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Recent Developments in the Significance and Therapeutic Relevance of Neuroactive Steroids – Introduction to the Special Issue

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Abstract

The special issue heralds an exciting time in the field when the significance of neuroactive steroids in the regulation of inhibitory transmission is being realized and translated to new treatments for intractable neurologic and psychiatric conditions. In the past year, the binding sites for neuroactive steroids on γ -aminobutyric acid type A (GABA_A) receptors have been discovered and clinical trials for epilepsy and traumatic brain injury have been successful. New data in animal models points to the potential value of neuroactive steroids in other central nervous system (CNS) disorders including depression, schizophrenia, alcoholism, multiple sclerosis and other neurodegenerative conditions. How can one class of compounds have so many potential applications? The answer may lie in the ability of neuroactive steroids to regulate synaptic and extrasynaptic inhibitory transmission across brain, hypothalamic-pituitary-adrenal (HPA) axis function, inflammatory processes and myelin formation. The manuscripts in this issue of Pharmacology and Therapeutics will bring you up to date on the sites of neurosteroid actions, the systemic and molecular consequences of these actions and the potential therapeutic relevance of neuroactive steroid effects for CNS disease. These studies point to the opportunity for more translational research into potential therapeutic applications and evaluation of potential untoward side effects following long-term treatment.

Keywords

GABAergic neuroactive steroids; epilepsy; alcoholism; depressive disorders; neuroinflammation; neurodegeneration

Neuroactive steroids are endogenous neuromodulators that can be synthesized de novo in the brain as well as adrenal glands, ovaries and testes (for review, see (Biggio and Purdy, 2001). The biosynthetic pathway for these steroids is shown in Figure 1. The inhibitory neuroactive steroids with potent GABA_A receptor positive modulatory effects are highlighted in green, while the excitatory neuroactive steroids with weak GABA_A receptor antagonist effects are highlighted in orange. Among these compounds, the 3 α ,5 α - and 3 α ,5 β - reduced metabolites of progesterone (Majewska et al., 1986; Morrow et al., 1987), deoxycorticosterone (DOC) (Majewska et al., 1986; Morrow et al., 1987), dihydroepiandrosterone (DHEA) (Frye et al.,

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1996; Park-Chung et al., 1999; Kaminski et al., 2005) and testosterone (Kaminski et al., 2005; Kaminski et al., 2006) enhance GABAergic neurotransmission and produce inhibitory neurobehavioral effects such as anxiolytic, anticonvulsant and sedative actions. The excitatory neuroactive steroids include the sulfated derivatives of pregnenolone and DHEA (Farb and Gibbs, 1996) as well as the 3 α ,5 α - and 3 α ,5 β - reduced metabolites of cortisol (Penland and Morrow, 2004).

Despite the presence of multiple endogenous GABAergic steroids, the physiological significance of these endogenous modulators remains mostly elusive. This gap in our knowledge may be attributed to the lack of readily available methods for detection and quantification of the 5 α and 5 β derivatives of each steroid as well as the need for specific antagonists of neurosteroid sites on GABA receptors. Studies using gas chromatography and mass spectroscopy (GC/MS) detection of neurosteroids have appeared, but are still restricted to a few of the GABAergic steroids (Marx et al., 2006; Purdy et al., 2006; Schule, 2006). The methods remain expensive and labor intensive, preventing large scale application to many research questions. Since there are very few studies of the 3 α ,5 α - and 3 α ,5 β -reduced metabolites of DOC, DHEA and testosterone, the magnitude of neurosteroid influence on GABAergic neurotransmission in the CNS is not yet known. Considering the abundance of precursors and the common metabolic enzymes, it is likely that the GABAergic metabolites of progesterone, DOC, DHEA and testosterone are both singularly and coordinately significant physiological regulators of CNS excitability.

Nomenclature

Neurosteroid nomenclature is often confusing for many investigators. Throughout this issue of P & T, we have used IUPAC steroid nomenclature for consistency. However, many of the original articles on neurosteroids as well as many of the articles cited in these reviews used conventional steroid nomenclature. The following table shows the common names of steroids with both forms of steroid nomenclature.

IUPAC name	Conventional (trivial) names	Abbreviation
3 α -hydroxy-5 α -pregnan-20-one	5 α -pregnane-3 α -ol-20one, allopregnanolone	3 α ,5 α -THP
3 α -hydroxy-5 β -pregnan-20-one	5 β -pregnane-3 α -ol-20one, pregnanolone	3 α ,5 β -THP
3 α ,21-dihydroxy-5 α -pregnan-20-one	5 α -pregnan-3 α ,21-diol-20-one tetrahydrodeoxycorticosterone	3 α ,5 α -THDOC
3 α ,21-dihydroxy-5 β -pregnan-20-one	5 β -pregnan-3 α ,21-diol-20-one	3 α ,5 β -THDOC

Neuroactive steroids interact with GABA_A Receptors

Systemic administration of the prototypical neurosteroids 3 α ,5 α -THDOC and 3 α ,5 α -THP induces anxiolytic, anticonvulsant and sedative-hypnotic effects, similar to those induced by other GABA_A receptor positive modulators (for review see Morrow et al., 2001). Neuroactive steroids interact with GABA_A receptors via specific binding sites on α subunits (Hosie et al., 2006); see review this issue. These steroids allosterically modulate binding to GABA and benzodiazepine recognition sites (Gee et al., 1987) and compete for [³⁵S]TBPS binding sites (Gee et al., 1987). Neurosteroid activation can enhance GABA-mediated Cl⁻ conductance and directly stimulate Cl⁻ conductance in voltage clamp studies and [³⁶Cl⁻] flux studies (Majewska et al., 1986; Harrison et al., 1987; Morrow et al., 1987). Neuroactive steroids appear to interact with multiple neurosteroid recognition sites (Morrow et al., 1990) that may represent different properties of recognition sites on singular (Puia et al., 1990, Hosie et al., 2006) or distinct GABA_A receptor subtypes (Puia et al., 1991; Mihalek et al., 1999), including extrasynaptic receptors (Chandra et al., 2006). Studies of the structural requirements for neurosteroid activity at GABA_A receptors include 3 α reduction and 5 α /5 β reduction of the A ring as well as

hydroxylation of C21 (Paul and Purdy, 1992). The 5 β -reduced metabolites of DOC and progesterone, 3 α ,5 β -THDOC and 3 α ,5 β -THP are equipotent modulators of GABAergic transmission (Callachan et al., 1987; Morrow et al., 1990; Xue et al., 1997). Humans synthesize the 5 β -reduced neuroactive steroids; moreover the concentrations of 3 α ,5 β -THP are physiologically relevant and comparable to those of 3 α ,5 α -THP in human plasma and cerebrospinal fluid (Romeo et al., 1998; Uzunova et al., 1998).

The effects neuroactive steroids on both synaptic and extrasynaptic GABA_A receptors are reviewed in detail in this issue. Given the ubiquitous expression of GABA_A receptors throughout the CNS, it might be expected that neurosteroids would exert global inhibitory actions across the brain. Herd et al. review the literature showing that neurosteroid action is highly specific, being both brain region and neuron dependent. The mechanisms of this selectivity involve GABA_A receptor subunit composition, the differential expression of steroid synthesising and metabolising enzymes, and local steroid metabolism and phosphorylation mechanisms. Further, extrasynaptic GABA_A receptors are highly sensitive to neurosteroids and appear to play a vital role in the neuronal plasticity changes that accompany stress, puberty and the ovarian cycle.

The precise neurosteroid binding sites on GABA_A receptors have recently been identified (Hosie et al., 2006). In this issue, Hosie et al. review the research over the past 20 years that has searched for the neurosteroid binding sites on GABA_A receptors. These data indicate a molecular basis for the presence of heterogeneous interactions of neurosteroids and may provide important drug target information for rationale therapies for CNS disease.

Medicinal chemical approaches for the identification of neurosteroid actions at GABA_A receptors are reviewed in this issue by Akk et al., missing. These studies have yielded data consistent with the likelihood of multiple binding sites on GABA_A receptors. Evidence for this comes from the finding that some steroids exhibit different concentration dependencies for distinguishable steroid effects on GABA_A channel function. More evidence comes from the differences in enantiomer selectivity of various steroid analogues. Enantiomers remain the cleanest means of distinguishing contributions of membrane effects from “site” effects. There is optimism that the recent identification of steroid binding sites through mutagenesis and future photoaffinity labeling will help clarify the observed complexity. Finally, although complexity of steroid actions poses challenges experimentally, there is hope that this same complexity, once it is better understood, can be exploited for developing specific analogues directed toward specific GABA_A receptor subunits and selective behavioral effects.

Neurosteroids Regulate GABA_A Receptor Expression

The effects of chronic exposure to neuroactive steroids such as 3 α ,5 α -THP and 3 α ,5 α -THDOC are reviewed in this issue by Smith et al., missing. Both chronic exposure and withdrawal from exogenous neurosteroids increase GABA_A receptor α 4 subunit expression to produce CNS hyperexcitability. Increases in α 4 subunit expression produce benzodiazepine insensitivity, one common factor in rodent models and the clinical presentation of premenstrual dysphoric disorder. The biophysical characteristics of the receptor adaptations can produce different effects depending on the direction of Cl⁻ current in target cell populations – explaining the molecular basis of paradoxical excitatory effects of neuroactive steroids. These results not only have important implications for hormonal cycle-associated changes in neuronal activation and behavior, but also suggest a common underlying mechanism for the role of inhibition in triggering compensatory changes in neuronal excitability states due to the plasticity of GABA_A receptor populations with different pharmacological and biophysical properties.

Neuroactive steroids modulate CNS development and repair following injury

The neurodevelopmental functions and mechanisms of action of four distinct neurosteroids – pregnenolone, progesterone, allopregnanolone and dehydroepiandrosterone are reviewed by Mellon (this issue). Absence or reduced concentrations of neurosteroids during development and in adults may be associated with neurodevelopmental, psychiatric, or behavioral disorders. Treatment with physiologic or pharmacologic concentrations of these compounds may also promote neurogenesis, neuronal survival, myelination, increased memory, and reduced neurotoxicity. This review highlights the therapeutic potential of neurosteroids in neurodevelopmental disease.

Progesterone and its metabolites also promote the viability of neurons in the adult brain and spinal cord. Schumacher et al. review their neuroprotective effects in traumatic brain injury, experimentally induced ischemia, spinal cord lesions and a genetic model of motoneuron disease. Progesterone plays an important role in developmental myelination and in myelin repair, and the aging nervous system appears to remain sensitive to some of progesterone's beneficial effects. Thus, the hormone may promote neuroregeneration by several different actions: by reducing inflammation, swelling and apoptosis, thereby increasing the survival of neurons, and by promoting the formation of new myelin sheaths. Recognition of the important pleiotropic effects of progesterone supports the therapeutic potential for the treatment of brain lesions and other diseases of the nervous system. These authors suggest a novel therapeutic strategy using ligands of the peripheral benzodiazepine receptor to increase the synthesis of steroids with neuroprotective and neuroregenerative properties.

Neuroactive steroids modulate the HPA axis and the effects of stress

The activation of the HPA axis in response to acute stress increases the release of corticotropin releasing factor from the hypothalamus, which stimulates the release of adrenocorticotrophic hormone (ACTH) from the pituitary, which in turn, stimulates the adrenal cortex to release glucocorticoids, neuroactive steroid precursors and GABAergic neuroactive steroids. Glucocorticoids, mainly cortisol in humans and non-human primates, and corticosterone in rodents, provide negative feedback upon the hypothalamus and pituitary. Likewise, GABAergic neuroactive steroids inhibit CRF production, release, ACTH release and subsequent corticosterone levels in rodents (Owens et al., 1992; Patchev et al., 1994; Patchev et al., 1996). The ability of neuroactive steroids to reduce HPA axis activation may play an important role in returning the animal to homeostasis following stressful events. This physiological coping response appears to be critical for mental health since it is dysregulated in various mood disorders including depression, post-traumatic stress disorder and premenstrual dysphoric disorder.

While stress increases neurosteroid levels in the short term, animal models of chronic stress and depression show lower brain and plasma neurosteroid concentrations, and alterations in neurosteroid responses to acute stressors. Girdler and Klatzin (this issue) review the potential role of neuroactive steroids in depressive disorders. In humans, neurosteroid depletion is consistently documented in patients with current depression and may reflect their greater chronic stress. Women with the depressive disorder, premenstrual dysphoric disorder (PMDD) and euthymic women exhibit blunted allopregnanolone responses to mental stress relative to non-PMDD controls or never depressed women. Girdler et al suggest that that failure to mount an appropriate allopregnanolone response to stress may reflect the price of repeated biological adaptations to the increased life stress that is well documented in depressive disorders, and altered allopregnanolone stress responsivity may also contribute to the dysregulation seen in HPA-axis function in depression.

Neurosteroids play a crucial role in stress, alcohol dependence and withdrawal, and other physiological and pharmacological actions by potentiating or inhibiting neurotransmitter action. The article by Biggio et al. (this issue) describes the effects of acute and chronic stress on peripheral and brain neurosteroid levels in rodents as well as GABA_A receptor gene expression and function. Further, these authors describe the interactions between stress and ethanol exposure on brain steroidogenesis and the plasticity of GABA_A receptors. These neurochemical and molecular mechanisms demonstrate the remarkable impact of neurosteroid actions through regulation of the HPA axis and GABA_A receptors.

Neuroactive steroids mediate ethanol actions

Systemic administration of moderate ethanol doses (1-2.5 g/kg) increases both plasma and brain levels of 3 α ,5 α -THP and 3 α ,5 α -THDOC in rodents (Morrow et al., 1998; Barbaccia et al., 1999; Morrow et al., 1999; VanDoren et al., 2000; O'Dell et al., 2004). Ethanol-induced elevations in neuroactive steroids reach physiologically relevant concentrations that are capable of enhancing GABAergic transmission. A large body of evidence from multiple laboratories suggests that ethanol-induced elevations of GABAergic neuroactive steroids contribute to many behavioral effects of ethanol in rodents. Neuroactive steroids have been shown to modulate ethanol's anticonvulsant effects (VanDoren et al., 2000), sedation (Khisti et al., 2003), impairment of spatial memory (Morrow et al., 2001; Matthews et al., 2002), anxiolytic-like (Hirani et al., 2005) and antidepressant-like (Hirani et al., 2002) actions. Each of these behavioral responses is prevented by pretreatment with the biosynthesis inhibitor finasteride and/or by prior adrenalectomy. The hypnotic effect of ethanol is partially blocked by adrenalectomy. Importantly, administration of the immediate precursor of 3 α ,5 α -THP restores effects of ethanol in adrenalectomized animals, showing that brain synthesis of neuroactive steroids modulates effects of ethanol (Khisti et al., 2003). However, neuroactive steroids do not appear to influence the motor incoordinating effects of ethanol, since neither finasteride administration or adrenalectomy diminish these actions (Khisti et al., 2004). Taken together, these studies suggest that elevations in neuroactive steroids influence many of the GABAergic effects of ethanol *in vivo* and the effects of neuroactive steroids may determine sensitivity to many behavioral effects of ethanol.

We have suggested that ethanol-induced elevations of GABAergic neurosteroids contribute to ethanol sensitivity and protect against the risk for ethanol dependence (Morrow et al., 2006). Neuroactive steroid responses to HPA axis challenges in ethanol naïve animals may predict future alcohol consumption. Dexamethasone suppresses DOC levels in monkey plasma and the degree of dexamethasone suppression measured in ethanol naïve monkeys was predictive of subsequent alcohol drinking in these monkeys (Porcu et al., 2006). Diminished elevations of GABAergic neuroactive steroids following ethanol exposure would result in reduced sensitivity to the anxiolytic, sedative, anticonvulsant, cognitive-impairing and discriminative stimulus properties of ethanol. Reduced sensitivity to ethanol is associated with greater risk for the development of alcoholism in individuals with alcoholism in their family (Schuckit, 1994; Schuckit and Smith, 1996). Moreover, individuals with the GABA_A receptor α 2 subunit polymorphism that is associated with alcohol dependence exhibit substantially reduced sensitivity to the subjective effects of ethanol compared to individuals that lack this polymorphism (Pierucci-Lagha et al., 2005). Likewise, rats and mice with low sensitivity to various behavioral effects of alcohol tend to self-administer greater amounts of ethanol in laboratory settings. Taken together, these observations suggest that ethanol-induced elevations of GABAergic neuroactive steroids in brain may underlie important aspects of ethanol sensitivity that may serve to prevent excessive alcohol consumption. The loss of these responses may promote excessive alcohol consumption to achieve the desired effects of ethanol. The lack of neurosteroid elevations in response to ethanol could underlie innate ethanol tolerance or ethanol tolerance induced by long-term ethanol use. Indeed, the observation that

finasteride did not alter the subjective effects of ethanol in subjects with the GABA_A receptor $\alpha 2$ subunit polymorphism associated with alcohol dependence (Pierucci-Lagha et al., 2005) is consistent with the idea that neurosteroid responses contribute to ethanol sensitivity and risk for alcoholism. Hence, neuroactive steroid supplementation may be therapeutic for recovery from alcoholism or helpful with symptoms of ethanol withdrawal.

Summary and Conclusions

While many investigators have suggested the therapeutic relevance of neuroactive steroids in neuropsychiatric disease, there are currently no neuroactive steroids in clinical practice. However, ganaxolone (3 α -hydroxy,3 β -methyl,5 α -pregnan-20-one) is under review by the FDA for childhood epilepsies. The use of neuroactive steroid precursors, such as progesterone and pregnenolone may prove useful in some conditions, particularly traumatic brain injury, as suggested by Schumacher et al. (this issue). Remarkably, the first trials of progesterone in traumatic brain injury revealed no significant untoward effects. Nonetheless, this issue remains a lingering concern for neuroactive steroid development. Since GABAergic neuroactive steroids regulate GABA_A receptor function and expression, the possibility of untoward effects during or following neurosteroid therapy must be explored. Despite this concern, there is great optimism in the field for the usefulness of the inhibitory, anti-inflammatory, and myelin promoting targets of neuroactive steroids for CNS disease.

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