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Research Report

Neuroanatomical distribution and colocalisation of nuclear receptor corepressor (N-CoR) and silencing mediator of retinoid and thyroid receptors (SMRT) in rat brain

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Abstract

The two structurally related nuclear receptor corepressor (N-CoR) and silencing mediator of retinoid and thyroid receptors (SMRT) proteins have been found to differentially affect the transcriptional activity of numerous nuclear receptors, such as thyroid hormone, retinoic acid and steroid receptors. Because of the numerous effects mediated by nuclear receptors in brain, it is of interest to extend these in vitro data and to explore the cellular distribution of both corepressors in brain tissue. We therefore examined, using in situ hybridisation, whether the relative abundance of these two functionally distinct corepressors differed in rat brain and pituitary. We find that although both N-CoR and SMRT transcripts are ubiquitously expressed in brain, striking differences in their respective levels of expression could be observed in discrete areas of brain stem, thalamus, hypothalamus and hippocampus. Using dual-label immunofluorescence, we examined in selected glucocorticoid sensitive areas involved in the regulation of the hypothalamus–pituitary–adrenal axis activity, the respective protein abundance of N-CoR and SMRT. Protein abundance was largely concurrent with the mRNA expression levels, with SMRT relatively more abundant in hypothalamus and brain stem areas. Colocalisation of N-CoR and SMRT was demonstrated by confocal microscopy in most areas studied. Taken together, these findings are consistent with the idea that the uneven neuroanatomical distribution of N-CoR and SMRT protein may contribute to the site-specific effects exerted by hormones, such as glucocorticoids, in the brain.

Theme: Cellular and molecular biology
Topic: Staining, tracing, and imaging techniques

Keywords: N-CoR; SMRT; Corticosteroid receptor; Brain; HPA-axis

1. Introduction

Nuclear receptors are ligand-inducible transcription factors that modulate gene expression by specifically binding to responsive elements in promoter regions of target genes. The nuclear receptor family consists of type I receptors (e.g. estrogen, progesterone, androgen, mineralocorticoid and glucocorticoid receptors (ER, PR, AR, MR and GR)), type II receptors (e.g. retinoic acid, thyroid hormone and vitamin D receptors (RAR, TR and VDR)) and orphan receptors [3]. These receptors are widely distributed in an uneven manner over many brain regions. The neuroanatomical distribution of the receptors does not satisfactorily explain site-specific effects elicited by their cognate ligand. Previously, we have reported an uneven distribution for the two splice variants of the most abundantly expressed p160 coactivator in the rodent brain that have distinct effects on for example the GR and MR [20,21].

Two structurally related but functionally distinct proteins, silencing mediator of retinoid and thyroid receptors (SMRT) and nuclear corepressors (N-CoR), have both emerged as

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key players in the mechanism of nuclear receptor mediated gene repression [17,24]. N-CoR and SMRT repress gene expression by binding directly to the nuclear receptor and facilitating the recruitment of chromatin remodelling enzymes. They are well-documented, structurally related corepressor proteins of approximately 270 kDa, which repress gene transcription by recruiting histone deacetylases to the proximity of the nuclear receptor and forming corepressor complexes [9].

While both corepressors originally were defined as repressors of type II nuclear receptors, such as thyroid hormone receptor (TR) and retinoic acid receptor (RAR), they have now been shown to alter the transcriptional activity of steroid receptors in vitro [19,29,30]. For example, the dose–response curve of agonist-activated GR and MR is shifted to the right in the presence of corepressor proteins. This was observed for endogenous as well as for synthetic ligands [27].

Consequently, it has been proposed that at subsaturating levels of steroids, the presence of corepressor proteins will influence the genomic effects of the ligand-activated nuclear receptors. Although N-CoR and SMRT share structural similarities, it is noteworthy that they have been shown to differentially affect nuclear receptor signalling [29]. Therefore, we hypothesise that their relative abundance might determine the effects of steroids on gene transcription. Because of the numerous effects mediated by nuclear receptors in the brain, it is of interest to extend these in vitro data and to explore the cellular distribution of both corepressors in brain tissues.

We mapped the expression and the relative abundance of N-CoR and SMRT mRNA in rat brain and pituitary gland. Our particular interests are the site-specific effects elicited by glucocorticoid hormones in the brain (for review [11]) and the regulation of the hypothalamus–pituitary–adrenal axis (HPA-axis). Therefore, we demonstrated the respective levels of expression on protein level by means of immunofluorescence in GR and/or MR expressing areas that are important in the regulation of the HPA-axis activity.

2. Methods

2.1. Animals and tissue preparation

Adult male Wistar rats (240 g; $n = 8$) were obtained from Charles River Laboratories (Germany). All animals were group-housed ($n = 4$ per cage), had ad libitum access to food and water and were maintained under controlled conditions, on a 12:12 h light cycle (lights on from 0800 to 2000 h). One week after arrival, all rats were sacrificed in the morning (between 9:00 and 11:00 a.m.). The brains were snap frozen in isopentane (cooled in an ethanol-dry ice bath) and the pituitaries were frozen on dry ice. All tissues were stored at −80 °C until further use. Experiments were carried out with the approval of the Animal Care Committee of the Faculty of Medicine, Leiden University, The Netherlands (DEC nr. 03130).

2.2. In situ hybridisation

Brains of male Wistar rats were used for the in situ hybridisation procedure ($n = 4$). Thin sections of both brains (20 μm) and pituitaries (10 μm) were cut on a cryostat (Leica CM3050S), thaw-mounted on poly-L-lysine (Sigma) coated slices and stored at −80 °C. The sections were fixed for 30 min in freshly made 4% para-formaldehyde (Sigma) in phosphate-buffered saline (PBS, pH 7.4), rinsed twice in PBS, acetylated in triethanolamine (0.1 M, pH 8.0) with 0.25% acetic anhydride for 10 min, rinsed for 10 min in 2× SSC (SSC: 150 mM sodium chloride, 15 mM sodium citrate), dehydrated in an ethanol series, air dried and stored at room temperature until the in situ hybridisation. N-CoR and SMRT riboprobes were amplified by PCR on genomic DNA. For N-CoR, a segment of 482 nucleotides (Genbank Accession number U35312; nucleotide 4715 to 4654) and for SMRT, a segment of 381 nucleotides (Genbank Accession number AF113001; nucleotide 7217 to 7597), were inserted in a PGEMT-easy vector. These murine fragments contained minimal cross-homology and showed 96% identity with corresponding rat mRNA. Hybridisation mix consisted of 50% formamide, 20% dextran sulphate, 1.2 mM EDTA (pH 8.0), 25 mM sodium phosphate (pH 7.0), 350 mM sodium chloride, 100 mM DTT and 1% Denhardt's, 2% RNA–DNA mix, 0.2% nathiosulphate and 0.2% sodium dodecyl sulphate. A 100 μl aliquot of hybridisation mix containing 2.5×10^6 dpm of N-CoR or SMRT riboprobe was added to each section. Coverslips were brought on the slides which were hybridised overnight in a moist chamber at 55 °C. The next morning, coverslips were removed and the sections washed in graded salt/formamide at optimised temperature. After the washing steps, sections were dehydrated in a series of ethanol baths and air dried.

The N-CoR and SMRT hybridised sections were apposed to Kodak BioMax MR film for 13 and 7 days, respectively. After development of the films, the sections were counterstained with 0.5% cresyl violet for anatomical analysis. Control sections were treated identically to experimental sections except that sense riboprobes were used. Control sections did not give signal above background.

2.3. Immunofluorescence and confocal laser scanning microscopy

Brains of adult male Wistar rats were used for the immunohistochemistry experiments ($n = 4$). Thin brain sections (20 μm) were cut on a cryostat (Leica CM3050S), thaw-mounted on poly-L-lysine (Sigma) coated slices and stored at −80 °C until further use. Slides were allowed to thaw at 4 °C during 20 min prior to fixation. The sections were fixed during 10 min in prechilled methanol/acetone/water [40:40:20 (v/v/v)] solution at 4 °C. After fixation, sections were washed three times in 1× phosphate-buffered saline with 0.2% Tween (1× PBST pH 7.4) and blocked 60