Psychoneuroendocrinology (2009) **34S**, S48—S58

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Neurosteroids: Endogenous allosteric modulators of $GABA_A$ receptors

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Received 10 June 2009; received in revised form 4 August 2009; accepted 5 August 2009

KEYWORDS
$GABA_A$ receptor;
Neurosteroid;
Tonic conductance;
Extrasynaptic receptors;
Inhibitory neurotransmission

Summary In the mammalian central nervous system activation of the ionotropic $GABA_A$ receptor by the neurotransmitter GABA plays a crucial role in controlling neuronal excitability. This essential form of neuronal regulation may be subject to "fine tuning" by particular metabolites of progesterone and deoxycorticosterone, which bind directly to the $GABA_A$ receptor to enhance the actions of GABA. Originally such steroids were considered to act as endocrine messengers, being synthesised in peripheral glands such as the adrenals and ovaries and crossing the blood brain barrier to influence neuronal signalling. However, it is now evident that certain neurons and glia may produce such "neurosteroids" and that these locally synthesised modulators may act in a paracrine, or indeed an autocrine manner to influence neuronal activity. Neurosteroid synthesis may change dynamically in a variety of physiological situations (*e.g.* stress, pregnancy) and perturbations in their levels are implicated in a variety of neurological and psychiatric disorders. Here we will consider (1) evidence supporting the concept that neurosteroids act as local regulators of neuronal inhibition, (2) that extrasynaptic $GABA_A$ receptors appear to be a particularly important neurosteroid target and (3) recent advances in defining the neurosteroid binding site(s) on the $GABA_A$ receptor.

1. Introduction

In the 1940s Hans Selye demonstrated that certain pregnane steroids cause both sedation and anesthesia (Selye, 1941). However, it was to be more than 40 years later before a viable molecular mechanism emerged to explain the central depressant effects of these steroids. Harrison and Simmonds in a seminal paper reported that in a rat brain slice preparation, the synthetic steroidal anesthetic alphaxalone (5α-pregnan-3α-ol-11,20 dione) enhanced both stimulus-evoked inhibition and the effects of exogenously applied muscimol, a selective agonist of the ionotropic $GABA_A$ receptor. These effects occurred at relatively low aqueous concentrations of the steroid, and crucially an analogue of alphaxalone, the behaviourally inactive 3β-ol isomer betaxalone, was inert in this respect (Harrison and Simmonds, 1984). As the $GABA_A$

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0306-4530/$ — see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.psyneuen.2009.08.009

receptor is the major inhibitory receptor in mammalian brain and that drugs known to cause sedation such as certain barbiturates and benzodiazepines were already established at that time to enhance neuronal inhibition, the $GABA_A$ receptor appeared to be a logical candidate to mediate the depressant actions of these steroids.

Given the structure of alphaxalone, Harrison and Simmonds proposed that "*other steroid hormones and their metabolites might also interact with the $GABA_A$ receptor*". Indeed, electrophysiological, radioligand binding and tracer flux ($^{36}Cl^-$) studies soon established certain endogenously synthesised steroids such as the progesterone metabolites 5α-pregnan-3α-ol-20-one (5α3α-THPROG) and 5β-pregnan-3α-ol-20-one (5β3α-THPROG) and the deoxycorticosterone metabolite 5α-pregnan-3α,21-diol-20-one (5α3α-THDOC), to be potent positive allosteric modulators of the $GABA_A$ receptor (Barker et al., 1987; Callachan et al., 1987; Lambert et al., 1987; Harrison et al., 1987; Gee et al., 1987, 1988; Peters et al., 1988). Patch-clamp studies revealed that in the presence of GABA, low nM aqueous concentrations of these steroids acted primarily to increase the probability of the GABA-gated ion channel being in the open state, with no effect on the single channel conductance (Callachan et al., 1987; Lambert et al., 1987). Additionally, at concentrations in excess of those required for GABA-modulation (<100 nM) these steroids directly activated the $GABA_A$ receptor, *i.e.* a GABA-mimetic effect that was sufficient to suppress excitatory transmission (Lambert et al., 1990). The GABA-modulatory effects of these steroids were not influenced by the benzodiazepine antagonist flumazenil (Callachan et al., 1987; Cottrell et al., 1987). Furthermore, interaction studies with pentobarbital suggested the steroid site to be distinct from that of the barbiturates (Gee et al., 1988; Peters et al., 1988). Hence, a novel site on the $GABA_A$ receptor, which bound these synthetic and endogenous steroids was proposed (Lambert et al., 1987).

These early studies were performed on native $GABA_A$ receptors and were conducted prior to the cloning of the α1 and β1 subunits of the $GABA_A$ receptor. It is now recognised that the α1 and β1 subunits are part of a large family of $GABA_A$ receptor subunits, composed of 19 members (α1-6, β1-3, γ1-3, δ, ε, ρ1-3, θ, π) that are divided into subfamilies according to their amino acid homology (Barnard et al., 1998; Olsen and Sieghart, 2008). The receptor is composed of 5 subunits, and current estimates suggest the subunit repertoire underpins the expression of ~20–30 distinct subtypes of $GABA_A$ receptor, expressed heterogeneously within the mammalian CNS (Sieghart and Sperk, 2002; Fritschy and Brunig, 2003). The subunit composition impacts upon both the biophysical and pharmacological properties of the $GABA_AR$ and where in the cell these receptors are expressed (*e.g.* synaptic or extrasynaptic). For benzodiazepines and certain general anesthetics the key amino acids responsible for their subunit selective actions have been identified (Wieland et al., 1992; Belelli et al., 1997; Sigel and Buhr, 1997). Subsequently, mice harbouring mutations of these critical residues were engineered to render specific $GABA_AR$ isoforms insensitive to these allosteric modulators (Reynolds et al., 2003; Rudolph and Mohler, 2004). This genetic approach is proving invaluable in identifying which $GABA_AR$ isoforms mediate the constellation of behaviours induced by benzodiazepines and general anesthetics. Consistent with an action on $GABA_ARs$, the neuroactive pregnane steroids exhibit anxiolytic, anticonvulsant, analgesic, sedative and anesthetic activity (Lambert et al., 1995; Gasior et al., 1999; Rupprecht, 2003). Clearly, it is of interest to establish the identity of the $GABA_AR$ subtypes that mediate the behavioural effects of these steroids and to clarify where in the CNS such receptors are located. The identification of key residues on the $GABA_AR$ α subunit, that govern the steroid/$GABA_AR$ interaction should allow for a similar genetic dissection of behaviour to progress (Hosie et al., 2006, 2007, 2009).

Peripheral glands such as the adrenal cortex and the ovaries are established sources of $GABA_AR$-active steroids (Purdy et al., 1991; Paul and Purdy, 1992). Physiologically, raised levels of such neuroactive steroids (*e.g.* during stress or pregnancy) are envisaged to cross the blood brain barrier to influence mood and behaviour, *i.e.* the steroid in this scenario acts as an endocrine messenger. However, the demonstration that the brain and spinal cord contain all the necessary enzymatic machinery to synthesise these $GABA_AR$-active steroids (Purdy et al., 1991; Paul and Purdy, 1992; Schumacher et al., 2003; Poletti et al., 1999; Robel et al., 1999; Agís-Balboa et al., 2006) raised the prospect that they might additionally act in a paracrine, or autocrine manner, to locally influence neuronal inhibition and consequently these steroids are classed as "neurosteroids" – see below.

Given the growing evidence for the local production of steroids within the CNS, the plasma or cerebrospinal fluid levels of neurosteroids may be an inaccurate surrogate marker of the actual concentrations experienced by neuronal $GABA_ARs$ *in situ*. With that *proviso*, such measurements reveal neurosteroid levels to change dynamically in a variety of physiological scenarios such as stress, pregnancy and the ovarian cycle (Purdy et al., 1991; Paul and Purdy, 1992). Abnormal levels of neurosteroids may be a contributory factor to behaviours associated with certain psychiatric conditions including major depression, postpartum depression, premenstrual tension, panic attacks and schizophrenia and similar pertubations are implicated in catamenial epilepsy (Purdy et al., 1991; Smith, 2004; Sundstrom-Poromaa, 2004; Eser et al., 2006; Marx et al., 2006). Furthermore, a number of psychoactive drugs including ethanol, γ-hydroxybutyrate and fluoxetine perturb neurosteroid levels and it is conceivable that the consequent changes to $GABA_AR$ activity may contribute to their behavioural actions (Uzunova et al., 1998; Barbaccia, 2004; Kumar et al., 2004; Morrow et al., 2004; Finn et al., 2006).

As $GABA_ARs$ are ubiquitously expressed throughout the brain and spinal cord, the impact of neurosteroids might be presumed to be indiscriminate, a scenario difficult to reconcile with a physiological role. Furthermore, studies utilising recombinant $GABA_A$ receptor subtypes reveals (with one or two important exceptions) the neurosteroid/$GABA_AR$ interaction to be rather promiscuous (Belelli et al., 2002). However, by contrast the interaction of neurosteroids with native $GABA_ARs$ is highly selective. The basis of this specificity is dependent upon a number of factors including the activity of resident neuronal kinases and phosphatases, the differential expression of enzymes that locally synthesise, or metabolize neurosteroids and in some cases the subunit composition of the $GABA_A$ receptors (Belelli and Lambert, 2005; Herd et al., 2007). Here, we will focus on recent