CHAPTER 2

Physiological roles for the neurosteroid allopregnanolone in the modulation of brain function during pregnancy and parturition

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Abstract: Allopregnanolone is a well-established allosteric modulator of the GABA_A receptor but its physiological roles within the nervous system remain unclear. Derived principally from circulating progesterone, allopregnanolone achieves its highest concentrations within the nervous system during late pregnancy and recent studies have now begun to elucidate its roles at this time in the rat. At the molecular level it is clear that the regulation of GABA_A receptor subunit gene expression by progesterone and its derivatives occurs in a subunit- and a neuron-specific manner and that both progesterone and allopregnanolone are involved. At the cellular level, the increasing concentrations of allopregnanolone with advancing pregnancy can be shown to have important physiological actions in repressing the electrical activity of specific neuronal phenotypes such as the magnocellular oxytocin neurons. The marked fall in progesterone and allopregnanolone concentrations prior to parturition equally appears to have a substantial impact upon GABA_A receptor signaling in the hippocampus, frontal cortex and oxytocin neurons. Together, studies at a basic level suggest that the rise and fall in allopregnanolone concentrations during pregnancy are likely to exert a powerful regulatory influence upon neurotransmission in a variety of brain networks. The temporal correlation between these events and the observed cognitive, psychiatric and physiological changes associated with pregnancy and the peri-partum period in humans is striking and warrants close attention.

Introduction

Progesterone is one of the principal hormones secreted by the corpus luteum and placenta to ensure the survival and successful development of the embryo through to term and beyond. Accordingly, the gradually increasing concentrations of circulating progesterone over the course of pregnancy target a wide range of reproductive and non-reproductive tissues within the expectant mother. One such site of action is the nervous system where, not surprisingly, roles for progesterone in the regulation of the neural networks controlling parturition and pain, as well as a spectrum of reproductive behaviors, have been described (Pfaff et al., 1994; Crowley et al., 1995; Dawson and Gintzler, 1998; Numan et al., 1999). Additionally, for many women, pregnancy and the peri-partum period represent a time of marked change in their cognitive and psychological well-being with reduced memory capability, altered mood and anxiety levels (Pugh et al., 1963; O’Hara, 1986; Buckwalter et al., 1998; Keenan et al., 1998). Some of these parameters, and particularly those of post-partum mood, have been positively associated with the absolute levels, or the peri-partum decline, of plasma progesterone concentrations (Nott et al., 1976; Harris et al., 1994; Buckwalter et al., 1998).

While it seems reasonable to conclude that specific progesterone receptor-expressing neuronal pop-
Fig. 1. Schematic representation of the production and action of allopregnanolone on GABA<sub>A</sub> receptors. Allopregnanolone is formed in the brain by glial cells either de novo from cholesterol, or principally, from circulating progesterone originating from the gonads and placenta. Progesterone undergoes a two step reduction (enzymes in italics) within glial cells to become allopregnanolone, which then acts as an allosteric modulator at several different subtypes of GABA<sub>A</sub> receptors within the central and peripheral nervous system. Although the precise mechanism of action is not yet fully understood, it is clear that allopregnanolone modulates the receptor to result in increased channel open times following activation by GABA. This is observed experimentally in voltage clamp recordings of individual neurons (inset) where allopregnanolone (+ allo) is found to have no effect upon opening of the channel (current amplitude measured in pA) but increases the decay kinetic (duration measured in ms) of inhibitory post-synaptic currents.

It is now well established that allopregnanolone at low 'physiological' concentrations has a direct and highly selective allosteric modulatory action upon the γ-aminobutyric acid (GABA) type A ionotropic (GABA<sub>A</sub>) receptor (Majewska, 1992; Lambert et al., 1995; Rupprecht and Holsboer, 1999). Allopregnanolone exerts a pronounced effect upon neurons by enhancing GABA<sub>A</sub> receptor signaling, which is typically inhibitory in the adult brain. Allopregnanolone binds to the GABA<sub>A</sub> receptor in an as yet undetermined manner, to increase the mean channel open time of the receptor when activated by its ligand GABA (Fig. 1). This results in GABA<sub>A</sub> receptors remaining open longer and allowing more chloride ion flux in the presence of allopregnanolone. As chloride ion entry into a neuron normally hyperpolarizes the
Fig. 2. Schematic representation of the fluctuating levels of circulating allopregnanolone (black) and progesterone (gray) concentrations over the course of pregnancy and parturition (left) and their effects upon the profile of specific GABA<sub>A</sub> receptor subunits expressed in oxytocin, hippocampal, and cerebral cortex neurons. The putative physiological implications of these changes are given for each region (right). Data for the α<sub>4</sub> subunit in the hippocampus are extrapolated from pseudopregnant animals and the full profile over pregnancy is not available (Smith et al., 1998b). The α<sub>5</sub> and γ<sub>2</sub> data are from Follesa et al. (1998) and the α<sub>1</sub> results from Fenelon and Herbison (1996). While progesterone regulates subunit expression in oxytocin neurons, allopregnanolone influences subunit expression in the hippocampus and cerebral cortex.

cell, the net effect of allopregnanolone exposure is to enhance inhibitory GABA transmission. Although the mode of action of this progesterone derivative has been well established in several in vitro paradigms, many questions remain. In particular, because the pentameric GABA<sub>A</sub> receptor can be formed from among more than 15 different subunits (Barnard et al., 1998), it is not clear whether allopregnanolone acts at all GABA<sub>A</sub> receptor isoforms or only those receptors with a specific subunit stoichiometry (see Lambert et al., 1995). Furthermore, because of the lack of a specific antagonist for allopregnanolone’s action at the GABA<sub>A</sub> receptor, and the inherent technical difficulties in examining neurons within their native environment, it is unclear what physiological roles allopregnanolone may play within the brain. In this respect, the time of late pregnancy, when the highest physiological levels of allopregnanolone are achieved in the brain, appears to be an ideal setting within which to explore this issue.

Very recent studies have now hinted at the physiological roles of allopregnanolone in the control of the magnocellular oxytocin neurons, hippocampus and cerebral cortex during pregnancy. While there are undoubtedly many more brain regions and neuronal phenotypes to be discovered which are targeted by allopregnanolone in the ‘maternal brain’, an examination of these three networks is likely to be instructive in uncovering the ways in which progesterone can influence the nervous system through its derivative allopregnanolone.

**Allopregnanolone, the magnocellular oxytocin neurons and parturition**

The magnocellular oxytocin neurons reside within the supraoptic (SON) and paraventricular nuclei of the hypothalamus from where they project to the posterior pituitary and secrete oxytocin directly into the circulation. The electrical activity of these neurons undergoes substantial changes over the course of pregnancy and lactation (Summerlee, 1981; Leng et al., 1999), and progesterone is thought to have a particularly important role in synchronizing the activity of oxytocin neurons with other physiological processes during pregnancy (Negoro et al., 1973;
Crowley et al., 1995). The oxytocin neurons in the rat are known to exhibit a low level of firing during most of pregnancy, but then display an abrupt transition to synchronous bursting behavior, superimposed on elevated tonic firing, at the time of birth when the pulsatile release of oxytocin contracts the uterine myometrium to help expel the pups (Summerlee, 1981; Jiang and Wakerley, 1995). As the oxytocin neurons do not seem likely to possess progesterone receptors (Numan et al., 1999; Fenelon and Herbison, 2000), or respond directly in a non-genomic fashion to progesterone itself (Wang et al., 1995), it has not been clear how progesterone might influence this important neuronal phenotype.

The oxytocin neurons receive a substantial GABAergic input, estimated to comprise approximately 40% of all synapses on these cells (Majdoubi et al., 1997), and express GABA$_{A}$ receptors comprised principally of $\alpha_1$, $\alpha_2$, $\beta_2$, $\beta_3$ and $\gamma_2$ subunits (Fenelon et al., 1995). A potential role for progesterone in the regulation of GABA$_{A}$ receptors expressed by oxytocin neurons was first suggested by in situ hybridization studies which revealed that the expression of the $\alpha_1$ subunit transcript in oxytocin neurons fluctuated over the course of pregnancy in a highly selective manner (Fenelon and Herbison, 1996). The expression level of $\alpha_1$ subunit mRNA in putative oxytocin neurons was found to increase with advancing pregnancy and then fall by 30–40% over the last 2 days prior to birth (Fig. 2). This closely resembled the profile of progesterone secreted over pregnancy in vivo (Brussaard et al., 1997) and this correlates perfectly with the peak of allopregnanolone concentrations found in the brain on day 19 of pregnancy in rats (Concas et al., 1998). Together, these observations have led us to suggest that allopregnanolone modulation of oxytocin neuron GABA$_{A}$ receptor activity represents an important physiological feedback mechanism through which progesterone represses oxytocin neuron activity in late pregnancy to prevent premature delivery (Brussaard and Herbison, 2000).

More remarkably, however, we found that the sensitivity of the oxytocin neuron GABA$_{A}$ receptor to allopregnanolone declines dramatically over the last day of pregnancy so that it is reduced by over 10-fold on the day of parturition (Brussaard et al., 1997). As this decline in allopregnanolone facilitation of the GABA$_{A}$ receptor occurs in the face of unchanging GABA release upon the oxytocin neuron (Brussaard et al., 1999; Fenelon and Herbison, 2000), it seems likely to exert a powerful dis-inhibitory influence upon the electrical activity of the oxytocin neurons. Together with changes in other neurotransmitters such as glutamate, norepinephrine, and opioid peptides (Douglas et al., 1995; Herbison et al., 1997), the allopregnanolone dis-inhibition of oxytocin neurons in very late pregnancy is likely to be part of the mechanism enabling their transition to a synchronous bursting pattern of behavior necessary for parturition to proceed.

Although a reasonable case may be made for a physiologically relevant role for allopregnanolone in the regulation of the oxytocin neurons, the precise mechanisms underlying the peri-partum changes in the allopregnanolone sensitivity of the GABA$_{A}$ receptors expressed by oxytocin neurons are unclear. On one hand, the clear correlation between declining allopregnanolone sensitivity and reduced $\alpha_1$ subunit mRNA expression makes it tempting to suggest that this subunit is particularly important for the allosteric modulation of the native GABA$_{A}$ receptor by allopregnanolone (Brussaard et al., 1997). While evidence from some in vitro heterologous recombinant studies would support this contention (Shingai et al., 1991), others would not (Lambert et al., 1999) and it remains unclear what relationship-specific subunits
may have on the allopregnanolone sensitivity of native GABA<sub>A</sub> receptors. An alternative explanation may be that changes in the phosphorylation state of the receptor underlie the altered efficacy of allopregnanolone (Leidenheimer and Chapell, 1997; Brussaard et al., 2000; Fancisk et al., 2000). Nevertheless, it remains intriguing that progesterone itself, rather than allopregnanolone, is likely to be responsible for increasing α<sub>1</sub> subunit mRNA expression in oxytocin neurons (Fenelon and Herbison, 2000) and that this change may then possibly confer allopregnanolone sensitivity upon the GABA<sub>A</sub> receptor. If proven, this would represent a highly efficient mechanism through which progesterone could co-ordinate its own influence with that of the non-genomic actions of its derivative allopregnanolone.

**Allopregnanolone, the hippocampus, seizures and anxiety during pregnancy**

Like the benzodiazepine compounds, progesterone can exert anti-seizure and anxiolytic effects in humans (Craig, 1966; Freeman et al., 1993; Herzog, 1995). Work in the rat now clearly suggests that these actions of progesterone are attributable to the direct allopregnanolone modulation of GABA<sub>A</sub> receptors within the brain (Gallo and Smith, 1993; Bitran et al., 1995; Frye and Bayon, 1998). During pregnancy, as well as the menstrual cycle, there is evidence for cyclical changes in seizure threshold and anxiety with adverse symptoms occurring mostly in the post-partum period and late-luteal, early-follicular phase of the cycle when circulating progesterone concentrations have just declined (Dennerstein et al., 1985; Herzog, 1995; Wang et al., 1996; Rupprecht and Holsboer, 1999). Accordingly, it has been suggested that the relatively acute withdrawal of progesterone and allopregnanolone at these times may have important implications for GABA<sub>A</sub> receptor functioning and thus neuronal excitability within the brain.

Smith et al. (1998a,b) have very recently proposed a molecular mechanism within the hippocampus for understanding the increased anxiety and reduced seizure threshold of rats following progesterone withdrawal. Using either pseudopregnant or cyclical progesterone administration paradigms in rats, these investigators have shown that the acute withdrawal of progesterone, or blockade of central allopregnanolone production, results in increased seizure susceptibility and anxiety. In parallel, they found a decrease in total GABA<sub>A</sub> receptor current within the hippocampus and the presence of GABA<sub>A</sub> receptors which were now relatively insensitive to benzodiazepines as well as allopregnanolone. All of these parameters correlated with a marked increase in the expression of the α<sub>4</sub> subunit of the GABA<sub>A</sub> receptor within the hippocampus (Fig. 2).

Thus, Smith and colleagues have suggested that the fall in progesterone concentrations in late pregnancy or the luteal phase, results in lowered allopregnanolone concentrations within the brain and that this causes an increase in expression of the α<sub>4</sub> subunit within the hippocampus (Smith et al., 1998a,b). In turn, the subunit change produces GABA<sub>A</sub> receptors that exhibit relatively fast IPSCs, and consequently the inhibitory effect is shorter-lasting, while this form of the receptor is much less sensitive to enhancement by benzodiazepines and allopregnanolone. The combined result is that the progesterone withdrawal appears likely to result in hippocampal neurons with markedly reduced GABA<sub>A</sub> receptor 'tone' and the consequent elevated hippocampal excitability may, therefore, underlie the increased anxiety and reduced seizure threshold of the rats (Smith et al., 1998a,b).

However, it remains to be determined whether this represents a truly cyclical phenomenon whereby the rising allopregnanolone concentrations of advancing pregnancy would have the opposite effects of suppressing α<sub>4</sub> subunit mRNA expression and reducing anxiety and increasing seizure threshold during pregnancy. Studies by others certainly suggest a more complex scenario in the hippocampus during mid to late pregnancy as progesterone and/or allopregnanolone administration to rats can also modulate the expression of the α<sub>1</sub> and γ<sub>2</sub> subunits of the GABA<sub>A</sub> receptor within this structure (Weiland and Orchinik, 1995; Follesa et al., 1998). Although this, and other issues such as the hippocampal specificity and mechanism of allopregnanolone's influence upon α<sub>4</sub> gene expression are yet to be resolved, these studies are amongst the first to provide an insight into allopregnanolone action within the physiological context of fluctuating progesterone concentrations in pregnancy.
Allopregnanolone and the cerebral cortex during pregnancy

The first indication that GABA_A receptor functioning may be altered during pregnancy came from a study by Majewska et al. (1989) in which they demonstrated that whole forebrain GABA_A receptor binding affinity was markedly increased in mid- to late-pregnancy before it fell to normal levels at parturition in the rat. At the time, they suggested that this may have resulted from the presence of high levels of GABA_A receptor-facilitating neurosteroids in the brain of pregnant animals.

More recently, Follesa et al. (1998) have reported that the abundance of y2 and a5 subunit mRNA within the cerebral cortex declines with advancing pregnancy but then rapidly returns to control values over the last two days before birth (Fig. 2). They also demonstrated an identical temporal profile of changes in GABA_A receptor-induced chloride ion uptake in the cerebral cortex over pregnancy and parturition, which suggested that the subunit changes were of functional significance. Interestingly, in a further paper, these authors showed that the change in y2 subunit mRNA expression was likely to be dependent upon allopregnanolone, rather than progesterone, exposure (Concas et al., 1998). Thus, as is thought to be the case for the a4 subunit in the hippocampus (Smith et al., 1998a), allopregnanolone itself appears to be directly responsible for altering the expression of specific subunit mRNAs in a region-specific manner (Fig. 2). The mechanism through which allopregnanolone modulates transcript stability and/or gene transcription in a subunit selective manner is presently unknown. In vitro studies have suggested that chronic allopregnanolone exposure will eventually uncouple GABA_A receptor signaling at multiple levels (Yu et al., 1996). However, the almost perfect inverse relationship between subunit mRNA changes in the cerebral cortex and circulating allopregnanolone levels in these studies indicate a direct suppressive effect of allopregnanolone on subunit expression (Concas et al., 1998; Follesa et al., 1998).

These new in vivo observations clearly suggest that the changing gonadal steroid levels of pregnancy are having a substantial impact upon GABA_A receptor signaling within the cerebral cortex. The direction of these changes in subunit mRNA expression and GABA_A receptor agonist-induced chloride uptake are, however, opposite in direction to the GABA_A ligand binding results reported initially by Majewska et al. (1989). As those workers examined the whole forebrain, while Follesa et al. (1998) analyzed the cerebral cortex, it seems reasonable to conclude that regional differences in the direction of GABA_A receptor modulation must exist within the brain during pregnancy. Indeed, this is already clear from the opposite direction of subunit mRNA changes in the SON (Fenelon and Herbison, 1996) and cerebral cortex over pregnancy and parturition.

The marked increase in GABA_A receptor expression within the cerebral cortex of rats over the last 2 days of pregnancy is notable not only as a reversal of previous events but perhaps more importantly, for the rapidity with which it occurs. Two studies in women have clearly indicated that the degree of progesterone decline in the peri-partum period is the most important association with post-partum negative mood (Nott et al., 1976; Harris et al., 1994), and it is quite possible that this results from the rapid changes in GABA_A receptor expression within the brain at this time. While post-natal depression and other neuropsychological events of pregnancy are very likely multi-factorial, and hormones such as estrogen may also be involved (Gregoire et al., 1996), the role of fluctuating allopregnanolone modulation and potentially widespread GABA_A receptor changes deserves further attention.

Conclusions

There is clear evidence for a substantial influence of elevated progesterone and allopregnanolone concentrations during late pregnancy upon GABA_A receptor functioning in the brain of the rat. However, as we already have evidence for both cell type- and GABA_A receptor subunit-specific effects, this phenomenon is not simple or homogeneous in nature within the brain. While the elevated concentrations of allopregnanolone in late pregnancy selectively reduce a4/y2, and a5/y2, subunit expression in the hippocampus and cerebral cortex, respectively, progesterone enhances a1 subunit mRNA expression in oxytocin neurons (Fig. 2). Thus, the response of any particular neuronal phenotype may be unique and reflect more
the underlying physiology of the neuronal network in which it operates.

From an experimental view-point, it will be difficult to ascribe robust physiological meaning to the reported changes in the hippocampus and cortex, although clear hypotheses have been presented. In terms of the magnocellular oxytocin neurons, a reasonable physiological mechanism can be based upon the allopregnanolone-enhancement of inhibition in late pregnancy, when these neurons must be restrained, and the subsequent allopregnanolone- and GABA_A receptor-mediated dis-inhibition of these cells, when they must be activated at parturition. However, the clinical relevance of these observations to the control of the timing of parturition in humans have yet to be determined.

It is important to note that allopregnanolone is not the only neurosteroid derived from cholesterol and that many other related steroidal molecules have been discovered to have neuromodulatory actions. For example, pregnenolone, the immediate precursor to progesterone, can exert its own actions upon excitatory and inhibitory amino acid receptors once sulfated, and its dehydroepiandrosterone derivatives are also neuroactive (Lambert et al., 1995; Baulieu, 1998; Rupprecht and Holsboer, 1999). Thus, the state of elevated gonadal steroid synthesis in pregnancy is likely to expose the brain to a variety of different neurosteroids. Whether these neurosteroids are of physiological significance remains to be determined as, in general, high micromolar concentrations are usually required for them to be active in vitro (Lambert et al., 1995; Rupprecht and Holsboer, 1999). From another perspective, we already know that these other neurosteroids can act selectively; for example pregnanolone sulfate does not appear to influence oxytocin neurons (Richardson and Wakerley, 1998). While much is yet to be done to investigate each of these compounds, one of the future challenges will be to provide a coherent picture of co-ordinated neurosteroid action within defined neuronal networks.

In terms of the cognitive and psychiatric changes associated with pregnancy and the post-partum period in humans, there would appear to be sufficient data in the rat to consider seriously the hypothesis that changes in both the GABA_A receptor and allopregnanolone concentrations are at least partly involved. Although studies suggest roles for progesterone in certain facets of brain dysfunction during pregnancy, there is little hard data to distinguish between the actions of progesterone and allopregnanolone in the human. This situation seems unwarranted given the present state of basic science and potential impact of the substantial changes which occur peri-partum in the rat. In the laboratory, further work is clearly required to establish the precise nature of allopregnanolone’s actions upon the GABA_A receptor, as well as the physiological relevance of its actions during pregnancy in multiple neuronal networks. Ultimately, however, the highly region- and subunit-specific nature of the GABA_A receptor changes over pregnancy will require the development of agents selective for specific GABA_A receptor isoforms to enable any degree of specificity in the treatment of unwanted ‘side-effects’ of pregnancy on brain function.

### Abbreviations

- Allopregnanolone
- GABA
- GABA_A receptor
- ITPCs
- SON

- 3α-hydroxy-5α-pregnant-20-one
- or 5α-pregnant-3α-ol-20-one
- γ-aminobutyric acid
- γ-aminobutyric acid type A ionotropic receptor
- inhibitory post-synaptic current
- supraoptic nucleus

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### References


