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The fetal brain: role of progesterone and allopregnanolone

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Abstract: Progesterone and allopregnanolone have crucial and different roles in brain development, function and recovery after injury. Pregnancy is characterized by an increased synthesis of progesterone and its neuro-active metabolites by the placenta, maternal and fetal brain. This supports the critical role of these steroids in maternal brain adaptation during pregnancy and development of the fetal brain. Moreover, allopregnanolone may play a brain-protective role during complications of pregnancy, complications of pregnancy, such as preterm delivery or intrauterine growth restriction (IUGR), by reducing the impact of hypoxia and excitotoxic brain damage or impairment myelination. Behavioral consequences of altered progesterone/allopregnanolone fetal brain programming have also been hypothesized, although further evidence is needed. New potential applications of allopregnanolone as a treatment strategy have also been proposed, addressing unmet clinical needs in perinatal care.

Keywords: allopregnanolone; fetal brain; progesterone.

Introduction

The brain is a target and a source of progesterone synthesis and action. The synthesis of progesterone in the nervous system has been demonstrated in several species and the enzymes required for progesterone and allopregnanolone synthesis are widely distributed throughout the brain and spinal cord. The behavioral and neuroprotective effects of progesterone and allopregnanolone have been widely recognized in traumatic brain injury, ischemic stroke and neurodegenerative disease, at least in in vitro and in vivo studies [1].

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Multiple receptors or associated proteins may contribute to the progesterone effects: classical nuclear receptors (PRs), membrane progesterone receptor component 1 (PGRMC1), membrane progesterone receptors (mPRs), and γ-aminobutyric acid type A (GABAA) receptors after conversion to allopregnanolone.

Progesterone and allopregnanolone are the most important neuroactive steroids during pregnancy, as they are found in remarkably high concentrations in the fetal and maternal circulation and brain. Increased local biosynthesis of pregnenolone, progesterone and 5α-dihydroprogesterone may be a part of an endogenous adaptive mechanism in maternal and fetal brain during pregnancy [2].

Here, we describe the potential mechanisms involved in the action of progesterone (and its metabolite, allopregnanolone) in the brain during pregnancy.

Mechanism of actions of progesterone in the brain

A comprehensive account of the molecular and cellular activities of progesterone on the central nervous system (CNS) is beyond the scope of this article, and several recent reviews of the subject are available [1, 3]. However, in an attempt to describe the biological plausibility of the hypotheses that progesterone and progestins greatly affect brain function, the mechanisms that seem most relevant will be briefly described here.

The different physiological effects of progesterone can be mediated by both PRs and membrane receptors [4].

The most common isoforms of PRs are PR-A and PR-B, which are responsible for the transcriptional effects of progesterone. Although both PRs are generated from a single gene [5], PR-B differs from PR-A by an additional 164 amino acid sequence in the N-terminal region [6]; thus the different structure gives them diverse transactivational properties observed both in vitro [7, 8] and in vivo [9, 10]. Interestingly, a third isoform, PR-C, has also been identified, which is thought to modulate the transcriptional activity of PR-A and PR-B [11, 12]. The differential structure of the PR isoforms confers distinct tissue-specific

responses to progesterone, through post-translational modifications, dimerization and recruitment of cofactor proteins contributing to the differential transactivation properties of each isoform. Consequently, these events lead to the regulation of distinct substrates of progesterone-dependent target genes [3].

In the CNS, PR-A and PR-B were identified, although their biological properties are not yet completely defined [13–15]; instead, there is no evidence for the existence of PR-C isoform to date [3]. Reverse transcription-polymerase chain reaction (RT-PCR) analyses revealed the expression of both the PR-A and PR-B mRNA transcripts in all regions of the brain where the neural PRs are known to be present [3]. Their co-localization in several brain districts such as amygdala, hippocampus, cortex, basal forebrain, cerebellum, locus coeruleus, midbrain rafe nuclei, glial cells and gray matter could confirm the involvement of progesterone in the control of well-being, cognitive functions and memory processes in physiological as well as pathological conditions [16]. Furthermore, in the adult female rat brain, estradiol (E2) and progesterone differentially regulate the isoforms in distinct regions of the brain [17]. In the hypothalamus, estrogen can up-regulate PR-A and PR-B expression as PRs are expressed in the same areas of the estrogen receptors, while progesterone itself down-regulates them. In the hippocampus, only PR-A expression is up-regulated by E2, while progesterone has no influence in both PR expressions [17]. However, in the cerebellum and the frontal cortex, neither E2 nor progesterone has any effect on PR isoforms' mRNA expression. Moreover, the transcription of PR isoforms varies with the estrous cycle in a region-specific manner; for example, studies on E2-treated rhesus macaques indicate a region-specific regulation of the PR isoforms, with PR-B expression being predominant in the hypothalamus and PR-A in the pituitary [18, 19].

The increasing in vitro and in vivo evidence of differential transcriptional activities and co-regulator interactions between PR-A and PR-B predict that these two isoforms could have distinct roles in mediating additional and/or alternate signaling pathways within steroid-sensitive neurons [3]. In addition, genetic variations of the common progesterone receptor gene described in the endometrium and the breast tissue might be associated with functional differences inside the brain. Similarly, an association between receptor transcriptional silencing and the methylation status of PR-A and PR-B promoter regions is well documented, and hypermethylation of PR-B induces a down-regulation of the receptor [20]. However, no data is currently available for the methylation status of brain PR-A and PR-B. In ongoing clinical trials, premature infants have been treated with a continuous infusion of P and estradiol for the initial few weeks of life: premature infants treated with hormones achieved normal psychomotor development earlier than untreated premature infants [21].

Concerning non-classical pathways, several studies have demonstrated that progesterone is able to interact with membrane receptors such as the PGRMC1, σ1 receptor and GABAA receptor, through allopregnanolone; PGRMC1 is localized on the membrane of hypothalamic and spinal neurons [22, 23] and its expression was shown to be induced by E2 treatment, thus suggesting a role in the activation of female sex behavior [22]. Moreover, a role of PGRMC1 in mediating protective effects of progesterone in the nervous system is also supported by the observation that its mRNA and protein were up-regulated by progesterone treatment in dorsal horn neurons of spinal cord-injured male rats [23]. Another membrane receptor of progesterone is the σ1 receptor that is involved in the neuronal aging processes [24, 25]. The receptor is involved in the potentiation of the N-methyl-D-aspartate (NMDA) response of hippocampal neurons and the NMDA-evoked norepinephrine release but the presence of progesterone leads to a reduction of σ1 activity [26, 27].

Furthermore, progesterone plays a role in the control of other transmission systems like opioidergic, serotoninergic and cholinergic. The nicotinic receptor of acetylcholine is a target of progesterone as well, and progesterone inhibits the activity of the receptor independently of the membrane potential [28, 29].

Studies in vitro have shown that PR, like other steroid receptors, can be modulated by compounds other than steroids in a "ligand-independent manner". These molecules include cyclic nucleotides that increase intracellular kinase activity [30], as well as extracellular compounds that interact with membrane receptors and stimulate intracellular phosphorylation pathways, including growth factors and neurotransmitters like dopamine.

Thus, the "ligand-dependent" (genomical/classical and non-genomical/non-classical) and the ligand-independent mechanisms of PRs activation and sensitization allow steroids to widely affect the regulation of cerebral activities.

Allopregnanolone synthesis

Neurons and glia cells possess all the enzymes necessary for progesterone, testosterone and estradiol metabolism [aromatase, 5α-reductase (5α-R) mainly in neurons, (3α-hydroxysteroid dehydrogenase, 3α-HSD) mainly

in type 1 astocytes]. Allopregnanolone (3-hydroxy-5-pregnan-20-1), is a 3,5-reduced metabolite of progesterone produced by the enzyme 5α-R and 3α-HSD. Allopregnanolone is a neurosteroid produced by the CNS, adrenals and ovaries [11]. Allopregnanolone is a potent endogenous steroid that rapidly affects the excitability of neurons and glial cells through direct modulation of GABAA receptor activity [12, 13]. In addition, allopregnanolone exhibits neurotrophic/neuroprotective actions, reducing cell death, gliosis, and functional deficits after traumatic brain injury in rats and in experimental models of Alzheimer's disease [14]. Experimental data suggested a direct functional association between allopregnanolone brain content, neurosteroids and sex steroid concentration in experimental models of ovarian function withdrawal.

Effects of progesterone and allopregnanolone in the developmental brain

The critical role of progesterone in brain activity has been postulated as evidence that Purkinje cells (a typical cerebellar neuron) have been shown to express P450scc and 3β-HSD during postnatal development and in adulthood, and were demonstrated to be a source of progesterone and allopregnanolone particularly during the neonatal period when enzymatic activities increase [31].

Progesterone may also affect oligodendrocyte differentiation. Oligodendrocytes and their precursors differentially express enzymes needed for progesterone and other neurosteroid production, suggesting that these compounds may be involved in oligodendrocyte progenitor proliferation and differentiation during development [32]. Oligodendrocyte pre-progenitors, precursors, and fully differentiated oligodendrocytes differentially express 3β-HSD, 5α reductase and 3α-HSD. Pre-progenitors have highest expression of 3β-HSD and 3α-HSD, and can convert pregnenolone to progesterone. 3α-HSD activity is highest in oligodendrocyte pre-progenitors, but is also found in oligodendrocyte precursors and mature oligodendrocytes. In contrast, mature oligodendrocytes have the highest levels of expression of 5α-reductase but are unable to convert pregnenolone to progesterone, suggesting a lack of 3β-HSD expression [32, 33].

Progesterone also stimulates myelination in the CNS [33, 34]. In slice cultures of 7-day-old rat and mouse cerebella, high concentrations of progesterone (20–50 μM) increased expression of myelin basic protein about fourfold. This effect may be mediated through progesterone receptors as the selective progesterone receptor agonist R5020 also increased expression of myelin basic protein while the progesterone receptor antagonist RU-486 abolished the effect of progesterone. The involvement of the progesterone receptor was confirmed using cerebellar slice cultures from progesterone receptor knockout mice. In those animals, progesterone had no significant effect on myelin basic protein expression. In addition to direct effects of progesterone on its nuclear receptor, some effects on expression of myelin basic protein were likely mediated through neurosteroid metabolites of progesterone (allopregnanolone). A 5α-reductase inhibitor partially inhibited the effect of progesterone, and allopregnanolone significantly increased expression of myelin basic protein, although this stimulation was less than that found with progesterone treatment. In addition, the GABAA receptor antagonist bicuculline inhibited the effect of allopregnanolone on increasing expression of myelin basic protein. Thus, progesterone affects myelination not only in the peripheral nervous system but also in the CNS as well through mechanisms that involve both the progesterone receptor and the GABAA receptor [35].

The role of progesterone and allopregnanolone in fetal brain

Progesterone and allopregnanolone are the most important neuroactive steroids during pregnancy, as they are found in remarkably high concentrations in the fetal circulation and brain [36, 37]. Besides contributing to the maintenance of pregnancy, these hormones are also important to facilitate adaptations of maternal brain needed for timely parturition, lactation and for expression of appropriate maternal behavior postpartum [36, 37]. During gestation, increased levels of allopregnanolone are observed both in maternal and fetal circulation [38]. This could probably be explained by the fact that the maternal brain has an increased capacity to generate neurosteroids during pregnancy [38]. Neurosteroid levels drop quickly after birth and this event may reduce neuroprotection and cause some problems for preterm newborns [39]. Studies carried out on sheep have shown that allopregnanolone levels in the fetal brain are increased during gestation, particularly near term [36, 39]. Moreover, it has also been shown that the GABAA receptor is expressed in fetal sheep brain and its expression rises with advancing gestation [2, 40]. Several research works indicate that some interactions between placenta and brain, due to increasing expression of 5α-reductase in these two organs, may regulate concentrations of allopregnanolone in the fetal and maternal brain [40]. 5α-Reductase activity may be the

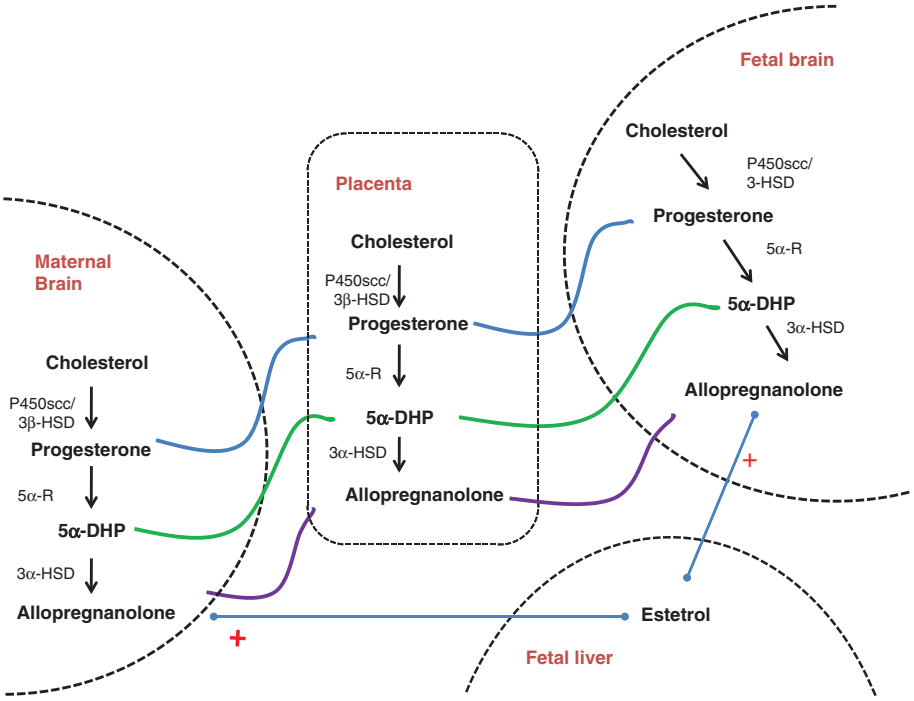

Figure 1: Multi-level synthesis of allopregnanolone in the maternal-placental-fetal system during human pregnancy.

major determinant of the levels of neuroactive steroids found locally within regions of the brain. Total activity may be a product of the activities of the two 5α-reductase isoforms, type-1 and type-2. Both isoforms are expressed in fetal sheep and guinea pig brains throughout late gestation.

We previously demonstrated that estetrol, E4, a naturally occurring estrogen only produced by the human fetal liver, increased allopregnanolone levels in the brain and in the circulation, underlying the crucial role of allopregnanolone during human fetal brain development [41] (Figure 1).

Recent findings suggest that the neurosteroid milieu deeply influences behavioral state and exerts a tonic suppression of CNS excitability in the fetus [42]. This is supported by findings that the inhibition of neuroactive steroid synthesis during pregnancy, either by lowering placental progesterone synthesis, using a 3β-HSD inhibitor, or blocking the metabolism of progesterone to allopregnanolone, markedly increases arousal-like behavior and excitation in the ovine fetus. Furthermore, this indicates that allopregnanolone levels markedly influence behavioral states during fetal life and may have a major impact on brain development. The hypothesis according to which the fetal brain itself plays a key role in regulating levels of allopregnanolone is supported by the evidence that its concentrations in the fetal brain rise further after hypoxic stress [40, 42].

There is now increasing evidence that neurosteroids improve outcomes following hypoxic/ischemic brain injury in adults by aiding tissue repair. These processes involve increased production of allopregnanolone and its interaction with GABAA receptors. Suppression of allopregnanolone production alone increases apoptotic cell numbers in the fetal brain in the absence of any injurious process. Reduced allopregnanolone levels negatively affect the number of brain cells. These effects of inhibiting neuroactive steroid synthesis were blocked by the co-infusion of alfaxalone, suggesting that allopregnanolone in the fetal brain is required to maintain constitutive levels of cell death and proliferation in late development.

Previous findings show that neuroactive steroids stimulate myelination, an action thought to involve neuroactive steroid-induced stimulation of GABAA receptors, which appears to indirectly affect oligodendrocytes. This idea is supported by a recent study where recovery from acute perinatal hypoxic injury involved increased proliferation of oligodendrocyte progenitor cells and their maturation into mature oligodendrocytes [35].

Allopregnanolone responses to chronic placental insufficiency are mediated by an increase of 5α-reductase expression in the placenta and brain, especially in the hippocampus [40]. Changes in brain allopregnanolone levels seem to be observed also after birth in lambs [36]. After exposure to hypoxia, these newborn animals show

a rise in brain allopregnanolone concentrations [2, 36] and plasma cortisol [2]. These observations suggest that allopregnanolone may also play a neuroprotective role in newborns, not only during gestation [2].

Conclusions

Progesterone and allopregnanolone have crucial and somewhat different roles in brain development, function and recovery after injury. Pregnancy is characterized by an increased synthesis of progesterone and its neuroactive metabolites by the placenta, and the maternal and fetal brain. This supports the critical role of these steroids in maternal brain adaptation during pregnancy and development of fetal brain. Moreover, allopregnanolone may play a brain-protective role during complications of pregnancy, such as preterm delivery or intrauterine growth restriction (IUGR), reducing the impact of hypoxia and excitotoxic brain damage or impairment myelination. Behavioral consequences of altered progesterone/allopregnanolone fetal brain programming have also been hypothesized, although further evidence is needed. New potential applications of allopregnanolone as a treatment strategy have been proposed, addressing unmet clinical needs in perinatal care.

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