Pleiotropic actions of allopregnanolone underlie therapeutic benefits in stress-related disease

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ABSTRACT

For several years, research from around the world has suggested that the neuroactive steroid (3α,5α)-3-hydroxy-5α-pregnan-20-one (allopregnanolone) may have therapeutic potential for treatment of various stress-related diseases including post-traumatic stress disorder (PTSD), depression, alcohol use disorders (AUDs), as well as neurological and psychiatric conditions that are worsened in the presence of stress, such as multiple sclerosis, schizophrenia, and seizure disorders. In this review, we make the argument that the pleiotropic actions of allopregnanolone account for its ability to promote recovery in such a wide variety of illnesses. Likewise, the allopregnanolone precursors, pregnenolone and progesterone, share many actions of allopregnanolone. Of course, pregnenolone and progesterone lack direct effects on GABA A receptors, but these compounds are converted to allopregnanolone in vivo. This review presents a theoretical framework for understanding how endogenous neurosteroids that regulate 1) γ-aminobutyric acid (GABA) A receptors, 2) corticotropin releasing factor (CRF) and 3) pro-inflammatory signaling in the innate immune system and brain could play a key role in both the prevention and treatment of stress-related disease. We further discuss cautions and limitations of allopregnanolone or precursor therapy as well as the need for more clinical studies.

1. Introduction

This review primarily addresses the rationale for using the neuroactive steroid allopregnanolone for treatment of various stress-related diseases based on its pleiotropic actions upon γ-aminobutyric acid (GABA) A receptors, corticotropin releasing factor (CRF) signaling and pro-inflammatory signaling in the innate immune system. We present theories that stress-related disease results from 1) the loss of central nervous system (CNS) inhibition due to adaptations in GABA A receptor-mediated neurotransmission across brain, 2) excessive CRF signaling that dysregulates the hypothalamic-pituitary-adrenal (HPA) axis and extrahypothalamic CRF circuits across brain, and 3) excessive neuroimmune signaling through Toll-like receptors (TLRs) in the innate immune system and the brain. Next, we review the evidence that allopregnanolone counteracts these stress-induced adaptations to restore GABAergic inhibition, moderate CRF signaling across brain and inhibit pro-inflammatory TLR signaling in the innate immune system and brain. Pregnenolone and progesterone are precursors of allopregnanolone that increase its concentration in serum and brain and also directly inhibit inflammatory signaling through TLR receptors. We argue that all of these endogenous neurosteroids may represent protective factors that prevent the development of stress-related disease vulnerability and could be valuable therapeutics as well. The pleiotropic actions of these neurosteroids provide an attractive strategy to simultaneously address three major domains of stress-related disease in its treatment. In addition, this rationale summarizes evidence that allopregnanolone, pregnenolone or progesterone address many of the co-occurring symptoms of stress-related neuropsychiatric conditions, including anxiety, depression, sleep disturbance, and pain, all of which may facilitate the management of disease.

2. The impact of stress on GABAergic transmission

An abundance of evidence shows that GABAergic transmission is impacted by stress at multiple levels. Clinical studies of stress-related psychiatric disorders suggest alterations in GABAergic transmission, including decreased GABA levels, reductions in the density of GABAergic interneurons and changes in GABA A receptor subunit expression (Baer et al., 2000; Sanacora et al., 2004; Hasler et al., 2007; Rajkowska et al., 2007; Sequeira et al., 2009; Luscher et al., 2011).
agreement, numerous preclinical studies have highlighted the role of GABAergic transmission in the HPA response to stress, and how chronic stress exposure disrupts these regulatory responses. Local GABAergic neurons in the paraventricular nucleus (PVN) of the hypothalamus and in adjacent forebrain areas send a GABAergic inhibitory signal to CRF neurons in response to various stressors (Cullinan et al., 2008). This regulatory response is disrupted under stress conditions. Indeed, several paradigms of acute stress (inescapable tail-shock, mild foot-shock, carbon dioxide inhalation, forced swim, handling) induce a rapid and reversible downregulation of GABAergic transmission in several brain regions of male rats, as assessed by binding assays of GABA and GABA_A receptor modulators, as well as GABA-stimulated chloride flux in synaptoneurosomes (Drugan et al., 1989; Biggio et al., 2007). However, sex and species differences in the effects of stress on GABA_A receptor function have also been highlighted (Akinci and Johnston, 1993; Chadda and Devaud, 2004).

Another mechanism that contributes to the stress-induced loss of GABAergic inhibition involves a dephosphorylation and down-regulation of the K^+ /Cl^- co-transporter KCC2, which has been reported to occur in the hypothalamus of male rats and following acute stress; such changes switch the GABAergic transmission on CRF neurons to occur in the hypothalamus of male rats and mice following acute stress (Cullinan et al., 2008). During stress, the main neurotransmitters released in PVN are norepinephrine and glutamate (Herman et al., 2002). In basal conditions, the CRF neurons are under the influence of peripheral hormonal influences, during stress. The afferents inputs release various neurotransmitters such as norepinephrine, glutamate, GABA, angiotensin II, CRF itself and other peptides in the PVN. These molecules interact with their receptors located in the CRF neurons and regulate CRF synthesis, release and intracellular signal-transduction pathways. Hypothalamic CRF neurons receive inhibitory afferents from hippocampus and, indirectly, from prefrontal cortex, as well as excitatory inputs from central amygdala and from the bed nucleus of stria terminalis (BNST), indirectly (Ziegler and Herman, 2000; Ulrich-Lai and Herman, 2009). In basal conditions, the CRF neurons are under the inhibitory influence of GABAergic interneurons located in the PVN (Cullinan et al., 2008). During stress, the main neurotransmitters released in PVN are norepinephrine and glutamate (Herman et al., 2002). In stress-related psychiatric disorders, chronic stress disrupts the equilibrium between excitatory/inhibitory inputs, causing a decrease in inhibition and an increase in excitatory signals that results in hyperactivity of the HPA axis.

CRF and its receptors (CRFR1/2) are widely distributed across the brain and by binding its high affinity receptor, CRFR1, CRF is able to coordinate autonomic and behavioral stress responses. Thereby, dysregulation of the CRF/CRFR1 system has been linked to stress-related
Supporting the role of CRF hyperactivity in stress-related neuropsychopathologies, studies in rodents show that CRF injections into the ventral hippocampus increase anxiety-like behavior in rats (Pentkowski et al., 2009). Furthermore, central and basolateral amygdala infusion of CRF increases anxiety-related and freezing behavior and reduces social interaction in rats (Swiergel et al., 1993; Sajdý et al., 1999). Intra-BNST administration of CRF dose-dependently increases anxiety-like behavior in rats (Sahuque et al., 2006). In nucleus accumbens, CRF regulates appetitive behavior through regulation of dopamine release. In this case, severe stress produces a persistent dysregulation of CRF-dopamine interactions, causing a switch from appetitive to aversive behavioral actions (Lemos et al., 2012). In ventral tegmental area, the dysregulation of the CRF/CRFR1/2 system has been correlated with AUDs and other substance use disorders (Grieder et al., 2014; Zorrilla et al., 2014; Henckens et al., 2016). Finally, modification in the noradrenergic system of the locus coeruleus caused by CRF hyperactivity has been proposed to underlie pathological hyperarousal observed in numerous stress-related psychiatric disorders (Bissette et al., 2003). The circuit adaptations described here are shown in Fig. 2 along with the hypothetical role of neuroactive steroids in restoring excessive CRF signaling in the brain.

4. The impact of stress on pro-inflammatory neuroimmune signaling

Neuroimmune signaling through TLRs is an important contributor to various inflammatory systemic, neurological and psychiatric conditions, including traumatic hemorrhagic shock (Reino et al., 2011), AUDs (He and Crews, 2008; Qin et al., 2008; Crews et al., 2017a,b), other addictions (Lacagnina et al., 2017), depression (Dantzer et al., 2008; Bhattacharya et al., 2016), traumatic brain injury (He et al., 2004a) and epilepsies (Maroso et al., 2011). The innate immune cells in the brain, microglia, as well as neurons and other glial cells, signal via TLRs to promote innate immune gene expression and produce pro-inflammatory cytokines and chemokines in a progressive and feed-forward fashion (Pavlov and Tracey, 2017) that is particularly long-lasting in the brain (Crews et al., 2017a,b).

4.1. Evidence for stress-induced activation of TLR signaling

TLRs recognize all types of microbes that invade the human body as well as endogenous signals that are released in response to tissue damage, cellular stress and psychological stress. The activation of TLRs can protect against external invasions of pathogens to fight infection, but they can also arise in response to tissue injury such as ischemia (Aguirre et al., 2013), cellular oxidative stress (Akhter et al., 2019) and psychological stressors such as chronic restraint (Zhang et al., 2008). Using TLR4-deficient mice, Zhang and colleagues demonstrated that activation of TLR4 is responsible for the stress-induced increase in pro-inflammatory cytokines tumor necrosis factor (TNF-α) and interleukin-1β, as well as a decrease in the anti-inflammatory cytokines interferon-γ and interleukin-2. Moreover, TLR4 contributed to the lymphocyte reduction induced by chronic stress (Zhang et al., 2008).

The activation of TLRs in response to endogenous signals can be harmful and often causes more problems than the initial cell or tissue injury (Hennessy et al., 2010). This process is known as pro-inflammatory innate immune signaling in the periphery and neuroimmune signaling in the brain. One example of stress activation of TLR signaling is evident from recent studies showing that the endogenous TLR4 agonist endotoxin is increased in circulation following acute alcohol and restraint stress in rats (Walter et al., 2017). The effect is similar in magnitude to the elevation of cortisol in the circulation and may represent an important connection between stress and TLR4 signaling. Furthermore, TLR activity is known to be enhanced by CRF in both macrophages (Tsatsanis et al., 2006) and brain (Whitman et al., 2013), indicating another mechanism of stress enhancement of pro-inflammatory signaling.
The combination of these pleiotropic actions may restore losses in GABAergic transmission, CRF overactivation and contribute to the pathological effects of stress. Together, the production of pro-inflammatory signaling and the re-expression of TLR2, TLR3, TLR4, and TLR7, and members of the pathways activated by these pathogen-activated receptors (Crews et al., 2017a,b). TLR signaling initiates with complex formation and activation of myeloid differentiation response protein (MyD88)-dependent and independent pathways. These include transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), cyclic AMP response element binding protein (CREB), activator protein-1 (AP-1), and interferon regulatory factor (IRF) that translocate to the nucleus and control expression of chemokines and cytokines. Ethanol activates the TLR-MyD88-NFkB pathways as well as the TLR3-regulated TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway. Intermittent alcohol drinking during adolescence upregulates the expression of TLR4 in the adult hippocampus, TLR3 and TLR4 in the adult prefrontal cortex, and TLR1-TLR8 in the adult cerebellum (Breese and Knapp, 2016; Knapp et al., 2016; Harper et al., 2017), suggesting distinct immune activation and function at distinct brain sites.

Production of pro-inflammatory cytokines appears to be involved in altered brain activity (Gruol, 2013). Indeed, neuroimmune signaling via the classic pro-inflammatory cytokines in brain enhances glutamatergic transmission and reduces GABAergic transmission (Stellwagen et al., 2005; Ferguson et al., 2008; Stuck et al., 2012; Pribiag and Stellwagen, 2013), rather than produce other signs of inflammation such as swelling or macrophage infiltration. On the other hand, TLR7 signaling in brain leads to neurodegeneration, involving the production of high motility group box 1 (HMGB1) and the microRNA let-7 (Coleman et al., 2017). The following sections will describe the evidence that neurosteroids may restore losses in GABAergic transmission, CRF overactivation and excessive TLR signaling. The combination of these pleiotropic actions may be key to their therapeutic benefits.

5. Evidence that GABAergic neuroactive steroids abrogate the loss of GABAergic inhibition in stress-related disease

Neuroactive steroids that are 3α,5α-reduced derivatives of progesterone, deoxycorticosterone, dehydroepiandrosterone and testosterone, are endogenous positive modulators of all GABA_{A} receptors (Puia et al., 1991; Kokate et al., 1994; Belelli et al., 2002; Reddy and Bogawski, 2002; Herd et al., 2007). Allopregnanolone and alloxytrahydrodeoxycorticosterone (3α,5α-THDOC) exhibit high potency at most subtypes of GABA_{A} receptors (Puia et al., 1990, 1993), although they are more potent at some extrasynaptic GABA_{A} receptors containing the δ subunit (Belelli and Lambert, 2005; Carver and Reddy, 2013). For example, low concentrations of 3α,5α-THDOC, which have no effect on phasic conductance mediated by synaptic GABA_{A} receptors, selectively enhance tonic currents mediated by δ subunit containing GABA_{A} receptors in mouse dentate gyrus and cerebellar granule cells (Stell et al., 2003). Likewise, allopregnanolone and the synthetic neuroactive steroids SGE-516 and ganaxolone also increase tonic currents in dentate gyrus granule cells (Modgil et al., 2017). Although the neuroactive steroid binding sites are located at the interface of the α and β subunits of synaptic receptors (Hosie et al., 2006), the δ subunit is thought to be responsible for the enhanced sensitivity to neuroactive steroids. In fact, δ subunit knockout mice have blunted tonic currents along with reduced sensitivity to neuroactive steroid modulation of GABA_{A} receptors (Mihalek et al., 1999; Spigelman et al., 2003; Stell et al., 2003). Furthermore, δ subunit knockout mice also display a loss of δ subunits and blunted tonic inhibition (Chandra et al., 2006), along with reduced GABAergic potentiation by alphaxalone (Liag et al., 2008).

Neuroactive steroids might represent a useful therapeutic approach to restore deficits in GABAergic inhibition observed in patients with stress-related disease. This argument is supported by data from animal models and the effects of neuroactive steroids in human conditions that involve the loss of inhibitory control in the CNS. Some examples are described below.

5.1. Anxiolytic and antidepressant actions in animals and humans

Administration of allopregnanolone exerts anxiolytic-like and antidepressant-like effects in rodents (Crawley et al., 1986; Bitran et al.,
1991; Khisti et al., 2000) and humans (Kanes et al., 2017; Meltzer-

agitation, anxiety and depressive symptoms during a 60 hour constant

travenous infusion of Brexanolone, a proprietary formulation of allo-

contribute to these actions. Previous evidence has documented that

showed that benzodiazepine sensitive synaptic GABAA receptors are

produce the loss of GABA A receptors in the CNS. By contrast, alcohol dependence reduces GABAergic inhibition across

deficits in sleep quality in stress-related disorders by restoring the

5.3. Effectiveness in status epilepticus

Status epilepticus is a neurological life-threatening condition char-

ceptors do not internalize and are functional (Goodkin et al., 2008),

Thus, representing a putative pharmacological target for status epi-

regions. Given that GABAergic neuroactive steroids have potent ant-

cortical GABA inhibition is compromised in patients with depression, since le-

5.4. Potential actions in sleep disturbances

Both synaptic and extrasynaptic GABA A receptors contribute to

regulate sleep and wakefulness. Benzodiazepines, traditionally used to

In summary, deficits in inhibitory transmission in the CNS are hallmarks of stress-related disease. The neuroactive steroid allo-

pregnanolone is capable of correcting deficits in inhibitory neuro-

pregnanolone in vivo (Marx et al., 2007; Porcu et al., 2009; Milivojevic et al., 2016), and therefore may also share the ability to abrogate the loss of inhibitory transmission in stress-related disease.
6. Evidence that allopregnanolone restores deficits precipitated by aberrant CRF signaling

Acute stress rapidly induces HPA axis activation with subsequent release of corticosterone, as well as allopregnanolone (Purdy et al., 1991). The increase in allopregnanolone content, which occurs approximately 30 min after acute stress, is thought to represent a homeostatic mechanism to restore the GABAergic inhibition upon the hypothalamic PVN, thus shutting down HPA axis activity (Biggio et al., 2007; Gunn et al., 2015). Both corticosterone and allopregnanolone exert a negative feedback upon the hypothalamus and pituitary. Specifically, allopregnanolone counteracts the anxiety-like behavior induced by CRF administration, although it does not appear to be involved in basal CRF release (Patchev et al., 1994). Moreover, allopregnanolone or 3α,5α-THDOC administration before stress attenuates the stress-induced increase in adrenocorticotropic hormone (ACTH) and corticosterone (Owens et al., 1992; Patchev et al., 1996). In agreement, intracerebroventricular administration of allopregnanolone antiserum enhanced the corticosterone response to stress in prepubertal and adult rats, without affecting its basal levels (Guo et al., 1995). In addition, systemic administration of allopregnanolone to adult male non-stressed rats increased hypothalamic CRF content as well as serum ACTH and corticosterone (Naert et al., 2007), supporting a regulatory role for this neuroactive steroid in HPA function, whereby allopregnanolone may increase hormone levels in basal conditions and decrease them in stress-induced perturbations to restore homeostasis. Systemic administration of pregnenolone, dehydroepiandrosterone and their sulfate metabolites also increased hypothalamic CRF and serum ACTH and corticosterone (Naert et al., 2007). All these effects were rapid and likely mediated by a direct action of neuroactive steroids on neurotransmission in the hypothalamus that regulate HPA axis activation.

Several lines of both clinical and basic science evidence suggest that neuroactive steroids may restore homeostasis in CRF signaling both at the hypothalamic and extrahypothalamic circuit levels. The anxiolytic effects of allopregnanolone are likely to be related to both hypothalamic and extrahypothalamic CRF levels since CRF circuits are tightly coupled to anxiety-like behaviors in rodents (vide supra). Some examples follow.

Affective disorders, including major depression, postpartum depression, PTSD and AUDs are characterized, among other features, by neuroendocrine alterations at the HPA axis level (Bixo et al., 1997; Adinoff et al., 2005a, 2005b, 2005c; Girdler and Klatzkin, 2007; Girdler et al., 2012; Baumeister et al., 2014; Schule et al., 2014; Rasmusson et al., 2017). These alterations generally involve excessive baseline cortisol and suppression of the HPA axis response to stress. Considering the ability of allopregnanolone to regulate the HPA axis at the level of the hypothalamus, it is possible that restoration of HPA axis balance is an important component of treatment. Indeed, the remarkable clinical efficacy of Brexanolone in the treatment of postpartum depression may be related to this property of allopregnanolone. While further studies are needed to determine the mechanism(s) of the antidepressant actions of Brexanolone, the rapid and long-lasting efficacy following a short course of 60 hours of treatment suggests a type of reset that is consistent with normalization of HPA axis function.

Furthermore, although classical treatments for depression such as selective serotonin reuptake inhibitors require several weeks to produce therapeutic actions, increased neurosteroidogenesis is a likely mechanism involved in their therapeutic actions. Several studies have shown that administration of antidepressant drugs restores neuroactive steroid concentrations in both patients and rodents (Uzunov et al., 1996; Romeo et al., 1998; Uzunova et al., 1998; Marx et al., 2006; Schule et al., 2014). Indeed, serotonin reuptake inhibitors promote the conversion of 5α-dihydroprogesterone to allopregnanolone via a direct effect on the neurosteroidogenic enzyme 3α-hydroxysteroid dehydrogenase (Griffin and Mellon, 1999). Thus, allopregnanolone may have great potential for the treatment of stress-related psychiatric disorders in general and specifically in those associated with dysregulation of the HPA axis.

PTSD patients show elevated CRF levels (Bremner et al., 1997), along with a dysregulated HPA axis function that leads to glucocorticoid hypersensitivity (Castro-Vale et al., 2016). In addition, concentrations of GABAergic neuroactive steroids are altered in PTSD patients: a reduction in cerebrospinal levels of allopregnanolone has been reported in premenopausal women and men, and such levels were negatively correlated with PTSD symptoms (Rasmusson et al., 2006, 2017). Indeed, both preclinical and clinical evidence suggests a role for allopregnanolone in PTSD (Rasmusson et al., 2017). Clinical trials are ongoing (NCT03799562; NCT00560781) to test the effectiveness of the precursor pregnenolone in targeting PTSD symptoms.

Social isolation in animal models of affective disorders each induce alterations in CRF and HPA axis function and each of these conditions is responsive to treatment with allopregnanolone or pregnenolone. Social isolation from weaning to adulthood is a model of chronic stress and PTSD that markedly decreases brain and plasma allopregnanolone levels in rats and mice (Serra et al., 2000; Dong et al., 2001). A blunted HPA axis activity has been hypothesized to account for such decrease (Biggio et al., 2014). In agreement, socially isolated male rats also show a reduction in basal ACTH and corticosterone levels (Serra et al., 2005; Pisu et al., 2016). However, socially isolated male rats are more sensitive to the steroidogenic effects of stress, as shown by the greater increase in allopregnanolone content following acute stress (Serra et al., 2000, 2003), suggesting that social isolation actually induces an hyperresponsiveness of the HPA axis. In agreement, central administration of CRF increased plasma corticosterone levels to a greater extent in socially isolated rats, compared to group-housed controls (Serra et al., 2005). Likewise, exposure to acute stress induced a greater and prolonged increase in CRF and ACTH content in socially isolated rats (Boero et al., 2018), supporting a dysregulation of the HPA axis. Indeed, social isolation also impairs the HPA negative feedback regulation, as suggested by the blunted corticosterone response to dexamethasone challenge, observed in socially isolated vs. group-housed male rats (Serra et al., 2005). Importantly, administration of allopregnanolone either from the onset of social isolation, or following six weeks of isolation, prevents or normalizes the corticosterone response to the dexamethasone suppression test, as well as depression- and anxiety-like behavior in male rats (Evans et al., 2012). Since allopregnanolone normalizes the HPA axis and behavioral abnormalities after social isolation, it could have similar effects in other conditions that dysregulate the HPA axis and produce abnormal anxiety and depression.

7. Evidence that neurosteroids inhibit TLR4 signaling and the production of pro-inflammatory molecules

Recent studies indicate that pregnenolone and allopregnanolone inhibit pro-inflammatory signaling by TLR4 receptors in cultured macrophage cells (RAW264.7 cells) and the brain of alcohol-prefering P rats that exhibit innate TLR4 activation (Balan et al., 2019). In the mouse macrophage cell line, lipopolysaccharide (LPS) increased the entire pathway of pro-inflammatory markers of TLR4 activation, and all of these effects were completely inhibited by both allopregnanolone and pregnenolone at 0.5 and 1.0 μM doses. Pregnenolone was more potent than allopregnanolone and was not converted to allopregnanolone in the cells. The mechanism of neuroactive steroid inhibition of TLR4 signaling appears to involve blockade of TLR4/lymphotoxin antigen 96 (MD-2) protein interactions in RAW246.7 cells, as both neurosteroids blocked the co-immunoprecipitation of TLR4 with MD-2, a reduction in cerebrospinal levels of allopregnanolone has been reported in premenopausal women and men, and such levels were negatively correlated with PTSD symptoms (Rasmusson et al., 2006, 2017). Indeed, both preclinical and clinical evidence suggests a role for allopregnanolone in PTSD (Rasmusson et al., 2017). Clinical trials are ongoing (NCT03799562; NCT00560781) to test the effectiveness of the precursor pregnenolone in targeting PTSD symptoms.

Systemic allopregnanolone administration (15 mg/kg, I.P.) also reduced expression of the TLR4 activation marker tumor necrosis factor
The endogenous neurosteroids pregnenolone, progesterone, and allopregnanolone have protective activity in neurological and psychiatric conditions that involve pro-inflammatory signaling. Significantly, progesterone and/or allopregnanolone have shown efficacy in clinical studies of traumatic brain injury (Wright et al., 2007; Stein and Sayeed, 2019), cocaine craving (Fox et al., 2013; Milivojevic et al., 2004), and as well as postpartum depression (Kanes et al., 2017; Meltzer-Brody et al., 2018). Further, allopregnanolone has therapeutic activity in animal models of traumatic brain injury (He et al., 2004b), multiple sclerosis (Schumacher et al., 2007; Noorbakhsh et al., 2014), and Alzheimer's disease (Brinton, 2013). This growing body of inflammatory conditions that respond to intervention with these neurosteroids supports the idea that inhibition of neuroimmune signaling may be an important component of their actions.

Pain is considered a neuroinflammatory condition. Preclinical studies suggest that neuroactive steroids play an important role in both inflammatory and neuropathic pain. Intracerebroventricular administration of allopregnanolone has analgesic effects in naive male rats (Kavaliers and Wiebe, 1987). Likewise, systemic and intrathecal administration of allopregnanolone, or its precursor progesterone, exerts anti-nociceptive properties in several models of neuropathic (see Gonzalez et al., 2019) for review) and inflammatory pain (Ocvirk et al., 2008). Thus, administration of neuroactive steroids might be beneficial to ameliorate pain symptoms. Indeed, a recent clinical study in 92 veterans showed that pregnenolone administration for 4 weeks reduced chronic pain symptoms by 20% while the patients in the placebo control group reported a decrease in pain symptoms of 4%. In addition, low back symptoms were inversely related to serum neuroactive steroid levels. Treatment of these disorders. Neuroactive steroids might represent a useful therapeutic approach to arrest detrimental neuroimmune signaling, observed in patients. This argument is supported by data from animal models of chronic stress and effects of neuroactive steroids in other conditions that involve excessive neuroimmune activation in the CNS. Some examples are described.

7.1. Therapeutic effects of pregnenolone, progesterone or allopregnanolone in multiple neuroinflammatory diseases

To dose, no potent and specific antagonist of neuroactive steroid effects on GABA receptors has been found. Several candidate antagonists have been reported, including the 3β-antagonists of certain pregnane steroids (Wang et al., 2002), the 3α,5β- or 3α,5α-reduced metabolites of cortisol (Penland and Morrow, 2004), and the steroid analog [3α,5α]17-phenylandrost-16-en-3-ol (17PA) (Mennerick et al., 2004; Kelley et al., 2007). However, none of these compounds exhibit sufficient potency, efficacy, or specificity to provide useful tools for the interrogation of neuroactive steroid actions.

Numerous investigators have employed the inhibition of steroid synthesis to interrogate the role of neuroactive steroids in disease models. The strategy has been to use finasteride to block the formation of allopregnanolone in order to determine its role in normal brain function and pathology. Endogenous allopregnanolone is formed by the reduction of progesterone in two steps. The classical pathway indicates that 5α-reductase converts progesterone to 5α-dihydroprogesterone...
and then 3α-hydroxysteroid dehydrogenase converts 5α-dihydroprogesterone to allopregnanolone (see the biosynthetic pathway in Porcu et al., 2009). However, an alternative pathway also exists where 3α-hydroxysteroid dehydrogenase converts progesterone to 3α-hydroxyprogesterone (also known as 3α-hydroxy-4-pregnen-20-one). This neuroactive steroid is also GABAergic and displays equal potency and efficacy as allopregnanolone and 3α,5α-THDOC in the Cl− flux assay of effects on GABA_A receptors (Morrow et al., 1987, 1990). 3α-Hydroxyprogesterone is found in brain in amounts that are approximately equal to allopregnanolone (Wiebe et al., 1997; Griffin and Mellon, 2001) and in male testes (Wiebe, 1982), but its physiologic and behavioral effects are largely unknown. In the presence of finasteride, this steroid would not be further metabolized to allopregnanolone. In addition, progesterone is converted by 5β-reductase to 5β-dihydroprogesterone and then to pregnanolone (3α,5β-3-hydroxyprogenn-20-one). This steroid is also an equipotent GABAergic allosteric modulator that is present in human serum in approximately the same quantity as allopregnanolone. Although it is normally undetectable in rodents, the administration of pregnenolone to rats induces the appearance of pregnanolone (Porcu et al., 2009). If 5c-reductase is inhibited or subjected to genetic deletion, pregnenolone and progesterone are increased and the role of 3α-hydroxyprogesterone and pregnanolone as substitute neuroactive steroids must be considered. If investigators presume that finasteride blocks the formation of allopregnanolone, and there is no compensation by other steroids, the interpretation of data may lead to inappropriate conclusions. In light of this consideration, the interpretation of effects of these interventions are very difficult at best. This unsolved conundrum in the field has resulted in data that promotes uncertainty and greater caution in the use of allopregnanolone for therapeutics.

8.2. Neuroactive steroid therapeutics may interact with socially acceptable drugs like nicotine or alcohol and/or lead to untoward effects including allopregnanolone dependence

It is known that ethanol and nicotine can increase GABAergic neuroactive steroids (VanDoren et al., 2000; Porcu et al., 2003, 2010; Concas et al., 2006) and these steroids may interact with ethanol or nicotine in ways that might be deleterious, such as when driving or operating machinery. Since addictions are chronic and characterized by both the loss of control of one’s behavior as well as the tendency to relapse, the concern over these potential interactions is understandable and might be fully justified. Alternatively, it is possible that patients with addictions may have different responses to allopregnanolone and/or pregnenolone compared to average healthy subjects. This might occur if patients are suffering from deficient GABAergic transmission, CRF regulation, and/or neuroimmune overactivity. In this case, the administration of the neuroactive steroids may normalize biological function and interactions with ethanol would not be unmanageable. In diabetes, for example, the administration of insulin normalizes metabolic function rather than causing excess energy production that would be detrimental in normal healthy control subjects. In every clinical trial reported to date with allopregnanolone or pregnenolone for neuropsychiatric conditions, there were no serious adverse effects in the patients (vide supra). Nonetheless, until we know more about neuroactive steroid effects in patients with diseases that might respond to therapy with allopregnanolone, trials could be limited to intravenous infusions of allopregnanolone in a medically supervised setting, drug interactions could be tested in laboratory settings, and oral treatments could be given for short periods to assess patient responses.

The rapid and long-lasting antidepressant effect of Brexanolone in patients with postpartum depression (Meltzer-Brody et al., 2018) suggests that allopregnanolone therapy might be useful for generalized depression, PTSD, alcohol or cocaine detoxification and might last up to a month or longer such that other treatments such as cognitive behavioral therapies could have a greater impact. Neuroactive steroid therapy might be considered like other anti-inflammatory therapies that are administered once monthly to control inflammatory conditions, such as the TNF-α antagonist, Remicade. The allopregnanolone precursors pregnenolone and progesterone may also be effective and further studies are clearly warranted. However, pregnenolone and progesterone also produce other metabolites that could have unexpected effects and more studies are needed to justify the use of these steroids in stress-related disease at this time.

Other animal studies have suggested that progesterone withdrawal and therefore by analogy, withdrawal from allopregnanolone can lead to anxiety, GABA_A receptor dysregulation and dysfunction (Costa et al., 1995; Moran et al., 1998; Moran and Smith, 1998; Smith et al., 1998). The data suggests a danger from rapid allopregnanolone withdrawal in human subjects as well. Again, this clinical concern has not borne out in any of the clinical studies conducted to date. Nonetheless, withdrawal signs should be monitored and prevented by gradual tapering if therapy is discontinued. However, therapy for chronic conditions may be lifelong, as in the case of cardiovascular disease, diabetes and many other conditions.

9. Final conclusion

We have previously suggested that allopregnanolone may be a protective factor in normal healthy controls that helps to prevent the development of AUDs (Morrow, 2007). We now suggest that it might also help to prevent many other stress-related diseases that involve the dysregulation of GABAergic transmission, excessive CRF signaling, and neuroimmune activation. Allopregnanolone is an endogenous factor that helps to maintain normal CNS inhibition, behavioral control, HPA axis homeostasis, and prevents the activation of neuroimmune signaling through various TLR receptors that initiate systemic inflammatory responses and excessive brain excitability. Acute stress and acute ethanol exposure increase neurosteroid levels in circulation along with endotoxin and cortisol/cortisosterone. The concurrent production of pregnenolone, progesterone and allopregnanolone helps to ensure the return to hypothalamic and extrahypothalamic CRF and HPA axis homeostasis and prevents the activation of TLR signaling and thus the production of pro-inflammatory molecules. This is a healthy response to alcohol and stress, and it may explain why most people can manage the stress of life without issues.

With chronic stress, the neurosteroids are depleted in serum and/or brain, HPA axis and CRF dysregulation ensue, GABA_A receptor function is dysregulated, and markers of neuroinflammation are elevated. In addition, there may be tolerance to the effects of acute stress challenge on the production of allopregnanolone. At this point, behavioral manifestations of disease are evident with anxiety, dysphoria and possibly drug craving/intake to relieve the symptoms of the adaptations caused by chronic exposure to stress. Fig. 3 illustrates how allopregnanolone can restore homeostasis of GABA inhibition, CRF signaling, and neuroimmune activation following chronic stress exposure.

Here we propose that inadequate GABAergic function, along with excessive CRF and neuroimmune signaling is central to stress-related disorders, and therefore patients would respond to treatment with allopregnanolone or its precursors pregnenolone/progesterone in a favorable manner. The pleiotropic actions of allopregnanolone are likely key to its therapeutic potential. Pregnenolone and progesterone have inhibitory properties on TLR signaling, and they are rapidly converted to allopregnanolone in vivo and thus may share its beneficial pleiotropic actions. Furthermore, these precursors are readily available and less expensive to obtain. It is time for clinical studies to address and to compare the potential of these neurosteroids in stress-related conditions.
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