Hormones and Cerebellar Development

16

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Contents

Abstract

Cerebellar development and plasticity involve various epigenetic processes that activate specific genes at different time points, including humoral influences from endocrine cells. Of the circulating hormones, a group of small lipophilic hormones including steroids (corticosteroids, progesterone, androgens, and estrogens) and thyroid hormones help mediate environmental influences on the cerebellum. Receptors for such lipophilic hormones are mainly located inside the cell nucleus (nuclear receptor, NR). They represent the largest family of ligand-regulated transcription factors. In the cerebellum, they are expressed in a

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specific temporal and spatial pattern. Of the lipophilic hormones, the impact of the thyroid hormone and gonadal steroids on cerebellar development has been studied extensively. Thyroid hormone deficiency during postnatal development results in abnormal morphogenesis and functional impairment. Estrogen and progesterone also play important roles in this process. In addition to the supply from circulation, several gonadal steroids are produced locally within the Purkinje cells (neurosteroids). This chapter discusses the effect of thyroid and steroid hormones on cerebellar development. Neurosteroids that are locally synthesized in the cerebellum are discussed in a different chapter.

Keywords

Environmental influences · Hormone receptors · Steroids · Thyroid hormone · Nuclear Receptor · Coactivator · Corepressor · Triiodothyronine · T3 · Thyroxine · T4 · TRα · TRβ · Retinoid X receptor · Thyroid hormone response element · Hypothyroidism · Perinatal hypothyroidism · Organic anion transporter · Monocarboxylate transporter · Iodothyronine deiodinase · Non-genomic thyroid hormone action · Steroid receptor cofactor-1(SRC-1) · Retinoic acid related orphan receptor (ROR) · Staggerer · Anti-thyroid drug · Pax8 · TR gene knockout · Mutant TR · Resistance to thyroid hormone · Thyrotropin · TSH · Sexual differentiation · Stress responses · Adrenal steroid hormone · Mineralocorticoids · Glucocorticoids · Hypothalamo-pituitary-adrenal (HPA) axis · GR · MR · Oxidative stress-induced cell death · Maternal deprivation (MD) · Non-genomic mechanism · Psychiatric disorders · Testosterone · Estradiol (E2) · Gonadal hormone · Aromatase · Perinatal critical period · Sexually dimorphic neurogenesis · ERα · ERβ · Androgens · Androgen receptor (AR) · Reelin · Neuronal protection

Introduction

Brain development involves epigenetic processes that activate specific genes at different time points. Epigenetic influences controlling neuronal development may originate from the neuronal cell itself or from outside of the brain. The former includes spatial and temporal patterning of gene expression that is tightly regulated by their intrinsic molecular programs. The latter includes sensory influence, mediated by the peripheral nervous system, and humoral influence from endocrine cells. Environmental influences, including stressors, social experiences, nutrients, drugs, and environmental chemicals, may affect such processes.

The cerebellar cortex forms well-organized structures: a highly specific and uniform arrangement of cells and microcircuitry (Leto et al. 2016). The cerebellum is one of the few sites in the brain where the pattern of intrinsic connections is known in considerable detail, making it ideal for studying the mechanisms of neural development and plasticity. Based on such advantages, a great deal of excellent work has been conducted at various levels, from basic science to clinical disorders.

On the other hand, although a number of hormone receptors are expressed in the cerebellum, and cerebellar development and function are greatly influenced by hormonal status, a relatively smaller number of studies have examined the role of hormonal signaling on the development and plasticity of the cerebellum.

Of the circulating hormones, a group of small lipophilic hormones, including steroids (corticosteroids, progesterone, androgens, and estrogens), and thyroid hormone may serve important roles in mediating environmental influences. Because of their chemical natures, these hormones may be able to cross the blood-brain barrier more easily than peptide hormones, although the existence of specific transporters has been proposed (Suzuki and Abe 2008). Receptors for such lipophilic hormones are mainly located in the cell nucleus (nuclear receptor, NR) and represent the largest family of ligand-regulated transcription factors (Mangelsdorf et al. 1995). Figure 1 is a schematic showing the molecular mechanisms of NR-mediated transcription. NRs are widely distributed in the central nervous system (CNS) as well as in other organs with a specific expression pattern (Bookout et al. 2006). In the cerebellum, NRs are expressed in a specific temporal and spatial pattern (Qin et al. 2007). However, the role of NRs in cerebellar function is not fully understood. NRs act by binding to specific coregulators, such as coactivators and corepressors, to regulate transcription of their target genes (Rosenfeld et al. 2006). Cofactors may alter chromatin architecture by enzymatically modulating histones, via acetylation and methylation, or remodeling chromatin structure. A genetically modified mouse lacking one of these coactivators shows aberrant cerebellar development (Nishihara 2008). This indicates that temporal and spatial expression of these coregulators also play an important role in mediating NR signaling.

Of the lipophilic hormones, the impact of thyroid hormone and gonadal steroids on cerebellar development and plasticity has been well studied. Thyroid hormone

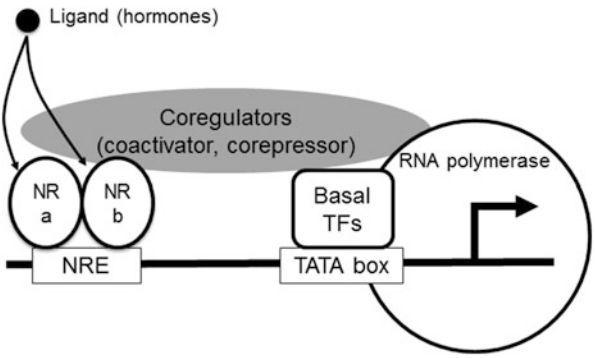

Fig. 1 Schematic figure showing the mechanisms of steroid/thyroid hormone receptor (nuclear receptor, NR)-mediated transcription. Nuclear receptor (NR) binds to specific nucleotide sequences known as hormone response element (HRE) as a homodimer or heterodimer with retinoid X receptor. Various coregulators bind to NRs in a ligand-dependent manner. Cofactors may alter chromatin structure by modulating histone acetylation/methylation or stabilization of basal transcriptional machinery (basal TFs). Usually, coactivator complex is recruited in the presence of ligand whereas corepressor complex in the absence of ligand

deficiency during postnatal development results in impaired cerebellar morphogenesis and function in mammals including humans and rodents (Koibuchi et al. 2003). Estrogen and progesterone also play important roles in this process (Dean and McCarthy 2008). In addition to being supplied from circulation, these gonadal steroids are produced locally within Purkinje cells (Tsutsui 2006). These steroids may not only act through NRs but also through membrane-associated receptors (Sakamoto et al. 2008; Hanzell et al. 2009). Although the functional significance of the rapid action of estrogen and progestin has not yet been fully understood, these steroids may modulate neurotransmitter action such as GABA and NMDA-receptor-mediated signaling (Belcher et al. 2005; Frye 2001). It should be noted that these thyroid/steroid hormone-mediated pathways may be disrupted by prescribed drugs and environmental chemicals (Nguon et al. 2005; Darras 2008).

Thyroid Hormone and Cerebellar Development

Molecular Mechanisms of Thyroid Hormone Action

The thyroid hormone (L-triiodothyronine, T3; L-tetraiodothyronine, thyroxine, T4) binds to the thyroid hormone receptor (TR) and regulates transcription of target genes (Vella and Hollenberg 2017). TR genes are encoded in two genetic loci, termed α and β, which are located at chromosomes 17 and 3 in humans and 11 and 14 in mice (Lazar 1993). Each locus produces at least two proteins, which are termed as TRα1 and α2 (or c-erbAα2) and TRβ1, TRβ2, and TRβ3. Furthermore, some introns – such as intron 7 of TRα gene – have a weak promoter activity. Thus, deleting upstream exons may result in the expression of additional TR-related proteins, which is limited under normal conditions (Chassande 2003). So far, at least three additional TR-related proteins may be generated. Such proteins, termed as TRΔα1, TRΔα2, and TRΔβ3, lack N-terminus and DNA-binding domains (DBD). TR and its related proteins, generated from α or β gene loci, are shown in Fig. 2.

TR forms a homodimer or heterodimer with a retinoid X receptor and binds to a thyroid hormone response element (TRE) located at the promoter region of target genes. TR binds to TRE regardless of the presence of T3 and regulates transcription in a ligand-dependent manner. In the presence of T3, it recruits protein complexes, called coactivators, to activate transcription, whereas in the absence of T3, it recruits corepressor complexes to repress transcription. Although TRα2 can bind to TRE, T3 cannot. TRα2 may act as an endogenous inhibitor for other TRs. Because of TR's bidirectional function, the phenotype of the TR gene knockout mouse is different from that of hypothyroid (thyroid hormone-deficient) animals (Koibuchi 2009). Thus, TR-gene knockout and hypothyroid animal models that are induced by thyroid dysgenesis or dyshormonogenesis are equally important for understanding the role of thyroid hormone system in the brain. Animal models to study thyroid hormone action in the developing cerebellum are discussed in more detail later in this chapter.

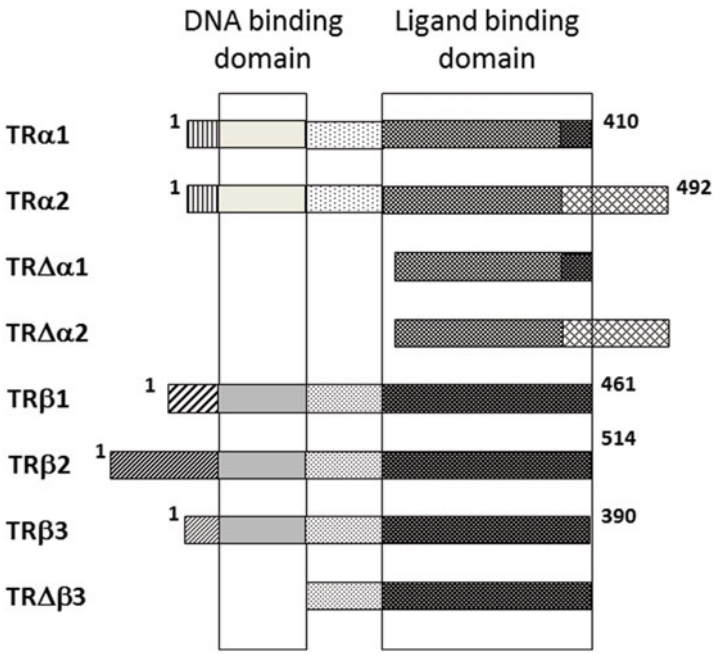

Fig. 2 Thyroid hormone receptor and its related proteins generated from α or β gene locus. Numbers indicate the number of amino acids. Hatched region with the same pattern indicates that the amino acid sequence is identical

The Effect of Thyroid Hormone on the Developing Cerebellum

As shown in Fig. 3, perinatal hypothyroidism dramatically affects cerebellar development. The growth and branching of Purkinje cell dendrites are greatly reduced by perinatal hypothyroidism (Nicholson and Altman 1972a). The number of synapses between Purkinje cell dendrites and granule cell axons is decreased (Nicholson and Altman 1972a, b). The disappearance of the external granule cell layer (EGL) and migration of granule cells into the internal granule cell layer (IGL) are delayed (Nicholson and Altman 1972c). Myelination is also delayed (Balázs et al. 1971). Furthermore, synaptic connections among cerebellar neurons and afferent neuronal fibers from other brain regions are also affected (Hajós et al. 1973). Such abnormal development cannot be rescued unless TH is replaced within the first 2 weeks of postnatal life in rodents (Koibuchi et al. 2003). As a consequence, various behavioral impairments such as motor coordination and cognitive disorders are caused even by mild hypothyroidism (Amano et al. 2018; Khairinisa et al. 2018). Such abnormal development may be transferred through generations, since maternal behavior is partly impaired by perinatal hypothyroidism (Khairinisa et al. 2018). In addition, a dispersed primary culture system has been developed from a newborn rat cerebellum (Ibhazehiebo et al. 2011a). Using this system, the effect of T4 treatment on Purkinje cell dendrite arborization was studied. As shown in Fig. 4, T4 treatment for 14 days

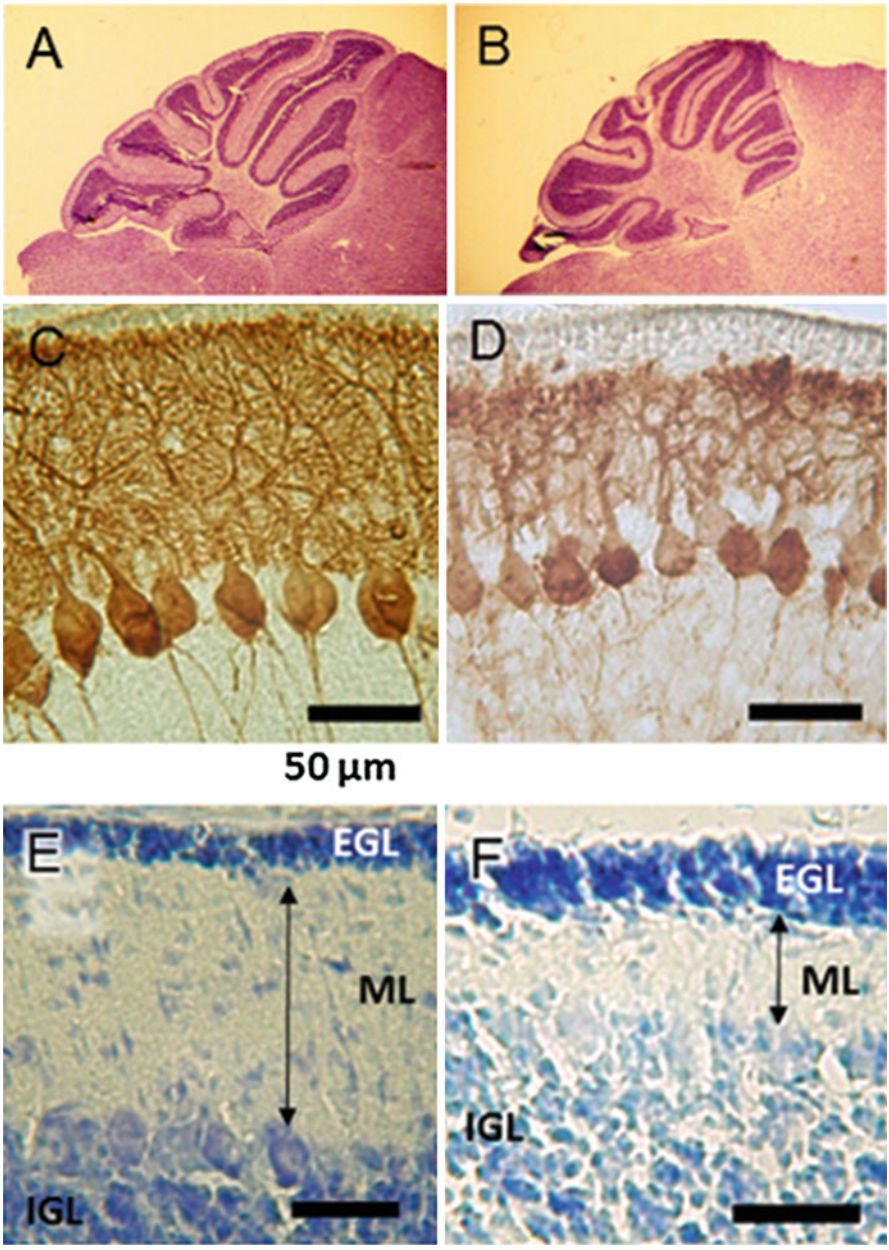

Fig. 3 The effect of altered thyroid hormone status during rat cerebellar development. Rats were rendered hypothyroid by administering antithyroid drug (propylthiouracil) starting from day 17 after conception. They were sacrificed at day 15 after birth. Compared with control rat (**a**, **c**, **e**), hypothyroid rat cerebellum is smaller (**b**). Retardation of dendrite arborization is evident in Purkinje cells, as shown by immunohistochemistry for calbindin (**d**). Proliferation and migration of granule cell from the external granule cell layer (EGL) to the internal granule cell layer (IGL) is also retarded (**f**). Also note the decrease in the width of the molecular layer (ML) by perinatal hypothyroidism (arrows in **e**, **f**)

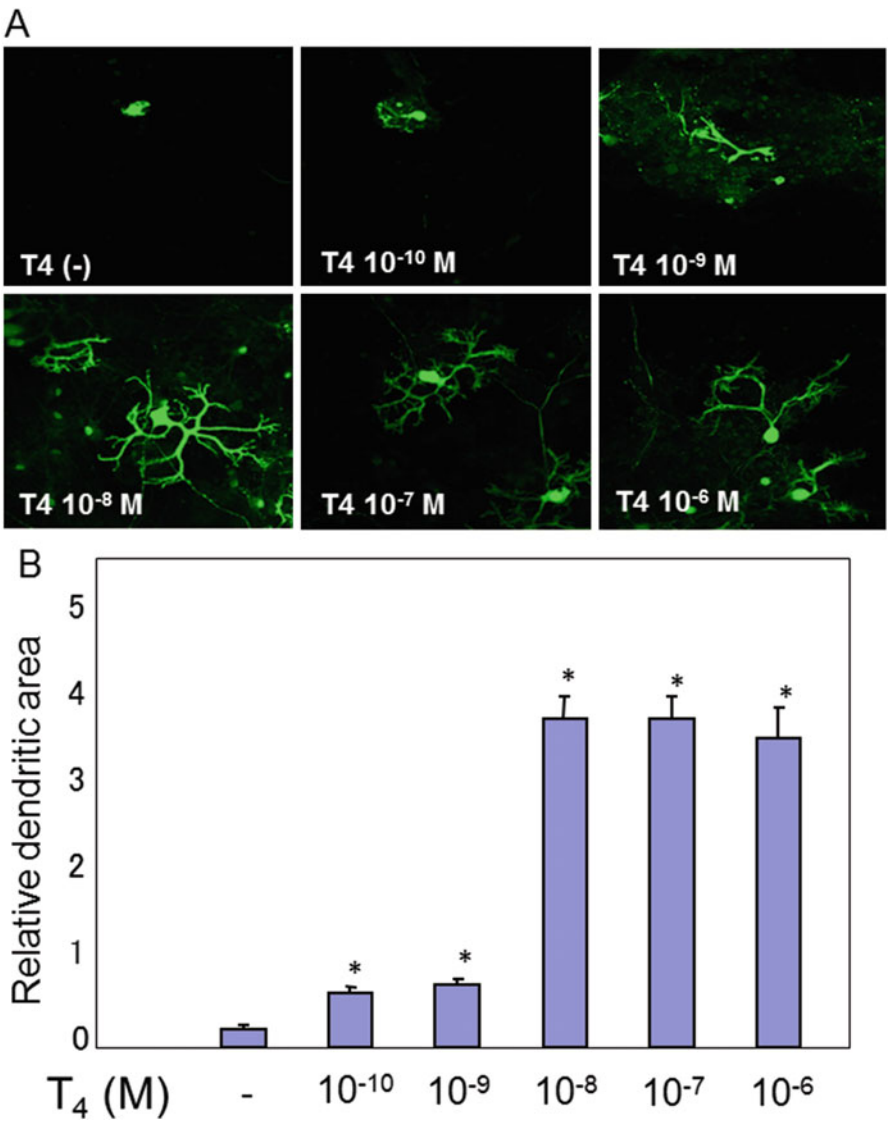

Fig. 4 The effect of thyroid hormone (T4) on Purkinje cell development in rat primary cerebellar culture. Newborn rats were sacrificed on postnatal day 2 to dissect out the cerebellum. Dispersed cells were plated and cultured for 14 days with indicated amount of T4. Immunocytochemistry was performed using anti-calbindin antibody. (**a**) Photomicrographs of Purkinje cells in culture. (**b**) Quantitative analysis for the dendritic area of Purkinje cells in culture)

dramatically increases Purkinje cell dendritic areas. Thyroid hormone can also increase neurite extension in purified cerebellar granule cell aggregate culture (Ibhazehiebo et al. 2011b). These findings indicate that thyroid hormone acts directly on cerebellar cells to promote development.

Thyroid hormone crosses the blood-brain barrier or blood-cerebrospinal fluid barrier through its specific transporters such as the organic anion transporter (Oatp) 1c1 and monocarboxylate transporter (MCT) 8 (Bernal 2005). T4 seemingly crosses such barriers more easily than T3 (Calvo et al. 1990). T4 is then taken up by astrocytes or tanycytes, in which it is converted to T3, an active form of thyroid hormone, by type 2 iodothyronine deiodinase, which is predominantly expressed in these cell types (Guadaño-Ferraz et al. 1997). T3 is then transferred to neurons or oligodendrocytes – mainly through MCT8 – and binds to TR. Impaired thyroid hormone transport, caused by MCT8 mutation, induces severe neurological disorders in humans (Dumitrescu et al. 2004) and model animals (Trajkovic et al. 2007). When T3 is in the neuron/oligodendrocyte, it is converted to T2 for inactivation by type 3 iodothyronine deiodinase, which is predominantly present in neuronal cells (Tu et al. 1999). In the human fetal cerebellum, T3 levels remain low during the first 20 weeks of pregnancy, followed by a gradual increase until birth (Kester et al. 2004). Such a change may occur because of the differential expression of types 2 and 3 deiodinases rather than a change in circulating thyroid hormone levels. In rats, type 3 deiodinase expression is relatively high during fetal life, whereas type 2 deiodinase activity continues to increase until early postnatal life (Bates et al. 1999). Such differential activities in deiodinases are consistent with changes in T3 levels found in the cerebellum. This pattern of changes in T3 content and deiodinase activities in the cerebellum is greatly different from other brain regions, i.e., the cerebral cortex, in which a striking increase in T3 content is seen during the first trimester in the human cerebellum with a very low level of type 3 deiodinase (Kester et al. 2004). Peeters et al. (2013) have shown that type 3 deiodinase-null mice displayed reduced foliation of the cerebellar cortex, accelerated disappearance of the eternal granule cell layer, and premature expansion of the molecular layer. This indicates that deiodinases titrate the thyroid hormone levels in the cerebellum for temporal regulation of the cerebellar development.

It has been generally accepted that the TR mediates most thyroid hormone actions in the brain, although non-genomic thyroid hormone actions have also been proposed (Leonard 2007). TRs are widely expressed in the developing and adult cerebella (Bradley et al. 1992). TRβ1 is predominantly expressed in Purkinje cells, whereas TRα1 is expressed in another subset of cells. C-erbAα2 or TRα2, which is produced from the TRα gene by alternative splicing – and which does not bind to thyroid hormone – is also widely expressed, although its physiological role has not yet been clarified.

Although TRs are widely expressed in the developing cerebellum, and their levels do not decrease in adults, thyroid hormone regulates many thyroid hormone-responsive genes, predominantly during the first 2 weeks of postnatal life in rodents. The expression of a wide variety of genes is altered by thyroid hormone status during cerebellar development (Dong et al. 2005). Such responsive genes include neurotrophic factors, adhesion molecules, cytoskeletal proteins, and transcription factors. Furthermore, a recent development of a genome-wide screening technique has enabled a comprehensive search specifically for thyroid hormone target genes (Gagne et al. 2013). Through temporal and spatial regulation of such genes, thyroid hormone may precisely control cerebellar development.

TR and cofactors, such as steroid receptor coactivator (SRC)-1, are strongly expressed in the adult and developing cerebella (Martinez de Arrieta et al. 2000; Yousefi et al. 2005). SRC-1 disruption results in abnormal cerebellar developments similar to those seen in hypothyroid animals (Nishihara et al. 2003). SRC-1 is predominantly expressed in Purkinje cells and the IGL, whereas only cells located in the premigratory zone express SRC-1 in the EGL (Yousefi et al. 2005). These results are consistent with previous data which show the thyroid hormone is insensitive to proliferating granule cells (Messer et al. 1984). Furthermore, cerebellar SRC-1 protein levels were greatest at P15, when thyroid hormone most strongly affects cerebellar development (Yousefi et al. 2005). These results indicate that the change in coactivator expression may play an important role in TH sensitivity in the cerebellum. Indeed, in addition to SRC-1, other coregulators expressed in the developing cerebellum may also be involved. One such candidate is hairless (Thompson and Bottcher 1997), which is expressed in the developing cerebellum and directly regulated by thyroid hormone (Thompson and Bottcher 1997). It interacts with several NRs, including TRs, and functions as either a corepressor – by recruiting histone deacetylase (Potter et al. 2002) – or as histone H3 lysine 9 demethylase (Liu et al. 2014). Furthermore, developmental alteration of DNA methylation may also play a role in altering thyroid hormone sensitivity (Zhou et al. 2016). These epigenetic processes not only control TR actions but also other NR actions, which also have a distinct critical period to control cerebellar development.

In addition to coactivators/corepressors, TR may interact with other nuclear receptors that regulate gene expression. One such example is retinoic acid-related orphan receptor (ROR) α, which is also a member of the steroid/thyroid receptor superfamily. It is strongly expressed in Purkinje cells and plays a critical role in cerebellar development. Cerebellar phenotype and alteration of neurotrophin expression of natural mutant mouse (*staggerer*) harboring an RORα mutation is similar to that in the hypothyroid mouse (Qiu et al. 2007), although its thyroid function is normal. This indicates that RORα may be involved in thyroid hormone-regulated gene expression in the developing cerebellum. In fact, thyroid hormone regulates RORα expression during the first 2 postnatal weeks (Koibuchi et al. 2001), indicating that thyroid hormone may alter gene expression critical for cerebellar development through RORα regulation. Furthermore, RORα augments TR-mediated transcription, whereas *staggerer*-type mutant RORα does not have such action (Qiu et al. 2007). Another study has shown that RORα may directly interact with TR without binding to TRE. The DNA binding domain of RORα may play a role in such interaction (Qiu et al. 2009). These results indicate that RORα is required for full TR function in the developing cerebellum.

Animal Models to Study Thyroid Hormone Action in the Developing Cerebellum

The cerebellum is the most common brain region used to study the mechanisms of thyroid hormone action on brain development. Since the rodent cerebellum develops mostly during the postnatal period, perinatal hypothyroidism causes various

cerebellar abnormalities, as discussed above. Perinatal hypothyroidism can be induced easily by administering antithyroid drugs, such as propylthiouracil and methimazole (methylmercaptoimidazole) (Koibuchi 2009), which inhibit thyroid hormone synthesis by inhibiting thyroid peroxidase activity. In addition to drug-induced hypothyroid animal models, many mutant or gene-modified animal models showing congenital hypothyroidism have been reported, some of which have been used to study thyroid hormone action in the cerebellum (Koibuchi 2009). One example is the *pax8* gene knockout mouse (Poguet et al. 2003). Pax8 is essential for thyroid follicular cell differentiation; thus, its knockout mouse shows a severe hypothyroidism. Morphological development and gene expression in the cerebellum are greatly affected in this mouse.

Regarding the TR gene knockout model, these animal models may not always be suitable for studying thyroid hormone action in the cerebellum. As discussed above, TR has bidirectional actions of transcriptional regulation of target genes. Without T3 it represses transcription, whereas with T3 it activates transcription. Since TR deletion abolishes the repressive action of TR, phenotypes of TR knockout mice are greatly different from those of mice harboring low thyroid hormone levels. However, TR knockout mice are essential to study the role of TR in organ development and function. Another issue that may be considered to generate TR knockout mice is that some introns, such as intron 7 of TRα gene, have a weak promoter activity. Thus, deleting upstream exons may result in expression of additional TR-related proteins, which may be limited under normal conditions (Chassande 2003). As discussed above, at least three additional TR-related proteins, TRΔα1, TRΔα2, and TRΔβ3, may be generated. Thus, phenotypes of TR knockout mice may result from combining deletion of a specific TR with overexpression of other TR species. Table 1 shows the list of TR-knockout mice. Possible remaining TR proteins in each animal are also indicated.

TRα1 deleted mice showing a limited alterations in behavior and neural circuit are also reported (Guadaño-Ferraz et al. 2003). However, except for aberrant maturation of astrocytes, their cerebellar phenotype appeared normal (Morte et al. 2004). More strikingly, TRα1 deletion prevented the structural alteration of the cerebellum in hypothyroidism induced by methimazole and perchlorate treatment (Morte et al. 2002). These results indicate that the abnormal cerebellar phenotype in thyroid dyshormonogenesis animals may result from the dominant-negative action of unliganded TRα proteins. Interestingly, deleting TRα1 also prevented structural alteration induced by deleting type 3 deiodinase, which inactivates thyroid hormone action by converting T3 to T2. These results indicate that, although the cerebellar phenotype of TRα1 deleted mice is limited, liganded TRα1 plays an important role in cerebellar development.

On the other hand, TRα2 knockout mice show both hyper- and hypothyroid phenotype in an organ-specific manner (Saltó et al. 2001). This may be a result of elevated TRα1 expressions in this mouse. TRα1 expression in the brain is also elevated, but the cerebellar phenotype was unclear. Deleting both TRα1 and TRα2 also shows only a limited phenotype in the cerebellum. However, besides the

Table 1 Thyroid hormone receptor (TR) gene knockout mouse models

Targeted gene	Targeted exon	References	Deleted TRs	Remained TRs	Representative phenotypes
TRα					
TRα1$^{-/-}$	exon 9	Guadaño-Ferraz et al. 2003; Morte et al. 2002, 2004	α1, Δα1	α2, Δα2, all β	Normal T3 with slightly reduced T4 level Prevention of hypothyroid phenotype in the cerebellum
TRα2$^{-/-}$	exon 10	Saltó et al. 2001	α2, Δα2	α1, Δα1, all β	Overexpression of TRα1, inducing both hyper- (high body temperature, increased heart rate) and hypothyroid phenotype (increased body fat)
TRα$^{-/-}$	exon 2	Fraichard et al. 1997	α1, α2	Δα1, Δα2, all β	Aberrant intestine and bone development
TRα$^{0/0}$	exon5-intron7	Gauthier et al. 2001; Macchia et al. 2001	all α	all β	Aberrant intestine and bone development, but the phenotype is less severe than those in TRα$^{-/-}$
TRβ					
TRβ2$^{-/-}$	exon 2	Abel et al. 1999; Ng et al. 2001	β2	β1, (β3, Δβ3) all α	Central resistance to thyroid hormone levels Elevated TSH, T3, and T4 levels Selective loss of M-cone in retina
TRβ$^{-/-}$	exon 3	Forrest et al. 1996; Sandhofer et al. 1998	all β	all α	Central resistance to thyroid hormone Elevated TSH, T3, and T4 levels Aberrant auditory functional development
TRα and *β*					
TRα1$^{-/-}$TRβ$^{-/-}$	See above	Göthe et al. 1999	α1, Δα1	α2, Δα2 all β	High T3 and T4 levels due to high TSH level Growth retardation. Abnormal bone maturation

(continued)

Table 1 (continued)

Targeted gene	Targeted exon	References	Deleted TRs	Remained TRs	Representative phenotypes
$TR\alpha^{-/-}TR\beta^{-/-}$	$TR\alpha^{-/-}$: see above $TRb^{-/-}$: exon 4–5	Gauthier et al. 1999	α1, α2	all β, Δα1, Δα2	Aberrant intestine and bone development (more severe than $TR\alpha^{-/-}$) Elevated TSH,T3 and T4 levels (more severe than $TR\beta^{-/-}$)
$TR\alpha^{0/0}TR\beta^{-/-}$	$TR\alpha^{0/0}$: see above $TR\beta^{-/-}$: exon 4–5	Gauthier et al. 2001	all α all β	None	Reduced body temperature and bone maturation (more severe than $TR\alpha^{-/-}$) Aberrant auditory function (more severe than $TR\beta^{-/-}$) Aberrant intestine development (milder than $TR\alpha^{-/-}$, or $TR\alpha^{-/-}$ $TR\beta^{-/-}$)

cerebellar phenotype, the existence of TRΔα1 and/or TRΔα2 shows altered phenotypes in various organs. When TRα1 and TRα2 are deleted but expressions of TRΔα1 and TRΔα2 are not inhibited ($TR\alpha^{-/-}$) (Fraichard et al. 1997), their phenotype is more severe than those of mice in which all TRα proteins are deleted ($TR\alpha^{0/0}$) (Gauthier et al. 2001; Macchia et al. 2001). The decrease in plasma thyroid hormone levels is greater, and there is a more severe impairment of bone and intestine development.

A more limited brain phenotype is observed in TRβ knockout mice. While TRβ1 is widely expressed including in the cerebellum – particularly in Purkinje cells (Bradley et al. 1992) – TRβ2 expression is confined to the pituitary, hypothalamus (TRH neuron), retina, and inner ear. TRβ2 knockout mice show central resistance to thyroid hormone with elevated T3, T4, and TSH levels in the serum (Abel et al. 1999). Furthermore, this deletion causes a selective loss of M-cones in the retina (Ng et al. 2001). However, the abnormal brain phenotype seems to be confined to the hypothalamus, and changes in cerebellar phenotype have not been reported. On the other hand, there is aberrant development of auditory function in addition to central hypothyroidism in TRβ knockout mice (Forrest et al. 1996). However, although TRβ is strongly expressed in the Purkinje cells, its deletion does not alter thyroid hormone-responsive genes in the cerebellum (Sandhofer et al. 1998).

In the case of TRα and β double knockout, because the function of one receptor cannot be substituted for the other, their phenotypes are more severe than those of single gene knockout. In $TR\alpha1^{-/-}TR\beta^{-/-}$ mice, delayed general growth and aberrant bone maturation, which are not seen in each single knockout mouse, are observed (Göthe et al. 1999). In $TR\alpha^{-/-}TR\beta^{-/-}$ mice, aberrant intestinal development, which is seen in $TR\alpha1^{-/-}$, and high T3, T4, and TSH levels – which is seen in

$TR\beta^{-/-}$ – are observed. Both of these are more severe than those of single knockout mice (Gauthier et al. 1999). However, in $TR\alpha^{0/0}TR\beta^{-/-}$ mice, while low body temperature and abnormal auditory function, which are more severe than those of $TR\alpha^{0/0}$ or $TR\beta^{-/-}$, respectively, are seen, aberrant intestinal development is milder than those of $TR\alpha^{-/-}TR\beta^{-/-}$ or $TR\alpha1^{-/-}$ (Gauthier et al. 2001). These results indicate the possible contribution of TRα variants (Δα1 and/or Δα2) in generating differential phenotypes. Altered brain development in these double knockout mice has not yet been studied in detail.

In addition to TR knockout mice, several knock-in mice, harboring mutant TRs, have been generated (Hashimoto et al. 2001; Fauquier et al. 2011). Such animals are considered models for human syndrome of resistance to thyroid hormone (RTH), which is characterized as reduced thyroid hormone actions in thyroid hormone target tissue (Ortiga-Carvalho et al. 2014). Although most human patients harbor a mutation in the TRβ gene (RTHβ), recent studies have revealed that the patient harboring TRα gene mutation (RTHα) also exists. While RTHβ patients and model animals show elevated serum levels of T3 and T4 with non-suppressed thyrotropin (TSH), RTHα patients and model animals show slightly lower or normal levels of T4, slightly elevated or normal levels of T3, and normal levels of TSH. Such differences may be a result of the difference in tissue distribution of TRα and β, particularly in the hypothalamus and anterior pituitary. Both RTH patients and animal models show various neurological phenotypes. Animal models for both RTHs show abnormal cerebellar development (Hashimoto et al. 2001; Fauquier et al. 2011). These mice show decreased arborization of Purkinje cell dendrites with aberrant locomotor activity and decreased expression of thyroid hormone-responsive genes in the cerebellum. The cerebellar phenotype of RTH animal models is more severe than that of TR knockout animals, indicating that the abnormal cerebellar development seen in hypothyroid animals may be induced mainly by unliganded TRs. Furthermore, the effect may not be a result of generalized TH resistance, but may be because of the cerebellar-cell specific action of TH resistance. This hypothesis is supported by studies using animal models expressing dominant-negative TRs in cerebellar cells (Fauquier et al. 2014; Yu et al. 2015), showing aberrant cerebellar development.

Steroid Hormones and Cerebellar Development

General Overview

Adrenal and gonadal steroid hormones are known as stress and sex hormones, respectively, and both play important roles in CNS development. They affect various developmental events of neurons, such as survival, differentiation, and remodeling of axons and dendrites. These effects are associated with brain organization, sexual differentiation, and stress responses. Steroid hormones bind cognate ligand-activated receptors, members of the steroid/thyroid superfamily of nuclear receptors, to modulate the transcription of hormone-responsive genes. This review summarizes the published data characterizing the actions of these hormones and the localization

of the receptors in the developing cerebellum. Most of the information presented here is from rodent studies.

Adrenal Steroid Hormones and Cerebellar Development

Mineralocorticoids and glucocorticoids are major adrenal steroid hormones synthesized in the adrenal cortex. Mineralocorticoids help maintain sodium and potassium levels, while glucocorticoids are involved in the stress response and in regulating carbohydrate metabolism. Their levels are controlled via the hypothalamo-pituitary-adrenal (HPA) axis by pituitary adrenocorticotropic hormone and hypothalamic corticotropin-releasing factor. Most of the effects on the brain are mediated via binding to intracellular receptors, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) (Rashid and Lewis 2005). Regulation at the genomic level is thought to be responsible for slow and long-lasting effects, such as the actions of corticosteroid hormones on neurogenesis, neuronal morphology, and function in response to chronic stress, while rapid effects (responses within minutes) are regulated by non-genomic action (Evanson et al. 2010). Membrane-associated glucocorticoid and mineralocorticoid receptors may be involved in mediating non-genomic rapid effects (Prager and Johnson 2009).

GRs, expressed in the adult brain, are associated with HPA axis activation to regulate physiological neuronal functions (Garabedian et al. 2017). Expression is first detected in the embryonic rat brain, and levels are high and similar as in the developing cerebellum and hippocampus (Lawson et al. 1992). Prenatal glucocorticoids influence Purkinje cell development (Rugerio-Vargas et al. 2007). Another study using chick embryos has reported the presence of GR mRNA in the embryonic cerebellum (Yamate et al. 2010). Furthermore, Yamate et al. also showed that effects after treatment with excess glucocorticoids are mediated via GRs and indirectly influence behavioral activity after hatching. In the mouse, GR immunoreactivity is intense in the EGL, the Purkinje cell layer, and white matter regions but weak in the molecular layer and IGL at postnatal day 7 (P7). These results suggest that glucocorticoids exert actions on cellular differentiation in the developing cerebellum, inducing multiple changes in peripheral responses and brain function.

Glucocorticoids can induce positive and negative effects on the developing brain depending on the developmental stage and exposure duration (Malaeb and Stonestreet 2014). In rats, cortisone treatment during prenatal (Velazquez and Romano 1987) and postnatal (Bohn and Lauder 1980) development resulted in fewer cerebellar granule cells. Ahlbom et al. (2000) further showed that cerebellar granule cells exposed to high levels of glucocorticoids during the prenatal period become more sensitive to oxidative stress-induced cell death. A single glucocorticoid injection into the neonatal mouse can also induce apoptotic changes in the cerebellum. This results in permanent reductions in the number of neurons within the internal granule layer, suggesting that there is a limited period of vulnerability to glucocorticoids during development (Noguchi et al. 2008). Stressful experiences, such as maternal deprivation (MD), in the early postnatal period in rats retard

development of cerebellar-dependent motor coordination and lead to behavioral abnormalities similar to schizophrenia (Llorente et al. 2009). Rats that are maternally separated during P4–P14, which corresponds to the stress-hyporesponsive period characterized by reduced responsiveness of the HPA axis (Walker et al. 2001), have elevated corticosterone levels at P13 and display behavioral alterations in adolescence and adulthood (Viveros et al. 2009). These results support the possibility that abnormally increased levels of glucocorticoids due to neonatal stress during development are associated with structural abnormalities in the cerebellum. In addition, another study using chick embryos suggested that apoptosis, induced in immature granule neurons by corticosteroids, may be mediated via a non-genomic mechanism (Aden et al. 2008). Based on these animal studies, these actions of glucocorticoids on the developing human cerebellum may be related to lower birth weight and may also be responsible for the emotional and behavioral problems observed in children whose mothers are treated with glucocorticoids for respiratory dysfunction during pregnancy (Noguchi et al. 2008). In addition, structural and functional cerebellar abnormalities, such as Purkinje cell loss, have been detected in many psychiatric disorders, such as autism and schizophrenia (Baldaçara et al. 2008; Martin et al. 2010).

Interestingly, some effects of neonatal MD are different between the sexes. Effects on cellular degeneration and astrocyte proliferation in the cerebellum of neonatal MD rats were greater in males than females (Llorente et al. 2009). This is attributed to males being more vulnerable to stress and/or a sex difference in the onset of sensitivity to stress. Furthermore, impaired eyeblink conditioning was observed only in MD males. This is thought to be associated with the sexually dimorphic pattern of developmental GR expression in the posterior region of the cerebellar interpositus nucleus, a key region for eyeblink conditioning (Wilber and Wellman 2009).

Gonadal Hormones and Cerebellar Development

Testosterone and estradiol (E2) are the two major gonadal steroids synthesized in the testis and the ovary. An important factor for the actions of these gonadal hormones is aromatase, an enzyme that is responsible for producing estrogens from androgens.

In the developing brain, gonadal steroids are well known for their functions in forming brain structures that are different between males and females (Lenz and McCarthy 2010). During a limited perinatal period from late embryonic development through the first few days of postnatal life, testosterone is produced in males by the testis, which is differentiated from the indifferent gonad during early embryonic development directed by the testis-determining gene Sry (Koopman et al. 1991). In the brain, testosterone is converted to E2 by aromatase, whereas female ovaries – whose differentiation occurs postnatally – do not secrete E2 during this period. Thus, during the perinatal critical period, significantly higher levels of E2 in males compared to females are thought to act on male brain development. Regulatory mechanisms of E2 action have been reviewed in detail elsewhere (Wright et al.

2010); however, briefly, E2 regulates apoptosis to produce sexually dimorphic cell numbers, dendritic spine formation, neuronal migration, and synaptic organization in hypothalamic regions, most of which are key regions for regulating male and female sexual functions in the adult brain. In the developing rat hippocampus, gonadal steroids control sexually dimorphic neurogenesis (Zhang et al. 2008). Because of the lack of estrogen exposure during the perinatal period, the female brain was thought to develop without E2. However, studies using knockout mice of the aromatase gene have suggested that E2 produced by the ovaries during a prepubertal period plays a role in the differentiation of the female-typical brain (Bakker and Brock 2010).

The two major estrogen receptors (ERs), ERα and ERβ, are expressed in hypothalamic regions where development occurs differently between the sexes. A degree of overlapping distribution and colocalization occurs between ERα and ERβ in some hypothalamic regions, such as the ventromedial hypothalamus, a key region in female reproduction, suggesting the two proteins may interact (Ikeda et al. 2003). Phenotypes of ERα and ERβ knockout mice are different, suggesting distinctive roles (Kudwa et al. 2006). It has also been suggested that rapid plasma membrane-mediated non-genomic actions of estrogen, which possibly interacts with classical transcriptional regulation (genomic mechanisms), also play important physiological roles in regulating the neural actions of estrogen in the brain (Raz et al. 2008; Vasudevan and Pfaff 2008; Kelly and Qiu 2010). These results suggest complicated interactions of ERα and ERβ by various signaling regulatory mechanisms. Furthermore, it has recently been suggested that epigenetic alterations of DNA methylation patterns on the promoters of ER genes, induced by estradiol during development, are important for sexually dimorphic brain organization. This may be a mechanism by which estradiol regulates ER gene expression to produce permanent masculinization of the brain (Wilson and Westberry 2009).

Androgens, directly acting on the androgen receptor (AR), are also thought to play a role in brain masculinization. This is based on studies of human patients with complete androgen insensitivity syndrome and patients with aromatase gene mutations, as well as on studies of rodents with the testicular feminization mutation, which produces a nonfunctional AR (Zuloaga et al. 2008).

Gonadal steroids also play an important role in the development of brain regions that are not significantly different between the sexes, including the cerebellum (Lenz and McCarthy 2010; Forger et al. 2016). Estradiol levels in the cerebellum are higher during the first postnatal week than later developmental stages (Sakamoto et al. 2003; Biamonte et al. 2009), and treating newborn rats with estradiol promotes dendritic growth and spine formation of Purkinje cells (Sakamoto et al. 2003). These studies indicate that estrogens play a role in cerebellar development. Higher expression levels of ERα compared to ERβ in the hypothalamus indicate that ERα is predominantly associated with reproduction, whereas ERβ is expressed in non-reproductive regions, such as in the cerebral cortex, hippocampus, cerebellum, and the dorsal raphe. It is thought to play an important role in brain morphogenesis (Fan et al. 2010). Cerebellar development occurs at late embryonic and early postnatal stages in rodents. Both ERα and ERβ are detected in an immature cerebellar granule cell line that was derived from late embryonic mouse cerebellum. Experiments with

ER-subtype selective agonists and overexpression of ERα and ERβ in this cell line have indicated that ERα, but not ERβ, mediates E2 actions in embryonic cerebellar granule cells (Gottfried-Blackmore et al. 2007). Quantitative RT-PCR studies have shown that both receptors are expressed in the cerebellum from birth through adulthood, but levels of ERβ mRNA in neonatal rats are significantly higher than those of ERα (Ikeda and Nagai 2006). These studies have also shown that ERα levels in the cerebellum during early postnatal development are significantly higher than in the adult; adult levels are only slightly higher than background. In contrast, no significant changes in the level of cerebellar ERβ mRNA occurred during development or in adulthood (Ikeda and Nagai 2006). However, this ERβ expression pattern differs somewhat from that previously studied using western blot analysis, in which the level of ERβ protein decreased transiently at P5 and P7 and then increased dramatically at P10 followed by a subsequent decrease (Jakab et al. 2001). Using in situ hybridization and immunohistochemistry, we have further shown that ERα-positive Purkinje cells are abundant during early postnatal stages, but these cells are reduced to only a few in adulthood. Since considerable outgrowth and differentiation of Purkinje cell dendrites occur during the first 3 postnatal weeks, these results suggest a possible role of ERα in Purkinje cell differentiation (Ikeda and Nagai 2006). ERβ immunoreactivity was detected in various neurons, including Golgi cells, Purkinje cells, and basket cells, and the expression in each cell type occurs at different postnatal days. Jakab et al. (2001) detected additional ERβ-immunoreactive cells, such as differentiating external granular layer cells and glial cells, although we failed to detect protein or mRNA for ERβ in these cells. The cellular localization of ERβ may reflect its role in cellular differentiation and maturation in cerebellar development. As shown in Fig. 5, the different expression profiles of ERα and ERβ suggest that E2 exerts its actions in a cell-type-specific manner via binding to the two ERs, which play distinctive roles in cerebellar development. The possibility of rapid estrogen signaling mechanisms in the developing cerebellum has been recently discussed elsewhere (Belcher 2008).

Although no sex differences in architecture have been reported in normal cerebellar development, there is a clear sex difference of cerebellar pathology in several developmental diseases in humans and corresponding animal models. This has been linked to alterations in circulating gonadal steroids during critical periods of cerebellar development (Dean and McCarthy 2008). Estrogens can protect neurons from oxidative stress-induced death (Daré et al. 2000) in several brain regions, including the cerebellum, by modifying neuronal vulnerability (Miñano et al. 2007). The antioxidative action of E2 may be relevant to sex differences in negative symptom scores of schizophrenia, which are higher in males (Goldstein and Link 1988). Another study of *reeler* mice, which have a mutation in the reelin gene – a candidate gene in neurodevelopmental disorders such as schizophrenia and autism (Fatemi 2001) – indicates that, by interacting with reelin, E2 acts on survival and maturation of Purkinje cells in female mice. Also, the female-specific regulation of the reelin promoter by E2 might be epigenetic (Biamonte et al. 2009). Thus, in addition to their neurotrophic actions in the developing cerebellum, estrogens may play important roles in neuronal protection.

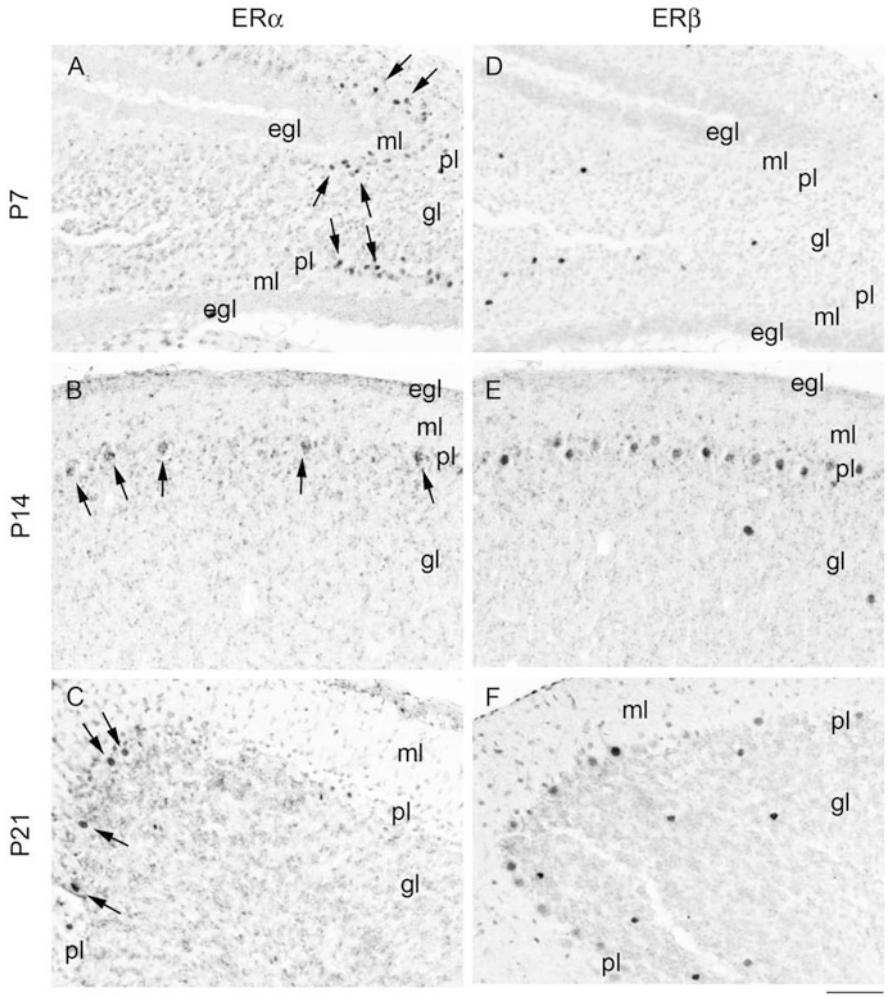

Fig. 5 Localization of ERα and ERβ proteins during postnatal cerebellar development. Representative images of immunohistochemistry for ERα (**a**–**c**) and ERβ (**d**–**f**) in the cerebellum at postnatal day (P) 7, P14, and P21. Arrows indicate representative ERα-immunoreactive Purkinje cells. *egl* external germinal layer, *gl* granular layer, *ml* molecular layer, *pl* Purkinje cell layer. Scale bar, 50 μm

Cerebellar testosterone levels are transiently higher in males than females at P5 (Biamonte et al. 2009). An in vitro study showed the presence of AR protein in cultured P7 cerebellar granule cells, demonstrating that cerebellar granule cells obtained from testosterone-treated neonatal rats are protected from cell death, induced by oxidative stress, via a mechanism mediated by the androgen receptor (Ahlbom et al. 2001). Autism spectrum disorders are more frequent in men than in women, and high fetal testosterone levels may be involved (Knickmeyer and Baron-Cohen 2006).

Several studies have reported that aromatase mRNA levels in the cerebellum during the early postnatal period are higher than in later stages (Sakamoto et al. 2003; Lavaque et al. 2006; Biamonte et al. 2009). Lavaque et al. (2006) detected a transient and marked elevation in aromatase mRNA levels at P10 in the male, but not female, cerebellum. They suggested this gene may contribute to the local synthesis of estrogen, which plays an important role in cerebellar development, combined with estradiol derived from the gonads. Higher vulnerability of males to MD stress might be associated with male-specific induction of aromatase, although such mechanisms remain unclear.

Conclusions and Future Directions

Although many nuclear hormone receptors are expressed in the developing cerebellum, only a limited amount of data is available regarding the effect of thyroid/steroid hormones on this process. This may be because nuclear receptors act as transcriptional factors to activate or repress the transcription of target genes. Under such circumstances, the response to hormone stimulation is rather slow compared to that mediated by membrane-associated receptors, and various signal transduction cascades may be involved to express their action as a specific phenotype. However, hormonal signaling plays an important role to mediate environmental influences on the developing brain. Thus, hormonal disruptions may cause cerebellar disorders leading to various psychosomatic diseases. This chapter will help increase the understanding of the role of thyroid/steroid hormones in the developing cerebellum.

Cross-References

- ▶ Analysis of Gene Networks in Cerebellar Development
- ▶ Endocrine Disorders
- ▶ Epigenetic Regulation of the Cerebellum
- ▶ Granule Cell Migration and Differentiation
- ▶ Specification of Granule Cells and Purkinje Cells
- ▶ Synaptic Remodeling and Neosynaptogenesis
- ▶ Synaptogenesis and Synapse Elimination

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