Frontiers in Neuroendocrinology 35 (2015) 28–48

Contents lists available at ScienceDirect

Frontiers in Neuroendocrinology

journal homepage: www.elsevier.com/locate/yfrne

Review

$GABA_A$ receptor-acting neurosteroids: A role in the development and regulation of the stress response

Benjamin G. Gunn [a], Linda Cunningham [a], Scott G. Mitchell [a], Jerome D. Swinny [b], Jeremy J. Lambert [a], Delia Belelli [a,*]

[a] Division of Neuroscience, Medical Research Institute, Dundee University, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK
[b] Institute for Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK

ARTICLE INFO

Article history:
Available online 12 June 2014

Keywords:
Allopregnanolone
$GABA_A$ receptor
HPA axis
Early-life stress
Depression

ABSTRACT

Regulation of hypothalamic–pituitary–adrenocortical (HPA) axis activity by stress is a fundamental survival mechanism and HPA-dysfunction is implicated in psychiatric disorders. Adverse early life experiences, e.g. poor maternal care, negatively influence brain development and programs an abnormal stress response by encoding long-lasting molecular changes, which may extend to the next generation. How HPA-dysfunction leads to the development of affective disorders is complex, but may involve $GABA_A$ receptors ($GABA_A$Rs), as they curtail stress-induced HPA axis activation. Of particular interest are endogenous neurosteroids that potently modulate the function of $GABA_A$Rs and exhibit stress-protective properties. Importantly, neurosteroid levels rise rapidly during acute stress, are perturbed in chronic stress and are implicated in the behavioural changes associated with early-life adversity. We will appraise how $GABA_A$R-active neurosteroids may impact on HPA axis development and the orchestration of the stress-evoked response. The significance of these actions will be discussed in the context of stress-associated mood disorders.

1. Introduction

1.1. Stress, $GABA_A$ receptors and neurosteroids

Stressful experiences engage a co-ordinated neuronal and hormonal response, orchestrated by the hypothalamic–pituitary–adrenocortical (HPA) axis *via* activation of corticotrophin releasing factor (CRF)-releasing parvocellular neurones of the hypothalamic paraventricular nucleus (PVN). The activity of the PVN is subject to regulation by GABA, the dominant inhibitory neurotransmitter in the hypothalamus (Decavel and Van den Pol, 1990; Miklos and Kovacs, 2002), which acts primarily *via* $GABA_A$ receptors ($GABA_A$Rs). The neurocircuitry regulating the activity of the PVN is highly complex, comprised of mono- and polysynaptic inputs from a number of different limbic and forebrain regions. $GABA_A$Rs are expressed throughout this circuit where they play an important role in modulating the functional activity, and hence output, of these brain regions. Thus, regulation of HPA axis activity through $GABA_A$R-mediated transmission not only occurs at the level of the PVN, but also at multiple levels of the stress neurocircuitry.

$GABA_A$Rs possess a pentameric structure formed from multiple subunits. To date, 19 subunits have been identified (α1-6, β1-3, γ1-3, δ, ε, θ, π and ρ1-3), which are divided into subfamilies based upon their amino acid homology (Olsen and Sieghart, 2008, 2009). These subunits exhibit discrete expression profiles, allowing for the expression of ~20–30 different $GABA_A$R isoforms within the CNS (Fritschy and Brunig, 2003; Olsen and Sieghart, 2008; Hortnagl et al., 2013; Fritschy and Panzanelli, 2014) with most native receptors comprising two α, two β and a single γ, δ or ε subunit. Importantly, $GABA_A$R isoforms containing the γ subunit are generally, albeit not exclusively (e.g. α5βγ2 isoforms) targeted to synapses where they mediate "phasic" GABAergic transmission, while δ-$GABA_A$Rs comprise a major class of peri- and extrasynaptic receptors that mediate a "tonic" (Farrant and Nusser, 2005; Belelli et al., 2009) and "spill-over" (Herd et al., 2013) form of GABAergic inhibition. The subunit composition not only determines the regional and cellular location of $GABA_A$Rs, but also influences their biophysical and pharmacological profile. For example, incorporation of the γ2 subunit in conjunction with specific α subunits (α1-3 and α5) conveys benzodiazepine (BDZ) sensitivity (Olsen and

* Corresponding author. Fax: +44 1382 383647.
E-mail address: d.belelli@dundee.ac.uk (D. Belelli).

http://dx.doi.org/10.1016/j.yfrne.2014.06.001
0091-3022/

Sieghart, 2009; Rudolph and Knoflach, 2011; Rudolph and Mohler, 2014).

Modulation of $GABA_AR$ function by endogenous ligands may provide a physiologically and pathologically relevant mechanism to regulate $GABA_AR$-associated functions and behaviour. In this respect, the positive allosteric actions of some endogenously occurring steroids have been identified to be of particular physiological and pharmacological significance over the course the past 3 decades. Specifically, following the pioneering discovery of the $GABA_AR$ potentiating actions of the synthetic anaesthetic steroid, Alphaxalone (5α-pregnan-3α-ol-11,20-dione Harrison and Simmonds, 1984) certain endogenous steroids, synthesised *de novo* in the brain and hence called neurosteroids (Baulieu, 1981) were shown to share this property. Such neurosteroids include the progesterone (PROG) metabolites 5α-pregnan-3α-tetrahydroprogesterone (5α3α-THPROG), 5β-pregnan-3α-tetrahydroprogesterone (5β3α-THPROG) and the deoxycorticosterone (DOC) metabolite 5α,3α-tetrahydrodeoxycorticosterone (5α3α-THDOC), which in common potently and stereo-selectively enhance $GABA_AR$ function in an allosteric fashion (Paul and Purdy, 1992; Belelli and Lambert, 2005). Intriguingly, the levels of such neurosteroids are rapidly elevated following acute stress (Purdy et al., 1991; Barbaccia et al., 2001; Morrow et al., 2009) and therefore, they may act to "fine-tune" the function of $GABA_ARs$ and consequently influence HPA axis activity. In support, neurosteroids inhibit CRF release and exhibit anxiolytic and stress-protective properties (Crawley et al., 1986; Patchev et al., 1994, 1996; Carboni et al., 1996; Bitran et al., 1999).

Electrophysiological recordings have demonstrated that neurosteroids, such as 5α3α-THPROG and 5α3α-THDOC, potentiate the response of GABA (i.e. GABA-modulatory) at nanomolar aqueous concentrations, whilst at higher concentrations these endogenous regulators directly activate (i.e. GABA-mimetic) the $GABA_AR$-channel complex (Callachan et al., 1987; Lambert et al., 1995; Shu et al., 2004). A significant body of evidence consistent with the presence of a specific neurosteroid binding site on the receptor has been provided during the past 25 years including: modulation of [^{3}H] muscimol binding in solubilised preparations with minimal lipid content (Bureau and Olsen, 1993); clear enantioselectivity (Wittmer et al., 1996) and antagonism of both the *in vitro* and *in vivo* actions of neurosteroids by selective ligands i.e. 3α5α-17-phenylandrost-16-en-3-ol (17PA – Mennerick et al., 2004). A more definitive validation arose from site-directed mutagenesis studies, which revealed that neurosteroids interact with two distinct groups of amino acid residues located within the transmembrane (TM) domains of the $GABA_AR$, which are both critical for their GABA-modulatory and the GABA-mimetic actions (Hosie et al., 2006). Subsequent reports have indicated that the neurosteroid binding pocket may possess a more complex structure than initially suggested with additional amino acid residues contributing to the modulatory actions of distinct, but structurally related steroid molecules (Akk et al., 2008; Chisari et al., 2010; Zorumski et al., 2013). Note that *in vitro* electrophysiological studies consistently report neuroactive steroids such as 5α3α to enhance $GABA_AR$ function at nM aqueous concentrations, suggesting the presence of a relatively high affinity binding site on the $GABA_AR$ protein. However, by virtue of their high lipid solubility, the actual concentration of neurosteroid achieved locally at the receptor protein will be in the micromolar range. Indeed, the differential accumulation of these steroids within the lipid membrane may serve to increase their local concentration, a suggestion which is in accord with a putative transmembrane docking site for neurosteroids. Such a scenario would enable and indeed facilitate neurosteroid access to a relatively low affinity binding site(s) located within the transmembrane spanning regions of the protein, *via* lateral diffusion through the membrane bilayer (Akk et al., 2005; Akk et al., 2007; Chisari et al., 2010).

A variety of factors have been shown to influence the apparent sensitivity of native $GABA_ARs$ to neurosteroid modulation, including the subunit composition, phosphorylation state of the receptor and local steroid metabolism (Belelli and Lambert, 2005; Lambert et al., 2009; Nani et al., 2013). A detailed discussion of the relative contribution by each of these molecular mechanisms can be found in recent reviews (Herd et al., 2007; Lambert et al., 2009; Gunn et al., 2011).

Importantly, from a physiological perspective, naturally occurring plasma and brain levels of neurosteroids are estimated to be within a range required to potentiate $GABA_AR$ function. Further, the synthesis of these neuromodulators is dynamically regulated in response to physiological and pathophysiological challenges e.g. stress thus supporting the proposal for a significant role as endogenous regulators of $GABA_AR$-mediated inhibitory transmission both in the central (CNS) and peripheral nervous system (PNS). In this review we will specifically appraise and focus on the documented and potential relevance of such actions for the early programming of the stress-neurocircuit and the regulation of stress-evoked responses. The potential pathological significance of such actions for stress-associated psychopathology will also be discussed.

2. Neurosteroids: endogenous modulators of $GABA_AR$ function

2.1. Neuronal and glia-mediated synthesis of neurosteroids

Although a significant proportion of neurosteroids are derived from peripheral sources, such as the adrenal cortex and ovaries (Paul and Purdy, 1992), the brain itself is a steroidogenic organ that is capable of the *de novo* synthesis of these neuromodulators (Purdy et al., 1991; Reddy, 2003; Barbaccia, 2004). Neurosteroids are synthesised from cholesterol *via* a series of steps that include the translocation of cholesterol across the mitochondrial membrane by the steroidogenic acute regulatory protein (StAR) and translocator protein 18 kDa (TSPO; formerly the mitochondrial peripheral BDZ receptor), the rate limiting step of steroid and neurosteroid synthesis. Within the mitochondria, cholesterol is converted to pregnenolone (PREG) by the P450 side-chain cleavage enzyme, CYP11A1 and then trafficked to the cytoplasm where it can be converted to a number of neurosteroids known to be active at the $GABA_AR$, including 5α3α-THPROG. PREG is converted to 5α3α-THPROG following three sequential reactions catalysed by 3β-hydroxysteroid dehydrogenase (3β-HSD), 5α-reductase (5α-R) and 3α-hydroxysteroid dehydrogenase (3α-HSD), with progesterone and 5α-dihydroprogesterone (5α-DHP) being the respective intermediates (Do Rego et al., 2009). Two of these enzymes, 5α-R and 3α-HSD, are also involved in the conversion of the peripherally derived glucocorticoid metabolite, deoxycorticosterone (DOC) into 5α3α-THDOC (Fig. 1 – Karavolas and Hodges, 1990). Although a detailed discussion is beyond the scope of this review (but see Do Rego et al., 2009), two isoforms of 5α-R (type I and II) exist. While type I is the most abundant in both human and rodent brain, type II can be hormonally (e.g. testosterone) induced (Torres and Ortega, 2003).

A variety of brain cells have been shown to synthesise neurosteroids. Early studies described a role for astrocytes and glia in neurosteroidogenesis (Melcangi et al., 1993; Mellon and Deschepper, 1993). However, more recent immunohistochemical studies have suggested that the synthetic machinery necessary for neurosteroidogenesis, including StAR (King et al., 2002), CYP11A1 (Kimoto et al., 2001), 3α-HSD and 5α-R (Agis-Balboa