Allopregnanolone: Regenerative therapeutic to restore neurological health

Gerson D. Hernandez, Roberta D. Brinton *

Center for Innovation in Brain Science, University of Arizona, Tucson, AZ, USA

ARTICLE INFO
Keywords:
- Neurosteroids
- Allopregnanolone
- Regenerative therapeutic
- Neurogenesis

ABSTRACT
Chronic stress has been proposed as a driver of altered brain structure and function, including the pathogenesis of neurodegenerative diseases and a driver of disease progression. A key outcome of stress in the brain is structural remodeling of neural architecture, which may be a sign of successful adaptation, whereas persistence of these changes when stress ends indicate failed resilience. Neuroendocrine homeostasis and stress response are mainly dependent upon the functioning of the hypothalamic–pituitary–adrenal axis. Neurosteroids will fluctuate depending on whether the stress is acute or chronic. Advancements in neurosteroid research have led to the identification of multiple targets for drug development, but the most promising innovative target may be neurogenesis, given its potential impact in neurodegenerative disorders like Alzheimer’s disease. Allopregnanolone is an endogenous pregnane neurosteroid and a reduced metabolite of progesterone, which acts as a potent allosteric modulator and direct activator of the GABA-chloride channel complex. Perhaps the most intriguing finding related to the potential therapeutic effects of allopregnanolone is its potential to promote neuroregeneration.

Prelude by Dr. Brinton: In honor of and gratitude for Bruce McEwen, Ph.D, my post-doctoral mentor whose innovative, bold and expansive approach to science provided the launch pad for me to ultimately address the challenge that in the 21st century, there is not a single cure for a single neurodegenerative disease. Bruce’s integrative genius preceded the age of omics and systems biology. His insights were the result of a deep understanding and appreciation of the complexities of organismic function from the molecular to the systems level.

While this special issue is published in Neurobiology of Stress, I should disclose that my first and only study of stress was when I initially joined Bruce’s laboratory in 1984. My singular stress study with Bruce was an expansion of my earlier analyses that began with my predoctoral mentor Dr. Hank Yamaura and Dr. Kelvin Gee when we mapped allopregnanolone sites of action in brain. This project formed the perfect platform to connect with Bruce’s interest in stress biology (Gee, 1988; Gee, Bolger, Brinton, Coirini and McEwen, 1988; Gee, Chang, Brinton and McEwen, 1987). At the time, evidence for neurosteroids in brain was just emerging and we had detected allopregnanolone binding sites in the hippocampus. With Bruce, we proceeded to determine whether allopregnanolone would modulate the stress response, and as reviewed below, it does indeed.

Thanks to Bruce’s integrative approach, my career as a scientist was transformed. Bruce’s laboratory was a vibrant world of discovery at every level from the molecular to the clinical. A case in point was the opportunity to be a blind observer of a clinical trial of estrogen in women with Alzheimer’s disease conducted by Howard Fillit, M.D. It was that experience in Bruce’s laboratory that transformed my career and expanded my horizon from mechanistic discovery science to translational science and ultimately to clinical science. Outcomes of these analyses have illuminated the bioenergetic crisis in brain following the decline of estrogen during midlife which sets into motion a cascade of events that ultimately can increase the risk of Alzheimer’s disease (R. D. Brinton, 2013). Based on several decades of mechanistic, translational, and clinical science, we have developed an estrogen receptor beta selective formulation to prevent Alzheimer’s prodromal pathology (G. Hernandez et al., 2018; Schneider et al., 2019; Y. Wang et al., 2020) that will now be tested in a phase 2 clinical trial. Bruce was a steadfast supporter of our endeavors at every step in this long journey.

And those allopregnanolone sites in the hippocampus … some are on neural stem cells where they promote neurogenesis and oligogenesis. Based on our mechanistic science (R. D. Brinton, 2013), translational research and phase 1b/2a clinical trial (G. D. Hernandez et al., 2020; Raikes et al., 2022), we are now conducting a phase 2 clinical trial of allopregnanolone in persons with Alzheimer’s disease, to regenerate the
degenerated brain (ClinicalTrials.gov ID: NCT04838301).

The arc of Bruce’s integrative scientific genius is both expansive and impactful leading to discoveries that illuminate mechanistic insights with clinical translational opportunities. The legacy of Bruce S. McEwen, Ph.D. is alive and well in those he mentored, in the generations of scientists to come and in those, worldwide, who will benefit from his invaluable contributions.

1. Introduction

The central role of the brain in the neurobiology of stress and adaptation has been documented by Bruce McEwen over many years (B. S. McEwen, 2006, 2007, 2016; Bruce McEwen et al., 2015; B. S. McEwen and Gianaros, 2010, 2011). Moreover, the relationship between stress, chronic inflammation and neurodegenerative disease is well established. Chronic stress has been proposed as a driver of altered brain structure and function, including the pathogenesis of neurodegenerative diseases and a driver of disease progression (Heneka et al., 2015; Herrera et al., 2015; Hiller, Quinn and Schmidt, 2017; Holmes, 2013; Justice, 2018; Madore, Yin, Leibovitz and Butovsky, 2020; Mishra and Brinton, 2018; Swaab, Bao and Lucassen, 2005; Vyas et al., 2016). According to McEwen and colleagues, a key outcome of stress in the brain is structural remodeling of neural architecture, which may be a sign of successful adaptation, whereas persistence of these changes when stress ends indicate failed resilience. The underlying mechanisms of plasticity and vulnerability of the brain provide the basis for understanding the efficacy of interventions for anxiety and depressive disorders as well as age-related cognitive decline. (Bruce S. McEwen et al., 2015).

2. Neurosteroids and stress

Neuroendocrine homeostasis and stress response are mainly dependent upon the functioning of the hypothalamic–pituitary–adrenal (HPA) axis. An essential regulator of the HPA axis is the corticotropin-releasing hormone (CRH) secreted by the hypothalamus, which promotes the secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary gland, which in turn promotes the secretion of corticosteroids by the adrenal glands (Taylor and Fishman, 1988). The main glucocorticoid, cortisol, acts on many organs and brain regions, mainly exerting excitatory effects of neurons and GABA neurotransmitter receptors (Baulieu and Robel, 1996; Corpéchot et al., 1983; Gee, 1988; Gee et al., 1988; Gee et al., 1987; Hu, Bourreau, Jung-Testas, Robel and Baulieu, 1987; Paul and Purdy, 1992; Purdy, Morrow, Moore and Paul, 1991). The term “neuroactive steroids” was then used to define both natural steroids produced peripherally or in brain and synthetic steroids that rapidly alter the excitability of neurons (Paul and Purdy, 1992).

3. Allopregnanolone and stress

Allopregnanolone (3α-hydroxy-5α-pregn-20-one) is an endogenous pregnane neurosteroid and a reduced metabolite of progesterone (Fig. 1), which acts as a potent allosteric modulator and direct activator of the GABA-chloride channel complex (Baulieu, Robel and Schumacher, 2001; R. Brinton, 1994). Neurosteroids bind to GABA_A receptor sites that differ from GABA, benzodiazepines, ethanol, and barbiturate binding sites and can act as positive or negative modulators of GABA_A receptor function (Irwin and Brinton, 2014). Allopregnanolone (Allo) has high affinity to GABA_A receptors and therefore, has shown to have anxiolytic, anticonvulsant, and hypnocic effects, which were first described in animals (Belelli, Bolger and Gee, 1989; Crawley, Glowa, Majewska and Paul, 1986; Mendelson et al., 1987).

It was demonstrated decades ago that neurosteroids increase acutely after various applied stressors in animal models (Barbaccia et al., 1996). Allo, in particular, has been shown to increase in animals following multiple stressors and seems to be involved in a negative feedback mechanism that reduces CRH, ACTH and corticosterone (Barbaccia et al., 1997; Guo et al., 1995; Patchev, Shoaihi, Holsober and Almeida, 1994; Purdy et al., 1991). Increases in neurosteroid levels have also been documented after acute stress exposure in humans and are suggested to be neuroprotective. Physiological stressors that demonstrate changes in allopregnanolone levels are pregnancy and parturition (Luisi et al., 2000a). During their reproductive years, women are chronically exposed to Allo concentrations ranging from less than 1 nmol/l (0.32 ng/ml) to over 4 nmol/l (1.27 ng/ml) during the luteal phase (Genazzani et al., 1998). During pregnancy, the increase in plasma progesterone results in a production rate of Allo that can reach 100 mg per day. The highest concentrations of Allo are reached during the third trimester of pregnancy at levels up to 157 nmol/l (50 ng/ml), which are not associated with adverse effects for either mother or fetus (Dombroski, Casey and MacDonald, 1997; Gago et al., 2004; Luisi et al., 2000b; Jun Ming Wang, Patrick B Johnston, Bret Gene Ball, & Roberta Diaz Brinton, 2005). Allo concentrations subsequently fall after parturition, a finding that has led to its correlation with post-partum behavioral disorders (Osborne, Betz, Yenokyan, Standeven and Payne, 2019). Moreover, allopregnanolone concentrations in the fetal brain are markedly increased in response to acute hypoxic stress caused by umbilical cord constriction, which indicates that a stressor such as prenatal hypoxemia initiates a neurosteroid response that may protect the fetal brain cells from hypoxia (Hirsh, Yawno, Nguyen and Walker, 2006). In obstetrics, transient compression of the umbilical cord or uteroplacental insufficiency can be detected in electronic fetal heart monitor tracing as heart decelerations, which are related to interruption of fetal oxygenation (Lee

![Fig. 1. Allopregnanolone biosynthetic pathway. Steroidogenesis of allopregnanolone starting from cholesterol metabolism. The brain must synthesize its own cholesterol from acetyl-CoA of peripherally circulating cholesterol. In the mitochondria it is converted into pregnenolone by the cytochrome P450 side-chain cleavage enzyme. It is then converted in the cytosol to progesterone. Allo is a reduced metabolite of progesterone synthesized in the adrenal cortex, gonads and central nervous system.](image-url)
and Hon, 1963; Macones, Hankins, Spong, Hauth and Moore, 2008). In the case of impaired uteroplacental perfusion, decelerations result from chemoreceptor responses to hypoxia, which in mild cases typically leads to decreased fetal movements or sleeping. It has been postulated that the reason for such a fetal response is to reduce oxygen consumption, but perhaps it is also due to increased levels of allopregnanolone which consequently causes sedation. Lastly, increased levels of Allo have been associated with one behavioral condition known as premenstrual dysphoric disorder, in which symptoms are related to a woman’s menstrual cycle. (Girdler, Straneva, Light, Pedersen and Morrow, 2001). A significant increase in Allo is seen especially during the luteal phase, however symptom severity is not positively correlated with Allo levels, but rather exhibits a biphasic response considering that women with greater mood symptoms have lower levels of allopregnanolone (T. Bäckström et al., 2011; Girdler et al., 2001).

Contrary to what is observed in acute stress, allopregnanolone levels seem to be decreased in chronic stress, which has been attributed to compensatory changes to regain the reduced hypersensitivity of GABA receptors (Bali and Jaggi, 2014; Matsumoto, Puia, Dong and Pinna, 2007; Serra, Pisu, Mostallino, Sanna and Biggio, 2008). Neurosteroid reduction in relation to disease-associated stressors has been demonstrated in persons with depression (Elena Romeo et al., 1998; Uzunova et al., 1998), migraines (Rustichelli et al., 2020), post-traumatic stress disorder (PTSD) (Rasmussen et al., 2006), and after blast-related traumatic brain injuries (TBI) (Marx et al., 2016). Patients with mood disorders, including major depression and PTSD, exhibit reduced levels of Allo, including its biosynthesis (Agis-Balboa, Guidotti and Pinna, 2014), in plasma, CSF and brain (Elena Romeo et al., 1998; Uzunova et al., 1998). Moreover, research has shown that treatment with selective serotonin reuptake inhibitors (SSRIs) increases Allo levels in the brain (Pinna, Costa and Guidotti, 2006) or reverses the stress induced decrease of Allo in both animal models and patients with depression (Elena Romeo et al., 1998; Uzunova et al., 1998). Interestingly, decreased neurosteroid levels was not detected in chronic stressors like work-related psychosocial stress and burnout (Torbjörn Bäckström, Bixo, Nyberg and Savic, 2013) perhaps because these are not necessarily disease associated stressors.

Collectively, the work cited above laid the foundation and prompted further research to develop neurosteroids as therapeutic agents. Consequently, a therapeutic development program recently yielded an exogenous formulation of allopregnanolone (Brexanolone) for affective disorders. Brexanolone has been granted a “breakthrough therapy designation” by the FDA for the treatment of post-partum depression (Azhary Y, 2020; Melzter-Brody et al., 2018). Two separate clinical trials testing brexanolone in women with postpartum depression used a continuous intravenous (IV) titration infusion regimen for up to 60 hours, which resulted in significant and clinically meaningful reductions in Hamilton Rating Scale (HAM-D) for Depression scores compared to placebo (Melzter-Brody et al., 2018). However, the pattern of adverse events leading to dose reductions or interruptions in the trials show that most were related to excessive sedation or loss of consciousness, which is expected with such a dosing regimen. Brexanolone has since been further developed as an oral formulation with early evidence for improving symptoms of depression, as measured by HAM-D scores, in a phase 3 clinical trial in women with postpartum depression (Kristina M. Deligiannidis et al., 2021).

4. Neurogenesis and allopregnanolone

Neurogenesis in the adult human hippocampus was demonstrated decades ago by Eriksson and colleagues (Eriksson et al., 1998) and others have confirmed that significant neurogenesis occurs in healthy, aging human brains (Boldrini et al., 2018; Spalding et al., 2013). The most prominent adult neurogenic niches are located in the hippocampal subgranular zone (SGZ) of the dentate gyrus and the subventricular zone (SVZ) of the lateral ventricles (Irwin and Brinton, 2014). In the hippocampal neurogenic niche, newborn dentate gyrus granule cells migrate to the granule cell layer and integrate into the adjacent molecular cell layer. As these cells mature, dendrites grow into the molecular cell layer to receive glutamatergic afferents from the entorhinal cortex and their axonal projections form mossy fiber synapses in the CA3 subfield to strengthen neural circuitry and function of the hippocampus (Irwin and Brinton, 2014). In addition to the classic neurogenic areas, newly born interneurons, most destined to be GABAergic, are dispersed throughout the relatively voluminous cortical space (Jurkowski et al., 2020; Lim, Mi, Llorca and Martin, 2018).

The adult neurogenic turnover rate in the neocortex is similar to that of the dentate gyrus, although with differences in cell type, function, and relative numeric ratios to surrounding mature cells (Heather A. Cameron and Dayer, 2008). The regenerative potential of these brain regions continues throughout the duration of life, but neuronal progenitor proliferation dramatically decreases with age in both animal models and humans (H. A. Cameron and McKay, 1999; Knoth et al., 2010; Kuhn, Dickinson-Anson and Gage, 1996). Impaired neurogenesis can result from factors that are associated with age-related degenerative diseases and dementia, such as HPA axis dysregulation, chronic inflammation and stress, and microglial activation (Becker, 2017).

Surget and Belzun reviewed previous theoretical and experimental work which supported the underlying role of adult hippocampal neurogenesis in stress response. Subsequently, they proposed that adult hippocampal neurogenesis, through its computational influences, is instrumental in shaping adaptation to environmental demands (Surget and Belzun, 2021). Neural networks require both plasticity and stability (Merrillod, Bugaiska, & BONIN, 2013). Thus, learning requires plasticity to be able to encode new knowledge and the stability to prevent previous knowledge from being forgotten. Hippocampal neurogenesis seems to influence specific functions of the hippocampus, specifically those that are related to cognitive processes like pattern separation, and those related to behavioral adaptation of stress (Anacker and Hen, 2017; Anacker et al., 2018; Clelland et al., 2009; Snyder, Soumier, Brewer, Pickel and Cameron, 2011). Interestingly, healthy aging has been associated with a decreasing ability of behavioral pattern separation that may be evidenced by episodic memory problems, and has been related to the aging dentate gyrus and concomitant CA3 subregion deficits (Yassa and Stark, 2011). This was demonstrated with diffusion tensor imaging of the perforant path fiber bundle during two behavioral pattern separation tasks, which showed an age-related decrease in diffusion signal (reduced fiber path integrity) (Yassa, Muffuler and Stark, 2010).

A great deal has been written about allopregnanolone’s psycho-therapeutic effects, but very little emphasis has been placed on allopregnanolone’s regenerative effects (Fig. 2). Perhaps the most intriguing finding related to the effects of Allo was that allopregnanolone rapidly induced cytoarchitectural regression in cultured fetal hippocampal neurons, which later was shown to be a prelude to mitogenesis (R. Brinton, 1994; J. M. Wang, P. B. Johnston, B. G. Ball, & R. D. Brinton, 2005). Subsequently, Brinton and colleagues demonstrated that allopregnanolone, in a dose-dependent manner, induced a significant increase in proliferation of neuroprogenitor cells (NPCs) derived from the rat hippocampus and human neural stem cells (hNSCs) derived from the cerebral cortex (J. M. Wang et al., 2005). Based on those findings, they later demonstrated that a single administration of Allo reversed both neurogenic and cognitive deficits in vivo in male 3xTgAD mice prior to the appearance of Alzheimer’s disease (AD) pathology (J. M. Wang et al., 2010). Important for therapeutic potential, Allo significantly increased neurogenesis and functionally reversed cognitive deficits in male 3xTgAD mice following the onset of AD pathology (Chen et al., 2011). In summary, Allo significantly increased the number of newly generated cells and increased their survival, restoring the regenerative potential of the brain to normal without exceeding normal (Singh et al., 2012; J. M. Wang et al., 2010). Such regenerative effect of Allo is dose-dependent and exhibits a classic U-shaped dose-response curve.
indicating that more is not better. Based on that principle high doses of Allo, that induce a sedative response, do not promote neurogenesis. The inverted-U shaped dose response profile of Allo is particularly important from a safety perspective. Greater than normal concentrations of Allo or continuous unrelenting exposure to Allo inhibit neurogenesis thereby protecting against uncontrolled cell proliferation (Irwin and Brinton, 2014).

The aforementioned body of preclinical work has led to the clinical development of Allo as a novel regenerative therapeutic for Alzheimer’s disease (Irwin and Brinton, 2014). Allo was administered for the first time in persons diagnosed with mild cognitive impairment due to AD or mild AD in a phase 1b/2a clinical trial (ClinicalTrials.gov Identifier: NCT02221622). Outcomes demonstrated that Allo was safe and well-tolerated after a single IV infusion and after weekly IV infusions for 12 weeks (G. D. Hernandez et al., 2020). A maximally tolerated dose of 6 mg was established using sedation as a threshold which interestingly demonstrated a sex difference in sedation tolerance. Women exhibited signs of sedation at doses ≥10 mg whereas men did at doses ≥ 6 mg. Therefore, to optimally target neurogenesis, the sub-sedative dose of 4 mg was chosen for the treatment regimen (G. D. Hernandez et al., 2020).

Exploratory analyses indicated that Allo has the potential to slow the rate of decline in hippocampal volume and may increase volume in participants who were APOE ε4 carriers. Furthermore, multiple measures of white matter integrity indicated evidence of preserved or improved integrity. Allo significantly increased fiber tract fractional anisotropy and quantitative anisotropy, primarily in the corpus callosum, bilateral thalamic radiations, and bilateral corticospinal tracts. Lastly, consistent with structural changes in the brain, Allo increased local, interregional, and network-level functional connectivity in AD-vulnerable regions, including the precuneus and posterior cingulate, and network connections between the default mode network and limbic system.

Interestingly, women with postpartum depression and low levels of Allo have shown to have a reduced resting-state functional connectivity within corticolimbic regions such as anterior cingulate cortex, bilateral amygdala, hippocampus and dorsolateral prefrontal cortices (K. M. Deligiannidis et al., 2013).

Using surrogate imaging biomarkers as indicators of regeneration, we hypothesize that the regenerative effect of Allo has the potential to reverse the altered brain structure and function caused by chronic stress and seen in the progression of neurodegenerative diseases (Fig. 3). Based on the preclinical and early clinical evidence, we reasoned that if Allo exerts a regenerative effect in the brain then regeneration of the hippocampus could be evident in MRI detectable structural change: either an increase in hippocampal volume relative to a participant’s baseline and to placebo-treated participants, or a decrease in the magnitude of hippocampal atrophy relative to a participant’s baseline and to placebo. Moreover, if Allo is promoting oligogenesis then indicators of white matter structural integrity could be detectable using diffusion tensor imaging. If Allo is improving or maintaining structural connectivity, we reasoned that functional connectivity could be an indicator of restored or regenerated synaptic neural networks. Lastly, if Allo is promoting neurogenesis and myelin-genesis then functional regeneration could be affected as indicated by an increase in local connectivity within functionally homogeneous regions (Raikes et al., 2022).

The development of Allo as a regenerative therapeutic (Fig. 4) continues with an upcoming phase 2, multi-center, double-blind, randomized, placebo-controlled, clinical trial assessing efficacy and further testing the safety of Allo (ClinicalTrials.gov Identifier: NCT04838301). This is a proof-of-concept trial of 18 months duration, with a parallel
group design for the initial 12 months followed by an open-label period of 6 months, which will utilize an imaging endpoint as a surrogate biomarker of regeneration. Based on outcomes from the previous phase 1b/2a study, male and female participants ranging in age from 55 to 80 years old with an APOE e4-positive genotype and diagnosed with probable AD. Participants will receive weekly, 30-min intravenous infusions of Allo 4 mg or placebo.

5. Conclusions

The neurobiology of stress plays an important role in chronic inflammation and the development of neurodegenerative diseases. Advancements in neurosteroid research have led to the identification of multiple targets for drug development, but the most promising innovative target may be neurogenesis. Chronic stress impairs brain structure and function, which has the potential impact in neurodegenerative disorders like Alzheimer’s disease. This target has enabled the development of allopregnanolone as a regenerative therapeutic to restore neurological health.
Gerson D. Hernandez: Writing – original draft, Visualization, Investigation, Conceptualization. Roberta D. Brinton: Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Roberta Brinton reports a relationship with Neutherapeutics that includes: board membership. Gerson Hernandez reports a relationship with Neutherapeutics that includes: consulting or advisory. Roberta Brinton has patented US#85963299282 issued to Roberta Brinton.

Acknowledgement

This research has been supported by the National Institute on Aging, grant/award numbers: U01AG031115, U01AG047222, P50AG05142, R01AG063826; and, Alzheimer Drug Discovery Foundation grant 20150701.01.

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