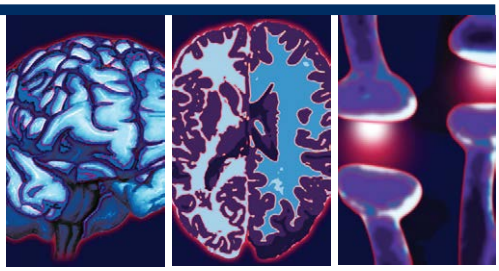
Review



Brain and Pineal 7α-Hydroxypregnenolone Regulating Locomotor Behavior: Discovery, Progress and Prospect

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Abstract

The brain synthesizes steroids *de novo* from cholesterol, which are called neurosteroids. The formation of neurosteroids in the brain is a conserved property across vertebrates, but it is still incompletely elucidated in vertebrates. In fact, 7α-hydroxypregnenolone (7α-OH PREG) was identified as a novel bioactive neurosteroid stimulating locomotor behavior in the brain of several vertebrates. Subsequently, the mode of action of 7α-OH PREG and the regulation of 7α-OH PREG synthesis in the brain have demonstrated by follow-up studies. Recently, it has been demonstrated that the pineal gland, an endocrine organ located close to the brain, is a major site of formation of bioactive neurosteroids. This is a new finding of the formation of neurosteroids because for a long time neurosteroids are believed to be produced in neurons and glial cells in the brain. In addition to the brain, the pineal gland actively produces 7α-OH PREG as a major pineal neurosteroid that acts on the brain to regulate locomotor rhythms. Thus, the discovery of 7α-OH PREG, a new bioactive neurosteroid, has provided a novel direction to investigate neurosteroid regulation of locomotor behavior. This review summarizes the discovery, progress and prospect of brain and pineal 7α-OH PREG regulating locomotor behavior in vertebrates.

Keywords

7α-hydroxypregnenolone (7α-OH PREG), Dopamine, Melatonin, Prolactin, Corticosterone, Brain, Pituitary gland, Pineal gland

Introduction

Extensive studies over the past thirty years have demonstrated that the brain produces steroids *de novo* from cholesterol (CHOL). Such steroids are called neurosteroids that regulate several brain functions, such as neuronal proliferation, activity and survival, and behavioral and neuroendocrine processes for reviews, see [1-9]. The formation of bioactive neurosteroids in the brain is a conserved property across vertebrates from mammals to fish [10-52]. However, the formation of bioactive neurosteroids in the brain is still incompletely elucidated in vertebrates for a review, see [5]. In fact, 7α-hydroxypregnenolone (7α-OH PREG) was identified as a novel bioactive neurosteroid stimulating locomotor behavior in the brain of various vertebrates, such as newts [41,53], quail [54] and fish [55] at the beginning of 2000s. The follow-up studies over the past decade have demonstrated that 7α-OH PREG produced in the brain of these vertebrates plays an important role in the regulation of locomotor behavior *via* the dopaminergic system for reviews [56-62].

On the other hand, it has been demonstrated that the pineal gland, an endocrine organ located close to the brain, actively produces various

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neurosteroids *de novo* from CHOL [63,64] at the beginning of 2010s. This is a new concept of neurosteroid formation because for the past thirty years neurosteroids were believed to be produced *de novo* from CHOL in neurons and glial cells in the brain for reviews [1-9]. Importantly, pineal 7α-OH PREG is actively secreted as a major pineal neurosteroid from the pineal gland and acts on the brain to control locomotor rhythms [63,64].

Herein we review the discovery, progress and prospect of brain and pineal 7α-OH PREG regulating locomotor behavior in vertebrates based on new findings obtained by recent studies.

Discovery of Brain and Pineal 7α-OH PREG Regulating Locomotor Behavior

■ Identification of brain 7α-OH PREG stimulating locomotor activity

At the beginning of 2000s, it was found that the brain of newts [41] and quail [54] actively produces 7α-OH PREG, a previously unknown neurosteroid, *de novo* from CHOL in these vertebrates, by biochemical techniques combined with high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). It was also found that the brain of salmon actively produces 7α-OH PREG *de novo* from CHOL [55]. Subsequently, cytochrome P450 7α-hydroxylase (cytochrome $P450_{7\alpha}$), which produces 7α-OH PREG from pregnenolone (PREG), was identified in the brain of these vertebrates [42,54,55] and demonstrated its enzymatic activity by HPLC and GC-MS using COS-7 cells transfected with the identified cytochrome $P450_{7\alpha}$ cDNA [42,54,55]. Because 7α-OH PREG is also produced in the brain of mammals [30,65-68], the production of 7α-OH PREG in the brain is considered to be a conserved property across vertebrates **(Figure 1)**.

Soon after the identification of 7α-OH PREG in the brain, the biological action of brain 7α-OH PREG was investigated in newts by analyzing locomotor behavior because 7α-OH PREG is actively synthesized in the diencephalon and rhombencephalon in the brain of newts [41] and quail [54]. Intracerebroventricular (ICV) injection of 7α-OH PREG increases locomotor activity of newts [41] and quail [54]. Based on these findings [41,54], it is considered that brain 7α-OH PREG acts on the brain to stimulate locomotor activity in newts and quail for reviews, see [56-61]. Subsequently, the biological action of brain 7α-OH PREG on upstream migratory behavior was investigated in salmon [55] because

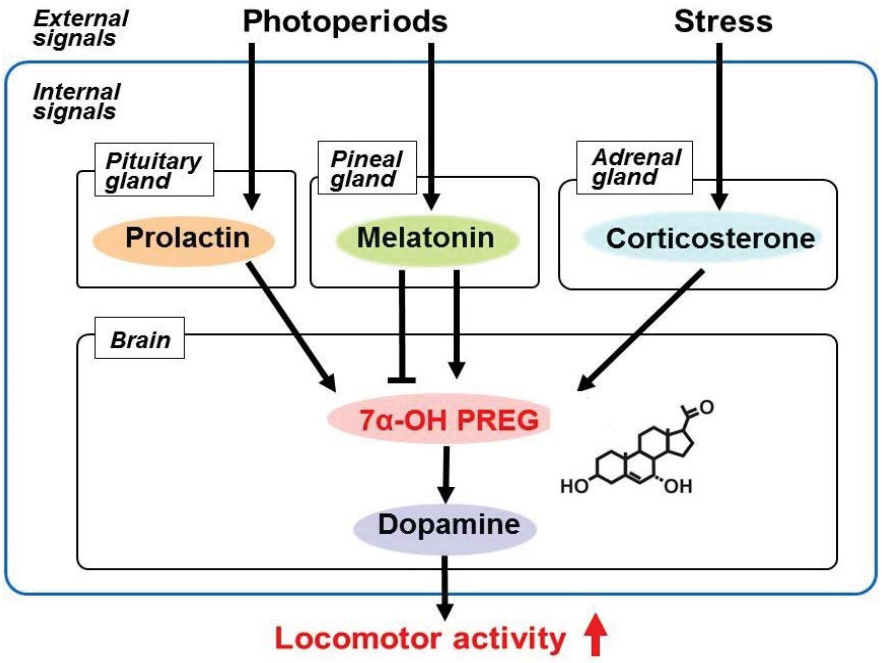

Figure 1: Mode of action and regulation of biosynthesis of brain 7α-OH PREG. 7α-OH PREG produced in the brain stimulates locomotor activity *via* the dopaminergic system in the brain. 7α-OH PREG synthesis in the brain is regulated by pineal melatonin, pituitary prolactin and adrenal corticosterone. Modified from Tsutsui *et al.*, Matsunaga *et al.*, Tsutsui *et al.* and Haraguchi *et al.* [41,53-61,89].

locomotor activity of salmon increases during upstream migration against an opposing current in their natal river [69-71]. Interestingly, 7α-OH PREG synthesis in the salmon brain increases during upstream migration and brain 7α-OH PREG stimulates upstream migratory behavior of salmon [55]. Thus, these findings indicate the stimulatory action of brain 7α-OH PREG on locomotor behavior in various vertebrates **(Figure 1)**.

■ Identification of pineal 7α-OH PREG regulating locomotor rhythms

It is well known that the pineal gland, an endocrine organ located close to the parietal region of the brain, transduces photoperiodic changes to the neuroendocrine system by rhythmic melatonin secretion in vertebrates for reviews, see [61,62,72]. Until recently, there was no report indicating the formation of neurosteroids in the pineal gland in any vertebrate. Importantly, at the beginning of 2010s, it has been demonstrated that the pineal gland is a major site of neurosteroidogenesis and actively produces a variety of neurosteroids *de novo* from CHOL in birds [63,64]. The discovery of pineal neurosteroids has built a new concept of the formation of neurosteroids because for the past thirty years neurosteroids are believed to be produced in neurons and glial cells in the brain for reviews, see [1-9].

In vertebrates, PREG, a common precursor of all steroid hormones, is formed by cleavage of the side-chain of CHOL by cytochrome P450scc. It was first found that the pineal gland of juvenile chickens and quail highly expresses cytochrome P450scc by RT-PCR analysis. It was further found that the pineal gland of these juvenile birds produces PREG from CHOL by HPLC and GC-MS analyses [63,64] **(Figure 2)**. In addition, immunohistochemical analysis showed that cytochrome P450scc is localized in pinealocytes [63].

It was further clarified that the pineal gland of juvenile chickens [64] and quail [63] expresses other steroidogenic enzymes, such as cytochrome $P450_{7\alpha}$, 3α- and 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3α- and 3β-HSD), 5α- and 5β-reductase, cytochrome P450 17α-hydroxylase/c17,20-lyase (cytochrome $P450_{17\alpha,lyase}$), 17β-hydroxysteroid dehydrogenase (17β-HSD) and cytochrome P450 aromatase (cytochrome P450arom). Based on molecular and biochemical analyses, the biosynthetic pathways of neurosteroids was demonstrated in the pineal gland of these juvenile birds [63,64]. Thus, the pineal gland produces a variety of neurosteroids *de novo* from CHOL, such as PREG, 7α- and 7β-OH PREG, progesterone (PROG), 3α,5α-tetrahydroprogesterone [3α,5α-THP/allopregnanolone (ALLO)], androstenedione (AD), testosterone (T), 5α- and 5β-dihydrotestosterone (5α- and 5β-DHT), and estradiol-17β (E2) in juvenile birds **(Figure 2)**.

By biochemical studies combined with HPLC and GC-MS analyses, it was further found that 7α-OH PREG and ALLO are major pineal neurosteroids. PREG is converted primarily into 7α-OH PREG and ALLO in the pineal gland and the production of these major pineal neurosteroids are higher in chicks than in adults. Surprisingly, 7α-OH PREG and ALLO are actively produced in the pineal gland and these major pineal neurosteroid are released from the

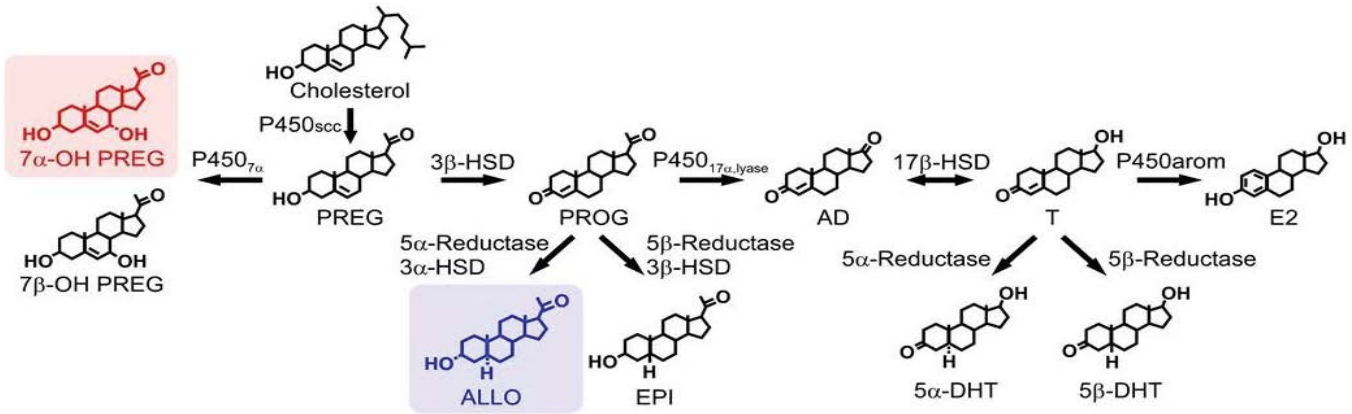

Figure 2: Biosynthetic pathways for pineal neurosteroids. The pineal gland expresses several key steroidogenic enzymes and produces various neurosteroids *de novo* from CHOL *via* PREG in juvenile birds. 7α-OH PREG and ALLO are major pineal neurosteroids that are secreted by the pineal gland and act on the brain. Modified from Haraguchi *et al.*, Haraguchi *et al.* and Tsutsui *et al.* [61-63,72].

pineal gland of juvenile birds [63,64] (Figure 2).

Subsequently, the biological action of pineal 7α-OH PREG, a major pineal neurosteroid, was investigated in juvenile birds [64]. It has been demonstrated that pineal 7α-OH PREG acts on the brain to regulate locomotor rhythms [54,64]. In addition, it has been demonstrated that the production of pineal 7α-OH PREG is stimulated by light depending on the time-of-day for the regulation of locomotor rhythms for reviews [61,62,72]. Mode of action of pineal 7α-hydroxypregnenolone regulating locomotor rhythms (Figure 3).

Progression of 7α-OH PREG Research

■ Regulation of the biosynthesis of brain 7α-OH PREG

The mechanisms that regulate the biosynthesis of 7α-OH PREG in the brain were then examined in vertebrates to understand the functional significance of brain 7α-OH PREG in the regulation of locomotor activity.

It was investigated whether 7α-OH PREG produced in the brain is involved in the regulation of diurnal locomotor changes because many vertebrates exhibit diurnal changes in locomotor activity for reviews, see [61,62,72]. Tsutsui et al. [54] found that male quail exhibit clear diurnal changes in locomotor activity and these changes occur in parallel with diurnal changes in 7α-OH PREG synthesis and concentration in the diencephalon. Tsutsui et al. [54] further found that administration of ketoconazole, an inhibitor of cytochrome P450s, to male quail abolishes diurnal changes in locomotor activity. Accordingly, it is possible that brain 7α-OH PREG controls diurnal changes in locomotor activity in male quail (Figure 1).

The pineal gland and eyes are known to regulate locomotor activity in birds [16,73-78]. Therefore, it was investigated whether melatonin regulates diurnal changes in brain 7α-OH PREG synthesis and thereby induces diurnal changes in locomotor activity in male quail. Tsutsui et al. [54] found that the synthesis and concentration of 7α-OH PREG increase in the brain of male quail after melatonin removal by pinealectomy (Px), combined with orbital enucleation (Ex). Conversely, the synthesis and concentration of 7α-OH PREG decrease in the brain of male quail after the administration of melatonin [54]. Thus, it appears that melatonin secreted by the pineal gland and eyes inhibits brain 7α-OH PREG synthesis in male quail [54] (Figure 1).

Quail is a diurnal animal and melatonin secretion is low during the light period. It is possible that the decrease in melatonin secretion during the light period induces an increase in brain 7α-OH PREG synthesis, and consequently locomotor activity increases during the light period in the quail for reviews [56-58,62]. By contrast, the

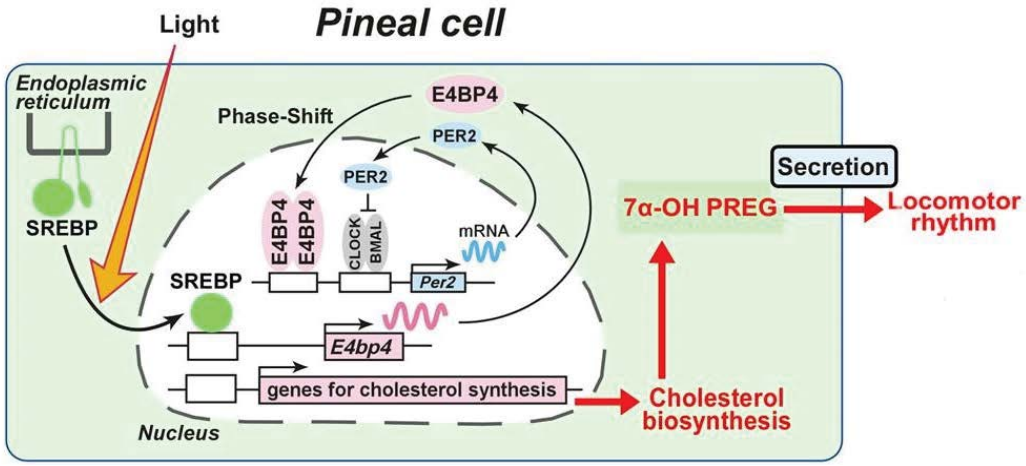

Figure 3: Mode of action and regulation of biosynthesis of pineal 7α-OH PREG. The circadian clock regulates daily locomotor rhythm in birds. The phase of the circadian clock is adjusted by the changes in light in a time-of-day-dependent manner in birds. A light pulse given at early night activates formation of the active form of SREBP, resulting in induction of a number of genes governing CHOL synthesis as well as a clock gene *E4bp4*. E4BP4 is a transcription factor that regulates the circadian clock through repression of clock gene *Per2*. SREBP-mediated induction of CHOL synthetic genes enhances the synthesis of CHOL and leads to the synthesis of 7α-OH PREG, a neurosteroid stimulating locomotor activity. Thus, the biosynthesis of pineal 7α-OH PREG is stimulated by light depending on the time-of-day for the regulation of locomotor rhythms. Adapted from Tsutsui *et al.*, Tsutsui *et al.*, Hatori *et al.*, and Tsutsui *et al.* [54,61-64,72].

synthesis and concentration of brain 7α-OH PREG in the newt, a nocturnal animal, increases during the dark period when locomotor activity is high for reviews [56-58,62]. In contrast to the quail, melatonin stimulates the synthesis and concentration of brain 7α-OH PREG in the newt for reviews [56-58,62]. Thus, the increase in melatonin secretion during the dark period increases brain 7α-OH PREG synthesis, and consequently locomotor activity increases during the dark period in the newt, a nocturnal animal for reviews [56-58,62] **(Figure 1)**.

Many migratory birds migrate at night, although most of them are diurnal outside the migratory seasons. Because during the migratory periods birds showing nocturnal migratory restlessness (Zugunruhe) have reduced melatonin concentrations at night than non-migratory periods, the involvement of melatonin on Zugunruhe has been hypothesized for reviews, see [58,61]. Because 7α-OH PREG appears to control diurnal locomotor rhythms by the interaction of melatonin in birds, it is possible that reduction of melatonin in migratory period birds at night may affect 7α-OH PREG synthesis in the brain to facilitate migratory activity of birds. Recently, it was founded that the concentration of brain 7α-OH PREG increases during migration in white-crowned sparrows [Tsutsui et al., unpublished]. More detail analyses are in progress.

Seasonal changes in the synthesis of 7α-OH PREG was further investigated in the brain of male newts [41,53]. It was found that 7α-OH PREG synthesis and cytochrome $P450_{7\alpha}$ mRNA expression in the brain increase during the breeding season when locomotor behavior increases in male newts [41,53]. It is known that plasma concentrations of prolactin (PRL) and gonadotropins (GTHs) increase in male newts during the breeding season [78-81]. Therefore, Haraguchi et al. [53] manipulated the levels of PRL and GTHs and investigated the changes in brain 7α-OH PREG synthesis in male newts. Hypophysectomy (Hypox) decreases 7α-OH PREG synthesis and concentration in the brain, suggesting the involvement of PRL and/or GTHs in the regulation of seasonal changes in brain 7α-OH PREG synthesis [53]. ICV injection of PRL but not GTHs to male newts increases brain 7α-OH PREG synthesis and concentration [53]. Thus, it appears that brain 7α-OH PREG synthesis is regulated by PRL secreted by the anterior pituitary gland in male newts **(Figure 1)**. Haraguchi et al. [53] further found that the neurons expressing cytochrome $P450_{7\alpha}$ in the diencephalon express PRL receptor in male newts. Accordingly, it is considered that pituitary PRL acts directly on neurons in the diencephalon to increase brain 7α-OH PREG synthesis during the breeding period when locomotor activity increases in male newts for reviews, see [56-58,62] **(Figure 1)**.

It is well known that in vertebrates acute stress increases the circulating glucocorticoid level [82-86] and changes locomotor behavior [87,88]. However, the mode of action of glucocorticoid on locomotor behavior is poorly understood. To clarify whether the increase in glucocorticoid concentration under acute stress changes the synthesis of brain 7α-OH PREG, a series of experiments was conducted in male newts. Restraint stress increases brain 7α-OH PREG synthesis and concentration concomitant with the increase in plasma concentrations of corticosterone (CORT) in newts [89]. Hypox decreases plasma CORT concentration and brain 7α-OH PREG synthesis, whereas administration of CORT to Hypox newts increases brain 7α-OH PREG synthesis [89]. Furthermore, glucocorticoid receptor (GR) is expressed in hypothalamic neurons expressing cytochrome $P450_{7\alpha}$ [89]. Thus, it is considered that acute stress increases brain 7α-OH PREG synthesis by the direct action of CORT on hypothalamic neurons *via* GR, thereby influences locomotor activity for a review [61] **(Figure 1)**.

■ Mode of action of brain 7α-OH PREG stimulating locomotor activity

Subsequently, the mode of action of brain 7α-OH PREG on the stimulation of locomotor behavior was elucidated in vertebrates. Matsunaga et al. [41] found that 7α-OH PREG increases dopamine concentration in the striatum involved in the regulation of locomotor behavior in the newt. 7α-OH PREG also increases the release of dopamine from cultured brain [41]. Matsunaga et al. [41] further found that administration of dopamine D_2 receptor antagonists (haloperidol and sulpiride) abolishes the stimulatory action of 7α-OH PREG on locomotor behavior [41]. Based on biochemical, morphological and behavioral analyses [41], it is considered that 7α-OH PREG synthesized in the brain, by acting on dopaminergic neurons, induces dopamine release from their terminals, and consequently increases locomotor activity of newts [41]. Tsutsui et al. [54] also found a similar mode of action of brain 7α-OH PREG on locomotor activity

in the quail. Recently, Haraguchi et al. [55] further found that brain 7α-OH PREG acts on dopamine neurons to induce dopamine release from their termini, and consequently stimulates upstream migratory behavior in the salmon. Thus, it appears that brain 7α-OH PREG acts on the brain to stimulate locomotor activity *via* the dopaminergic system in these vertebrates for reviews, see [56-61] **(Figure 1)**.

■ Mode of action of pineal 7α-OH PREG regulating locomotor rhythms

The circadian clock regulates daily locomotor rhythm in birds [90]. The phase of the circadian clock is adjusted by the changes in light in a time-of-day-dependent manner in birds [91]. The finding that the pineal gland of juvenile birds actively produces 7α-OH PREG has contributed to understand the molecular mechanisms underlying light-dependent regulation of the circadian clock [64].

Because 7α-OH PREG, a major pineal neurosteroid, is released from the pineal gland of juvenile birds [63,64] **(Figure 2)**, this pineal neurosteroid may play an important role in the regulation of daily locomotor rhythm. Therefore, a series of experiments was conducted in juvenile birds. The light pulse given at early night stimulates the formation of the active form of sterol regulatory element-binding protein (SREBP) transcription factor and induces SREBP-target genes involved in CHOL synthesis in the pineal gland [91]. Based on this photic induction of SREBP-target genes that are involved in CHOL synthesis, light may up-regulate CHOL synthesis in the pineal gland. Analysis of light-regulated genes in the chicken pineal gland gave the insight that the pineal gland actively produces and secretes 7α-OH PREG [64] that increases locomotor behavior in birds [54]. Importantly, pineal 7α-OH PREG synthesis is stimulated by a light pulse given at early night, but not at late night and daytime [64]. Furthermore, locomotor behavior of dark-reared juvenile birds is stimulated by light exposure more strongly at early night than at late night and daytime [64]. Furthermore, the light-dependent stimulation of locomotor behavior at early night is reduced by Px [64]. These findings [54,64] indicate that pineal 7α-OH PREG synthesis is stimulated by light depending on the time-of-day for the regulation of locomotor rhythms for reviews, see [61,62,72] **(Figure 3)**.

Future Prospects for 7α-OH PREG Research

■ Future prospects of brain 7α-OH PREG stimulating locomotor activity

Mode of action of brain 7α-OH PREG stimulating locomotor activity, brain 7α-OH PREG, a newly discovered bioactive neurosteroid, stimulates locomotor behavior in vertebrates. The stimulatory action of brain 7α-OH PREG on locomotor behavior is mediated by the dopaminergic system **(Figure 1)**. Because brain 7α-OH PREG stimulates locomotor behavior acutely, this bioactive neurosteroid may act on target cells by non-genomic mechanisms. The receptor for 7α-OH PREG remains unclear although PREG binds receptors for *N*-methyl-D-aspartate (NMDA) and γ -aminobutyric acid A ($GABA_A$) for reviews, see [61,62,72]. Future studies are needed to clarify whether the acute action of brain 7α-OH PREG on locomotor behavior is mediated through these receptors or an unknown membrane receptor for 7α-OH PREG.

■ Future prospects of pineal 7α-OH PREG regulating locomotor rhythms

Identification of pineal 7α-OH PREG regulating locomotor rhythms, neurosteroids are actively produced *de novo* from CHOL in the pineal gland of juvenile birds. This is a new concept of neurosteroidogenesis because we believed that neurons and glial cells in the brain and peripheral nervous system produce neurosteroids *de novo* from CHOL. Furthermore, mode of action of pineal 7α-OH PREG regulating locomotor rhythms, 7α-OH PREG, a major pineal neurosteroid, is considered to be a critical regulator of locomotor rhythms, connecting light-induced gene expression with locomotor behavior. Recent studies have demonstrated that the pineal gland of mice and zebrafish also produces 7α-OH PREG actively (Tashiro, K., Tokita, T., Haraguchi, S., Tsutsui, K., unpublished observation). Therefore, future studies are needed to demonstrate the biological action of pineal 7α-OH PREG in these vertebrates to clarify the generality of the findings in birds across vertebrates.

■ Application of basic research to clinical research

Since 7α-OH PREG synthesized in the brain increases locomotor activity by increasing dopamine release **(Figure 1)**, decreased synthesis of 7α-OH PREG in the brain may cause ataxia by decreasing locomotor activity. Furthermore,

failure in 7α-OH PREG synthesis in the brain is thought to be one of the causes of depression. On the other hand, as 7α-OH PREG synthesized in the pineal gland regulates locomotor rhythm **(Figure 3)**, the decrease in pineal 7α-OH PREG synthesis may cause biological dysrhythmia. A clinical research that identifies deficits in brain function caused by disorders in brain and pineal 7α-OH PREG synthesis is necessary. In addition, application of basic research in 7α-OH PREG may contribute to treatments of depression and biological dysrhythmia. It has been shown that circadian rhythm sleep depressive disorders have alterations in melatonin receptor expression and melatonin production [92]. It is therefore possible that 7α-OH PREG is the link between deficits in the melatonin signaling system and depression and biological dysrhythmia. It has also been known that disturbed rhythm and concentration of cortisol, an adrenal steroid, cause major depression disorder [93]. Studies of 7α-OH PREG thus open a new research field in neurosteroid regulation of biological rhythms and depression.

Conclusions

The discovery of brain and pineal 7α-OH PREG allows us to pursue new avenues in studies of behavioral neuroendocrinology across vertebrates. Based on extensive research, we now know that 7α-OH PREG is an important bioactive neurosteroid regulating locomotor behavior. This short-review summarized the discovery, progress and prospect of brain and pineal 7α-OH PREG regulating locomotor behavior in vertebrates. 7α-OH PREG produced in the brain acts as a novel bioactive neurosteroid to stimulate locomotor activity **(Figure 1)**. Interestingly, brain 7α-OH PREG stimulates not only locomotor behavior but also sexual behavior [94-96]. The pineal gland also produces 7α-OH PREG and ALLO as major pineal neurosteroids **(Figure 2)**. Pineal 7α-OH PREG acts on the brain and regulates locomotor rhythms **(Figure 3)**. It is also becoming clear that the other major pineal neurosteroid ALLO acts on the brain to survive cerebellar Purkinje cells by suppressing the expression of caspase-3, a crucial mediator of apoptosis, during development [63]. Therefore, we need to clarify the interaction of brain and pineal neurosteroids, 7α-OH PREG and ALL, in the regulation of brain development and functions. Furthermore, application of basic research to clinical research in these neurosteroids is needed in the field of Neuropsychiatry.

Acknowledgments

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