Mol Neurobiol (2008) 37:116–125
DOI 10.1007/s12035-008-8024-1

Neurosteroids in the Purkinje Cell: Biosynthesis, Mode of Action and Functional Significance

Kazuyoshi Tsutsui

Received: 23 February 2008 / Accepted: 25 April 2008 / Published online: 3 June 2008

Abstract Neurosteroids are synthesized de novo from cholesterol in the brain. To understand neurosteroid action in the brain, data on the regio- and temporal-specific synthesis of neurosteroids are needed. Recently the Purkinje cell, an important cerebellar neuron, has been identified as a major site for neurosteroid formation in vertebrates. This is the first demonstration of de novo neuronal neurosteroidogenesis in the brain. Since this discovery, organizing actions of neurosteroids are becoming clear by the studies using the Purkinje cell as an excellent cellular model. In mammals, the Purkinje cell actively synthesizes progesterone and estradiol de novo from cholesterol during neonatal life. Both progesterone and estradiol promote dendritic growth, spinogenesis, and synaptogenesis via each cognate nuclear receptor in the developing Purkinje cell. Such organizing actions that may be mediated by neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), contribute to the formation of cerebellar neuronal circuit during neonatal life. Allopregnanolone, a progesterone metabolite, is also synthesized in the cerebellum and acts on Purkinje cell survival in the neonate. This review summarizes the advances made in our understanding of the biosynthesis, mode of action and functional significance of neurosteroids in the Purkinje cell.

Keywords Neurosteroids · Progesterone · Allopregnanolone · Estradiol · Brain-derived neurotrophic factor · Neuronal growth · Synaptogenesis · Purkinje cell

K. Tsutsui (✉)
Laboratory of Integrative Brain Sciences, Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University,
2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480, Japan
e-mail: k-tsutsui@waseda.jp

Introduction

Steroid hormones supplied by the peripheral steroidogenic glands cross the blood–brain barriers, due to their chemically lipid solubility, and act on brain tissues through intracellular receptor-mediated mechanisms to regulate several important brain neuronal functions during development which persist into adulthood in vertebrates. Therefore, the brain has traditionally been considered to be a target site of peripheral steroid hormones. By contrast, new findings over the past decade have shown that the brain itself also has the capability of forming steroids de novo from cholesterol, the so-called "neurosteroids" (for reviews, see Refs. [1–5]). The formation of neurosteroids in the brain was originally demonstrated in mammals [6–15] and subsequently in other vertebrates, such as birds [16–29], amphibians [30–37] and fish [38] (for reviews, see Refs. [1–5]). Thus de novo neurosteroidogenesis in the brain from cholesterol is a conserved property of vertebrates.

To analyze neurosteroid biosynthesis and action in the brain, it is necessary to know which neurosteroids are synthesized in specific brain regions at specific times. Such information is essential to develop hypotheses predicting the potential roles of those neurosteroids in the developing and adult brains. Thus, the studies for this exciting area of neurohormonal research should be focused on the biosynthesis and action of neurosteroids produced locally in the identified neurosteroidogenic cells underlying important brain functions. The oligodendrocyte was first accepted to be the primary site for neurosteroid formation in the brain (for reviews, see Refs. [1 and 4]). Subsequently astrocytes [14] and some neurons [15] were shown to express some steroidogenic enzymes. However, whether neurons located in the brain produce neurosteroids remained unclear. Recently, we demonstrated that the Purkinje cell, an important cerebellar neuron, is a major site for neurosteroid formation in a variety of vertebrates [16, 17, 25, 33, 38–

41]. This is the first demonstration of de novo neuronal neurosteroidogenesis in the brain.

Our studies on mammals using the Purkinje cell have provided the opportunity to understand neuronal neurosteroidogenesis. Interestingly, this neuron possesses several kinds of steroidogenic enzymes, such as cytochrome P450 side-chain cleavage enzyme (P450scc) and 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3β-HSD), and actively produces progesterone during neonatal life [39, 40, 42] (Fig. 1). Allopregnanolone, a progesterone metabolite, is also synthesized in the neonatal cerebellum [43–46] (Fig. 1). Subsequently, important actions of progesterone [47–50] and the progesterone metabolite allopregnanolone [51] have become clear by the studies on mammals using the Purkinje cell which is known to play an essential role in the process of memory and learning. Furthermore, this neuron expresses a key enzyme of estrogen formation, cytochrome P450 aromatase (P450arom), and produces estradiol in the neonate [41, 44] (Fig. 1). Estradiol also contributes to important events in the developing Purkinje cell [41, 52].

This review summarizes the advances made in our understanding of the biosynthesis and action of neurosteroids in the Purkinje cell. Based on new findings obtained by the studies using this important neuron, this review also describes what are currently known about the mode of action and functional significance of neurosteroids. For detailed information of neurosteroids in glial cells, the reader is referred to other reviews [1, 4].

Progesterone and Allopregnanolone in the Purkinje Cell

Progesterone Formation and Metabolism

Progesterone is known to be a sex steroid and acts on brain tissues through nuclear progesterone receptors. In contrast

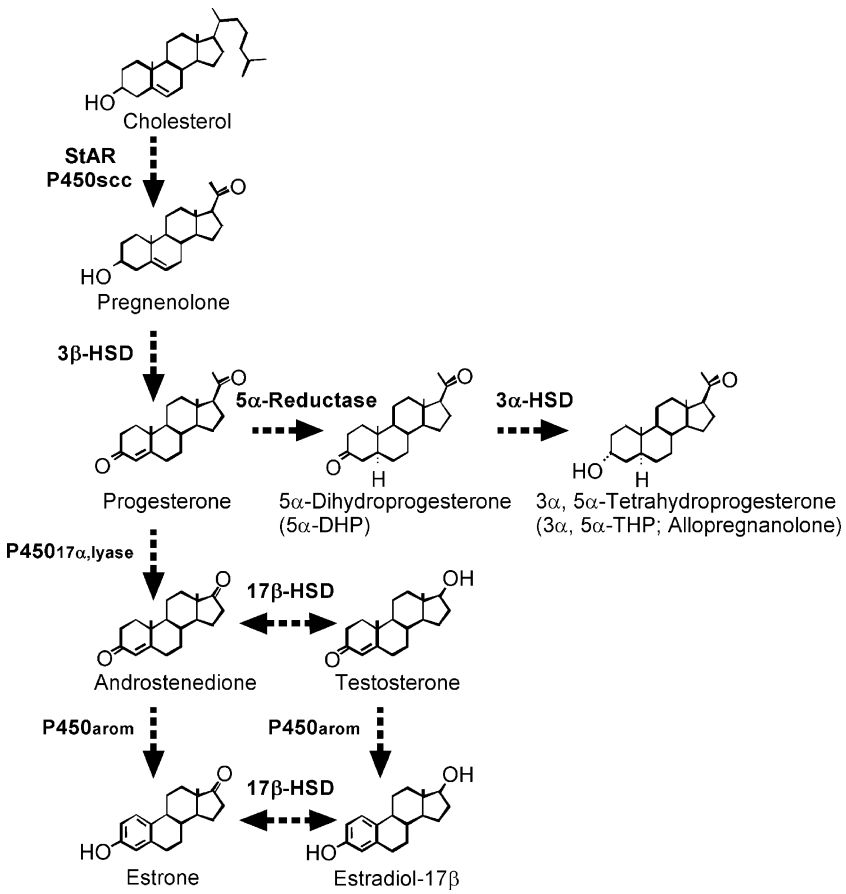

Fig. 1 Neurosteroid formation in the Purkinje cell. The Purkinje cell is a major site for neurosteroid formation in the brain. The rat Purkinje cell possesses several kinds of steroidogenic enzymes and produces pregnenolone and progesterone. The expression of P450scc remains during neonatal development and in adulthood, indicating the constant production of pregnenolone. This neuron also produces actively progesterone due to an increase of 3β-HSD activity only during neonatal life. Allopregnanolone is also metabolized by the enzymes 5α-reductase and 3α-HSD from progesterone during neonatal life. Estrogen formation in the Purkinje cell may also occur in the neonate because this neuron further expresses $P450_{17\alpha,lyase}$ and P450arom. *StAR* steroidogenic acute regulatory protein, *P450scc* cytochrome P450 side-chain cleavage enzyme, *3β-HSD* 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase, *$P450_{17\alpha,lyase}$* cytochrome P450 17α-hydroxylase/c17,20-lyase, *17β-HSD* 17β-hydroxysteroid dehydrogenase, *3α-HSD* 3α-hydroxysteroid dehydrogenase, *P450arom* cytochrome P450 aromatase

to this classical concept, the new concept of de novo progesterone formation from cholesterol in the Purkinje cell, a cerebellar neuron, derived from our observations made in the 1990s.

Pregnenolone is a precursor of progesterone secreted by peripheral steroidogenic glands and the formation of pregnenolone is initiated by the cleavage of the cholesterol side-chain by cytochrome P450scc, a rate-limiting mitochondrial enzyme (Fig. 1). As an initial step in the demonstration of progesterone formation in the Purkinje cell, it is therefore essential to demonstrate the biosynthesis of pregnenolone from cholesterol in this neuron. The first immunohistochemical study in quail using an antibody against P450scc reported that the striking observation of the distribution of intense immunoreactive cells in the cerebellar cortex [16, 17]. The distribution of immunoreactive cell bodies and fibers was coincident with the location of somata and dendrites of Purkinje cells [16, 17]. Western immunoblot analysis confirmed the presence of P450scc in Purkinje cells [16, 17]. These avian findings provided the first evidence for the neuronal location of cytochrome P450scc. We extended these findings further and investigated the presence of P450scc in rat Purkinje cells [39]. An antibody against inositol triphosphate (IP_3) receptor, a marker of the Purkinje cell, recognized P450scc-immunoreactive cerebellar cells that showed no immunoreaction with glial fibrillary acidic protein (GFAP), a specific marker of astrocytes [39]. Thus, immunoreaction with P450scc antibody was confined to the somata and dendrites of Purkinje cells in the rat cerebellum [39]. Interestingly, P450scc appeared in the rat Purkinje cell immediately after its differentiation, and the expression of this enzyme persisted during neonatal development into adulthood, indicating that Purkinje cells may produce pregnenolone throughout life [39]. In addition to higher vertebrates, our recent studies further identified P450scc in the Purkinje cell of amphibians [33]. Taken together, these findings obtained in both higher and lower vertebrates indicate that Purkinje cells possess P450scc and produce pregnenolone (Fig. 2). In addition, steroidogenic acute regulatory protein (StAR) was found in Purkinje cells [42] (Fig. 2). StAR is involved in the transport of cholesterol to the inner mitochondrial membrane, in which P450scc is localized, and, thus, plays a key role in acute steroid biosynthesis in peripheral steroidogenic glands [53]. StAR may also contribute to the regulation of pregnenolone formation in the Purkinje cell (Fig. 2).

Because the biosynthesis of progesterone from pregnenolone is performed by 3β-HSD (Fig. 1), the demonstration of the expression of 3β-HSD in the Purkinje cell is therefore essential to establish the concept of de novo progesterone formation from cholesterol in this neuron. We have further demonstrated that Purkinje cells express not only P450scc but also 3β-HSD (Fig. 2). RT-PCR and biochemical analyses showed the expression of 3β-HSD and its enzymatic activity in the rat cerebellum [40]. Using in situ hybridization of 3β-HSD mRNA, the site of 3β-HSD expression was localized in Purkinje cells and external granule cells [40]. Thus, both P450scc and 3β-HSD are expressed in Purkinje cells (Fig. 2). The colocalization of P450scc and 3β-HSD in external granule cells is still unclear. The expression of 3β-HSD in Purkinje cells was also evident in other vertebrates [2, 38]. Surprisingly, the expression of 3β-HSD increased during

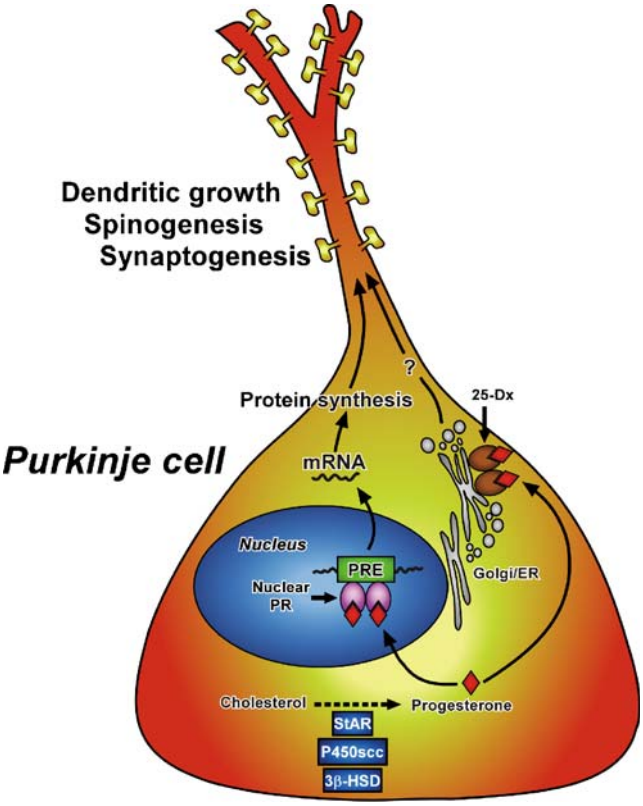

Fig. 2 A schematic model of organizing actions of progesterone in the Purkinje cell during neonatal development. The Purkinje cell actively produces progesterone due to an increase of 3β-HSD activity, only during neonatal life. Progesterone acts on the Purkinje cell through intranuclear receptor (PR)-mediated mechanisms that promote dendritic growth, spinogenesis, and synaptogenesis in this neuron by genomic mechanisms. Thus progesterone produced in the Purkinje cell may mediate its actions through an "intracrine" mechanism. Progesterone may induce the expression of some neurotrophic factors that directly promote Purkinje dendritic growth, spinogenesis, and synaptogenesis during neonatal life. Such genomic actions of progesterone contribute to the formation of the cerebellar neuronal circuit. However, we cannot rule out the possibility that progesterone might act on Purkinje cells through the mechanisms mediated by 25-Dx, which is associated with membrane structures of the endoplasmic reticulum and Golgi apparatus. *StAR* steroidogenic acute regulatory protein, *P450scc* cytochrome P450 side-chain cleavage enzyme, *3β-HSD* 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase, *PR* progesterone receptor, *PRE* progesterone response element, *Golgi* Golgi apparatus, *ER* endoplasmic reticulum

neonatal life, unlike P450scc [39, 40]. Such an age-dependent expression of 3β-HSD was confirmed by biochemical studies together with HPLC analysis, indicating an increase of progesterone formation during neonatal life [40]. Thus, this neuron actively produces progesterone as a product of an increase of 3β-HSD activity during neonatal life [40] (Fig. 2).

Biochemical analysis together with HPLC and TLC further revealed that the progesterone metabolite allopregnanolone is also found in the cerebellum during neonatal life [43–46]. Thus, 5α-reductase and 3α-HSD metabolize some of progesterone to allopregnanolone in the cerebellum during development (Fig. 1).

Organizing Actions of Progesterone

Because the Purkinje cell is a major site for progesterone formation and metabolism in the brain, this neuron has also served as an excellent cellular model for the study of actions of these neurosteroids. In mammals, this neuron actively produces progesterone during neonatal life, when cerebellar neuronal circuit formation occurs [39, 40, 42]. Because of this discovery of regio- and temporal-specific progesterone synthesis, organizing actions of progesterone have been demonstrated by the studies on mammals using the Purkinje cell.

Purkinje cells actively synthesize progesterone during the neonatal period, as the expression of 3β-HSD and its enzymatic activity increase in neonatal rats [40] (Fig. 2). Allopregnanolone, a progesterone metabolite, is also synthesized in the cerebellum of neonatal rats [43–46]. It is well known that in the rat marked, morphological changes occur in the cerebellum after birth during neonatal life. According to Altman [54, 55], rat Purkinje cells differentiate just after birth, and the formation of the cerebellar cortex becomes complete in the neonate through the processes of migration of external granule cells, neuronal and glial growth, and synaptogenesis. Thus, cerebellar development is dramatic during neonatal life, when cerebellar progesterone and allopregnanolone concentrations are high [40, 43, 44]. Therefore, progesterone and/or allopregnanolone may be involved in the formation of the cerebellar neuronal circuit by promoting neuronal growth and neuronal synaptic contact.

To test this hypothesis, we examined the effects of progesterone and allopregnanolone, produced as neurosteroids in the Purkinje cell during neonatal life, on neuronal growth, spinogenesis, and synaptogenesis in the rat cerebellum. In vitro studies using cultured cerebellar slices of newborn rats showed that progesterone promotes dendritic growth and dendritic spine formation of the Purkinje cell [47, 48] (Fig. 2). A similar result was obtained by in vivo studies [47, 48]. Electron microscopic analysis further revealed that progesterone induces an increase of the density of spine synapses on the Purkinje cell [47, 48] (Fig. 2). These effects were blocked by the progesterone receptor antagonist mifepristone (RU486) [47, 48]. In contrast to progesterone, there was no significant effect of allopregnanolone on these aspects of Purkinje development [47, 48]. These results indicate that progesterone promotes the dendritic growth, spinogenesis, and synaptogenesis of Purkinje cells (Fig. 2).

Mode of Action and Functional Significance of Progesterone

To understand the mode of action of progesterone, the expression of progesterone receptor (PR) in the cerebellum was then characterized in neonatal rats. Interestingly, intranuclear PR-A and PR-B were expressed in the Purkinje cell [47–49] (Fig. 2). It is therefore considered that progesterone acts directly on Purkinje cells through intranuclear receptor-mediated mechanisms to promote Purkinje dendritic growth, spinogenesis, and synaptogenesis [47–49] (Fig. 2). Such genomic actions of progesterone may be essential for the formation of the cerebellar neuronal circuit.

On the other hand, Purkinje cells express the putative membrane progesterone receptor, 25-Dx, during neonatal life [56]. RT-PCR and Western immunoblot analyses revealed the expressions of 25-Dx and its mRNA in the rat cerebellum, which increased during neonatal life [56]. By immunocytochemistry, the expression of 25-Dx was localized in the Purkinje cell and external granule cell layer [56]. At the ultrastructural level, 25-Dx immunoreactivity was associated with membrane structures of the endoplasmic reticulum and Golgi apparatus in the Purkinje cell [56] (Fig. 2). It is possible that progesterone may promote dendritic growth, spinogenesis, and synaptogenesis via 25-Dx as well as its nuclear receptor in the Purkinje cell in the neonate [57] (Fig. 2). This protein is now named ‘progesterone receptor membrane component 1’ (PGRMC1), and there is now strong evidence that PGRMC1 mediates the anti-apoptotic actions of progesterone in both rat granulose and luteal cells and associates with another membrane protein, such as plasminogen activator inhibitor RNA-binding protein-1 (PAIRBP1) [58–61]. Future studies are needed to demonstrate whether the promotion of Purkinje dendritic growth, spinogenesis, and synaptogenesis by progesterone is due to both genomic and nongenomic actions.

A series of our studies indicate that progesterone promotes Purkinje dendritic growth, spinogenesis, and synaptogenesis. Such organizing actions may contribute to the formation of the cerebellar neuronal circuit during neonatal life. Neurotrophins are attractive candidate regulators of Purkinje dendrite and spine development. It has

been reported that neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), are highly expressed in the developing cerebellum and are critical for proper development of Purkinje cells and granule cells [62–66]. Therefore, progesterone may induce the expression of some neurotrophic factors that directly promote Purkinje dendritic growth, spinogenesis, and synaptogenesis during neonatal life [67].

Neuroprotective Actions of Progesterone and Allopregnanolone

In addition to organizing actions of progesterone as described above, it has been reported that mifepristone (RU486), an anti-progesterone, protects Purkinje cells from developmental cell death, although progesterone does not possess any effect on Purkinje cell survival [50]. This protective effect of RU486 is considered to be independent on the activation of nuclear PRs [50]. Although 25-Dx is expressed in Purkinje cells [56], there is still no evidence for its role in mediating neurotrophic and/or neuroprotective effects of progesterone.

Purkinje cells metabolize some of progesterone to allopregnanolone during neonatal life [43–46]. Although allopregnanolone failed to promote the dendritic growth, spinogenesis, and synaptogenesis of Purkinje cells [47, 48], it has been shown that allopregnanolone is involved in Purkinje and granule cell survival [51]. The Niemann–Pick type C (NP-C) mouse has been used as an excellent animal model for understanding allopregnanolone action. NP-C is an autosomal recessive, childhood neurodegenerative disease characterized by defective intracellular cholesterol trafficking, resulting in Purkinje cell degeneration as well as neuronal degeneration in other regions. Brains from adult NP-C mice contain less allopregnanolone than wild-type brain [51]. Administration of allopregnanolone to neonatal NP-C mice increases Purkinje cell survival and delays neurodegeneration [51].

Estradiol in the Purkinje Cell

Estrogen Formation

Estradiol is also known to be a sex steroid and acts on brain tissues. Cytochrome P450arom is a key enzyme of estrogen formation in peripheral steroidogenic glands (Fig. 1). Recently, we further demonstrated the expression of P450arom in Purkinje cells during neonatal life [41] (Fig. 5). RT-PCR and in situ hybridization analyses showed that the expression of P450arom mRNA in the cerebellum is restricted to Purkinje cells and external granule cells in neonatal rats [41]. A specific enzyme immunoassay for estradiol further indicated that cerebellar estradiol concentrations in the neonate are higher than those in prepubertal and adult rats [41]. In addition, a recent study has shown the expression and activity of $P450_{17\alpha,lyase}$, which converts progesterone to androstenedione, an immediate precursor of estrogen formed by P450arom, in the Purkinje cell [25] (Figs. 1 and 5). These studies indicate estrogen formation in the Purkinje cell during neonatal life (Fig. 5).

Organizing Actions of Estradiol

To clarify the action of estradiol in Purkinje cells during neonatal life, we analyzed the effects of estradiol on dendritic growth and spine development of Purkinje cells by both in vitro and in vivo studies using newborn rats [41]. Both in vitro and in vivo studies with newborn rats showed that estradiol promotes dendritic growth of Purkinje cells [41]. Estradiol also increases the densities of Purkinje dendritic spines [41] and spine synapses [52]. These effects were inhibited by the estrogen receptor antagonist tamoxifen [41, 52]. Thus estradiol also promotes the dendritic growth, spinogenesis, and synaptogenesis of Purkinje cells.

Mode of Action and Functional Significance of Estradiol

It has been reported that in the neonatal rat, Purkinje cells express estrogen receptor-β (ERβ) [68, 69]. Therefore, it is likely that estradiol acts directly on Purkinje cells through intranuclear ERβ-mediated mechanisms to promote dendritic growth, spinogenesis, and synaptogenesis in Purkinje cells during neonatal development (Fig. 5). On the other hand, granule cells also express ERβ [68, 69] (Fig. 5). Involvement of ERβ in the brain function has also been reported in the rat hypothalamus [70, 71].

While ERβ appears to mediate effects of estradiol in Purkinje cell function, other receptors may also mediate effects of estradiol in other brain regions, such as hippocampus [72–77] and hypothalamus [78, 79]. The effect of estradiol on hippocampal CA1 pyramidal cell dendrite spine density requires the activation of *N*-methyl-D-aspartate (NMDA) receptors in adult female rats [75]. Such nongenomic estrogen actions may lead to alterations in gene expression. Hence, NMDA receptors may also mediate estradiol action in Purkinje cells. Future studies are needed to demonstrate whether the promotion of Purkinje dendritic growth, spinogenesis, and synaptogenesis by estradiol is due to both genomic and nongenomic actions.

To demonstrate the functional significance of estradiol in the Purkinje cell during neonatal life, we further investigated estrogen actions on dendritic growth, spinogenesis, and synaptogenesis in the Purkinje cell using cytochrome P450arom knock-out (ArKO) mice [52]. ArKO mice used in the study lack exons 1 and 2 and the proximal promoter

region of the P450arom gene *cyp19* (cytochrome P450, family 19) [80]. Estradiol deficiency in ArKO mice decreased dendritic growth, spinogenesis, and synaptogenesis in Purkinje cells in the neonate [52] (Figs. 3 and 4). In addition, administration of estradiol to ArKO mice increased Purkinje dendritic growth, spinogenesis, and synaptogenesis [52] (Figs. 3 and 4). These results indicate physiological actions of endogenous estrogen on the promotion of dendritic growth, spinogenesis, and synaptogenesis in the Purkinje cell during neonatal development.

To elucidate the mode of action of estradiol, we further examined the expression of BDNF and NT-3 in response to estrogen actions in the neonate [52], because these neurotrophic factors are known to be critical for proper development of Purkinje cells [62–66]. Estrogen administration to neonatal wild-type (WT) mice or ArKO mice increased the BDNF level in the cerebellum (Fig. 4), whereas the estrogen receptor antagonist tamoxifen decreased the BDNF level in WT mice similar to ArKO mice [52]. BDNF administration to tamoxifen-treated WT mice increased Purkinje dendritic growth [52]. In contrast to BDNF, estrogen administration did not influence the level of NT-3 in the cerebellum [52]. The NT-3 level also did not change in ArKO mice [52]. These results suggest that BDNF mediates estrogen action on the promotion of dendritic growth, spinogenesis, and synaptogenesis in the Purkinje cell during neonatal development (Fig. 5). In fact, the gene encoding BDNF contains a sequence similar to the canonical estrogen response element found in estrogen-target genes [81]. In addition, BDNF increases levels of synaptic vesicle proteins, such as synaptophysin and synapsin 1, which are reliable markers of synaptogenesis, in the spinal neurons [82]. Estrogen increases presynaptic and postsynaptic proteins, such as syntaxin, synaptophysin, and spinophilin, in the CA1 region of the primate hippocampus [83]. Furthermore, it has been reported that estrogen treatment induces these synaptic proteins in the CA1 region of the hippocampus and this effect is blocked by CI628, an antiestrogen of the tamoxifen type [84]. The expression of P450arom mRNA in the cerebellum is restricted to Purkinje cells and external granule cells in the neonatal rats [41, 85–87]. Both Purkinje cells and granule cells express BDNF [88, 89] and TrkB, a receptor for BDNF [90–92] (Fig. 5). It is therefore possible that estrogen induces the expression of BDNF, which acts on Purkinje cells and granule cells through TrkB-mediated mechanisms to promote Purkinje dendritic growth, spinogenesis, and synaptogenesis [52] (Fig. 5). Estradiol may also alter the expression of TrkB, which may affect the Purkinje cell development by altering the sensitivity of BDNF.

Neuroprotective and neurotrophic actions of estrogen have been reported by the studies using ArKO mice [93, 94]. Neuroprotective effects of estrogen on dentate gyrus neurons in the hippocampus were mediated by estrogen-induced insulin-like growth factor-I (IGF-I) [93], similar to neurotrophic effects of estrogen on Purkinje cells mediated by estrogen-induced BDNF [52].

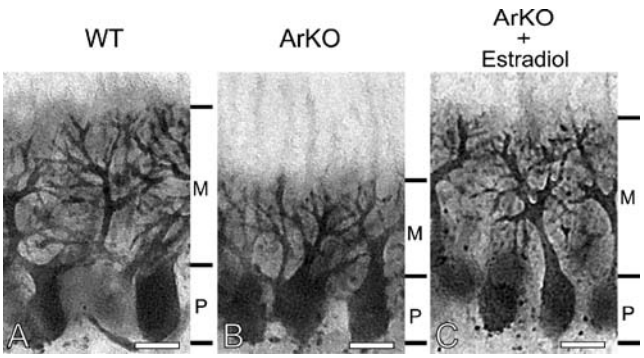

Fig. 3 Morphological comparison of Purkinje cell dendrites in wild-type (**A**, *WT*), cytochrome P450arom knock-out (**B**, *ArKO*), and estradiol-treated ArKO (**C**, *ArKO+Estradiol*) newborn mice. Parasagittal sections of cerebella were immunostained for calbindin. *Scale bars*, 10 μm. *M* molecular layer, *P* Purkinje cell layer

Related Findings in Other Neurons

In addition to Purkinje cells, the localization of neurosteroidogenic enzymes in other brain neurons has further characterized [95, 96]. In the rat hippocampus, the localization of cytochrome P450scc, $P450_{17\alpha,lyase}$, and P450arom has been found in pyramidal neurons in the CA1–CA3 regions as well as granule cells in the dentate gyrus [95, 96]. Thus, neurons as well as glial cells are now considered to play a major role in neurosteroid formation and metabolism in the brain. In addition to these brain neurons, cytochrome P450scc expression has been reported in neurons in the retinal ganglion, sensory neurons in the dorsal root ganglia and motor neurons in the spinal cord of the rat [15, 97].

Conclusions and Future Directions

De novo steroidogenesis from cholesterol is a conserved property of vertebrate brains, and such steroids synthesized de novo in the brain are called neurosteroids. Our studies provided an opportunity to understand the biosynthesis and action of neurosteroids in the Purkinje cell, a major site of neurosteroidogenesis in the brain. This neuron actively synthesizes progesterone and allopregnanolone de novo from cholesterol during neonatal life when cerebellar neuronal circuit formation occurs. This neuron may also produce estradiol in the neonate. Both progesterone and estradiol promote Purkinje dendritic growth, spinogenesis,

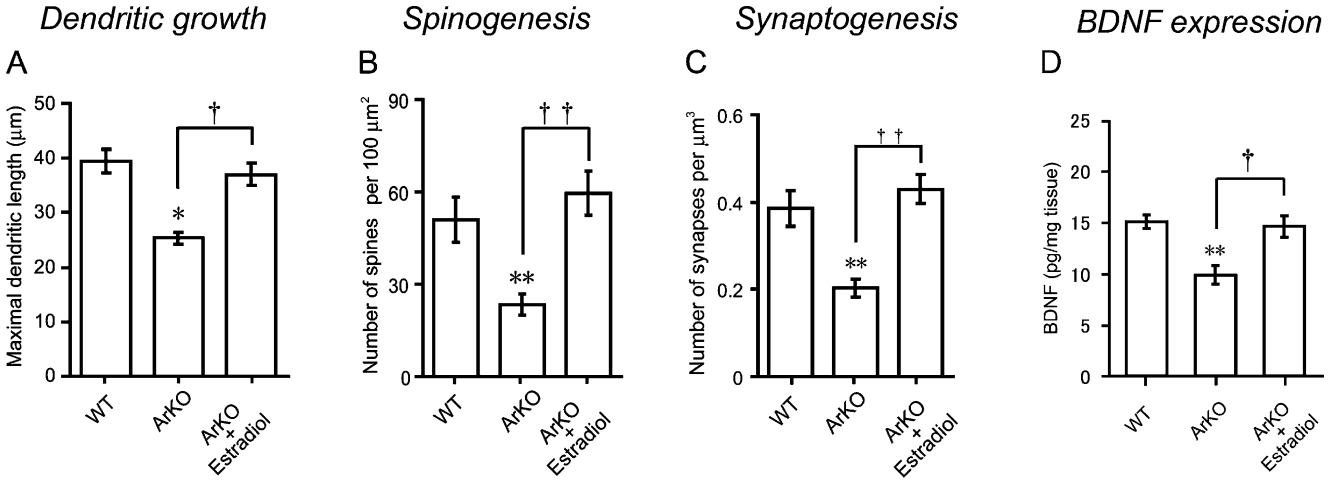

Fig. 4 Dendritic growth (**A**), spinogenesis (**B**), and synaptogenesis (**C**) in the Purkinje cell and BDNF expression (**D**) in the cerebellum of wild-type (*WT*), cytochrome P450arom knock-out (*ArKO*), and estradiol-treated ArKO (*ArKO+Estradiol*) newborn mice. Each *column* and *error bar* represent the mean±SEM (**A**–**C**, $n=5$ in each group; **D**, $n=8$ in each group). *$p<0.05$ and **$p<0.01$ versus *WT*; †$p<0.05$ and ††$p<0.01$ *ArKO* versus *ArKO+Estradiol* (by one-way ANOVA, followed by Duncan's multiple range test)

and synaptogenesis. Such organizing actions that may be mediated by neurotrophic factors, such as BDNF, contribute to the formation of cerebellar neuronal circuit during neonatal life. The progesterone metabolite allopregnanolone is also involved in Purkinje and granule cell survival. Thus, the discovery of neurosteroid formation in the Purkinje cell has opened avenues for a new research field in molecular neurobiology. Future directions for this

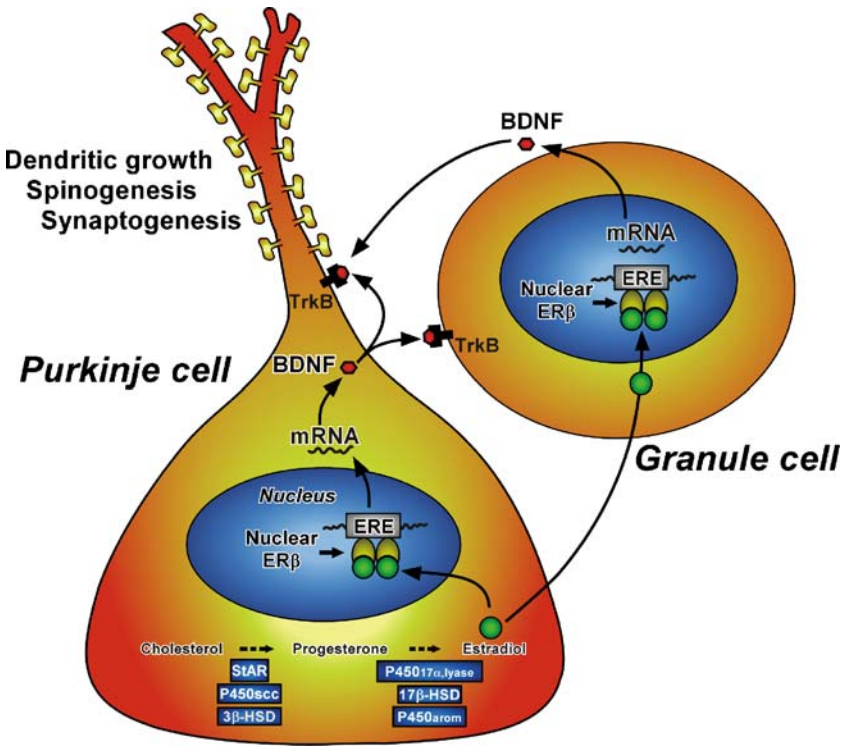

Fig. 5 A schematic model of organizing actions of estradiol in the Purkinje cell during neonatal development. The Purkinje cell may also actively produce estradiol due to an increase of P450arom activity during neonatal life. Estradiol acts on the Purkinje cell through intranuclear receptor (ERβ)-mediated mechanisms that promote dendritic growth, spinogenesis, and synaptogenesis in this neuron by genomic mechanisms. Both Purkinje cells and granule cells express BDNF and TrkB, a receptor for BDNF. Estradiol induces the expression of BDNF, which may act on Purkinje cells and granule cells through TrkB-mediated mechanisms to promote Purkinje dendritic growth, spinogenesis, and synaptogenesis. *StAR* steroidogenic acute regulatory protein, *P450scc* cytochrome P450 side-chain cleavage enzyme, *3β-HSD* 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase, *$P450_{17\alpha,lyase}$* cytochrome P450 17α-hydroxylase/c17,20-lyase, *17β-HSD* 17β-hydroxysteroid dehydrogenase, *P450arom* cytochrome P450 aromatase, *ERβ* estrogen receptor-β, *ERE* estrogen response element, *BDNF* brain-derived neurotrophic factor, *TrkB* BDNF receptor

exciting area of research should focus on physiological roles of neurosteroids, because Purkinje cells play an important role in the process of memory and learning. Therefore, behavioral studies using neurosteroidogenic enzyme knock-out animals and electrophysiological studies on the occurrence of long-term potentiation (LTP) and/or long-term depression (LTD) are needed.

Acknowledgments I thank Hirotaka Sakamoto, Mariko Usui, Hanako Shikimi, Katsunori Sasahara, Shogo Haraguchi, Minoru Takase and Kazuyoshi Ukena for their work cited in this manuscript. Supported by Grants-in-Aid (15207007, 16086206 and 18107002) for Scientific Research from the Ministry of Education, Science, and Culture, Japan (to KT).

Disclosure Statement The author has nothing to disclose.

References

1. Baulieu EE (1997) Neurosteroids: of the nervous system, by the nervous system, for the nervous system (review). Rec Progr Hormone Res 52:1–32
2. Tsutsui K, Ukena K, Takase M, Kohchi C, Lea RW (1999) Neurosteroid biosynthesis in vertebrate brains (review). Comp Biochem Physiol C 124:121–129
3. Tsutsui K, Ukena K, Usui M, Sakamoto H, Takase M (2000) Novel brain function: biosynthesis and actions of neurosteroids in neurons (review). Neurosci Res 36:261–273
4. Compagnone NA, Mellon SH (2000) Neurosteroids: biosynthesis and function of these novel neuromodulators (review). Front Neuroendocrinol 21:1–56
5. Mellon SH, Vaudry H (2001) Biosynthesis of neurosteroids and regulation of their synthesis (review). Int Rev Neurobiol 46:33–78
6. Corpéchot C, Robel P, Axelson M, Sjövall J, Baulieu EE (1981) Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. Proc Natl Acad Sci USA 78:4704–4707
7. Corpéchot C, Synguelakis M, Talha S, Axelson M, Sjovall J, Vihko R, Baulieu EE, Robel P (1983) Pregnenolone and its sulfate ester in rat brain. Brain Res 270:119–125
8. Robel P, Baulieu EE (1985) Neuro-steroids, 3β-hydroxy-Δ^5-derivatives in the rodent brain. Neurochem Int 7:953–958
9. Lanthier A, Patwardhan VV (1986) Sex steroids and 5-en-3β-hydroxysteroids in specific regions of the human brain and cranial nerves. J Steroid Biochem 25:445–449
10. Robel P, Corpéchot C, Clarke C, Groyer A, Synguelakis M, Vourc'h C, Baulieu EE (1986) Neuro-steroids: 3β-hydroxy-Δ^5-derivatives in the rat brain. In: Fink G, Harmar AJ, McKerns KW (eds) Neuroendocrine Molecular Biology. Plenum, New York, pp 367–377
11. Robel P, Bourreau E, Corpéchot C, Dang DC, Halberg F, Clarke C, Haug M, Schlegel ML, Synguelakis M, Vourch C, Baulieu EE (1987) Neuro-steroids: 3β-hydroxy-Δ^5-derivatives in rat and monkey brain. J Steroid Biochem 27:649–655
12. Jo DH, Abdallah MA, Young J, Baulieu EE, Robel P (1989) Pregnenolone, dehydroepiandrosterone, and their sulfate and fatty acid esters in the rat brain. Steroids 54:287–297
13. Mathur C, Prasad VVK, Raju VS, Welch M, Lieberman S (1993) Steroids and their conjugates in the mammalian brain. Proc Natl Acad Sci USA 90:85–88
14. Mellon SH, Deschepper CF (1993) Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain. Brain Res 629:283–292
15. Compagnone NA, Bulfone A, Rubenstein JLR, Mellon SH (1995) Steroidogenic enzyme P450c17 is expressed in the embryonic central nervous system. Endocrinology 136:5212–5223
16. Tsutsui K, Yamazaki T (1995) Avian neurosteroids. I. Pregnenolone biosynthesis in the quail brain. Brain Res 678:1–9
17. Usui M, Yamazaki T, Kominami S, Tsutsui K (1995) Avian neurosteroids. II. Localization of a cytochrome P450scc-like substance in the quail brain. Brain Res 678:10–20
18. Vanson A, Arnold AP, Schlinger BA (1996) 3β-Hydroxysteroid dehydrogenase/isomerase and aromatase activity in primary cultures of developing zebra finch telencephalon: dehydroepiandrosterone as substrate for synthesis of androstenedione and estrogens. Gen Comp Endocrinol 102:342–350
19. Tsutsui K, Yamazaki T, Usui M, Furukawa Y, Ukena K, Kohchi C, Kominami S (1997) P450scc activity in the brain. In: Harvey S, Etches RJ (eds) Perspectives in Avian Endocrinology. Journal of Endocrinol Ltd, Bristol, pp 427–436
20. Tsutsui K, Usui M, Yamazaki T, Ukena K, Kominami S (1997) Neurosteroids in the avian brain. In: Maitra SK (ed) Frontiers in Environmental and Metabolic Endocrinology. Burdwan, Burdwan, pp 151–159
21. Ukena K, Honda Y, Inai Y, Kohchi C, Lea RW, Tsutsui K (1999) Expression and activity of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase in different regions of the avian brain. Brain Res 818:536–542
22. Schlinger BA, Lane NI, Grisham W, Thompson L (1999) Androgen synthesis in a songbird: a study of cyp17 (17α-hydroxylase/c17,20-lyase) activity in the zebra finch. Gen Comp Endocrinol 113:46–58
23. Freking F, Nazairians T, Schlinger BA (2000) The expression of the sex steroid-synthesizing enzymes CYP11A1, 3β-HSD, CYP17, and CYP 19 in gonads and adrenals of adult and developing zebra finches. Gen Comp Endocrinol 119:140–151
24. Ukena K, Honda Y, Lea RW, Tsutsui K (2001) Developmental changes in progesterone biosynthesis and metabolism in the quail brain. Brain Res 898:190–194
25. Matsunaga M, Ukena K, Tsutsui K (2001) Expression and localization of cytochrome P450 17α-hydroxylase/c17, 20-lyase in the avian brain. Brain Res 899:112–122
26. Tsutsui K, Schlinger BA (2001) Steroidogenesis in the avian brain. In: Dawson A, Chaturvedi CM (eds) Avian Endocrinology. Narosa, New Delhi, pp 59–77
27. Matsunaga M, Ukena K, Tsutsui K (2002) Androgen biosynthesis in the quail brain. Brain Res 948:180–185
28. Soma KK, Alday NA, Hau M, Schlinger BA (2003) DHEA metabolism by 3β-HSD in adult zebra finch brain: sex difference and rapid effect of stress. Endocrinology 145:1668–1677
29. Tsutsui K, Matsunaga M, Ukena K (2003) Biosynthesis and biological actions of neurosteroids in the avian brain (review). Avian Poultry Biol Reviews 14:63–78
30. Mensah-Nyagan AG, Feuilloley M, Dupont E, Do-Rego JL, Leboulenger F, Pelletier G, Vaudry H (1994) Immunocytochemical localization and biological activity of 3β-hydroxysteroid dehydrogenase in the central nervous system of the frog. J Neurosci 14:7306–7318
31. Mensah-Nyagan AG, Feuilloley M, Do-Rego JL, Marcual A, Lange C, Tonon MC, Pelletier G, Vaudry H (1996) Localization of 17β-hydroxysteroid dehydrogenase and characterization of testosterone in the brain of the male frog. Proc Natl Acad Sci USA 93:1423–1428
32. Mensah-Nyagan AG, Do-Rego JL, Feuilloley M, Marcual A, Lange C, Pelletier G, Vaudry H (1996) In vivo and in vitro evidence for the biosynthesis of testosterone in the telencephalon of the female frog. J Neurochem 67:413–422
33. Takase M, Ukena K, Yamazaki T, Kominami S, Tsutsui K (1999) Pregnenolone, pregnenolone sulfate and cytochrome P450 side-

chain cleavage enzyme in the amphibian brain and their seasonal changes. Endocrinology 140:1936–1944
34. Inai Y, Nagai K, Ukena K, Oishi T, Tsutsui K (2003) Seasonal changes in neurosteroids in the urodele brain and environmental factors inducing their changes. Brain Res 959:214–225
35. Matsunaga M, Ukena K, Baulieu EE, Tsutsui K (2004) 7α-Hydroxypregnenolone acts as a neuronal activator to stimulate locomotor activity of breeding newts by means of the dopaminergic system. Proc Natl Acad Sci USA 101:17282–17287
36. Mensah-Nyagan AG, Do-Rego JL, Beaujean D, Luu-The V, Pelletier G, Vaudry H (1999) Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system (review). Pharmacol Rev 51:63–81
37. Mensah-Nyagan AG, Beaujean D, Luu-The V, Pelletier G, Vaudry H (2001) Anatomical and biochemical evidence for the synthesis of unconjugated and sulfated neurosteroids in amphibians (review). Brain Res Rev 37:13–24
38. Sakamoto H, Ukena K, Tsutsui K (2001) Activity and localization of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase in the zebrafish central nervous system. J Comp Neurol 439:291–305
39. Ukena K, Usui M, Kohchi C, Tsutsui K (1998) Cytochrome P450 side-chain cleavage enzyme in the cerebellar Purkinje neuron and its neonatal change in rats. Endocrinology 139:137–147
40. Ukena K, Kohchi C, Tsutsui K (1999) Expression and activity of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase in the rat Purkinje neuron during neonatal life. Endocrinology 140:805–813
41. Sakamoto H, Mezaki Y, Shikimi H, Ukena K, Tsutsui K (2003) Dendritic growth and spine formation in response to estrogen in the developing Purkinje cell. Endocrinology 144:4466–4477
42. Furukawa A, Miyatake A, Ohnishi T, Ichikawa Y (1998) Steroidogenic acute regulatory protein (StAR) transcripts constitutively expressed in the adult rat central nervous system: Colocalization of StAR, cytochrome P-450scc (CYP XIA1), and 3β-hydroxysteroid dehydrogenase in the rat brain. J Neurochem 71:2231–2238
43. Tsutsui K, Ukena K (1999) Neurosteroids in the cerebellar Purkinje neuron and their actions (review). Int J Mol Med 4:49–56
44. Tsutsui K, Sakamoto H, Ukena K (2003) Biosynthesis and action of neurosteroids in the cerebellar Purkinje neuron. J Steroid Biochem Mol Biol 85:311–321
45. Tsutsui K, Ukena K, Sakamoto H (2003) A novel aspect of the cerebellum: biosynthesis of neurosteroids in the Purkinje cell (review). Cerebellum 2:215–222
46. Tsutsui K, Sakamoto H, Shikimi H, Ukena K (2004) Organizing actions of neurosteroids in the Purkinje neuron (review). Neurosci Res 49:273–279
47. Sakamoto H, Ukena K, Tsutsui K (2001) Effects of progesterone synthesized de novo in the developing Purkinje cell on its dendritic growth and synaptogenesis. J Neurosci 21:6221–6232
48. Sakamoto H, Ukena K, Tsutsui K (2002) Dendritic spine formation in response to progesterone synthesized de novo in the developing Purkinje cell in rats. Neurosci Lett 322:111–115
49. Sakamoto H, Shikimi H, Ukena K, Tsutsui K (2003) Neonatal expression of progesterone receptor isoforms in the cerebellar Purkinje cell in rats. Neurosci Lett 343:163–166
50. Ghoumari AM, Dusart I, El-Etr M, Tronche F, Sotelo C, Schumacher M, Baulieu EE (2003) Mifepristone (RU486) protects Purkinje cells from cell death in organotypic slice cultures of postnatal rat and mouse cerebellum. Proc Natl Acad Sci USA 100:7953–7958
51. Griffin LD, Gong W, Verot L, Mellon SH (2004) Niemann–Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. Nat Med 10:704–711
52. Sasahara K, Shikimi H, Haraguchi S, Sakamoto H, Honda S, Harada N, Tsutsui K (2007) Mode of action and functional significance of estrogen-inducing dendritic growth, spinogenesis, and synaptogenesis in the developing Purkinje cell. J Neurosci 27:7408–7417
53. Clark BJ, Wells J, King SR, Stocco DM (1994) The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 269:28314–28322
54. Altman J (1972) Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. J Comp Neurol 145:353–397
55. Altman J (1972) Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and of the molecular layer. J Comp Neurol 145:399–463
56. Sakamoto H, Ukena K, Takemori H, Okamoto M, Kawata M, Tsutsui K (2004) Expression and localization of 25-Dx, a membrane-associated putative progesterone-binding protein, in the developing Purkinje cell. Neuroscience 126:325–334
57. Sakamoto H, Ukena K, Kawata M, Tsutsui K (2008) Expression, localization and possible actions of 25-Dx, a membraneassociated putative progesterone-binding protein, in the developing Purkinje cell of the cerebellum: A new insight into the biosynthesis, metabolism and multiple actions of progesterone as a neurosteroid (review). Cerebellum, in press. DOI 10.1007/s12311-008-0007-2
58. Peluso JJ, Pappalardo A, Losel R, Wehling M (2006) Progesterone membrane receptor component 1 expression in the immature rat ovary and its role in mediating progesterone's antiapoptotic action. Endocrinology 147:3133–3140
59. Peluso JJ (2006) Multiplicity of progesterone's actions and receptors in the mammalian ovary. Biol Reprod 75:2–8
60. Engmann L, Losel R, Wehling M, Peluso JJ (2006) Progesterone regulation of human granulosa/luteal cell viability by an RU486-independent mechanism. J Clin Endocrinol Metab 91:4962–4968
61. Cahill MA (2007) Progesterone receptor membrane component 1: an integrative review. J Steroid Biochem Mol Biol 105:16–36
62. Rocamora N, Garcia-Ladona FJ, Palacios JM, Mengod G (1993) Differential expression of brain-derived neurotrophic factor, neurotrophin-3, and low-affinity nerve growth factor receptor during the postnatal development of the rat cerebellar system. Brain Res Mol Brain Res 17:1–8
63. Ernfors P, Lee KF, Jaenisch R (1994) Mice lacking brain-derived neurotrophic factor develop with sensory deficits. Nature 368:147–150
64. Neveu I, Arenas E (1996) Neurotrophins promote the survival and development of neurons in the cerebellum of hypothyroid rats in vivo. J Cell Biol 133:631–646
65. Schwartz PM, Borghesani PR, Levy RL, Pomeroy SL, Segal RA (1997) Abnormal cerebellar development and foliation in BDNF−/− mice reveals a role for neurotrophins in CNS patterning. Neuron 19:269–281
66. Bates B, Rios M, Trumpp A, Chen C, Fan G, Bishop JM, Jaenisch R (1999) Neurotrophin-3 is required for proper cerebellar development. Nat Neurosci 2:115–117
67. Tsutsui K (2008) Progesterone biosynthesis and action in the developing neuron (review). Endocrinology 149:2757–2761
68. Price RH, Handa RJ (2000) Expression of estrogen receptor-β protein and mRNA in the cerebellum of the rat. Neurosci Lett 288:115–118
69. Jakab RL, Wong JK, Belcher SM (2001) Estrogen receptor β immunoreactivity in differentiating cells of the developing rat cerebellum. J Comp Neurol 430:396–409
70. Orikasa C, Kondo Y, Hayashi S, McEwen BS, Sakuma Y (2002) Sexually dimorphic expression of estrogen receptor beta in the anteroventral periventricular nucleus of the rat preoptic area: implication in luteinizing hormone surge. Proc Natl Acad Sci USA 99:3306–3309
71. Ikeda Y, Nagai A, Ikeda MA, Hayashi S (2003) Sexually dimorphic and estrogen-dependent expression of estrogen receptor

beta in the ventromedial hypothalamus during rat postnatal development. Endocrinology 144:5098–5115
72. Gould E, Woolley CS, Frankfurt M, McEwen BS (1990) Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. J Neurosci 10:1286–1291
73. Woolley CS, Gould E, Frankfurt M, McEwen BS (1990) Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J Neurosci 10:4035–4039
74. Woolley CS, McEwen BS (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12:2549–2554
75. Woolley CS, McEwen BS (1994) Estradiol regulates hippocampal dendritic spine density via an *N*-methyl-D-aspartate receptor-dependent mechanism. J Neurosci 14:7680–7687
76. McEwen BS, Akama K, Alves S, Brake WG, Bulloch K, Lee S, Li C, Yuen G, Milner TA (2001) Tracking the estrogen receptor in neurons: implications for estrogen-induced synapse formation (review). Proc Natl Acad Sci USA 98:7093–7100
77. Murphy DD, Segal M (1996) Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones. J Neurosci 16:4059–4068
78. Pérez J, Luquín S, Naftolin F, García-Segura LM (1993) The role of estradiol and progesterone in phased synaptic remodelling of the rat arcuate nucleus. Brain Res 608:38–44
79. Langub MC, Maley BE, Watson RE (1994) Estrous cycle-associated axosomatic synaptic plasticity upon estrogen receptive neurons in the rat preoptic area. Brain Res 641:303–310
80. Honda S, Harada N, Ito S, Takagi Y, Maeda S (1998) Disruption of sexual behavior in male aromatase-deficient mice lacking exons 1 and 2 of the cyp19 gene. Biochem Biophys Res Commun 252:445–449
81. Sohrabji F, Miranda RC, Toran-Allerand CD (1995) Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. Proc Natl Acad Sci USA 92:11110–11114
82. Wang T, Xie K, Lu B (1995) Neurotrophins promote maturation of developing neuromuscular synapses. J Neurosci 15:4796–4805
83. Choi JM, Romeo RD, Brake WG, Bethea CL, Rosenwaks Z, McEwen BS (2003) Estradiol increases pre- and post-synaptic proteins in the CA1 region of the hippocampus in female rhesus macaques (*Macaca mulatta*). Endocrinology 144:4734–4738
84. Brake WG, Alves SE, Dunlop JC, Lee SJ, Bulloch K, Allen PB, Greengard P, McEwen BS (2001) Novel target sites for estrogen action in the dorsal hippocampus: an examination of synaptic proteins. Endocrinology 142:1284–1289
85. Tsutsui K (2006) Biosynthesis and organizing action of neurosteroids in the developing Purkinje cell. Cerebellum 5:89–96
86. Tsutsui K (2006) Biosynthesis, mode of action and functional significance of neurosteroids in the developing Purkinje cell (review). J Steroid Biochem Mol Biol 102:187–194
87. Tsutsui K, Mellon SH (2006) Neurosteroids in the brain neuron: biosynthesis, action and medicinal impact on neurodegenerative disease (review). Central Nerv Syst Agents Med Chem 6:73–82
88. Hofer M, Pagliusi SR, Hohn A, Leibrock J, Barde YA (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 9:2459–2464
89. Borghesani PR, Peyrin JM, Klein R, Rubin J, Carter AR, Schwartz PM, Luster A, Corfas G, Segal RA (2002) BDNF stimulates migration of cerebellar granule cells. Development 129:1435–1442
90. Klein R, Parada LF, Coulier F, Barbacid M (1990) TrkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. EMBO J 8:3701–3709
91. Segal RA, Pomeroy SL, Stiles CD (1995) Axonal growth and fasciculation linked to differential expression of BDNF and NT3 receptors in developing cerebellar granule cells. J Neurosci 15:4970–4981
92. Carter AR, Chen C, Schwartz PM, Segal RA (2002) Brain-derived neurotrophic factor modulates cerebellar plasticity and synaptic ultrastructure. J Neurosci 22:1316–1327
93. Azcoitia I, Sierra A, Garcia-Segura LM (1999) Neuroprotective effects of estradiol in the adult rat hippocampus: Interaction with insulin-like growth factor-I signalling. J Neurosci Res 58: 815–822
94. Carswell HVO, Dominiczak AF, Garcia-Segura LM, Harada N, Hutchison JB, Macrae IM (2005) Brain aromatase expression after experimental stroke: topography and time course. J Steroid Biochem Mol Biol 96:89–91
95. Kimoto T, Tsurugizawa T, Ohta Y, Makino J, Tamura H, Hojo Y, Takata N, Kawato S (2001) Neurosteroid synthesis by cytochrome p450-containing systems localized in the rat brain hippocampal neurons: *N*-methyl-D-aspartate and calcium-dependent synthesis. Endocrinology 142:3578–3589
96. Hojo Y, Hattori TA, Enami T, Furukawa A, Suzuki K, Ishii HT, Mukai H, Morrison JH, Janssen WG, Kominami S, Harada N, Kimoto T, Kawato S (2004) Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017α and P450 aromatase localized in neurons. Proc Natl Acad Sci USA 101:865–870
97. Guarneri P, Guarneri R, Cascio C, Pavasant P, Piccoli F, Papadopoulos V (1994) Neurosteroidogenesis in rat retinas. J Neurochem 63:86–96