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Kobayashi award: Discovery of cerebellar and pineal neurosteroids and their biological actions on the growth and survival of Purkinje cells during development (review)☆

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ABSTRACT

The brain has traditionally been considered to be a target site of peripheral steroid hormones. On the other hand, extensive studies over the past thirty years have demonstrated that the brain is a site of biosynthesis of several steroids. Such steroids synthesized *de novo* from cholesterol in the brain are called neurosteroids. To investigate the biosynthesis and biological actions of neurosteroids in the brain, data on the regio- and temporal-specific synthesis of neurosteroids are needed. In the mid 1990s, the Purkinje cell, an important cerebellar neuron, was discovered as a major cell producing neurosteroids in the brain of vertebrates. It was the first demonstration of *de novo* neuronal biosynthesis of neurosteroids in the brain. Subsequently, neuronal biosynthesis of neurosteroids and biological actions of neurosteroids have become clear by the follow-up studies using the Purkinje cell as an excellent cellular model. Progesterone and estradiol, which are known as sex steroid hormones, are actively synthesized *de novo* from cholesterol in the Purkinje cell during development, when cerebellar neuronal circuit formation occurs. Importantly, progesterone and estradiol synthesized in the Purkinje cell promote dendritic growth, spinogenesis and synaptogenesis *via* their cognate nuclear receptors in the Purkinje cell. Neurotrophic factors may mediate these neurosteroid actions. Futhermore, allopregnanolone (3α,5α-tetrahydroprogesterone), a progesterone metabolite, is also synthesized in the cerebellum and acts on the survival of Purkinje cells. On the other hand, at the beginning of 2010s, the pineal gland, an endocrine organ located close to the cerebellum, was discovered as an important site of the biosynthesis of neurosteroids. Allopregnanolone, a major pineal neurosteroid, acts on the Purkinje cell for the survival of Purkinje cells by suppressing the expression of caspase-3, a crucial mediator of apoptosis. I as a recipient of Kobayashi Award from the Japan Society for Comparative Endocrinology in 2016 summarize the discovery of cerebellar and pineal neurosteroids and their biological actions on the growth and survival of Purkinje cells during development.

1. Introduction

For a long time, the brain has been considered as a target site for peripheral steroid hormones because these hormones cross the blood-brain barrier and act on the brain through intracellular receptor-mediated mechanisms that control important brain functions. On the other hand, extensive studies over the past thirty years have demonstrated that the brain itself also produces several steroids *de novo* from cholesterol, so-called “neurosteroids” (for reviews, see Baulieu, 1997; Tsutsui et al., 1999; Compagnone and Mellon, 2000; Tsutsui et al.,

☆ The author describes this review as a recipient of Kobayashi Award from the Japan Society for Comparative Endocrinology in 2016. The author was awarded Kobayashi Award by a series of his studies over the past three decades. The author has contributed to the advancement of Comparative Endocrinology and Neuroendocrinology by his extensive studies focused on the discovery of novel neurohormones, such as neuropeptides and neurosteroids, and the demonstration of their biological actions and functional significances. For example, the author discovered novel neuropeptides, such as gonadotropin-inhibitory hormone (GnIH) inhibiting reproduction, and neurosteroids, such as 7α-hydroxypregnenolone inducing locomotor activity, from a novel standpoint in collaboration with excellent researchers throughout the globe. Among extensive studies of the author, this review summarizes the discovery of cerebellar and pineal neurosteroids and their biological actions on the growth and survival of Purkinje cells during development. For detailed information of the discoveries of GnIH that inhibits reproductive physiology and behavior, 7α-hydroxypregnenolone that induces locomotor activity, and orthers, the reader is referred to his reviews (for reviews, see Tsutsui Profiles in Comparative Endocrinology, 2016; Tsutsui, 2016 and others).

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1999, 2000, 2003a, 2006; Tsutsui and Mellon, 2006; Mellon and Vaudry, 2001; Do-Rego et al., 2009). The biosynthesis of neurosteroids in the brain was originally discovered in mammals based on the first seminal observation by Baulieu and his colleagues who found that certain steroid hormones, such as pregnenolone, dehydroepiandrosterone and their sulfate esters, are present in higher amounts in the brain than in the plasma and their levels in the brain do not change after adrenalectomy and castration (Corpéchot et al., 1981; Corpéchot et al., 1983; Robel and Baulieu, 1985; Lanthier and Patwardhan, 1986; Robel et al., 1986; Robel et al., 1987; Jo et al., 1989; Mathur et al., 1993; Compagnone et al., 1995; Baulieu and Robel, 1998). Subsequently, the biosynthesis of neurosteroids in the brain has been demonstrated in other vertebrates, such as birds, amphibians, fish from the studies of several groups of Tsutsui, Schlinjer, Soma, Kah and Vaudry as follows (*in birds*: Tsutsui and Yamazaki, 1995; Usui et al., 1995; Vanson et al., 1996; Tsutsui et al., 1997a,b, 1999, 2003a; London et al., 2003, 2006, 2010; London and Schlinger, 2007; Schlinger et al., 1999; Ukena et al., 1999a, 2001; Freking et al., 2000; Matsunaga et al., 2001, 2002; Tsutsui and Schlinger, 2001; Soma et al., 2004; *in amphibians:* Beaujean et al., 1999; Bruzzone et al., 2010; Do-Rego et al., 2007; Mensah-Nyagan et al., 1994, 1996a, 1996b, 1999, 2001; Takase et al., 1999, 2002, 2011; Inai et al., 2003; Matsunaga et al., 2004a, 2004b; *in fish*: Brion et al., 2012; Diotel et al., 2011; Menuet et al., 2005; Sakamoto et al., 2001a). Thus, the biosynthesis of neurosteroids from cholesterol in the brain is highly conserved across vertebrate species, from fish to mammals.

To understand the biosynthesis and biological actions of neurosteroids in the brain, identification of neurosteroidogenic cells is essential. Data on temporal- and cell-specific synthesis of neurosteroids are useful for the investigation of the potential actions of neurosteroids in the brain. Glial cells, such as oligodendrocytes and astrocytes, were first accepted to be the primary site for the biosynthesis of neurosteroids in the brain of mammals (for reviews, see Baulieu, 1997; Compagnone and Mellon, 2000). On the other hand, neuronal biosynthesis of neurosteroids in the brain was unknown for a while in any vertebrate.

In the mid 1990s, Tsutsui and his colleagues discovered that the Purkinje cell, an important cerebellar neuron, is a major neurosteroidogenic cell in the brain of various vertebrates (*in birds*: Tsutsui and Yamazaki, 1995; Usui et al., 1995; *in mammals*: Ukena et al., 1998; Ukena et al., 1999b; Sakamoto et al., 2003a; *in amphibians:* Takase et al., 1999; Matsunaga et al., 2001; *in fish*: Sakamoto et al., 2001a). The Purkinje cell is known to play an important role as a cerebellar neuron in the process of memory and learning and other cerebellar functions (Manto et al., 2018). Discovery of the biosynthesis of neurosteroids in the Purkinje cell was the first finding of *de novo* neuronal neurosteroidogenesis in the brain. In mammals, the Purkinje cell expresses several key 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 *de novo* from cholesterol during cerebellar development (Furukawa et al., 1998; Ukena et al., 1998; Ukena et al., 1999b). The Purkinje cell also produces allopregnanolone (3α,5α-tetrahydroprogesterone), a progesterone metabolite, by the steroidogenic enzymes, 5α-reductase and 3α-HSD, during cerebellar development (Tsutsui and Ukena, 1999; Tsutsui et al., 2003b; Tsutsui et al., 2003c; Tsutsui et al., 2004). Subsequently, studies of the Purkinje cells have demonstrated the biological actions of progesterone on the growth of Purkinje cells (Sakamoto et al., 2001b; Sakamoto et al., 2002; Ghoumari et al., 2003; Sakamoto et al., 2003b) and of allopregnanolone on the survival of Purkinje cells during cerebellar development (Griffin et al., 2004). Furthermore, the Purkinje cell expresses cytochrome P450 aromatase (P450arom), a key enzyme for estrogen formation, and produces estradiol during cerebellar development (Sakamoto et al., 2003a; Tsutsui et al., 2003b). Biological actions of estradiol on the growth of Purkinje cells during development have also been demonstrated (Sakamoto et al., 2003a; Sasahara et al., 2007). Thus, the biosynthesis and biological actions of neurosteroids in the Purkinje cell is now established in vertebrates.

At beginning of the 2010s, Tsutsui and his colleagues further discovered the biosynthesis of various neurosteroids *de novo* from cholesterol in the pineal gland, an endocrine organ located close to the cerebellum (Haraguchi et al., 2012; Hatori et al., 2011). The discovery of pineal neurosteroids has built a new concept of the biosynthesis of neurosteroids because, for the past thirty years, it was generally believed that only neurons and glial cells in the central and peripheral nervous systems produce neurosteroids (for reviews, see Baulieu, 1997; Compagnone and Mellon, 2000; Do-Rego et al., 2009; Mellon and Vaudry, 2001; Tsutsui and Mellon, 2006; Tsutsui et al., 1999, 2000, 2003b, 2003c, 2006). Importantly, allopregnanolone, a progesterone metabolite, is produced as a major pineal neurosteroid and secreted from the pineal gland during cerebellar development (Haraguchi et al., 2012; Hatori et al., 2011). Follow-up studies have demonstrated that pineal allopregnanolone is involved in the survival of Purkinje cells by suppressing the activity of caspase-3, a crucial mediator of apoptosis during cerebellar development (Haraguchi et al., 2012; for a review, see Tsutsui, 2016).

Herein the author describes the advances made in our understanding of the biosynthesis and biological actions of cerebellar and pineal neurosteroids on Purkinje cells during cerebellar development by new findings obtained by extensive studies over the past three decades.

2. Discovery of the cerebellar Purkinje cell as a major site for the biosynthesis of neurosteroids in the brain

Up to now, knowledge of cerebellar functions is increasing considerably. Studies of the cerebellum are now a central focus in neurobiology. During the last four decades, many laboratories worldwide have dedicated their researches to understand various roles of the cerebellum. It has been established that the Purkinje cell is an important cerebellar neuron that contributes to several cerebellar functions. In the mid 1990s, Tsutsui and his colleagues discovered *de novo* neuronal biosynthesis of neurosteroids from cholesterol in the Purkinje cell using immunohistochemical and biochemical techniques. It is well known that pregnenolone, a 3β-hydroxy-Δ^5-steroid, is the main precursor of steroid hormones and the biosynthesis of pregnenolone is initiated by the cleavage of the cholesterol side-chain by cytochrome P450scc, a rate-limiting mitochondrial enzyme. Immunohistochemical studies in quail using an antibody against cytochrome P450scc indicated the location of intense immunoreactive somata and dendrites of Purkinje cells (Tsutsui and Yamazaki, 1995; Usui et al., 1995). The presence of cytochrome P450scc was also found in Purkinje cells by Western immunoblot analysis (Tsutsui and Yamazaki, 1995; Usui et al., 1995). This is the first evidence for the neuronal location of cytochrome P450scc in the brain.

To put avian findings into a broader perspective, Tsutsui and his colleagues demonstrated the expression of cytochrome P450scc in the Purkinje cell of mammals (Ukena et al., 1998) (Fig. 1). Cytochrome P450scc is expressed in the Purkinje cell immediately after its differentiation, and the expression of this enzyme persists during neonatal development until adulthood in rats, indicating constant production of pregnenolone in Purkinje cells throughout life (Ukena et al., 1998). In addition to birds and mammals, Tsutsui and his colleagues further found the expression of cytochrome P450scc in the Purkinje cell of amphibians (Takase et al., 1999). It thus appears that the Purkinje cell expresses cytochrome P450scc and produces pregnenolone from cholesterol across vertebrates (Fig. 1). Purkinje cells also express steroidogenic acute regulatory protein (StAR) (Furukawa et al., 1998) (Fig. 1). StAR is known as an important factor for the transport of cholesterol to the inner mitochondrial membrane that localizes cytochrome P450scc (Clark et al., 1994). Accordingly, StAR contributes to the regulation of pregnenolone biosynthesis in the Purkinje cell during neonatal development. These findings provide new concept of the

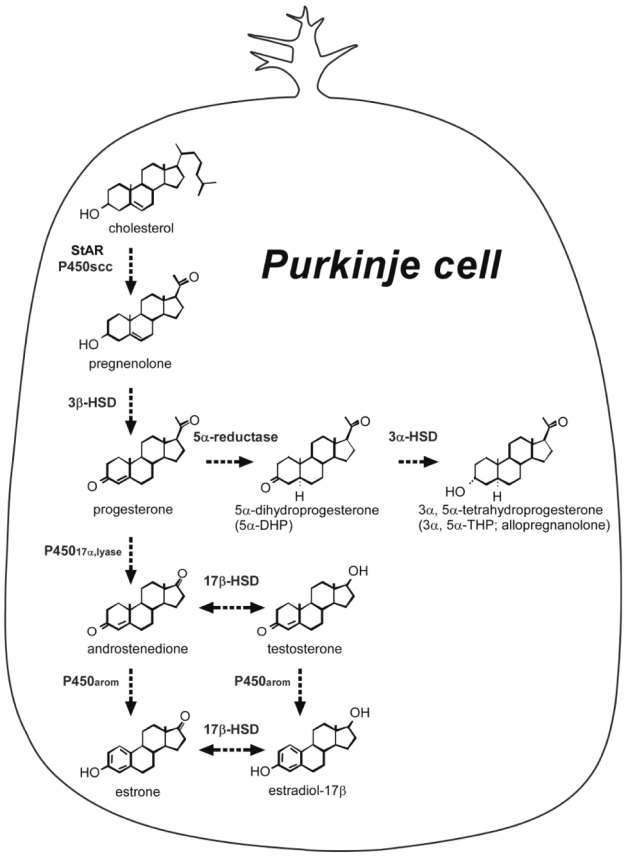

Fig. 1. Biosynthesis of neurosteroids in the cerebellar Purkinje cell. The Purkinje cell, an important cerebellar neuron, is a major site for the biosynthesis of neurosteroids in the brain. The Purkinje cell exresses several steroidogenic enzymes that produces a variety of neurosteroids. The Purkinje cell produces progesterone from pregnenolone actively due to an increase in 3β-HSD activity during neonatal development. Allopregnanolone is also metabolized by the enzymes 5α-reductase and 3α-HSD from progesterone during neonatal development. The Purkinje cell further expresses $P450_{17\alpha,lyase}$ and P450arom, and actively produces estradiol-17β during neonatal development. 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. See the text for details.

biosynthesis of pregnenolone, a main precursor of steroids, in brain neurrons.

3. Biosynthesis of neurosteroids in the cerebellar Purkinje cell during development

3.1. Biosynthesis of progesterone in the Purkinje cell

After the discovery of pregnenolone biosynthesis in the Purkinje cell, it has been demonstrated that the Purkinje cell is a major site of the biosynthesis of various neurosteroids in the brain of vertebrates including mammals. From the extensive studies on mammals, the colocalization of several kinds of key steroidogenic enzymes in this neuron during development has been demonstrated. Progesterone is traditionally known as a sex steroid hormone produced in the gonad and acting on the brain. However, in the late 1990s, Tsutsui and his colleagues demonstrated *de novo* progesterone biosynthesis from cholesterol in the Purkinje cell of rats during neonatal development. Because the biosynthesis of progesterone from pregnenolone is catalyzed by 3β-HSD, a membrane-bound mitochondrial enzyme, the demonstration of the expression of 3β-HSD in the rat Purkinje cell is essential to establish the concept of *de novo* progesterone biosynthesis from cholesterol in the Purkinje cell in mammals. Tsutsui and his colleagues found that, in rats, Purkinje cells express not only cytochrome P450scc but also 3β-HSD by RT-PCR, biochemical analyses and *in situ* hybridization (Ukena et al., 1999b). Thus, the Purkinje cell of rats expresses both cytochrome P450scc and 3β-HSD (Fig. 1). The expression of 3β-HSD in the Purkinje cell is also evident in other vertebrates (Tsutsui et al., 1999; Sakamoto et al., 2001a). Surprisingly, the expression of 3β-HSD in rat Purkinje cells increases during neonatal development, unlike a constant expression of cytochrome P450scc (Ukena et al., 1998; Ukena et al., 1999b). An increase in the biosynthesis of progesterone in Purkinje cells during neonatal development was also demonstrated by biochemical studies together with HPLC analysis in rats (Ukena et al., 1999b). Based on these findings, it appears that the Purkinje cell actively produces progesterone as a product of an increase in 3β-HSD activity during neonatal development (Ukena et al., 1999b) (Fig. 1).

3.2. Biosynthesis of allopregnanolone in the Purkinje cell

In addition, the biosynthesis of allopregnanolone (3α,5α-tetrahydroprogesterone), a progesterone metabolite, in the cerebellum was demonstrated by biochemical analyses such as HPLC and TLC (Tsutsui and Ukena, 1999; Tsutsui et al., 2003a; Tsutsui et al., 2003b; Tsutsui et al., 2003c; Tsutsui et al., 2004) (Fig. 1). Importantly, biochemical and immunohistochemical analyses showed that the Purkinje cell metabolizes progesterone to allopregnanolone by 5α-reductase and 3α-HSD during neonatal development (Fig. 1). Taken together, the Purkinje cell expresses cytochrome P450scc, 3β-HSD, 5α-reductase and 3α-HSD, and produces allopregnanolone *de novo* from cholesterol during neonatal development (Fig. 1).

3.3. Biosynthesis of estradiol in the Purkinje cell

After the demonstration of the biosyntheses of pregnenolone, progesterone and allopregnanolone in the Purkinje cell, the biosynthesis of estradiol in the Purkinje cell was further investigated. Estradiol is also known to be a sex steroid that acts on the brain. Cytochrome P450arom is a key steroidogenic enzyme of estrogen biosynthesis. It was found that in rats the Purkinje cell expresses cytochrome P450arom during neonatal development (Sakamoto et al., 2003a) (Fig. 1). RT-PCR and *in situ* hybridization analyses also showed the expression of cytochrome P450arom mRNA not only in Purkinje cells but also in external granule cells in the cerebellum of neonatal rats (Sakamoto et al., 2003a). It was further found that the Purkinje cell expresses cytochrome P450 17α-hydroxylase/c17,20-lyase ($P450_{17\alpha,lyase}$), which converts progesterone to androstenedione, an immediate precursor of estrogen formed by cytochrome P450arom (Matsunaga et al., 2001; Sakamoto et al., 2003a) (Fig. 1). Accordingly, these findings indicate the biosynthesis of estradiol in the Purkinje cell during neonatal development (Fig. 1).

4. Biological actions of progesterone, allopregnanolone and estradiol produced in the cerebellar Purkinje cell on the growth and survival of Purkinje cells during development

4.1. Biological actions of progesterone produced in the Purkinje cell

To analyze biological actions of neurosteroids in the brain, data on the regio- and temporal-specific synthesis of neurosteroids are essential. Because Purkinje cell is a major site for the biosynthesis of neurosteroids in the brain, this neuron has served as an excellent cellular model for the study of biological actions of neurosteroids. As mentioned above, the Purkinje cell of rats actively synthesizes progesterone during neonatal development (Ukena et al., 1999b) (Fig. 1). The Purkinje cell also synthesizes the progesterone metabolite allopregnanolone in neonatal rats (Tsutsui and Ukena, 1999; Tsutsui et al., 2003b; Tsutsui et al.,

2003c; Tsutsui et al., 2004). Marked morphological changes are known to occur in the cerebellum of rats during neonatal development (Altman, 1972a; Altman, 1972b). Based on the findings of Altman, the Purkinje cell differentiates around birth and the formation of the cerebellar cortex becomes complete during neonatal development through the processes of neuronal and glial growth, synaptogenesis and migration of external granule cells in rats (Altman, 1972a; Altman, 1972b). Thus, cerebellar development is remarkable during neonatal life, when the biosynthesis of progesterone and allopregnanolone in the Purkinje cell increases in neonatal rats (Tsutsui and Ukena, 1999; Ukena et al., 1999b; Tsutsui et al., 2003b; Tsutsui et al., 2003c; Tsutsui et al., 2004). Based on these observations, Tsutsui and his colleagues hypothesized that progesterone and/or allopregnanolone play important roles in the formation of cerebellar neuronal circuits by promoting neuronal growth and neuronal synaptic contact in the cerebellum during neonatal development.

To test this hypothesis, Tsutsui and his colleagues investigated the biological actions of progesterone and allopregnanolone, which are actively produced as major neurosteroids in the Purkinje cell during neonatal development, on neuronal growth, spinogenesis and synaptogenesis in the developing cerebellum. *In vitro* studies using cultured cerebellar slices of neonatal rats showed that progesterone promotes dendritic growth and dendritic spine formation of the Purkinje cell (Sakamoto et al., 2001b; Sakamoto et al., 2002) (Fig. 2). A similar result was obtained by *in vivo* studies using neonatal rats (Sakamoto et al., 2001b; Sakamoto et al., 2002). It was also found that administration of the progesterone receptor (PR) antagonist mifepristone (RU486) to neonatal rats inhibits dendritic growth and dendritic spine formation of the Purkinje cell (Sakamoto et al., 2001b; Sakamoto et al., 2002). Electron microscopic analysis further showed that progesterone induces an increase of dendritic spine synapses on the Purkinje cell of neonatal rats (Sakamoto et al., 2001b; Sakamoto et al., 2002) (Fig. 2). In contrast to progesterone, allopregnanolone does not affect the growth of Purkinje cells during neonatal development (Sakamoto et al., 2001b; Sakamoto et al., 2002). These findings demonstrate that progesterone synthesized in the Purkinje cell promotesthe dendritic growth, spinogenesis and synaptogenesis of Purkinje cells during cerebellar development (Fig. 2). On the other hand, it has been reported the protection of Purkinje cells from developmental cell death by the anti-progesterone RU486 that suggests a role of progesterone in the protection of Purkinje cells (Ghoumari et al., 2003). However, progesterone did not possess any effect on the survival of Purkinje cells in this study (Ghoumari et al., 2003).

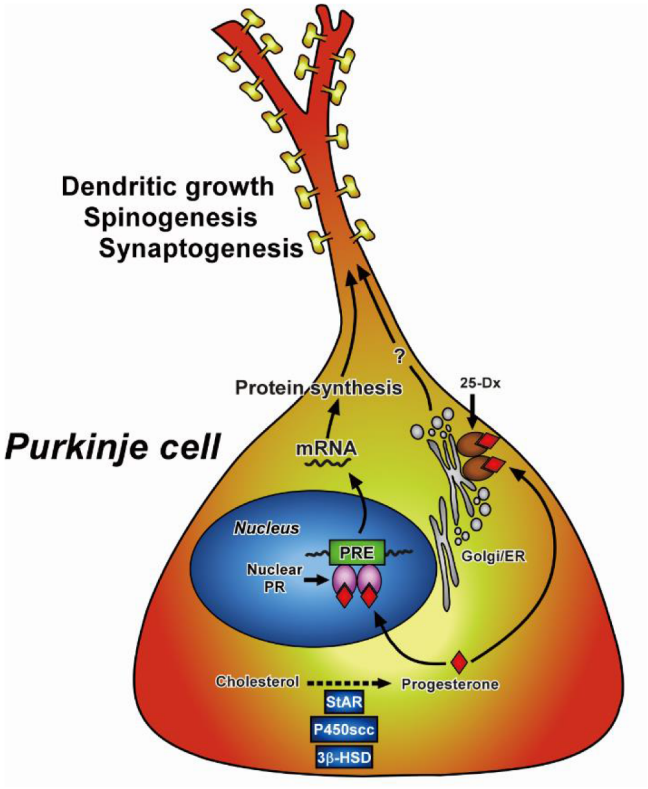

Fig. 2. Mode of action of progesterone produced in the Purkinje cell on the growth of Purkinje cells during cerebellar development. Progesterone produced in the Purkinje cell acts on the Purkinje cell through intranuclear receptor (PR)-mediated mechanisms that promote dendritic growth, spinogenesis and synaptogenesis in this neuron by genomic mechanisms during neonatal development. Progesterone may induce the expression of some neurotrophic factors that directly promote Purkinje dendritic growth, spinogenesis and synaptogenesis during neonatal development. Progesterone may also act on Purkinje cells through the mechanisms mediated by 25-Dx, a putative membrane progesterone receptor, which is associated with the 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. Adapted from Tsutsui, 2008a. See the text for details.

4.2. Biological actions of allopregnanolone produced in the Purkinje cell

As mentioned above, the Purkinje cell metabolizes progesterone to allopregnanolone during cerebellar development (Tsutsui and Ukena, 1999; Tsutsui et al., 2003b; Tsutsui et al., 2003c; Tsutsui et al., 2004). It has been reported that allopregnanolone, a progesterone metabolite, is involved in the survival of Purkinje cells and granule cells (Griffin et al., 2004), although allopregnanolone does not promote the dendritic growth, spinogenesis and synaptogenesis of Purkinje cells during cerebellar development (Sakamoto et al., 2001b; Sakamoto et al., 2002). For investigating the biological action of allopregnanolone, the Niemann-Pick type C (NP-C) mouse has been used as an excellent animal model (Griffin et al., 2004). NP-C is an autosomal recessive, childhood neurodegenerative disease characterized by defective intracellular cholesterol trafficking, resulting in the degeneration of Purkinje cells. Importantly, the brain of NP-C mice contains less allopregnanolone than that of wild-type mice (Griffin et al., 2004). In addition, administration of allopregnanolone to neonatal NP-C mice increases Purkinje cell survival and delays neurodegeneration (Griffin et al., 2004). Thus, allopregnanolone is considered to be an important neurosteroid for Purkinje cell survival during cellebellar development (Fig. 6).

4.3. Biological actions of estradiol produced in the Purkinje cell

The Purkinje cell highly expresses cytochrome P450arom, a key steroidogenic enzyme of estrogen formation, during cellebelar development, as mentioned above (Sakamoto et al., 2003a). Therefore, Tsutsui and his colleagues investigated biological actions of estradiol produced in the Purkinje cell on the growth of this neuron by *in vitro* and *in vivo* studies using neonatal rats. It was found that administration of estradiol to neonatal rats promotes the dendritic growth of Purkinje cells (Sakamoto et al., 2003a). Administration of estradiol to neonatal rats also increases Purkinje dendritic spines (Sakamoto et al., 2003a) and spine synapses (Sasahara et al., 2007). Tamoxifen, an estrogen receptor (ER) antagonist, inhibits these effects of estradiol (Sakamoto et al., 2003a; Sasahara et al., 2007). Thus, not only progesterone but also estradiol promotes the dendritic growth, spinogenesis and synaptogenesis of the Purkinje cell during cellebelar development (Fig. 3).

4.4. New concept of the biological action of progesterone and estradiol in the brain

For a long time, it was generally believed that the brain is a target

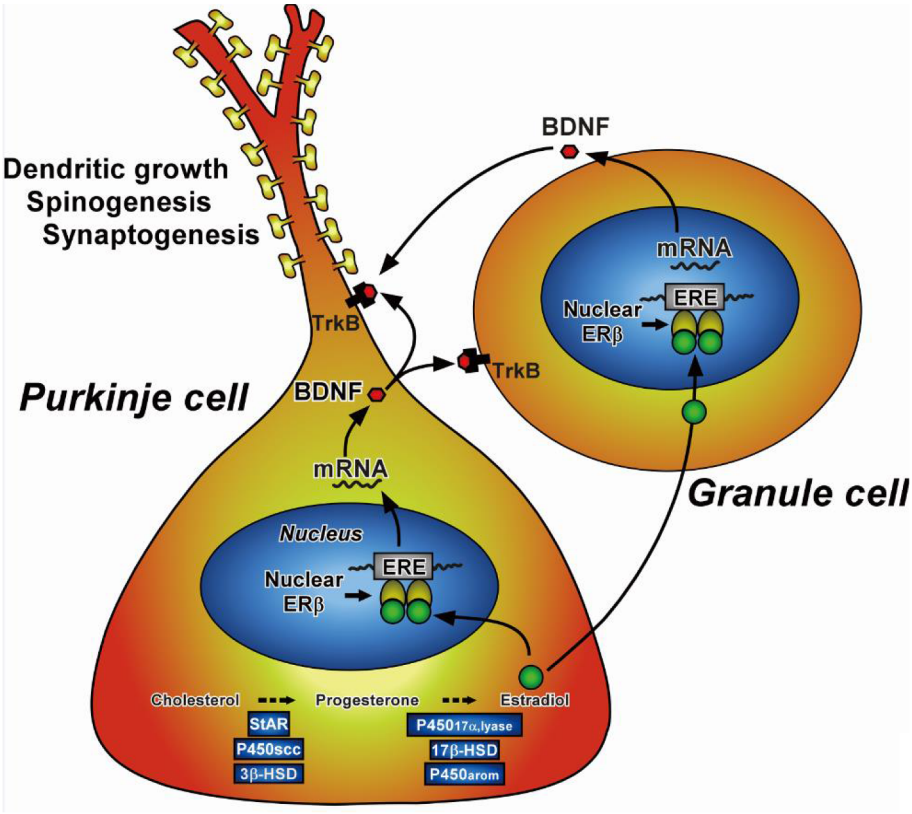

Fig. 3. Mode of action of estradiol produced in the Purkinje cell on the growth of Purkinje cells during cerebellar development. Estradiol produced in the Purkinje cell acts on the Purkinje cell through intranuclear receptor (Erβ)-mediated mechanisms that promote dendritic growth, spinogenesis and synaptogenesis in this neuron by genomic mechanisms during neonatal development. 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 during neonatal development. 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. Adapted from Tsutsui, 2008b. See the text for details.

site of peripheral sex steroids, such as progesterone and estradiol. The discovery of the Purkinje cell as a major site for the biosynthesis of progesterone and estradiol as neurosteroids has changed our classical understanding of the biosynthesis and biological actions of these sex steroids. Progesterone and estradiol produced in the Purkinje cell act directly on this neuron to promote dendritic growth, spinogenesis and synaptogenesis. These intriguing findings provide a new concept of the biological action of progesterone and estradiol produced in the brain neuron.

5. Mode of action and functional significance of progesterone and estradiol produced in the cerebellar Purkinje cell on the growth of Purkinje cells during development

5.1. Mode of action and functional significance of progesterone produced in the Purkinje cell

To elucidate the mode of action of progesterone produced in the Purkinje cell, Tsutsui and his colleagues characterized the expression of PR in the cerebellum during cerebellar development. Interestingly, it was found that the Purkinje cell expresses intranuclear PR-A and PR-B in neonatal rats (Sakamoto et al., 2001b; Sakamoto et al., 2002; Sakamoto et al., 2003b). Accordingly, progesterone can act directly on the Purkinje cell through intranuclear receptor-mediated mechanisms to promote Purkinje dendritic growth, spinogenesis, and synaptogenesis during cerebellar development (Sakamoto et al., 2001b; Sakamoto et al., 2002; Sakamoto et al., 2003b) (Fig. 2) The biosynthesis of progesterone and the expression of intranuclear PR-A and PR-B PR in the Purkinje cell indicate the 'intracrine' action of progesterone (Sakamoto et al., 2001b; Sakamoto et al., 2002; Sakamoto et al., 2003b) (Fig. 2). The 'intracrine' concept of androgens was originally developed by Labrie et al (for a review, see Labrie et al., 2003).

On the other hand, the putative membrane progesterone receptor, 25-Dx, is also expressed in the Purkinje cell during cerebellar development (Sakamoto et al., 2004). In neonatal rats, 25-Dx is localized in membrane structures of the endoplasmic reticulum and Golgi apparatus in the Purkinje cell (Sakamoto et al., 2004) (Fig. 2). Therefore, progesterone may promote dendritic growth, spinogenesis and synaptogenesis *via* 25-Dx as well as intranuclear PR-A and PR-B in Purkinje cells during cerebellar development (Sakamoto et al., 2008) (Fig. 2). 25-Dx is now named 'progesterone receptor membrane component 1' (PGRMC1). There are several reports showing that PGRMC1 mediates the anti-apoptotic actions of progesterone that associate with plasminogen activator inhibitor RNA-binding protein-1 (PAIRBP1) (Engmann et al., 2006; Peluso, 2006; Peluso et al., 2006; Cahill, 2007). Future studies are needed to determine whether the promotion of Purkinje dendritic growth, spinogenesis and synaptogenesis by progesterone is mediated by both genomic and nongenomic mechanisms.

It is known that neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), are highly expressed in the cerebellum during development and are involved in the growth of Purkinje cells and granule cells (Rocamora et al., 1993; Ernfors et al., 1994; Neveu and Arenas, 1996; Schwartz et al., 1997; Bates et al., 1999). These findings indicate that BDNF and NT-3 are attractive candidate regulators of Purkinje dendrite and spine development. Accordingly, these neurotrophic factors may mediate the biological action of progesterone on Purkinje dendritic growth, spinogenesis and synaptogenesis during cerebellar development (for reviews, see Tsutsui, 2008a; Tsutsui, 2008b). In addition, it is considered that the biological action of progesterone on the promotion of Purkinje dendritic growth, spinogenesis and synaptogenesis is involved in the formation of the cerebellar neuronal circuit.

5.2. Mode of action and functional significance of estradiol produced in the Purkinje cell

To elucidate the mode of action of estradiol produced in the Purkinje cell, the identification of estrogen receptors (ERs) in the cerebellum is also essential. There are important reports showing that intranuclear Erβ is expressed in the Purkinje cell of neonatal rats (Price

and Handa, 2000; Jakab et al., 2001). Accordingly, estradiol can also act directly on the Purkinje cell through intranuclear ERβ-mediated mechanisms to promote Purkinje dendritic growth, spinogenesis and synaptogenesis during cerebellar development (Fig. 3).

Importantly, tamoxifen, an ER antagonist, inhibits the action of estrogen on Purkinje cells during cerebellar development (Sakamoto et al., 2003a; Sasahara et al., 2007). It is known that this anti-estrogen binds to ERs (ERα and ERβ) and activates transcription *via* activating protein-1 response elements (Webb et al., 1995) and blocks transcriptional activation through the classical estrogen response element (McDonnell et al., 1995; Paech et al., 1997). Accordingly, it is possible that the anti-estrogen tamoxifen blocks transcriptional activation of ERβ in the Purkinje cell during cerebellar development.

In addition to Purkinje cells, granule cells express ERβ in the cerebellum (Price and Handa, 2000; Jakab et al., 2001) (Fig. 3). Therefore, estradiol may act not only on Purkinje cells but also granule cells through intranuclear ERβ to promote dendritic growth, spinogenesis and synaptogenesis in Purkinje cells during cerebellar development (Fig. 3). In addition to these findings in the cerebellum, involvement of ERβ in the btrain function has been reported in the hippocampus of rats (Orikasa et al., 2002; Ikeda et al., 2003).

While ERβ appears to mediate the actions of estradiol in the Purkinje cell, there are reports showing that other receptors may also mediate estrogen actions in the hippocampus (Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992; Woolley and McEwen, 1994; Murphy and Segal, 1996; McEwen et al., 2001) and the hypothalamus (Pérez et al., 1993; Langub et al., 1994). The activation of *N*-methyl-D-aspartate (NMDA) receptors is required for the action of estradiol on hippocampal CA1 pyramidal cell dendrite spine density in adult female rats (Woolley and McEwen, 1994). Such nongenomic estrogen actions may lead to alterations in gene expression. Accordingly, it is considered that NMDA receptors may also mediate estrogen actions in the Purkinje cell. Future study will clarify whether the promotion of Purkinje dendritic growth, spinogenesis and synaptogenesis by estradiol is due to both genomic and nongenomic mechanisms.

Because BDNF and NT-3 are known to be critical factors for the development of Purkinje cells (Rocamora et al., 1993; Ernfors et al., 1994; Neveu and Arenas, 1996; Schwartz et al., 1997; Bates et al., 1999), Tsutsui and his colleagues therefore investigated the expressions of these neurotrophic factors in the cerebellum in response to estrogen actions during cerebellar development (Sasahara et al., 2007). Estrogen administration to neonatal wild-type (WT) mice or cytochrome P450arom knock-out (ArKO) mice provoked an increase of BDNF expression in the cerebellum, whereas the anti-estrogen tamoxifen caused a decrease in BDNF expression in WT mice similar to ArKO mice (Sasahara et al., 2007). ArKO mice used in the study (Sasahara et al., 2007) lack exons 1 and 2 and the proximal promoter region of the P450arom gene *cyp19* (cytochrome P450, family 19) (Honda et al., 1998). BDNF administration to tamoxifen-treated WT mice further showed an increase in Purkinje dendritic growth (Sasahara et al., 2007). In contrast to BDNF, estrogen administration failed to induce the change in the expression of NT-3 in the developing cerebellum (Sasahara et al., 2007). The NT-3 expression also does not change in ArKO mice (Sasahara et al., 2007). These findings indicate that estrogen actions on the promotion of dendritic growth, spinogenesis and synaptogenesis in the Purkinje cell are mediated by BDNF during cerebellar development (Fig. 3). In fact, the gene encoding BDNF contains a sequence similar to the canonical estrogen response element in estrogen-target genes (Sohrabji et al., 1995). In addition to the cerebellum, it has been reported that, in mammalian spinal neurons, BDNF increases the levels of synaptic vesicle proteins, such as synaptophysin and synapsin 1, which are reliable markers of synaptogenesis (Wang et al., 1995). In the CA1 region of the primate hippocampus, estrogen is known to increase presynaptic and postsynaptic proteins, such as syntaxin, synaptophysin and spinophilin (Choi et al., 2003). In addition, it has been reported that estrogen administration induces these synaptic proteins in the CA1 region of the hippocampus, and this effect is blocked by CI628, an anti-estrogen of the tamoxifen type (Brake et al., 2001). In the cerebellum, the expression of cytochrome P450arom is restricted to Purkinje cells and external granule cells during cerebellar development (Sakamoto et al., 2003a; Tsutsui, 2006a; Tsutsui, 2006b; Tsutsui and Mellon, 2006). There are several reports showing that both Purkinje cells and granule cells express BDNF (Hofer et al., 1990; Borghesani et al., 2002) and TrkB, a receptor for BDNF (Klein et al., 1990; Segal et al., 1995; Carter et al., 2002) (Fig. 3). Taken together, it is therefore considered 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 during cerebellar development (Sasahara et al., 2007; Tsutsui, 2008b) (Fig. 3).

To demonstrate the functional significance of estradiol in the Purkinje cell during cerebellar development, Tsutsui and his colleagues further investigated estrogen actions on dendritic growth, spinogenesis and synaptogenesis in the Purkinje cell using ArKO mice (Sasahara et al., 2007). Decreases of dendritic growth, spinogenesis and synaptogenesis in Purkinje cells during cerebellar development were shown by estradiol deficiency in ArKO mice (Sasahara et al., 2007). In contrast, estradiol administration to ArKO mice caused increases in Purkinje dendritic growth, spinogenesis and synaptogenesis during cerebellar development (Sasahara et al., 2007). Consequently, these findings support a physiological role for endogenous estrogens on the promotion of dendritic growth, spinogenesis and synaptogenesis in the Purkinje cell during cerebellar development.

In other brain regions, neurotrophic and neuroprotective actions of estrogens have been reported by studies using ArKO mice (Azcoitia et al., 1999; Carswell et al., 2005). For example, neuroprotective actions of estrogens on dentate gyrus neurons in the hippocampus are mediated by estrogen-induced insulin-like growth factor-1 (IGF-1) (Azcoitia et al., 1999), similar to neurotrophic actions of estrogens on Purkinje cells in the cerebellum mediated by estrogen-induced BDNF (Sasahara et al., 2007).

6. Discovery of the pineal gland as a major site for the biosynthesis of neurosteroids

Next, this review describes our recent discovery of pineal neurosteroids. It is well established that the pineal gland is a photosensitive endocrine organ located close to the cerebellum and transduces photoperiodic changes to the neuroendocrine system by rhythmic melatonin secretion in vertebrates. Until recently, the biosynthesis of neurosteroids in the pineal gland remained unknown in any vertebrate. However, at the beginning of 2010s, the pineal gland was discovered as a major site for the biosynthesis of neurosteroids that actively produces various neurosteroids *de novo* from cholesterol in birds by a series of experiments using molecular and biochemical techniques (Haraguchi et al., 2012; Hatori et al., 2011) (Fig. 4). The discovery of neurosteroids in the pineal gland has built a new concept of the biosynthesis of neurosteroids because for the past thirty years it was generally believed that neurosteroids are produced in neurons and glial cells in the brain and peripheral nervous system (for reviews, see Baulieu, 1997; Compagnone and Mellon, 2000; Do-Rego et al., 2009; Mellon and Vaudry, 2001; Tsutsui and Mellon, 2006; Tsutsui et al., 1999, 2000, 2003b, 2003c, 2006).

It was first found that cytochrome P450scc is highly expressed in the pineal gland of juvenile chickens (Hatori et al., 2011) and quail (Haraguchi et al., 2012) by RT-PCR analysis. The biosynthesis of pregnenolone from cholesterol was also found in the pineal gland of these juvenile birds by HPLC and GC–MS analyses (Hatori et al., 2011; Haraguchi et al., 2012). Immunohistochemical analysis further showed the localization of cytochrome P450scc in pinealocytes in the pineal gland (Haraguchi et al., 2012). Subsequently, it was found that other key steroidogenic enzymes, such as cytochrome $P450_{7\alpha}$, 3α- and 3β-

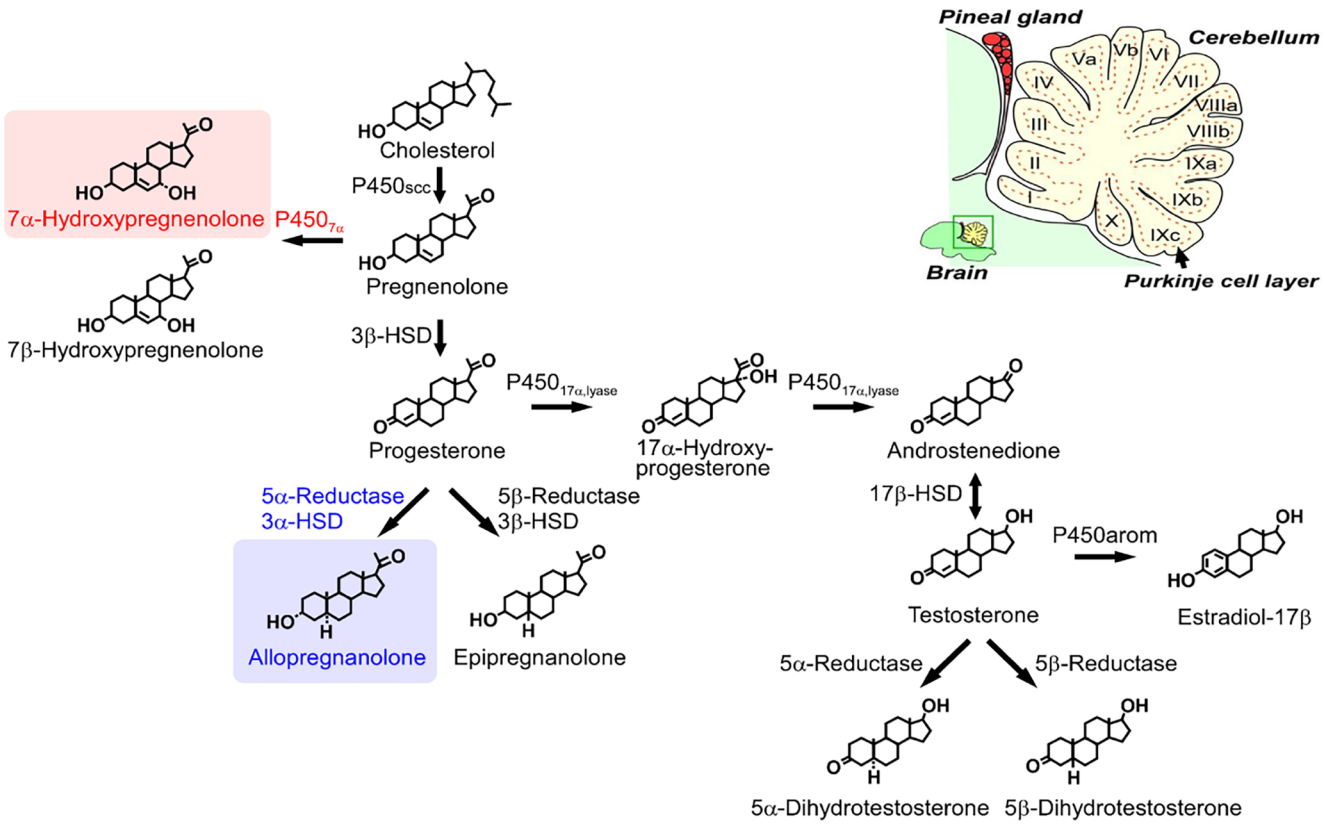

Fig. 4. Biosynthesis of neurosteroids in the pineal gland. The pineal gland, an endocrine organ located close to the cerebellum, expresses several steroidogenic enzymes. The pineal gland produces a variety of neurosteroids, such as pregnenolone, 7α- and 7β-hydroxypregnenolone (7α- and 7β-OH pregnenolone), progesterone, allopregnanolone, androstenedione, testosterone, 5α- and 5β-dihydrotestosterone, and estradiol-17β. Allopregnanolone and 7α-OH pregnenolone are the major pineal neurosteroids that are secreted from the pineal gland. P450scc, cytochrome P450 side-chain cleavage enzyme; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase; P450$_{17α,lyase}$, cytochrome P450 17α-hydroxylase/c17,20-lyase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; P450arom, cytochrome P450 aromatase. See the text for details.

HSD, 5α- and 5β-reductase, cytochrome P450$_{17α,lyase}$, 17β-hydroxysteroid dehydrogenase (17β-HSD) and cytochrome P450arom are expressed in the pineal gland of juvenile chickens (Hatori et al., 2011) and quail (Haraguchi et al., 2012) (Fig. 4). Based on tremendous amounts of data obtained by molecular and biochemical analyses using these juvenile birds, the biosynthetic pathways of neurosteroids in the pineal gland have been demonstrated in birds (Hatori et al., 2011; Haraguchi et al., 2012) (Fig. 4). It now appears that a variety of neurosteroids, such as pregnenolone, 7α- and 7β-hydroxypregnenolone (7α- and 7β-OH pregnenolone), progesterone, allopregnanolone, androstenedione, testosterone, 5α- and 5β-dihydrotestosterone, and estradiol-17β, are produced in the pineal gland (Fig. 4). These new findings provide the first evidence for *de novo* neurosteroidogenesis in the pineal gland in any vertebrate class.

7. Identification of major pineal neurosteroids during development

To clarify the biological actions of neurosteroids produced in the pineal gland, it is importnato to identify the major pineal neurosteroids. After the discovery of the biosynthesis of neurosteroids in the pineal gland, Tsutsui and his colleagues further demonstrated that allopregnanolone and 7α-OH pregnenolone are major neurosteroids produced in the pineal gland by biochemical studies combined with HPLC and GC–MS analyses (Haraguchi et al., 2012) (Fig. 4). Pregnenolone is converted primarily into allopregnanolone and 7α-OH pregnenolone in the pineal gland and the biosynthesis of these major pineal neurosteroids are higher in chicks than in adults (Haraguchi et al., 2012). Interestingly, the biosynthesis of allopregnanolone and 7α-OH pregnenolone is higher in the pineal gland than in the brain (Haraguchi et al., 2012). Importantly, the pineal gland releases significant amounts of pineal allopregnanolone and 7α-OH pregnenolone (Haraguchi et al., 2012; Hatori et al., 2011). It is therefore considered that allopregnanolone and 7α-OH pregnenolone are the major pineal neurosteroids that are secreted from the pineal gland of juvenile birds during development (Fig. 4).

8. Biological action of allopregnanolone produced in the pineal gland on the survival of cerebellar Purkinje cells during development

Bacause the two major pineal neurosteroids, allopregnanolone and 7α-OH pregnenolone, are abundantly released from the pineal gland during development (Haraguchi et al., 2012), these major pineal neurosteroids may play important roles in the avian brain during development. It has been reported that, in birds and mammals, pinealectomy (Px) induces cell loss in the brain including Purkinje cells during development (Kilic et al., 2002; Tunç et al., 2006). Based on this observation, Tsutsui and his colleagues investigated the biological action of allopregnanolone and 7α-OH pregnenolone produced in the pineal gland on the survival of Purkinje cells during cellebelar development by the follow-up studies using juvenile birds. The decrease in allopregnanolone concentration in the cerebellum and the induction of apoptosis of Purkinje cells were found by Px (Haraguchi et al., 2012). In addition, administration of allopregnanolone to Px birds increased allopregnanolone concentration in the cerebellum and prevented apoptosis of

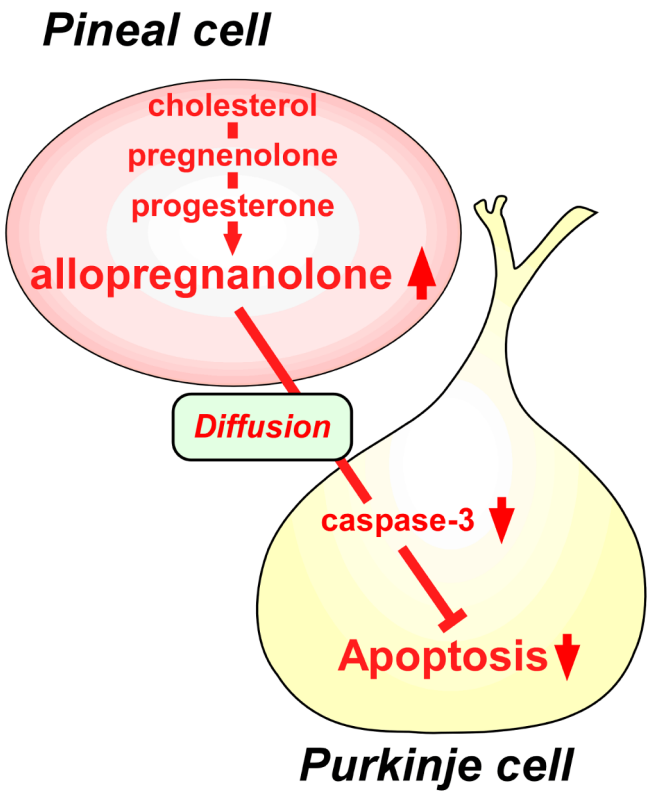

Fig. 5. Mode of action of allopregnanolone produced in the pineal gland on the survival of Purkinje cells during cerebellar development. Allopregnanolone that is actively produced in the pineal gland may affect adjacent cerebellar Purkinje cells by diffusion, and saves Purkinje cells from apoptosis in the developing cerebellum. Secreted pineal allopregnanolone inhibits the expression of active caspase-3 that facilitates apoptosis of Purkinje cells during cerebellar development. See the text for details.

Purkinje cells (Haraguchi et al., 2012). Injection of ^{3}H-allopregnanolone close to the pineal lumen showed that pineal allopregnanolone reaches Purkinje cells in the cerebellum by diffusion (Fig. 5) (Haraguchi et al., 2012). It thus appears that allopregnanolone secreted by the pineal gland acts, as a key factor, on Purkinje cell survival during cellebellar development (Fig. 5). This is a new function of the pineal gland for the prevention of Purkinje cell death in the developing cerebellum. In contrast to allopregnanolone, there was no effect of the other major pineal neurosteroid 7α-OH pregnenolone on the survival of Purkinje cells (Haraguchi et al., 2012). The discovery of the biological action of pineal allopregnanolone on the survival of cerebellar Purkinje cells during development has broadened the horizon of Neuroendocrinology.

9. Mode of action of allopreg nanolone produced in the pineal gland on the survival of cerebellar Purkinje cells during development

Subsequently, Tsutsui and his colleagues investigated the factor that mediates the neuroprotective action of pineal allopregnanolone on Purkinje cells to understand the mode of action of this neurosteroid on Purkinje cell survival. It is known that caspase-3 plays an important role as a crucial mediator of apoptosis in Purkinje cell death in vertebrates (Puig and Ferrer, 2001; Matsunaga et al., 2004a; Olkowski et al., 2008). Interestingly, Px increases the number of Purkinje cells that express active caspase-3 in juvenile birds and administration of allopregnanolone to Px birds decreases the number of Purkinje cells expressing active caspase-3 (Haraguchi et al., 2012). These findings indicate that the neuroprotective action of pineal allopregnanolone on Purkinje cells is accompanied with the decrease in caspase-3 activity during cerebellar development. It is therefore considered that, allopregnanolone produced in the pineal gland exerts an antiapoptotic action on Purkinje cells in the cerebellum by suppressing caspase-3 activity during development (Fig. 5).

It is unclear whether the action of pineal allopregnanolone on caspase-3 activity in the Purkinje cell is rapid (i.e., mediated through a membrane receptor) or slow (i.e., involving transcriptional activation). Similarly, the intracellular signaling pathway exerting a neuroprotective action of allopregnanolone produced in the brain remains unclear. However, the biological action of allopregnanolone produced in the brain is likely mediated through interaction with the pathway of γ-aminobutyric acid type A ($GABA_A$) receptor, because allopregnanolone is considered to be a potent allosteric modulator of $GABA_A$ receptor (Paul and Purdy, 1992; Lambert et al., 1995). Characterization of the mode of action of pineal allopregnanolone on the suppression of caspase-3 activity in the Purkinje cell during cerebellar development is now in progress to demonstrate the intracellular signaling pathway exerting a neuroprotective action of allopregnanolone in the Purkinje cell (Haraguchi et al., unpublished observation).

10. Functional significance of allopregnanolone produced in the pineal gland on the survival of cerebellar Purkinje cells during development

Investigating the regulatory mechanisms of the biosynthesis of pineal allopregnanolone has important implications for the understanding of the functional significance of pineal allopregnanolonein in the cerebellum. It is known that environmental light conditions influence brain development of vertebrates. For example, disruption by light stimuli affects cerebellar development. However, little is known about the molecular mechanisms behind how environmental light conditions affect cerebellar development. As described above, the pineal gland, a photosensitive organ located close to the cerebellum, actively produces allopregnanolone during cerebellar development. Recently, we found that the environmental light conditions change the biosynthesis of pineal allopregnanolone to mediate cerebellar development (Haraguchi et al., unpublished observation). It is therefore considered that pineal allopregnanolone contributes as important internal factor depending on the environmental light conditions to affect cerebellar development in vertebrates.

Nocturnal secretion of melatonin by the pineal gland is well known in photoperiodic vertebrates (Bronson, 1990). There is important evidence that melatonin modifies neurosteroid milieu in the brain (Tsutsui et al., 2008). Tsutsui and his colleagues has demonstrated that melatonin regulates the biosynthesis of 7α-OH pregnenolone in the diencephalon of birds (Tsutsui et al., 2008). Concomitant Px and orbital enucleation (Ex) provoke a marked increase in the biosynthesis of 7α-OH pregnenolone and stimulate the expression of cytochrome $P450_{7\alpha}$ producing 7α-OH pregnenolone in the quail diencephalon (Tsutsui et al., 2008). Reciprocally, melatonin administration to Px/Ex quail decreases the biosynthesis of 7α-OH pregnenolone and inhibits the expression of cytochrome $P450_{7\alpha}$ in the quail diencephalon (Tsutsui et al., 2008). The inhibitory effect of melatonin on the biosynthesis of 7α-OH pregnenolone is abolished by luzindole, a melatonin receptor antagonist (Tsutsui et al., 2008). These findings indicate that melatonin acts as an inhibitory factor for the biosynthesis of 7α-OH pregnenolone in the quail diencephalon (for a review, see Tsutsui et al., 2013a,b). Melatonin action on the biosynthesis of 7α-OH pregnenolone was also found in the newt diencephalon (for a review, see Tsutsui et al., 2013a). Therefore, these findings suggest that melatonin regulates the biosynthesis of allopregnanolone in the pineal gland. To establish this new molecular mechanisms of environmental light conditions affecting the survival of Purkinje cell during cerebellar development, future studies focused on the interaction of pineal allopregnanolone and melatonin are needed.

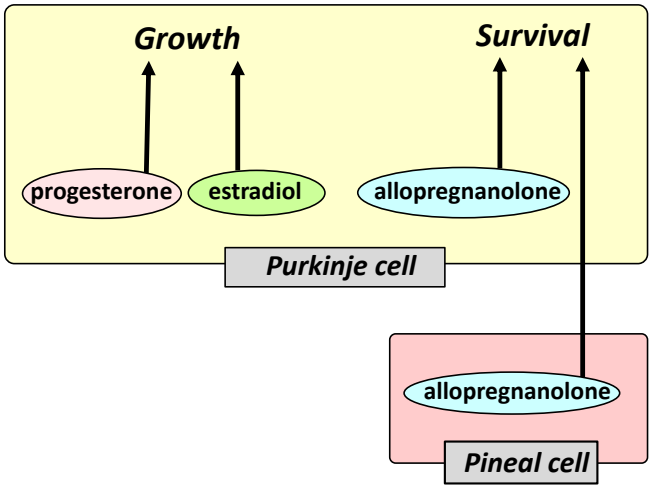

Fig. 6. Summay of biological actions of cerebellar and pineal neurosteroids on the growth and survival of Purkinje cells during cerebellar development. Progesterone and estradiol produced in the Purkinje cell promote dendritic growth, spinogenesis and synaptogenesis *via* each cognate nuclear receptor in the developing Purkinje cell. These neurosteroid actions that may be mediated by neurotrophic factors contribute to the formation of cerebellar neuronal circuit during cerebellar development. On the other hand, allopregnanolone, a major pineal neurosteroid, also acts on the cerebellum to maintain Purkinje cells by suppressing the activity of caspase-3, a crucial mediator of apoptosis, during cerebellar development. See the text for details.

11. Conclusions and future directions

The author described the discovery of cerebellar and pineal neurosteroids and their biological actions on the growth and survival of Purkinje cells during development in this review. The Purkinje cell, an important cerebellar neuron, is discovered as a major site for the biosynthesis of neurosteroids in the brain. This neuron actively synthesizes progesterone, allopregnanolone and estradiol *de novo* from cholesterol during cerebellar development when cerebellar neuronal circuit formation occurs (Fig. 6). Both progesterone and estradiol promote the dendritic growth, spinogenesis and synaptogenesis of the Purkinje cell during cerebellar development (Fig. 6). These actions of progesterone and estradiol are mediated by neurotrophic factors, such as BDNF, and may contribute to the formation of cerebellar neuronal circuit during cerebellar development. Allopregnanolone is also involved in the survival of Purkinje and granule cells (Fig. 6). Accordingly, the discovery of neurosteroids in the Purkinje cell has led us to re-evaluate our understanding about the mechanisms regulating neuronal development and survival in the cerebellum. Studies of neurosteroids produced in the Purkinje cell are currently of great interest to many researchers.

On the other hand, the pineal gland was also discovered as a major site for the biosynthesis of neurosteroids. The pineal gland actively produces neurosteroids *de novo* from cholesterol. This is a new concept of the biosynthesis of neurosteroids because for the past thirty years we believed that neurosteroids are produced only in neurons and glial cells in the brain and peripheral nervous system. Importantly, allopregnanolone, a major neurosteroid produced in the pineal gland, acts on the cerebellum to prevent the death of Purkinje cells in the cerebellum by suppressing the activity of caspase-3, a crucial mediator of apoptosis, during cerebellar development (Figs. 5 and 6). Accordingly, it is important to clarify the interaction of cerebellar and pineal allopregnanolone in the regulation of neuronal development and survival in the cerebellum during development. Future studies are also needed to investigate previously unknown interactions of other neurosteroids produced in the cerebellum and the pineal gland.

This review summarized new findings in the field of Comparative Endocrinology and Neuroendocrinology by the discoveries of cerebellar and pineal neurosteroids. These new findings that the author described in this review may become old with time. Therefore, it is necessary to continuously build new concepts through generations for the progress of this research field.

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Disclosure statement

The author has nothing to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2018.10.014.

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