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Review Article

Nicola Pluchino[1] / Yveline Ansaldi[1] / Andrea R. Genazzani[2]

Brain intracrinology of allopregnanolone during pregnancy and hormonal contraception

[1] University Hospital of Geneva, Division of Gynecology and Obstetrics, Genéve, Switzerland, E-mail: nicola.pluchino@hcuge.ch
[2] Division of Gynecology and Obstetrics, University of Pisa, Pisa, Italy

Abstract:
Allopregnanolone (ALLO) has a crucial role in brain development and remodeling. Reproductive transitions associated with endocrine changes affect synthesis and activity of ALLO with behavioral/affective consequences. Pregnancy is characterized by an increased synthesis of progesterone/ALLO by the placenta, maternal and fetal brains. This suggests the critical role of these steroids in maternal brain adaptation during pregnancy and the development of the fetal brain. ALLO is brain protective during complications of pregnancy, such as preterm delivery or intrauterine growth restriction (IUGR), reducing the impact of hypoxia, and excitotoxic brain damage. Negative behavioral consequences of altered progesterone/ALLO maternal brain adaptation have been also hypothesized in the post-partum and targeting ALLO is a promising treatment. Hormonal contraception may alter ALLO action, although the effects are mostly related to a specific class of progestins. Understanding the interactions between ALLO and the endocrine environment is crucial for more effective and tailored hormonal treatments.

Keywords: Allopregnanolone, brain, progesterone, progestins
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Introduction

Allopregnanolone (ALLO) is a neuroactive metabolite of progesterone whose synthesis in the nervous system has been demonstrated in several species. The enzymes required for progesterone and ALLO synthesis are widely distributed throughout the brain and spinal cord. Progesterone and ALLO behavioral and neuroprotective effects are widely recognized in traumatic brain injury, ischemic stroke and neurodegenerative disease, demonstrated in in-vitro and in-vivo studies [1], [2].

Progesterone and ALLO are the most important neuroactive steroids during pregnancy, as they are found in high concentrations in the fetal and maternal circulations, and in the brain. They represent an endogenous adaptive mechanism in the maternal and fetal brain during pregnancy [1]. Endogenous variation in circulating sex steroids, as well as sex steroids treatment, have a critical impact on the pathway leading to ALLO synthesis and brain receptivity to neurosteroids. Multiple receptors and associated proteins contribute to the effect of progesterone and ALLO. These include: classical nuclear receptors (PR), membrane progesterone receptor component 1 (PGRMC1), membrane progesterone receptors (mPR) and γ-aminobutyric acid type A (GABA-A). GABA-A is a ligand-gated ion channel primarily associated with the inhibition or fine-tuning of excitatory neurotransmission, which can be modulated by progesterone metabolites including 3α-hydroxy-5β-pregnan-20-one (pregnanolone); 3α-hydroxy-5β-pregnan-20-one (ALLO; 3α-hydroxy-5α-pregnan-20-one) and its 21-hydroxylated derivative: tetrahydro-DOC (THDOC) derived from the A-ring reduction of deoxycorticosterone. Of these metabolites, ALLO is amongst the most potent endogenous allosteric modulators of GABA-A [3].

Mechanism of actions of ALLO in the brain

A comprehensive account of the molecular and cellular activities of ALLO on the CNS is beyond the scope of this article, and several recent reviews of this subject are available [4], [5]. However, in the attempt to describe the

Nicola Pluchino is the corresponding author.

biological effects of specific phases of reproductive transitions, characterized by significant hormonal changes (e.g. pregnancy and postpartum), and of hormonal manipulation (hormonal contraception), on brain function, the mechanisms that seem most relevant, and that are mediated through the actions of ALLO, will briefly be described here.

ALLO synthesis and action

In the central and peripheral nervous systems, ALLO synthesis occurs primarily in glial cells – astrocytes, oligodendrocytes and Schwann cells, as well as in many neuronal cell types including neural progenitors. The enzymes necessary for progesterone, DHEA and estradiol metabolism are all present in neuronal and glial cells: aromatase and 5α-reductase (5α-R) are primarily found in neurons whilst 3α-hydroxysteroid dehydrogenase 3α-HSD is mainly located in type 1 astocytes. ALLO, 3-hydroxy-5-pregnan-20-one, is a 3,5-reduced metabolite of progesterone produced by the enzymes 5α-R and 3α-HSD. ALLO is also produced by the adrenals, placenta and ovaries, as direct metabolism of progesterone and blood concentration are correlated to progesterone levels [5], [6], [7]. At a cellular level, ALLO 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 by acting on GABA-A [7], [8], [9], [10]. The mechanism of action of ALLO-activated cell cycle gene expression in neural stem cells is mediated by binding to $GABA_AR$, which elicits efflux of chloride and a concomitant influx of calcium that contributes to the induction of cell signaling events, leading to gene transcription of mitotic genes and downregulation of anti-mitotic genes. At nanomolar concentrations, ALLO enhances the apparent affinity of GABA for GABA-A, and at micromolar concentrations, it can directly activate $GABA_AR$ chloride channels. ALLO binds to two transmembrane sites of the heteropentameric $GABA_AR$ assembled from eight subunit families. GABA-A binding sites have the general subunit stoichiometry $2\alpha{:}2\beta{:}1\gamma$. GABA-A channel complexes that occur at the synaptic cleft have a higher threshold for activation and display phasic conductance. In contrast to synaptic GABA-A, a subset of extrasynaptic GABA-A, contains the neurosteroid-sensitive δ subunit making it pharmacologically distinct. It displays a tonic conductance pattern [3], [11], [12], [13], [14], [15], [16], [17], [18], [19], [20]. Experimental data suggested a direct functional association between ALLO brain content, neurosteroids and sex steroid concentration in experimental models of ovarian function withdrawal.

ALLO in the fetal brain

Progesterone and ALLO are the most important neuroactive steroids during pregnancy, as they are found in high concentrations in the fetal circulation and brain [21], [22]. During gestation, increased levels of ALLO are observed both in the maternal and fetal circulations [22], [23]. A possible explanation for this may be related to the maternal brain's increased capacity to generate neurosteroids during pregnancy [22], [23]. Neurosteroid levels drop quickly after birth and this event may reduce neuroprotection and have an impact on preterm newborns [24]. Studies carried out on sheep have shown that ALLO levels in the fetal brain are increased during gestation, particularly near term [23], [24]. Moreover, it has been shown that the GABA-A receptor is expressed in fetal sheep brain and its expression rises with advancing gestation [21], [25]. Research had indicated that placental and brain interactions, and the increased expression of 5α-reductase in these two organs, may regulate concentrations of ALLO in the fetal and maternal brains [26]. 5a-Reductase activity may be the major determinant of the levels of neuroactive steroids found locally within regions of the brain, with the total activity of 5α-reductase being a product of the activity of the two 5a-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 E4 is produced in the placenta from a 15α-hydroxylated hepatic androgenic precursor, increased ALLO in the brain and in the circulation, underlining the crucial role of ALLO during human fetal brain development [27].

Recent findings suggest that the neurosteroid milieu deeply influences the fetal behavioral state and exerts a tonic suppression of CNS excitability [24]. This is supported by findings that inhibition of neuroactive steroid synthesis during pregnancy, either by lowering placental progesterone synthesis in a pregnant sheep, using a 3β-hydroxysteroid dehydrogenase inhibitor, or blocking the metabolism of progesterone to ALLO, increases arousal-like behavior and excitation (reduction of sleep phases) in the fetus. Furthermore, this indicates that ALLO 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 ALLO is supported by the evidence that its concentrations in the fetal brain rise further after hypoxic stress [28], [29].

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 ALLO and its inter-

action with GABAA receptors. Suppression of ALLO production alone increases apoptotic cell numbers in the fetal brain in the absence of any injury process. Reduced ALLO negatively affects the number of brain cells. The effects of inhibiting neuroactive steroid synthesis were blocked by co-infusion of alfaxalone, suggesting that ALLO in the fetal brain is required to maintain constitutive levels of cell death and proliferation in late development.

Previous findings have shown that neuroactive steroids stimulate myelination. This is thought to be mediated by the neuroactive steroid-induced stimulation of GABAA receptors, which appears to indirectly affect oligodendrocytes. Recovery from acute perinatal hypoxic injury involves increased proliferation of oligodendrocyte progenitor cells and their maturation into mature oligodendrocytes [30].

ALLO responses to chronic placental insufficiency are mediated by an increase of 5α-reductase expression in the placenta and the brain, especially in the hippocampus [25]. Changes in brain ALLO levels seem to also be observed after birth in lambs [21], [24], [25]. After exposure to hypoxia, these newborn animals showed a rise in brain ALLO concentrations [21], [24], [25], [26] and in plasma cortisol [28]. These observations suggest that ALLO may also play a neuroprotective role after birth and not only during gestation [25], [26], [27], [28].

ALLO in the maternal brain: pregnancy and post-partum

Besides contributing to the maintenance of pregnancy, ALLO also facilitates the adaptations of the maternal brain required for timely parturition, lactation and the expression of appropriate maternal behavior postpartum [22]. In rodent pregnancy, ALLO specifically suppresses the maternal hypothalamic-pituitary-adrenal (HPA) axis response to stress through increased opioid and GABAergic inhibition of corticotropin-releasing hormone (CRH) release [31]. Exploratory analyses suggested that the women who were homozygous for the minor allele of the haplotype tag single nucleotide polymorphisms in the aldo-keto reductase family 1, members C2 (AKR1C2 polymorphism rs1937863) had nominally lower ALLO levels and lower depression scores in gestational week 17, but also the highest increase in depression scores between weeks 17 and 32 using the Edinburgh Postnatal Depression Scale [32]. Preclinical and clinical studies have shown that neuroactive steroids might have an important role in the pathophysiology of post-partum depression. During pregnancy in mice, expression of the γ-aminobutyric acid type A ($GABA_A$) receptor δ subunit is downregulated as ALLO concentrations increase, and at parturition, expression of the $GABA_A$ receptor δ subunit is recovered as ALLO concentrations drop steeply. $GABA_A$ receptor δ-subunit-deficient mice do not adapt to the substantial changes in ALLO concentration during pregnancy and parturition and showed depression-like and anxiety-like behavior as well as other abnormal maternal behavior that was reversed by the administration of ALLO [22], [29] These findings lend support to the hypothesis that changes in ALLO concentrations during pregnancy and postpartum are capable of provoking affective dysregulation. Recently, in a Phase II study, an infusion of brexanolone, an intravenous formulation of ALLO, was administered to women with severe post-partum depression, which resulted in a significant and clinically meaningful reduction in depression, compared with placebo [29].

Thus, lower ALLO during pregnancy appears to predict post-partum depression, suggesting that the measurement of ALLO during pregnancy may have a clinical role as a screening tool in tailored antenatal and post-partum care.

ALLO during hormonal contraception: role of progestins

Hormonal treatment modifies circulating and brain ALLO levels and it may affect the likelihood of developing negative mood changes during treatment. Although the reduction of ALLO circulating levels is the consequence of the inhibition of the gonadal axis, brain ALLO changes are the result of modification of CNS metabolism and GABA-A activity.

The psychological side effects are a contributing factor to non-compliance to hormonal contraception. Recent studies show that negative mood changes were given as a reason for discontinuation in 5% of combined oral contraceptive (COC) users [33], but that among those women who felt most moody, only 27% continued using this method at 6 months [34]. More recently, a nationwide prospective cohort study in Denmark demonstrated that hormonal contraception, particularly among adolescents, was associated with subsequent use of antidepressants, a first diagnosis of depression and suicide [35], [36].

The effects of hormonal contraception on mood may depend more on the type of progestin than on the dosage used. A randomized trial comparing the effect in women using COC with either levonorgestrel or desogestrel demonstrated less positive affect changes in levonorgestrel pill users while those using the desogestrel

pill had significantly greater positive effect changes in comparison to pre-treatment score on relaxation, mental tension and irritability [37].

Follesa et al. showed that long-term administration of combined EE and levonorgestrel (LNG), induces a marked decrease in the concentrations of ALLO and its precursors progesterone and pregnenolone in female rat cerebral cortex, hippocampus and plasma [38]. More recently, we showed that the long-term administration of EE alone or LNG alone also markedly decreased ALLO concentrations in the rat cerebral cortex and hippocampus. Administration of LNG, but not EE, increased the abundance of the γ2 subunit peptide in the cerebral cortex and hippocampus by 38 and 59%, respectively, and increased anxiety-like behavior. These results suggest that alterations in $GABA_A$ receptor subunit expression induced by long-term treatment with combined EE/LNG appear to be caused by LNG, resulting in anxiety-like behavior [38], [39].

According to their molecular profile, progestins might induce divergent biological effects compared to natural progesterone. The different chemical structures might induce a different metabolism; in contrast to progesterone, not all progestins could be converted into ALLO. Some of the 19-norprogestins, in particular, 19-nortestosterone-derived progestins, may have the potential to be converted to neuroactive metabolites, as they are extensively converted to 3α,5α- and 3β,5α-tetrahydro derivatives [40]. Norethisterone acetate and medroxyprogesterone acetate (MPA) were shown to produce some anxiolytic-like effects when rats were tested in the "elevated plus maze" test and the "shock-probe burying" test. However, whether the neuroendocrine effect of these progestins are directed or mediated by their metabolism into ALLO is still unknown [22]. In ovariectomized rats, micronized progesterone and other different progestins, (medroxyprogesterone acetate, dydrogesterone, drospirenone and nomegestrol acetate) showed distinctive effects on ALLO levels in specific brain areas (frontal and parietal lobe, hippocampus, hypothalamus, anterior pituitary) and in serum, while they did not have any effect on adrenal ALLO concentrations [39]. Moreover, those findings further varied when progestins were administered in association with an estrogen molecule, evidencing how a specific estro-progestin combination has a selective effect on central steroidogenesis [40], [41]. These findings represent the endocrine background for understanding the effects of hormonal contraception on mood in the clinical setting.

Conclusions

ALLO has a crucial role in brain development and remodeling. Reproductive transitions associated with endocrine changes affect synthesis and activity of ALLO with behavioral/affective consequences. Pregnancy is characterized by an increased synthesis of progesterone/ALLO by the placenta, maternal and fetal brains. This suggests the critical role of these steroids in maternal brain adaptation during pregnancy and development of the fetal brain. ALLO is brain protective during complications of pregnancy, such as preterm delivery or intrauterine growth restriction (IUGR), reducing the impact of hypoxia and excitotoxic brain damage. Negative behavioral consequences of altered progesterone/ALLO maternal brain adaptation have been also hypothesized in the post-partum and targeting ALLO is a promising treatment. Hormonal contraception affects ALLO brain concentration in the animal model and the effect of synthetic progestins differs from micronized progesterone. Understanding the interactions between ALLO and the endocrine environment is crucial for more effective and tailored hormonal treatments.

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