rodent brain, SRC-1 (72) and CBP (66) are expressed differentially and are important in hormone-dependent sexual differentiation of the sexually dimorphic nucleus and development of reproductive behaviour (54,66). In addition, SRC-1 modulates hormone-dependent gene expression, brain plasticity and behaviour in adult quail brain (73). Finally, the p160 coactivators function in glucocorticoid receptor action in glial cells (74). These findings indicate that SRC-1 and CBP have profound effects on hormone action in the brain and the regulation of behaviour.

For nuclear receptor coactivators to function with steroid receptors *in vivo*, both coactivator and receptor must be expressed in the same cell. As discussed above, SRC-1 is involved in ER-mediated transactivation of the PR gene in the VMN (55,70). However, in mammary epithelium, SRC-1 was not detected in oestradiol-induced PR cells, suggesting that SRC-1 is not necessary for ER-mediated induction of PR gene expression in this tissue (75). In the present study, we used dual-label immunofluorescence to test the hypothesis that oestradiol-induced PR containing cells express the nuclear receptor coactivators SRC-1 and CBP in behaviourally relevant brain regions. Identification of these coexpressing neurones would represent potential sites of functional interaction between ovarian steroid receptors and nuclear receptor coactivators in hormone-dependent gene expression in brain and behaviour.

Materials and methods

Animals

Female Sprague-Dawley rats, weighing 175-200 g, were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA, USA) and group-housed for 1 week under a 14 : 10 h light/dark cycle with food and water freely available. One week after arrival, animals were ovariectomised under ketamine/xylazine (100 mg ketamine and 18 mg xylazine/0.75 ml/kg in saline). Ten days following ovariectomy, rats were anaesthetised with sodium pentobarbital (89 mg/kg) and chloral hydrate (425 mg/kg) dissolved in saline prior to sacrifice. All animal procedures were approved by the Institutional Animal Care and Use Committee of Skidmore College (Saratoga Springs, NY, USA).

Western blot analysis of SRC-1 and CBP

Animals were sacrificed by decapitation and hypothalamic brain tissue was excised, placed in chilled microfuge tubes, snap frozen on dry ice and stored at -80 °C. Tissue was homogenised in TEDG (consisting of 10 mM Tris-base, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol 400 mM NaCl, pH = 7.4) and protease inhibitors (1 µg/ml of aprotinin, leupeptin, and pepstatin) using a Teflon homogeniser. After tissue homogenisation, samples were centrifuged at 12 000 g for 30 min at 4 °C to sediment the cellular debris. The supernatant fraction was collected, and the protein concentration was determined by Bradford assay. Eighty µg of total protein from each tissue sample was gel electrophoresed on 7.5% polyacrylamide gels containing 1% SDS and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). Samples were analysed by Western blot as described previously (71) for detection of SRC-1. Briefly, SRC-1 from brain was probed for using a mouse monoclonal antibody generated against amino acids 477-947 of human SRC-1(1135-H4, 0.5 µg/ml, kindly provided by Dean Edwards, Bert O'Malley, Ming Tsai and Sergio Oñate, Baylor College of Medicine). Membranes were incubated in a horseradish peroxidase linked sheep-anti-mouse secondary antibody (1 : 6000, Amersham, Piscataway, NJ, USA) for 1 h. Immunoreactive bands were detected with an enhanced chemiluminescence kit (ECL; New England Biolabs, Ipswich, MA, USA) and membranes exposed to film (Blue Sensitive X-ray film, Laboratory Products Sales, Rochester, NY, USA). Membranes were stripped with stripping buffer (62.5 mM Tris, pH = 6.7, containing 100 mM 2-mercaptoethanol and 2% SDS) for 3 h, and then re probed for CBP

by incubation in a rabbit polyclonal antibody, PA1-847, generated against amino acids 162-176 of human CBP peptide (1 : 500, Affinity BioReagents, Golden, CO, USA). Membranes were incubated in sheep-anti-rabbit (1 : 5000, Amersham) and immunoreactive bands were visualised as described above.

Dual-label immunofluorescence for progesterin receptors and nuclear receptor coactivators

A dual-label immunofluorescent technique was used to identify PR-containing neurones that express SRC-1 or CBP in behaviourally relevant brain regions. One week following ovariectomy, rats were injected subcutaneously with either 10 µg of 17β-oestradiol benzoate (EB, dissolved in 0.1 ml sesame oil) or vehicle at 48 and 24 h prior to being sacrificed. Ovariectomised rats treated with EB (n = 5) or vehicle (n = 4) were anaesthetised and perfused with 4% paraformaldehyde. Five thousand units of sodium heparin dissolved in 1 ml of saline were injected into the left ventricle. Saline (0.15 M, 25 ml) preceded the flow of 250 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH = 7.2) at a flow rate of 25 ml/min for 10 min. Brains were removed from the cranium, blocked and stored in 0.1 M sodium phosphate buffer (pH = 7.2) containing 20% sucrose at 4 °C overnight. Forty µm sections from the preoptic area through the midbrain region were cut on a freezing rotary microtome and stored in cryoprotectant at -20 °C until immunohistochemistry.

Brain sections were incubated in 0.05 M TBS containing 1% bovine serum albumin and 20% normal goat serum to reduce nonspecific binding. To detect PR and SRC-1, the sections were incubated in a cocktail containing a rabbit polyclonal antibody generated against the DNA binding domain of human PR (1 : 700, PR DAKO, Sweden) and the SRC-1 monoclonal antibody, 1135/H4 (1 µg/ml). Sections were incubated in a cocktail of fluorescently labelled secondary antisera containing CY3-labelled goat anti-rabbit serum (1.5 µg/ml, Jackson ImmunoResearch, West Grove, PA, USA) for visualisation of PR and fluorescein isothiocyanate (FITC)-labelled goat anti-mouse serum (10 µg/ml, Jackson ImmunoResearch) for detection of SRC-1.

To detect PR and CBP, MAB-462, a mouse monoclonal antibody generated against amino acids 922-933 of human PR (1 : 4000, Chemicon, Temecula, CA, USA) and the CBP polyclonal antibody PA1-847 (1 : 1000) were used. Sections were incubated in a cocktail of fluorescently labelled secondary antisera containing CY3-labelled goat anti-mouse serum (1 µg/ml, Jackson ImmunoResearch) for visualisation of PR and FITC-labelled goat anti-rabbit serum (3.5 µg/ml, Jackson ImmunoResearch) for detection of CBP. For immunolabelling of PR-containing neurones that express SRC-1 or CBP in the MCG, the same procedure described above was used with the following modifications of PR antibody concentrations to optimise visualisation of PR in the MCG: PR DAKO at 1 : 500 and PR MAB-462 at 1 : 500. Following the immunohistochemical procedure, the brain sections were mounted on glass slides, coverslipped with Vectashield (Vector Laboratories, Burlingame, CA, USA) and stored at 4 °C.

Controls for this dual-label technique included the omission of the primary or secondary antibodies. In addition, primary antibodies were preadsorbed with CBP peptide (PEP-052, Affinity BioReagents) or the appropriate recombinant proteins. SRC-1 and human PR-A and PR-B recombinant proteins were obtained from whole cell extracts of Sf9 insect cells, infected with the appropriate recombinant transfer plasmids, from the Tissue Culture CORE Facility of the University of Colorado Cancer Center as described previously (45,76).

Statistical analysis

The MPOA, VMN, ARC and MCG, which are rich in oestradiol-induced PR, were analysed with the experimenter blind to treatment groups. Images of immunofluorescence from the left side of one matched section per brain region for each rat (77) were captured at × 200 using an

Olympus Fluoview FV300 confocal microscope. Images of PR-immunoreactive cells (PR-IR) were printed on transparencies and the corresponding images of coactivator (SRC-1 or CBP) immunoreactivity were printed on paper and all immunoreactive cells were counted manually. To determine if oestradiol influenced expression of coactivators, images of matched sections from oestradiol-treated and control animals were analysed for total cell count, mean optical density and area of immunoreactivity for SRC-1 and CBP using Image-Pro Plus 4.5.1 (Media Cybernetics, Silver Spring, MD, USA) as described previously (71). The differences in coactivator-IR or PR-IR between oestradiol and the control groups were compared using a two-tailed t-test (Sigma Stat 2.03, SPSS, San Rafael, CA, USA) and $P < 0.05$ was considered statistically significant.

Results

Western blot analysis of nuclear receptor coactivator expression in the brain

To confirm the specificity of the antibodies for SRC-1 and CBP in brain tissue, an analysis by Western blot was performed. Western blot analysis using the SRC-1 monoclonal antibody, 1135/H4, revealed that SRC-1 protein is expressed in the preoptic area/hypothalamus of female rats at its known molecular mass of 160 kDa (Fig. 1, lane 1). In addition, CBP was expressed at its expected molecular mass of 265 kDa in the rat preoptic area/hypothalamus (Fig. 1, lane 2).

Expression of nuclear receptor coactivators in oestradiol-induced progesterin receptor containing cells in the brain

Coexpression of SRC-1 and PR—Consistent with previous studies, very little to no PR-immunoreactive (PR-IR) cells were detected in vehicle-treated animals, oestradiol caused a dramatic increase in the expression of PR-containing cells in all the brain regions analysed (13,14,18-26). Consistent with our previous findings and those of others (50-55,59), SRC-1-immunoreactive (SRC1-IR) cells were observed in many brain regions, including high levels of expression in the preoptic area, hypothalamus and midbrain. Specifically, we detected strong SRC-1 immunostaining in the VMN (Fig. 2B), MPOA (Fig. 3B), ARC and MCG. Taken together, these data indicate that SRC-1 protein is expressed in brain regions known to regulate female reproductive behaviour.

We found that many of the cells containing oestradiol-induced PR also expressed SRC-1. The majority of the oestradiol-induced PR cells in the VMN (Fig. 2) and ARC expressed SRC-1 (Table 1). In addition, high levels of coexpression of PR and SRC-1 were observed in the MPOA (Fig. 3) and more than one-third of the PRIR cells in the MCG also expressed SRC-1 (Table 1). By contrast to the high proportion of PR cells that expressed SRC-1, the majority of SRC1-IR cells did not contain oestradiol-induced PR (Table 1). Although almost half of the SRC1-IR cells in the VMN expressed PR, most of the SRC1-IR cells in the MPOA, ARC and MCG did not contain E-induced PR (Table 1).

Coexpression of CBP and PR—Because SRC-1 and CBP have been shown to function in concert to increase transcriptional activity of ER and PR *in vitro* (44) and in the brain (55), we investigated if PR-containing neurones coexpress CBP in these behaviourally relevant brain regions. As in the present study with SRC-1, little to no PR-IR cells were observed in the VMN, MPOA, ARC or MCG of vehicle-treated animals. By contrast, oestradiol treatment resulted in a large increase in PR-IR in these brain regions (Fig. 4A,D). It should be noted that the two primary antibodies used to detect PR, the rabbit polyclonal (DAKO) used in the SRC-1 studies and the mouse monoclonal (MAB-462) used in the CBP studies, recognise both PR-A and PR-B isoforms of the receptor. These antibodies were used at concentrations that yielded similar

levels of oestradiol-induced PR-IR cells in these studies (compare total number of PR-IR cells in Tables 1 and 2).

CBP-immunoreactivity was expressed throughout the female rat brain, including the MPOA, VMN, ARC (Fig. 4B), and MCG, dorsal medial hypothalamus, and the paraventricular and supraoptic nuclei. By contrast, sparse CBP labelling was observed in the lateral hypothalamus. This neuroanatomical distribution of CBP expression is consistent with previous immunohistochemical studies (65).

Similar to the present findings with SRC-1, high levels of coexpression of oestrogen-induced PR and CBP in behaviourally relevant brain regions were observed. In the VMN, MPOA and ARC (Fig. 4), most of the oestradiol-induced PR containing cells also expressed CBP (Table 2). To a somewhat lesser extent, the majority of PR-IR cells in the MCG also expressed CBP (Table 2). Most of the CBP-IR cells in the VMN also expressed PR (Table 2). Although not a majority, many CBP expressing cells in the MPOA, ARC and MCG also expressed oestradiol-induced PR (Table 2).

Controls for dual-label immunohistochemistry

A variety of controls was performed to confirm the specificity of our dual-label immunofluorescent technique. Omission of the primary monoclonal antibody for SRC-1, 1135/H4, from the immunohistochemical procedure resulted in no detectable SRC-1 immunoreactive cells in any brain region. In addition, preadsorption of 1135/H4 with a 20-fold excess of human SRC-1 protein resulted in no detectable SRC-1 immunoreactive cells (Fig. 5A,B). Omission of the primary antibody for CBP (PA1-847) from the immunohistochemical procedure or preadsorption of PA1-847 with a 20-fold excess of CBP peptide resulted in no CBP-IR cells (Fig. 5C,D). Omission of either primary antibody for PR (MAB-462 or DAKO) from the immunohistochemical procedure or preadsorption of these antibodies with a 20-fold excess of human PR-A and PR-B protein resulted in no PR-IR cells. In further confirmation of the specificity of the double label immunofluorescent technique, intensely labelled PR-IR cells devoid of SRC-1 or CBP-IR were observed, as well as SRC1-IR or CBP-IR cells that lacked PR-IR.

Regulation of nuclear receptor coactivator expression by oestradiol

To determine if oestradiol benzoate alters the expression of SRC-1 or CBP expression in the VMN, MPOA, ARC or MCG, sections from EB- and vehicle-treated animals were compared. No differences were detected in the number of SRC1-IR cells between EB- and vehicle-treated animals in the VMN (EB: 128 ± 36 versus vehicle: 129 ± 30 , $P = 0.99$), MPOA (228 ± 80 versus 176 ± 44 , $P = 0.61$), ARC (214 ± 75 versus 170 ± 34 , $P = 0.29$) or MCG (27 ± 6 versus 37 ± 4 , $P = 0.33$). In addition, no differences were detected in the total area or mean optical density of SRC-1 immunoreactivity between EB- and vehicle-treated animals in these same brain regions. Similarly, no differences in the number of CBP-IR cells were detected between EB- and vehicle-treated animals in the VMN (EB: 109 ± 41 versus vehicle: 97 ± 7 , $P = 0.69$), MPOA (109 ± 28 versus 90 ± 29 , $P = 0.68$), ARC (146 ± 27 versus 140 ± 37 , $P = 0.85$) or 0.30). No differences were MCG (27 ± 6 versus 37 ± 6 , $P = 0.30$). No differences were detected in total area or mean optical density of CBP immunoreactivity between EB- and vehicle-treated animals in these same brain regions. These data suggest that SRC-1 and CBP expression are not altered by EB treatment in the these brain regions as detected by immunofluorescence under the present experimental conditions.

Discussion

A variety of studies indicates that the nuclear receptor coactivators, SRC-1 and CBP, function together to regulate ER and PR function and activity *in vitro* (44,45). Moreover, recent work from our laboratory, as well as others, indicates that SRC-1 and CBP function in the brain to modulate hormone-dependent gene expression, development and behaviour (54,55,66,70,71, 73). For example, SRC-1 and CBP function in the VMN to influence ER-mediated transactivation of the PR gene (55) and ER- and PR-dependent aspects of female sexual behaviour (71). For nuclear receptor coactivators to interact directly with ovarian steroid receptors in the brain, both the coactivator and receptor must be expressed within the same cells in the brain. Therefore, the present study investigated if oestradiol-induced PR-containing cells in behaviourally relevant brain regions also express the nuclear receptor coactivators, SRC-1 or CBP. Double-label immunofluorescence revealed that the majority of E-induced PR cells in the VMN (Fig. 2), MPOA (Fig. 3) and ARC, and many cells in the MCG, express SRC-1 (Table 1). In addition, most of the cells containing E-induced PR in these same brain regions also express CBP (Fig. 4 and Table 2). Given that a high percentage of E-induced PR-IR cells expresses either SRC-1 or CBP, it is likely that a significant proportion of these PR-containing cells express both SRC-1 and CBP. Other studies reveal that virtually all hypothalamic oestradiol-induced PR cells also express *Egr* (27,28). Thus, the present studies indicate that a subpopulation of cells in behaviourally relevant brain regions coexpress ovarian steroid receptors (ER α and PR) and nuclear receptor coactivators (SRC-1 and CBP). Although it is important that future studies investigate the subnuclear distribution of these regulatory proteins (78-80), the present findings provide neuroanatomical evidence that nuclear receptor coactivators play a role in the action of ovarian hormones in behaviourally relevant brain regions. It is important to note that not all cells containing oestradiol-induced PR express SRC-1 or CBP (Tables 1 and 2). Although our immunohistochemical technique may not have been sensitive enough to detect very low levels of SRC-1 or CBP in all PR-containing cells, it may be that PR and ER α of some of these cells use other nuclear receptor coactivators (e.g. SRC-2 (70). Furthermore, many cells expressing SRC-1 or CBP do not express PR in the brain regions investigated. In these cells, SRC-1 and CBP may function with other nuclear receptors, such as ER or glucocorticoid receptors (71,74).

The SRC-1 antibody used in the present experiments was generated against the mid-region of SRC-1 (81) and is likely to recognise both the SRC-1a and SRC-1e isoforms. These isoforms, which differ only at the extreme C-terminus (32,82,83), appear to have different functions (82,84). Interestingly, the mRNAs of these SRC-1 isoforms are differentially expressed in some regions of the rat hypothalamus, including the VMN (53). In future studies, it will be important to investigate the differential expression of these SRC-1 isoforms by steroid receptor-containing cells.

In the present study, the possible regulation of SRC-1 or CBP expression by oestradiol was investigated. SRC-1 immunostaining in the VMN, MPOA, ARC and MCG was not altered by oestradiol treatment, suggesting that oestradiol does not influence SRC-1 expression in these brain regions under our experimental conditions. These findings are consistent with findings that SRC-1 mRNA expression is not influenced by oestradiol in the rat hypothalamus (50). However, other studies in rats have detected changes in SRC-1 protein over the oestrous cycle (58) and oestradiol regulation of SRC-1 mRNA in the hypothalamus (59). The lack of an effect of oestradiol on SRC-1 in the present study may be due to the use of ovariectomised, rather than intact (58), animals and a lower dose of oestradiol and/or investigating protein levels rather than mRNA (59). Furthermore, no changes in CBP-immunoreactivity following oestradiol treatment were detected in any of the brain regions analysed, which is consistent with data showing no effect of oestradiol on hypothalamic CBP protein (85) or mRNA levels of the closely related p300 coactivator (50). However, it should be noted that it is possible that the

present immunofluorescent technique was not sensitive enough to detect subtle changes in SRC-1 and/or CBP expression in these brain regions.

The present findings showing that many cells coexpress SRC-1 and PR are in contrast to a study in rat mammary tissue (75). Using a double-label immunohistochemical technique, SRC-1 was not detected in mammary epithelial cells that contained oestradiol-induced PR, suggesting that SRC-1 is not necessary for oestradiol-induction of PR in rat mammary gland (75). However, the present findings are supported by a variety of other studies. SRC-1 expression was significantly related to PR expression in hormone-responsive meningiomas (86). In addition, SRC-1 mRNA expression had a high correlation with PR mRNA in normal and malignant endometrium (87). Furthermore, a functional study in mice provides evidence that SRC-1 functions in uterine tissue with PR in a cell-type specific manner (88). These studies, taken together with the present findings, indicate that SRC-1 functions in a cell-type and tissue specific manner.

The heterogeneity of steroid responsiveness of individual neurones within a brain region is a fundamental issue of steroid hormone action in the brain. The presence or absence, as well as the overall ratio, of different coactivators within particular neurones may be one mechanism for fine-tuning steroid responsiveness within individual neurones. The neurones identified in the present study, which coexpress ovarian steroid receptors (PR and ERα) and coactivators (SRC-1 and CBP), are potential sites of functional interaction between receptors and coactivators with respect to hormone action in the brain. In addition, these neurones, located in the behaviourally relevant VMN and MPOA, ARC and MCG may be sites of nuclear receptor coactivator function in hormone-dependent female reproductive behaviour. The present studies provide strong neuroanatomical evidence for SRC-1 and CBP function with respect to ovarian hormone action in the brain. Future studies will need to continue to address the functional significance of these important regulatory proteins in hormone-dependent behaviour and physiology.

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Fig. 1. Steroid receptor coactivator-1 (SRC-1) and CREB-binding protein (CBP) are expressed in rat brain. Western blot analysis shows that SRC-1 (lane 1) and CBP (lane 2) proteins are expressed in the female rat preoptic area/hypothalamus.

Fig. 2.

Coexpression of oestradiol-induced progesterone receptor (PR) and steroid receptor coactivator-1 (SRC-1) in cells of the ventromedial nucleus of the hypothalamus. The ventromedial nucleus of the hypothalamus of oestradiol benzoate-treated animals were immunostained simultaneously for (A) PR (red) and (B) SRC-1 (green). (C) Overlaid image show cells expressing both PR and SRC-1 (yellow). Red arrow points to cell containing PR immunoreactive cells (-IR) only, green arrow points to cell containing SRC1-IR only and yellow arrow points to one of many cells containing both PR-IR and SRC1-IR.

Fig. 3.

Cells in the medial preoptic area coexpress oestradiol-induced progesterone receptor (PR) and steroid receptor coactivator-1 (SRC-1). The medial preoptic area of oestradiol benzoate-treated animals were immunostained simultaneously for (A) PR (red) and (B) SRC-1 (green). (C) Overlaid image show cells expressing both PR and SRC-1 (yellow). Yellow arrow points to one of many cells containing both PR immunoreactive cells (-IR) and SRC1-IR.

Fig. 4.

Coexpression of oestradiol-induced progesterone receptor (PR) and CREB-binding protein (CBP) in arcuate nucleus cells. Arcuate nucleus of animals treated with (A-C) oestradiol benzoate or (D-E) vehicle were immunostained simultaneously for (A and D) PR (red) and (B and E) CBP (green). (C) Overlaid image show cells expressing both PR and CBP (yellow). Red arrow points to cell containing PR immunoreactive cells (-IR) only, green arrow points to cell containing CBP-IR only and yellow arrow points to one of many cells containing both PR-IR and CBP-IR.

Fig. 5. Controls of steroid receptor coactivator-1 (SRC-1) and CREB-binding protein (CBP) immunostaining in rat brain. (A) Immunohistochemical staining of SRC-1 in the medial preoptic area (MPOA) using the monoclonal antibody, 1135/H4. (B) The MPOA stained with 1135/H4 preadsorbed with a 20-fold excess of human SRC-1 protein. (C) Immunostaining of CBP in the ventromedial nucleus (VMN) using PA1-847. (D) The VMN stained with PA1-847 preadsorbed with a 20-fold excess of CBP peptide.

Table 1

Cells Immunostained for Progesterone Receptor (PR), Steroid Receptor Coactivator-1 (SRC-1), or Both in the Ventral Medial Nucleus of the Hypothalamus (VMN), Medial Preoptic Area (MPOA), Arcuate Nucleus (ARC), and Midbrain Central Grey (MCG).

PR and SRC-1 coexpression in oestradiol benzoate-treated females				
Brain region	Total PR cells	Total SRC-1 cells	% PR cells expressing SRC-1	% SRC-1 cells expressing PR
VMN	56 ± 10	128 ± 36	89.6	48.6
MPOA	42 ± 13	228 ± 80	63.0	13.0
ARC	71 ± 24	214 ± 75	82.6	28.8
MCG	10.6 ± 3.4	26.7 ± 5.8	35.6	17.0

Total number of cells are shown as the mean ± SEM.

Table 2

Cells Immunostained for Progesterone Receptor (PR), CREB-Binding Protein (CBP), or Both, in the Ventromedial Nucleus of the Hypothalamus (VMN), Medial Preoptic Area (MPOA), Arcuate Nucleus (ARC), and Midbrain Central Grey (MCG).

PR and CBP coexpression in oestradiol benzoate-treated females				
Brain region	Total PR cells	Total CBP cells	% PR cells expressing CBP	% CBP cells expressing PR
VMN	78 ± 21	109 ± 41	78.3	77.5
MPOA	41 ± 10	109 ± 28	83.1	34.6
ARC	48 ± 9	146 ± 27	83.6	28.0
MCG	4.5 ± 1.6	27.4 ± 6.2	59.8	25.4

Total number of cells are shown as the mean ± SEM.