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Authors

Belelli, Delia
Hogenkamp, Derk
Gee, Kelvin W
[et al.](#)

Publication Date

2020-05-01

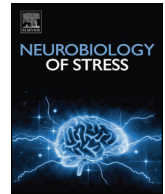
DOI

10.1016/j.ynstr.2019.100207

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Realising the therapeutic potential of neuroactive steroid modulators of the GABA_A receptor

Delia Belelli^a, Derk Hogenkamp^b, Kelvin W. Gee^b, Jeremy J. Lambert^{a,*}

^a Systems Medicine, Neuroscience, Mail Box 6, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, United Kingdom

^b Department of Pharmacology, 110C Med Surge1, Mail Code 4625, University of California, Irvine, School of Medicine, Irvine, CA, 92697, USA

ARTICLE INFO

Keywords:

Allopregnanolone
GABA_A receptor
Neurosteroid
Phasic inhibition
Tonic inhibition

ABSTRACT

In the 1980s particular endogenous metabolites of progesterone and of deoxycorticosterone were revealed to be potent, efficacious, positive allosteric modulators (PAMs) of the GABA_A receptor (GABA_AR). These reports were followed by the discovery that such steroids may be synthesised not only in peripheral endocrine glands, but locally in the central nervous system (CNS), to potentially act as paracrine, or autocrine “neurosteroid” messengers, thereby fine tuning neuronal inhibition. These discoveries triggered enthusiasm to elucidate the physiological role of such neurosteroids and explore whether their levels may be perturbed in particular psychiatric and neurological disorders. In preclinical studies the GABA_AR-active steroids were shown to exhibit anxiolytic, anticonvulsant, analgesic and sedative properties and at relatively high doses to induce a state of general anaesthesia. Collectively, these findings encouraged efforts to investigate the therapeutic potential of neurosteroids and related synthetic analogues. However, following over 30 years of investigation, realising their possible medical potential has proved challenging. The recent FDA approval for the natural neurosteroid allopregnanolone (brexanolone) to treat postpartum depression (PPD) should trigger renewed enthusiasm for neurosteroid research. Here we focus on the influence of neuroactive steroids on GABA-ergic signalling and on the challenges faced in developing such steroids as anaesthetics, sedatives, analgesics, anticonvulsants, antidepressants and as treatments for neurodegenerative disorders.

1. Introduction

Almost 80 years ago Hans Selye demonstrated that particular pregnane steroids can induce rapid sedation and a state of unconsciousness (Selye, 1941). Approximately 40 years elapsed before a viable molecular mechanism emerged to explain these rapid behavioural effects. Electrophysiological studies revealed the intravenous synthetic steroidal general anesthetic alphaxalone (5 α -pregnane-3 α -ol-11, 20-dione) to enhance the inhibitory effects of GABA in guinea pig olfactory cortex (Scholfield, 1980) and in cat spinal cord neurons (Lodge and Anis, 1984). Extracellular recordings in the rat cuneate nucleus demonstrated alphaxalone to potently enhance responses mediated by GABA_A receptor (GABA_AR) agonists (Harrison and Simmonds, 1984). Supporting the GABA_AR as a locus for the behavioural effect, betaxalone, a 3 β -ol isomer of alphaxalone, had no effect on GABA_ARs and was behaviourally inert (Harrison and Simmonds, 1984). In their seminal paper they recalled that alphaxalone was structurally related to certain endogenous pregnane steroids, raising the prospect that the activity of the major inhibitory receptor in the

mammalian central nervous system (CNS), the GABA_AR, may be subject to physiological, or pathophysiological regulation (Harrison and Simmonds, 1984). In confirmation, subsequent electrophysiological and radioligand binding approaches revealed particular metabolites of progesterone (e.g., 5 α -pregnan-3 α -ol-20-one, also known as allopregnanolone and now brexanolone) and of deoxycorticosterone (5 α -pregnan-3 α , 21-diol-20-one, [5 α -THDOC]) to also be highly efficacious, stereoselective, positive allosteric modulators (PAMs) of the GABA_AR at nM concentrations (Majewska et al., 1986; Barker et al., 1987; Callachan et al., 1987; Cottrell et al., 1987; Gee et al., 1988; Peters et al., 1988). Radioligand binding and electrophysiological studies established that the GABA facilitatory actions of these steroids were not mediated by binding to the proposed benzodiazepine site on the GABA_AR (Harrison and Simmonds, 1984; Cottrell et al., 1987; Gee et al., 1988). In common with barbiturates, these steroids promoted the open conducting state of the GABA_AR associated anion channel, thereby enhancing the actions of GABA and at higher concentrations, they directly activated the receptor (Majewska et al., 1986; Barker et al., 1987; Callachan et al., 1987; Cottrell et al., 1987; Peters et al., 1988), leading

* Corresponding author.

E-mail address: j.j.lambert@dundee.ac.uk (J.J. Lambert).

<https://doi.org/10.1016/j.ynstr.2019.100207>

Received 21 November 2019; Accepted 19 December 2019

Available online 23 December 2019

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Abbreviations

Ad	Alzheimer's disease
allopregnanolone	5 α -pregnan-3 α -ol-20-one
CNS	central nervous system
DGGCs	dentate gyrus granule cells
EEG	electroencephalogram
FDA	food and drug administration
GABA _A R	GABA _A receptor
HPA	hypothalamus, pituitary, adrenal axis
IPSC	inhibitory postsynaptic current
im	intramuscular
iv	intravenous
MOA	mechanism of action

nRT	nucleus reticularis
NREM	non rapid eye movement
ORG	ORG OD02-0
PAM	positive allosteric modulator
PD	pharmacodynamic
pGPCR	progesterone G-protein coupled receptor
PK	pharmacokinetic
PKA	protein kinase A
PKC	protein kinase C
po	oral administration
PPD	postpartum depression
REM	rapid eye movement
SE	status epilepticus
VB	ventrobasal

to their description as “barbiturate-like” modulators (Harrison and Simmonds, 1984; Majewska et al., 1986), although subsequently barbiturate/steroid interaction studies implied these GABA_AR modulators acted *via* distinct sites (Cottrell et al., 1987; Gee et al., 1988; Peters et al., 1988). The potency and stereoselectivity of their actions suggested involvement of a high affinity steroid binding site located on the GABA_AR. However, the apparent affinity derived from functional studies may be misleading as these steroids are highly lipophilic, causing their accumulation to relatively high local concentrations in the membrane *i.e.* in close apposition to a proposed transmembrane binding site for the steroid on the GABA_AR protein (Hosie et al., 2006; Chisari et al., 2010).

In this review we use the term neurosteroid for endogenous steroids synthesised in the CNS and the term neuroactive steroid for exogenous GABA_AR-active steroids.

2. Neuroactive steroids and GABA_AR subtypes

The GABA_AR is a member of the *cys*-loop transmitter-gated ion channel family and is composed of five transmembrane crossing subunits. Studies in the late 1980s and early 1990s revealed an unexpected diversity of GABA_AR subunits (α 1-6, β 1-3, γ 1-3, δ , ϵ , θ , π and ρ 1-3), a repertoire supporting the expression of ~20–30 subtypes of GABA_AR (Olsen and Sieghart, 2009), that exhibit a distinct expression pattern within the mammalian CNS. The majority of GABA_ARs are composed of two α subunits, two β subunits and a γ subunit (predominantly, but not exclusively the γ 2 subunit). For some receptors the δ subunit (δ -GABA_AR) replaces the γ subunit. The GABA_AR subunit composition influences *i*) where in the CNS the receptors are expressed and their subcellular location within a neuron *e.g.* synaptic *vs* extra/peri-synaptic expression *ii*) the biophysical properties of the receptor, including rates of deactivation, desensitization, and GABA affinity *iii*) the effect of enzymes such as kinases and phosphatases upon receptor function and expression), and *iv*) receptor pharmacology (Jacob et al., 2008; Olsen and Sieghart, 2008; Fritschy and Panzanelli, 2014; Nakamura et al., 2015).

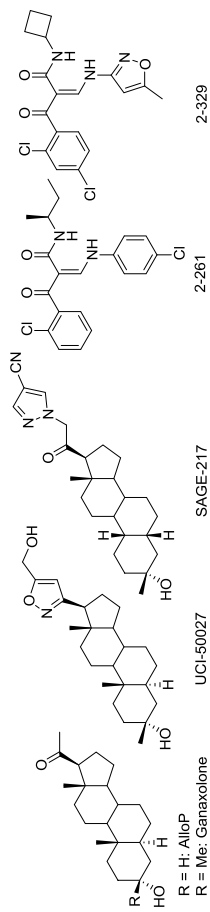
In common with established PAMs of the GABA_AR (*e.g.* diazepam), neuroactive steroids exhibit anxiolytic, analgesic, anticonvulsant and sedative effects, but additionally at greater doses they induce a state of general anaesthesia (Belelli and Lambert, 2005; Reddy and Estes, 2016). Although such steroids are highly selective PAMs of the GABA_AR, they exhibit only limited specificity for particular GABA_AR isoforms (Belelli et al., 2009; Brickley and Mody, 2012). A possible exception are δ -GABA_AR, where neurosteroids appear highly efficacious, although this apparent selectivity is probably secondary to the limited efficacy of GABA acting at δ -GABA_AR (Brickley and Mody, 2012). Table 1 utilises the voltage-clamp technique to compare the GABA_AR enhancing effects of allopregnanolone, with structurally related synthetic steroids across representatives of synaptic and extrasynaptic

GABA_AR. In agreement with previous studies (Belelli et al., 2009; Brickley and Mody, 2012) inspection of Table 1 reveals little GABA_AR isoform specificity of these steroids. Given this GABA_AR promiscuity a key question concerns the identification of the GABA_AR subtypes that mediate the behavioural repertoire neurosteroids. Experiments with “knock-in” mice genetically engineered to express diazepam-insensitive and etomidate-insensitive GABA_AR have revealed components of their behavioural effects to be mediated by particular GABA_AR (Rudolph and Knoflach, 2011). Key transmembrane amino acids have been identified that impair neurosteroid modulation of GABA_AR, permitting equivalent mouse behavioural studies, although to date there has not been a comprehensive behavioural profile of such mice (Newman et al., 2016). In the absence of such studies, components of the behavioural effects neuroactive steroids may exhibit a similar receptor profile to those of other GABA_AR modulators. However, whereas the effects of diazepam are mediated by enhancing the effects of GABA at γ -GABA_AR, importantly neurosteroids additionally act as PAMs of δ -GABA_AR. Indeed, behavioural studies of mice with deletion of the genes encoding for the δ subunit and the α 4 subunit (often partnered with the δ subunit) are proving instructive (see below).

Receptors incorporating the γ 2 subunit, are primarily, although not exclusively, expressed within inhibitory synapses, where they mediate fast phasic responses (under voltage-clamp conditions recorded as inhibitory postsynaptic currents [IPSCs]) upon activation by the neurally-evoked vesicular release of GABA (Farrant and Nusser, 2005; Brickley and Mody, 2012). In common, both benzodiazepines such as diazepam and neuroactive steroids prolong the IPSC duration, thereby enhancing phasic inhibition. Certain neurons (*e.g.* hippocampal dentate gyrus granule cells [DGGCs]; thalamocortical ventrobasal [VB] neurons) additionally express neurosteroid-sensitive δ -GABA_AR (Brickley and Mody, 2012). These receptors are insensitive to benzodiazepines and are predominantly expressed out with the inhibitory synapse in peri-synaptic, or extrasynaptic locations (Brickley and Mody, 2012). In these neurons extrasynaptic δ -GABA_AR mediate a tonic, persistent current, either in response to low ambient levels of GABA, or due to spontaneous gating of the receptor-channel complex (Brickley and Mody, 2012; Włodarczyk et al., 2013). Neuroactive steroids, in common with GABA_AR-active general anaesthetics, such as etomidate and propofol, will interact with synaptic γ 2-GABA_AR to prolong the duration of phasic inhibition, but additionally with extrasynaptic δ -GABA_AR to increase tonic inhibition (Stell et al., 2003; Belelli and Lambert, 2005; Peden et al., 2008; Brickley and Mody, 2012) – Fig. 1. By contrast, benzodiazepines will primarily influence phasic inhibition, although certain neurons do express benzodiazepine-sensitive γ 2 subunit containing GABA_AR (*e.g.* α 5 β γ 2), that mediate a tonic conductance (Caraiscos et al., 2004);

Considering how neurons respond during physiological rates of presynaptic stimulation, this division of fast phasic and tonic inhibition may be an over simplification. For example thalamocortical VB neurons

Table 1
 The potency and maximal efficacy of neuroactive steroids and non-steroidal modulators on the function of human GABA_AR subtypes expressed in *Xenopus laevis* oocytes. The GABA EC₁₀ (the concentration of GABA that evokes a response 10% of the maximal response to GABA) was determined for each oocyte expressing the receptor subtype of interest. All compounds were tested with a 30 s pre-treatment prior to co-application with an EC₁₀ concentration of GABA. The effect of the test drug upon the GABA EC₁₀ response by the test drug was expressed as a percentage and calculated as: $([I - GABA\ EC_{10}] + \text{modulator}/[GABA\ EC_{10}] \times 100)$, where I = current. The concentration-effect data were fitted to a four-parameter logistic equation (GraphPad Software, San Diego, CA). Data represent the mean \pm S.E.M. (n = 3). # Data from Hogenkamp et al. (2014) and Carter et al. (1997); AlloP = allopregnanolone; EC₅₀ = the concentration of steroid producing half the maximum response of that compound. Max Mod = the maximum modulation of the GABA EC₁₀ response produced by the test compound.



Compound	GABA _A Receptor Subtype		α1β2γ2				α2β1γ2				α2β2γ2				α4β3δ				
	EC ₅₀ ± SEM (μM)	Max Mod ± SEM control (%)	EC ₅₀ ± SEM (%)	EC ₅₀ ± SEM (μM)	Max Mod ± SEM control (%)	EC ₅₀ ± SEM (μM)	EC ₅₀ ± SEM (%)	EC ₅₀ ± SEM (μM)	Max Mod ± SEM control (%)	EC ₅₀ ± SEM (μM)	EC ₅₀ ± SEM (%)	EC ₅₀ ± SEM (μM)	EC ₅₀ ± SEM (%)	EC ₅₀ ± SEM (μM)	EC ₅₀ ± SEM (%)	EC ₅₀ ± SEM (μM)	EC ₅₀ ± SEM (%)	Max Mod ± SEM control (%)	Max Mod ± SEM control (%)
AlloP	0.09 ± .02	817 ± 62																1401 ± 88	
Ganaxolone	0.13 ± 0.04	1006 ± 241	0.11 ± 0.01	0.11 ± 0.01	909 ± 68	0.27 ± 0.20	0.11 ± 0.04	0.11 ± 0.04	485 ± 257	0.25 ± 0.06	0.11 ± 0.04	0.11 ± 0.04	485 ± 257	0.54 ± .09	0.11 ± 0.04	0.11 ± 0.04	1401 ± 88		
UCI-50027	0.63 ± 0.05	472 ± 84	0.79 ± 0.63	1.33 ± 0.25	587 ± 91	0.1 ± 0.06#	0.19 ± 0.07	0.19 ± 0.07	646 ± 254	0.34 ± 0.18	0.19 ± 0.07	0.19 ± 0.07	646 ± 254	0.25 ± 0.06	0.19 ± 0.07	0.19 ± 0.07	1710 ± 534		
SAGE-217	0.08 ± 0.02	488 ± 33	1.33 ± 0.25	0.32 ± 0.16	823 ± 353	0.34 ± 0.18	1.41 ± 0.47	1.41 ± 0.47	595 ± 176	0.18 ± 0.04	1.41 ± 0.47	0.38 ± 0.06	1355 ± 173	1.04 ± 0.45	1.41 ± 0.47	0.38 ± 0.06	919 ± 229		
2-261	1.2 ± 0.5	24 ± 8	0.36 ± 0.05	0.044 ± 0.003	1073 ± 194	0.95 ± 0.14	0.16 ± 0.04	0.16 ± 0.04	921 ± 47	0.95 ± 0.14	0.16 ± 0.04	0.16 ± 0.04	921 ± 47	0.15 ± 0.03	0.16 ± 0.04	0.15 ± 0.03	783 ± 122		
2-329	1.7 ± 0.2	843 ± 136	0.044 ± 0.003	0.044 ± 0.003	947 ± 244	0.41 ± 0.07	0.018 ± 0.008	0.018 ± 0.008	559 ± 151	0.41 ± 0.07	0.018 ± 0.008	0.018 ± 0.008	559 ± 151	0.45 ± 0.1	0.018 ± 0.008	0.45 ± 0.1	1771 ± 294		
																		2775 ± 99	

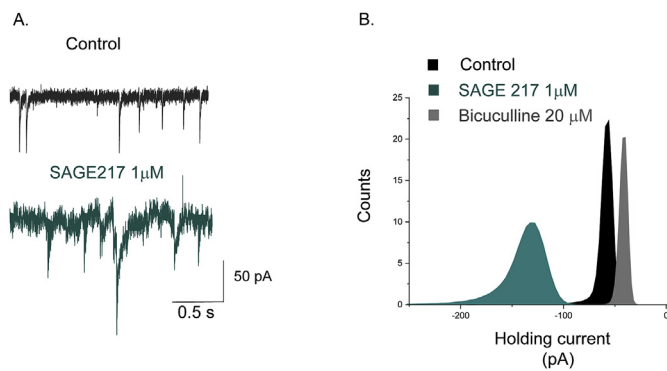


Fig. 1. *SAGE-217 greatly enhances both phasic and tonic inhibition GABA_AR in mouse thalamic VB neurons.* **A)** GABA_AR mIPSCs recorded at -70 mV from a representative VB neuron of a neonatal (P21) mouse horizontal slice before (Control, black trace) and following 10 min of SAGE 217 ($1 \mu\text{M}$, green trace). The mIPSCs appear as downward deflections from the baseline. Note the prolongation of the mIPSC decay by SAGE 217 and the simultaneous increase of the baseline noise. **B)** An all-points histogram of the holding current from the same recording depicted in **A)** under control conditions (black), following SAGE 217 ($1 \mu\text{M}$, green) and co-application of bicuculline ($20 \mu\text{M}$, grey). Note the large inward shift in the holding current after SAGE 217 ($1 \mu\text{M}$) and the reversal beyond the control holding current following bicuculline ($20 \mu\text{M}$), revealing a clear GABA_AR tonic conductance. See [Brown et al., 2015](#) for recording conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

are innervated by GABA-ergic nucleus reticularis (nRT) neurons. Murine VB neurons express synaptic ($\alpha 1\beta 2\gamma 2$) and extrasynaptic ($\alpha 4\beta 2\delta$) GABA_ARs, that mediate fast phasic and tonic inhibition respectively ([Peden et al., 2008](#); [Herd et al., 2013](#)). During drowsiness and non-rapid eye movement (NREM) sleep these presynaptic nRT neurons fire with high frequency bursts, consequently releasing substantial amounts of GABA onto VB neurons, which in addition to engaging synaptic GABA_ARs, spills over from the synapse to activate peri- and extrasynaptic GABA_ARs. Under voltage-clamp this spillover GABA results in a greatly prolonged IPSC. The slow component of the IPSC is absent in $\alpha 4^{-/-}$ mice and is prolonged by DS2, a δ -GABA_AR-selective PAM ([Herd et al., 2013](#)), confirming it to be mediated by activation of extrasynaptic GABA_ARs ($\alpha 4\beta 2\delta$). For VB neurons allopregnanolone and related GABA_AR-active steroids greatly prolong fast phasic inhibition and increase the tonic current ([Brown et al., 2015](#)) – **Fig. 1**. Although the effects of neurosteroids on this spillover component of the IPSC remains to be determined, it is probable that allopregnanolone, in common with DS2 and etomidate (an intravenous general anesthetic), will greatly prolong such events ([Herd et al., 2013, 2014](#)). By contrast, benzodiazepines would be inert in this respect, causing only a relatively modest prolongation *via* the synaptic ($\alpha 1\beta 2\gamma 2$) component of the IPSC ([Peden et al., 2008](#)). Therefore, in some neurons the neurosteroid effect upon neural inhibition may be considerably greater than that of benzodiazepines ([Weir et al., 2017](#)).

A recent report highlights a further important, but indirect action of neurosteroids upon tonic inhibition ([Parakala et al., 2019](#)). In addition to binding to GABA_ARs, certain steroids, including allopregnanolone and progesterone activate a family of progesterone G-protein coupled receptors (p-GPCRs) located on the membrane surface ([Pang et al., 2013](#); [Schumacher et al., 2014](#); [Parakala et al., 2019](#)). The steroid ORG OD 02-0 (ORG) selectively activates these p-GPCRs, with no effect upon nuclear progesterone receptors and importantly, in contrast to allopregnanolone, has no direct interaction with GABA_ARs ([Parakala et al., 2019](#)). Activation of p-GPCRs by ORG has no immediate effect upon phasic, or tonic inhibition of dentate granule neurons. However, prolonged p-GPCR activation by this steroid produced a delayed, selective and sustained increase of the tonic current, due to an increased cell surface expression of extrasynaptic GABA_ARs, with no effect on phasic

inhibition ([Parakala et al., 2019](#)). This metabotropic effect of ORG and allopregnanolone on GABA_AR trafficking resulted from phosphorylation of particular serine residues of the GABA_AR $\beta 3$ subunit *via* an involvement of PKA and PKC ([Parakala et al., 2019](#)). Importantly, although the neuroactive steroid ganaxolone acutely increased the tonic current of hippocampal DGGNs, in contrast to allopregnanolone it did not produce a sustained enhancement of this conductance ([Modgil et al., 2017](#)).

In summary, allopregnanolone interacts directly with GABA_ARs to acutely prolong phasic and enhance tonic inhibition, but may exert an additional, metabotropic effect to increase tonic inhibition on a slower time scale, by selectively influencing extrasynaptic GABA_AR trafficking. This form of sustained GABA_AR plasticity may be important in understanding the therapeutic and physiological effects of allopregnanolone. In particular, it may underpin behavioural effects of allopregnanolone that remain following elimination of the steroid from the CNS. As highlighted by the differential profile of ganaxolone ([Modgil et al., 2017](#)), for future drug discovery programs, consideration of the activity of synthetic steroids upon the family of p-GPCRs may be warranted, in addition to the current focus on their direct interaction with the GABA_AR. In certain neurons, including DGGNs, the extrasynaptic δ -GABA_ARs incorporate the $\beta 2$ subunit (e.g. $\alpha 4\beta 2\delta$) – ([Peden et al., 2008](#); [Herd et al., 2008](#)). It will be of interest to explore if this metabotropic effect extends to such receptors (see [Modgil et al., 2017](#)).

We will now consider attempts to develop neuroactive steroids as novel therapeutics, focussing primarily on indications where the GABA_AR is the likely site of action and where possible clinical findings are available for evaluation.

3. Developing clinical applications of neuroactive steroids

3.1. General anesthetics

The initial clinical use of neuroactive steroids was as general anesthetics. Following the discovery that progesterone exhibits both sedative and anticonvulsant efficacy in preclinical models ([Selye, 1941](#)), it was tested in humans intravenously (iv.) at 500 mg, a dose that produced an anesthetic effect ([Merryman et al., 1954](#)). This observation provided the impetus to examine the basis for this effect of progesterone and whether it had applications in developing new medicines. Based on pioneering work by Syntex on hypnotic steroids ([Gyermeik et al., 1968](#)), in the 1970s Glaxo developed “Althesin” a combination of the steroids alphaxalone and alfadalone ([Child et al., 1971](#)). These steroids are analogues of allopregnanolone, the former has an 11-carbonyl substitution and the latter an 11-carbonyl, 21-hydroxyl substitution. As described (Introduction), alphaxalone is an efficacious GABA_AR PAM. Clinically, Althesin was tested as a potential iv. induction agent undergoing clinical trials in the United States in the 1970s, but did not receive FDA approval for this application, although it was approved by regulatory agencies in Europe.

Althesin was formulated in a surfactant polyethylated castor oil (Cremophor). The attractive properties of Althesin, that distinguished it from other induction agents, were a lack of adverse cardiovascular, or respiratory effects, coupled with a limited post-recovery hangover due to a short half-life ([Towler et al., 1982](#)). However, Althesin caused anaphylaxis, resulting in withdrawal from human use, although it is used in veterinary practice ([Barletta, 2019](#)). The anaphylaxis was caused by histamine release induced by Cremophor and not the active components of Althesin ([Johnson et al., 2011](#)). An additional adverse effect of Althesin was hyperexcitability observed during induction and seizure activity during recovery ([Towler et al., 1982](#)). Minaxolone, a 2 β -ethoxy, 11 α -dimethylamino substituted analogue of allopregnanolone was also developed, but never marketed ([Kharasch and Hollmann, 2015](#)). Organon improved upon the poor solubility by creating a series of water-soluble neuroactive steroids with anesthetic properties, which were highly efficacious GABA_AR PAMs ([Hill-Venning et al., 1996](#);

Sneyd et al., 1997). These steroids included two candidates (Org-20599 and Org-21465) based on morpholinyl substitutions at the 2 β -position on the steroid A ring and were advanced into clinical trials. In common with Althesin, their excitatory actions led to discontinuation of the trials (Gasior et al., 1999). Interestingly, a reformulated version of alphaxalone (Phaxan), which uses a cyclodextrin as a carrier, is currently in clinical development by Drawbridge Pharmaceuticals. This reformulation potentially eliminates the toxic effects of the original Cremophor formulation, but whether post-recovery excitability can be mitigated remains to be established.

The discovery of the GABA-modulatory effects of allopregnanolone encouraged exploring additional medical applications, common to established GABA_AR modulators e.g. benzodiazepines.

3.2. Sedatives

Satisfactory sleep is crucial for physical and mental health (Krause et al., 2017). Various neuropsychiatric disorders including depression and schizophrenia are accompanied by disrupted sleep architecture. Benzodiazepines and zolpidem are widely prescribed for sleep disorders, but their side effects, including tolerance and withdrawal, make them far from ideal (Wafford and Ebert, 2008). Investigations of sedative applications of the allopregnanolone-related steroids have been sporadic, despite sedation being their most apparent behavioural effect. Clinical trials of Althesin and alphaxalone revealed sedative effects (Ramsay et al., 1974; Stewart et al., 1983). Neuroactive steroids have desirable attributes at sedative doses including a short duration of action, no hangover, no effects on sleep architecture, limited effects on cognition and an absence of cardiovascular and respiratory depression (Towler et al., 1982).

Although the neuroactive steroids were described as “barbiturate like” (Harrison and Simmonds, 1984; Majewska et al., 1986) they were subsequently shown to act upon a distinct site on the GABA_AR (Introduction). This finding opened a path to patent (US patent No. 5,120,723) the use this novel site on the GABA_AR, by Gee and colleagues for the treatment of CNS disorders amenable to modulation by neuroactive steroids. This patent and others led to the development of synthetic neuroactive steroids related to allopregnanolone as drug candidates by CoCensys (Belelli et al., 1990; McNeil et al., 1992; Gee et al., 1995). They developed several orally bioavailable steroids for evaluation as sedative-hypnotics (Edgar et al., 1997; Vanover et al., 2001). In particular, CCD-3693, was more efficacious than benzodiazepines in promoting NREM sleep. In rats CCD-3693 did not interfere with rapid eye movement (REM) sleep and was more selective in reducing electroencephalogram (EEG) wakefulness, with less impairment of locomotor activity during waking than triazolam, or zolpidem (Edgar et al., 1997). In contrast to the benzodiazepines, neuroactive steroids do not produce a distinct “rebound” wakefulness following their NREM sleep-promoting effect. CCD-3693 was progressed into clinical development by CoCensys in collaboration with Searle in the 1990s, where it demonstrated similar properties on human sleep. Although CCD-3693 had a superior pharmacodynamic (PD) profile to that of benzodiazepines, it was not developed then because of pharmacoeconomic considerations.

Regarding mechanism, the sedative effects of the benzodiazepines are primarily mediated by $\alpha 1\beta\gamma 2$ GABA_ARs (Wafford and Ebert, 2008; Rudolph and Knoflach, 2011) and neuroactive steroids are effective PAMs of such receptors (Table 1; Fig. 1). However, the sleep times induced by allopregnanolone and alphaxalone are reduced in $\delta^{-/-}$ subunit mice, implicating extrasynaptic δ -GABA_ARs (Mihalek et al., 1999). Similarly, the sedative effects of gaboxadol, a δ -GABA_AR-selective agonist (Belelli et al., 2005) are impaired in $\delta^{-/-}$ mice (Boehm et al., 2006; Wafford and Ebert, 2008; Herd et al., 2009). Although these studies identify a credible target for developing steroid based sedatives, their poor oral bioavailability remains a major impediment.

3.3. Anticonvulsant

The demonstration that a single iv. infusion of Althesin suppressed seizures in 7 of 9 patients with status epilepticus (SE) provided the first clinical evidence that steroids related to allopregnanolone have anticonvulsant activity (Munari et al., 1979). Clearly a logical and obvious indication for allopregnanolone was epilepsy. In support, allopregnanolone exhibits anticonvulsant efficacy in a number of preclinical models of seizures (Belelli et al., 1989; Luntz-Leybman et al., 1990; Kokate et al., 1994, 1996; Devaud et al., 1995; Kaminski et al., 2004; Reddy and Rogawski, 2012; Wu and Burnham, 2018). However, neither Althesin, nor allopregnanolone, were viable as orally active anticonvulsants, having plasma half-lives of < 1 h, limited solubility and oral bioavailability (Irwin et al., 2015). Side-stepping this impediment, a recent study described the successful treatment of two patients suffering from super-refractory SE following a two day continuous infusion with allopregnanolone (Vaitkevicius et al., 2017). Recently Sage Therapeutics, Inc. (Sage) took an i.v. formulation of allopregnanolone (brexanolone) into clinical testing for the treatment of drug refractory SE. The preparation, designated SAGE-547, showed promise in phase II trials, but failed to meet clinical endpoints in phase III (Rosenthal et al., 2017; Cox, 2017). Why SAGE-547 failed in phase III is not known. Perhaps the heterogeneous aetiology of SE undermines a response to the drug alone, or poor solubility prevented an optimal concentration from being achieved in the formulation used. Alternatively, a sustained allopregnanolone infusion may cause rapid PD tolerance (Turkmen et al., 2011). The clinical efficacy of allopregnanolone in large scale trials remains to be established in epilepsy, despite activity in pre-clinical models, or the anecdotal evidence of clinical activity of Althesin and other neuroactive steroids (Munari et al., 1979).

Ganaxolone, a synthetic analogue of allopregnanolone, was synthesised by CoCensys in the early 1990s to improve oral bioavailability, prevent *in vivo* conversion to progesterone and to increase the biological half-life (Carter et al., 1997). The introduction of a 3 β -methyl group in ganaxolone significantly improved half-life, while retaining the GABA_AR efficacy of allopregnanolone (Carter et al., 1997) –Table 1. This steroid was advanced by CoCensys into clinical trials in 1991 and showed promise as a potential antiepileptic (Reddy and Estes, 2016; Sperling et al., 2017). Ganaxolone was redirected for the treatment of acute migraine based on an uncertain rationale, but failed to demonstrate efficacy in phase II trials. Despite this costly detour, ganaxolone is currently under clinical development by Marinus Pharmaceuticals for treatment of various indications directed at seizure disorders, including cyclin-dependent kinase-like 5 deficiency disorder, protocadherin 19-related epilepsy and refractory SE (NCT03572933, NCT03865732, NCT03350035).

Recently, a series of novel (WO 2013/019711 A2) allopregnanolone-related steroids were designed to improve the aqueous solubility, bioavailability and side-effect profile of established neuroactive steroids and tested for antiepileptic activity (Hogenkamp et al., 2014). These 17 β -heteroaryl steroids are represented by UCI-50027, the prototype in this series, 3-[3 α -hydroxy-3 β -methyl-5 α -androstan-17 β -yl]-5-(hydroxymethyl)isoxazole. UCI-50027 is an orally active anticonvulsant and anxiolytic, which overcomes the general dosing limitations of other neuroactive steroids. UCI-50027 has an improved preclinical pharmacokinetic (PK) and side-effect profile, which makes it more potent and tolerable as an anxiolytic and anticonvulsant than ganaxolone. For example, UCI-50027 has a greater anticonvulsant/anxiolytic therapeutic index than ganaxolone in rodent seizure models. Ganaxolone and UCI-50027 produce a robust enhancement of submaximal GABA-evoked currents in oocytes expressing human recombinant $\alpha 1\beta 2\gamma 2L$ and $\alpha 4\beta 3\delta$ GABA_ARs (Table 1). Both compounds are potent modulators of $\alpha 2\beta 1\gamma 2L$ GABA_ARs, but whereas ganaxolone greatly enhanced the response to GABA irrespective of the β subunit isoform, UCI-50027 produced only a relatively modest maximum modulation of $\alpha 2\beta 1\gamma 2L$, c.f. $\alpha 2\beta 2\gamma 2L$ GABA_ARs (Table 1). The differential influence of the β

subunit appeared selective for $\alpha 2$, but not the $\alpha 1$ subunit-containing GABA_AR (Table 1). This reduced activity at the $\beta 1$ -subunit subtype may diminish the propensity to cause sedative/ataxic side effects *in vivo* at therapeutically relevant doses (Gee et al., 2010; Hogenkamp et al., 2014), although note the sedative effects of etomidate are mediated by GABA_ARs incorporating the $\beta 2$ -subunit (Reynolds et al., 2003).

Preclinical and clinical evidence suggests that allopregnanolone and related analogues should be useful in the treatment of various epilepsies, but to date none have received regulatory approval. Perhaps a more targeted approach, that requires a precise matching of the underlying pathophysiology of the various seizure disorders with the mechanism of action (MOA) of neurosteroids, may be a prerequisite for success. In that regard, numerous genetic mutations of GABA_AR subunits, implying impairments to phasic and tonic inhibition, associate with particular epilepsies, including mutations of the δ -subunit (Macdonald et al., 2010). Some clinical anticonvulsants increase ambient levels of GABA, suggesting manipulations to influence tonic inhibition may be beneficial in certain conditions (Brickley and Mody, 2012). However, note that enhancement of tonic inhibition in models of absence epilepsy aggravates the condition by promoting thalamic burst firing (Cope et al., 2009; Errington et al., 2011).

3.4. Post-partum depression (PPD)

Blockade of neurosteroid production by inhibition of 5 α -reductase with finasteride increases depression-like behaviors in experimental animal models (Walf et al., 2006; Beckley and Finn, 2007) and in some men treated for male pattern hair loss *i.e.* “finasteride syndrome” (Locci and Pinna, 2017; Melcangi et al., 2017). Allopregnanolone levels are negatively correlated with depression-like behaviors (Walf et al., 2006). Moreover, in humans antidepressant treatments increase allopregnanolone levels, which may be an integral component of their therapeutic effect (Romeo et al., 1998; Uzunova et al., 1998; Schule et al., 2007, 2011; Locci and Pinna, 2017). These findings are consistent with the documented role of the hypothalamus, pituitary, adrenal axis (HPA) dysfunction in the aetiology of depression (Lupien et al., 2009) and the dynamic regulation of neurosteroid levels in response to stressful experiences (Purdy et al., 1991; Tomaselli and Vallee, 2019) to influence HPA axis activity (Patchev et al., 1994; Gunn et al., 2015).

Mirroring changes in progesterone, allopregnanolone levels rise steadily through pregnancy until shortly before childbirth, when they begin to fall, with a steep decline occurring immediately after birth (Luisi et al., 2000; Gilbert Evans et al., 2005; Paoletti et al., 2006; Locci and Pinna, 2017). One hypothesis suggests enhanced allopregnanolone levels during pregnancy may reduce expression of extrasynaptic $\alpha 4\beta 8$ GABA_ARs, which remain depressed among patients who develop PPD (Maguire and Mody, 2008; Licheri et al., 2015; Osborne et al., 2017). In support, homozygous ($\delta^{-/-}$) and heterozygous ($\delta^{+/-}$) mice, impaired in their ability to decrease and upregulate δ -GABA_AR expression during pregnancy and postpartum respectively, developed depression-like and abnormal maternal behaviors, resulting in reduced pup survival (Maguire and Mody, 2008), although see Gunn et al. (2013). These symptoms were reversed in the heterozygous $\delta^{+/-}$ mouse by the δ -GABA_AR selective agonist gaboxadol (Maguire and Mody, 2008). If a similar dysregulation of receptor dynamics postpartum occurs in PPD then depressive symptoms may be exacerbated by fluctuating allopregnanolone levels and reduced δ -GABA_AR expression.

Evidence relating perinatal mood and anxiety symptoms with absolute levels of allopregnanolone has been equivocal, especially in larger population studies. Reduced allopregnanolone levels are inversely correlated with depression scores in women during late pregnancy (Hellgren et al., 2014), but not in the second trimester (Hellgren et al., 2017). Interestingly, in this study a polymorphism in a gene involved in allopregnanolone synthesis, aldo-keto reductase family 1, which results in reduced allopregnanolone levels, was associated with an increase in depression scores. In another study second trimester

cortisol, progesterone, pregnanolone and allopregnanolone levels were correlated with negative emotional symptoms (Crowley et al., 2016). Additional investigations demonstrated altered levels of GABA and neurosteroids in association with PPD (Deligiannidis et al., 2016). In contrast, mean allopregnanolone levels at 6 weeks postpartum in over 1500 ethnically diverse women showed no difference between healthy postpartum women and those with PPD (Guintivano et al., 2018). Low second trimester allopregnanolone levels were reported to predict subsequent PPD in a study of 60 pregnant women with mood disorders, where each additional ng/ml of this steroid reduced the probability of developing PPD by 63% (Osborne et al., 2017). However, there was no correlation when based on third trimester levels, consistent with another small study (Deligiannidis et al., 2013). It is not evident why levels during the third trimester showed no association. Studies reporting an association not with concurrent, but with subsequent symptoms, suggest that interactions with other systems, or other disease modifying changes (*e.g.*, receptor plasticity, gene transcription/expression, or changes to rate-limiting enzymes - Agis-Balboa et al., 2014) and other pleiotropic events may contribute to the complex interactions between allopregnanolone and perinatal symptoms.

Regardless, recent treatment trials of the synthetic *iv.* formulation of allopregnanolone (brexanolone), in phase III trials on PPD resulted in a statistically significant difference in remission rates between placebo and two different doses of brexanolone (Meltzer-Brody et al., 2018). Although not new since CoCensys (U.S. patent No. 5,120,723, issued 1992) anticipated the use of allopregnanolone for PPD, Sage was awarded breakthrough status and FDA approval for brexanolone as the first drug approved specifically for PPD. The steady-state plasma levels of brexanolone based on the C_{max} were in the 150–300 nM range during treatment infusion of PPD, corresponding to half the human EC_{50} for enhancement of $\alpha 4\beta 3\delta$ GABA_AR function by allopregnanolone (Brown et al., 2002). This magnitude of GABA_AR facilitation would presumably be near maximal if the brain concentration is $\times 3$ that in plasma, as observed in rodents (Irwin et al., 2015). Sage has rediscovered CoCensys' neuroactive steroids with a follow-on compound to brexanolone, SAGE-217, a synthetic orally-active allopregnanolone analogue that is covered generically in CoCensys patents (U.S. 6,143,736 issued Nov. 7, 2000 and U.S. 6,277,838 issued Aug. 21, 2001). They reported positive results with the compound in their phase II study when tested at 30 mg *p.o.* (once a day for 14 days). In common with allopregnanolone, SAGE-217 is a potent PAM of recombinant receptors, representative of both synaptic ($\alpha 1/2\beta 1/2\gamma 2$) and extrasynaptic ($\alpha 4\beta 3\delta$) GABA_ARs (Table 1) – Martinez Botella et al. (2017). In agreement with this profile, for mouse thalamic VB neurons we find SAGE-217 (1 μ M) to greatly prolong the duration of miniature inhibitory postsynaptic currents (mIPSCs) mediated by synaptic ($\alpha 1\beta 2\gamma 2$) GABA_ARs and to enhance the tonic current mediated by extrasynaptic ($\alpha 4\beta 2\delta$) GABA_ARs – Fig. 1.

Marinus is testing ganaxolone as an orally bioavailable treatment for PPD (NCT03228394, NCT03460756) with preliminary findings reported in the trade press as not meeting primary endpoints at an oral (*p.o.*) dose of 1175 mg (once per day for 28 days). Given the similar GABA_AR profile of these neuroactive steroids (Table 1) why is one steroid clinically active and the other inert? For example are they distinguished by their ability to activate the metabotropic progesterone receptor, leading to differences of δ -GABA_AR trafficking (Modgil et al., 2017; Parakala et al., 2019)? Alternatively, the answer may reside in analysis of their pharmacokinetics, or other properties, revealed when these results are reported in peer-reviewed literature.

The intriguing possibility that neuroactive steroids have wider applications in clinical depression beyond PPD is now under investigation (Luscher and Mohler, 2019). A recent phase II study reported oral SAGE-217 treatment for 14 days to be effective in reducing depressive symptoms in patients with moderate to severe major depressive disorder (Gunduz-Bruce et al., 2019). The main side effects included dizziness and somnolence/sedation, not surprising given the GABA_AR

profile of this steroid (Table 1, Fig. 1).

If the apparent therapeutic effect is due primarily to acute facilitation of extrasynaptic δ -GABA_AR function then other agents with a similar activity profile, may be useful in the treatment of PPD and other depressive disorders. Note benzodiazepines, which have no activity at δ -GABA_ARs, are not considered clinically useful in the treatment of depression *per se* (Baldwin et al., 2013), whereas clinically available drugs such propofol and etomidate, which in common with allopregnanolone, acutely enhance both phasic and tonic inhibition may be of interest (Belevi et al., 2005; Jia et al., 2007; Herd et al., 2013; Brown et al., 2015). However, note compounds such as propofol are unlikely to activate p-GPCRs to elicit a sustained effect upon δ -GABA_AR-mediated tonic inhibition, distinguishing them from allopregnanolone (Abramian et al., 2014; Parakala et al., 2019).

Finally, studies investigating the impact of depressive disorders on not only levels of allopregnanolone, but on the steroid metabolome, may prove instructive and identify additional steroid targets (Sharp et al., 2018; Tomaselli and Vallee, 2019).

3.5. Analgesia

The thalamus is associated with the processing of nociceptive signals (Steriade and Deschenes, 1984; McCormick, 1992; Oliveras and Montagne-Clavel, 1994). Thalamic sensory relay neurons receive GABA-ergic projections from sensory nuclei and the thalamic reticular nucleus (Steriade and Deschenes, 1984; McCormick, 1992). Microinjection of the GABA_AR antagonist picrotoxin into the rat thalamic VB complex results in “pain-like” behavior (Oliveras and Montagne-Clavel, 1994). As discussed (Section 2), electrophysiological and immunohistochemistry studies reveal mouse VB neurons to express neurosteroid-sensitive synaptic ($\alpha 1\beta 2\gamma 2$) and extrasynaptic ($\alpha 4\beta 2\delta$) GABA_ARs (Peden et al., 2008; Herd et al., 2013; Brown et al., 2015 – Fig. 1). Allopregnanolone is active in numerous models of acute and pathological pain states, with evidence of a neuroprotective effect (Kavaliers and Wiebe, 1987; Svensson et al., 2013; Borowicz et al., 2011; Rey and Coirini, 2015). The antinociceptive effects of allopregnanolone and ganaxolone are impaired in the $\delta^{-/-}$ mice (Mihalek et al., 1999). The δ -GABA_AR agonist gaboxadol is antinociceptive in rodents and humans (Reyes-Vazquez and Dafny, 1983; Grognet et al., 1983; Lindeburg et al., 1983; Kjaer and Nielsen, 1983), but analgesic efficacy is attenuated in the $\alpha 4^{-/-}$ mouse (Chandra et al., 2006). Collectively, these studies implicate extrasynaptic $\alpha 4\beta 2\delta$ GABA_ARs in mediating, or contributing to the antinociceptive effects of neuroactive steroids, possibly by enhancing thalamic burst firing (Cheong et al., 2008) and identify a putative target for the development of pain therapeutics (Whissell et al., 2015). Clinically, allopregnanolone and related analogues have not been evaluated for activity in pain states. The lack of reports on self-administration and tolerance to the analgesic effects of allopregnanolone related drugs, in contrast to opioids, makes them worthy of consideration as analgesics.

3.6. Alzheimer's disease (AD) and neurodegenerative disorders

The role of allopregnanolone and related steroids in AD remains to be established as to whether it has a causal contribution, or is an epiphenomenon of the disease. It may be important to distinguish between the action of allopregnanolone as a PAM of the GABA_AR *versus* actions at other sites (e.g., pregnane X receptor, membrane bound p-GPCR – Section 2). In preclinical AD models allopregnanolone promotes neurogenesis, restores cognitive function and reduces pathology (Wang et al., 2010; Irwin et al., 2014; Ratner et al., 2019). The levels of allopregnanolone are reduced in the prefrontal cortex of AD patients (Marx et al., 2006). Perhaps GABA_AR actions may contribute to the protective effects of allopregnanolone on neurodegeneration in AD models, but full efficacy may require a broader scope of interactions. However, the nature and extent of this contribution is unclear and may

be minimal since ganaxolone (up to 30 mg/kg at 4 h post-injury) and pregnanolone (20 mg/kg) showed little effect in a preclinical model of cerebral hematoma, as measured by neurological and histological outcomes up to 3 months post-hematoma (Lyden et al., 2000). Additionally, progesterone failed to meet primary criteria in phase III clinical trials for the treatment of moderate to severe traumatic brain injury, although the contribution of allopregnanolone and related steroids, is unknown (Wright et al., 2014; Skolnick et al., 2014). Nevertheless, *i.v.* allopregnanolone has been tested in traumatic brain injury with equivocal results (NCT01673828).

Given the short metabolic half-life and absence of any reported PK/PD relationship at the GABA_ARs in various AD models of cognitive deficits, among the most compelling argument for its MOA is a pleiotropic effect of allopregnanolone and related steroids, perhaps including an action upon p-GPCRs to influence GABA_AR expression (Parakala et al., 2019, Section 2). Allopregnanolone is currently in clinical trials for the treatment of mild cognitive impairment due to AD (NCT02221622). It was reported to be safe and well-tolerated in the 2–16 mg intramuscular (im.) range in a small blinded- and placebo-controlled trial of 24 patients (both male and female, age 55 and above), with mild cognitive impairment due to AD, or mild AD (Brinton et al., 2018). In this study the CogState battery scores showed high variability, with no significant differences in cognitive measures between groups, though scores trended towards improvement with treatment. Although anecdotal, MRI studies reported allopregnanolone (4 mg dose, intramuscular im.) to improve functional connectivity between the right parietal lobe and posterior cingulate cortex and to exhibit a sustained effect on hippocampal volume over the 3 weeks of treatment (Brinton et al., 2018). These encouraging preliminary results will require confirmation by larger trials to determine the validity of the drug target in AD. Interestingly, the acute administration of allopregnanolone and other GABA_AR-active steroids to healthy subjects inhibits learning and memory function in a manner similar to the benzodiazepines (Bruins Slot et al., 1999; Johansson et al., 2002).

3.7. Beyond neurosteroids: non-steroid compounds selectively targeting $\alpha 4\beta 2\delta$ GABA_AR subtypes

The clinical development of ganaxolone and allopregnanolone as drugs has relied on the use of patented formulations, which is driven by their poor physicochemical properties *e.g.* high lipophilicity (logP), which influences the bioavailability of the steroid. This major shortcoming is due to the intrinsic requirement of the androstane, or pregnane steroid core structure for efficacy (Hogenkamp et al., 2007). Although care is warranted regarding the interpretation of mouse gene deletion studies, the impaired behavioural efficacy of neuroactive steroids for either $\delta^{-/-}$ or $\alpha 4^{-/-}$ mice identify extrasynaptic $\alpha 4\beta 2\delta$ GABA_ARs as a target of interest in exploring the therapeutic potential of neuroactive steroids. As discussed (Section 2), the discovery that certain neuroactive steroids additionally activate p-GPCRs (Pang et al., 2013), leading to increased cell surface expression of δ -GABA_ARs (Parakala et al., 2019) may warrant determining the efficacy of novel steroids upon this target, in addition to profiling their effect upon GABA_AR isoforms.

Alternatively, structural chemotypes with PAM activity at $\alpha 4\beta 2\delta$ GABA_ARs, but improved physicochemical properties can be evaluated for indications for which neurosteroids have potential and demonstrated therapeutic utility. Currently there are few non-steroidal, small molecules that are selective for δ -GABA_AR subtypes. DS2 is a highly selective PAM for extrasynaptic δ -GABA_ARs, exhibiting no activity on synaptic GABA_ARs, but it has poor CNS penetration and was not dosed orally for pharmacological evaluation (Wafford et al., 2009; Jensen et al., 2013). Nevertheless, DS2 is reported to improve recovery in a mouse model of stroke by an anti-inflammatory mechanism (Neumann et al., 2019). AA29504 (Hoestgaard-Jensen et al., 2010), has a favorable ratio of brain to plasma levels, but was not dosed orally, nor is it a

pure PAM since it also has direct agonist activity (Vardya et al., 2012). Furthermore, as a carbamate, AA29504 may be too unstable to be absorbed through the gut after oral dosing. The selective δ -GABA_AR agonist gaboxadol (in clinical development by Ovid Pharmaceuticals for treatment of Angelman and Fragile X syndromes) may be worthy of consideration (Brown et al., 2002). However, the activity of δ -GABA_AR PAMs are governed in part by physiological firing patterns (Section 2), whereas this is presumably less so for direct agonists such as gaboxadol (Herd et al., 2013; Weir et al., 2017).

The non-steroidal small molecules 2–261 and 2–329 (Table 1), act as GABA_AR PAMs via a binding site distinct from that of neurosteroids, or benzodiazepines (Gee et al., 2010). They compare favorably to the neurosteroids, including those currently in clinical development, in terms of potency, efficacy and selectivity for GABA_AR subtypes including the $\alpha 4\beta 3\delta$ isoform (Table 1). They display unique pharmacological properties, which can be readily manipulated through their novel SAR to produce desirable side effect/safety profiles based on GABA_AR receptor subunit-selectivity. For example, the orally bioavailable prototype, 2–261, retains anxiolytic activity without ataxia, cognitive impairment, ethanol potentiation, rewarding effects (linked to addiction), tolerance, or withdrawal (Gee et al., 2010; Yoshimura et al., 2014). Furthermore, in rodents 2–261 has a neurosteroid-like profile in blocking electrical- and chemical-kindled seizures and organophosphate-induced SE, and exhibits activity in chronic pain (Reddy et al., 2018; Johnstone et al., 2019a, b). Thus, retention of therapeutically desirable effects of the GABA_AR-active steroids, but not their side effects, is clearly advantageous. The freedom to operate in the patent space provided by small molecules yields a more flexible approach to drug development, that builds upon the extensive body of knowledge accumulated during the last three decades on neuroactive steroids and GABA_ARs, but avoids their poor physicochemical properties and the prior art of the neurosteroid patent minefield.

CRedit author statement

Delia Belelli – Original writing, subsequent reviewing and editing.

Kelvin Gee - Original writing, subsequent reviewing and editing.

Derk Hogenkamp - Original writing, subsequent reviewing and editing.

Jeremy Lambert – Original writing, subsequent reviewing and editing.

References

- Abramian, A.M., Comenencia-Ortiz, E., Modgil, A., Vien, T.N., Nakamura, Y., Moore, Y.E., Maguire, J.L., Terunuma, M., Davies, P.A., Moss, S.J., 2014. Neurosteroids promote phosphorylation and membrane insertion of extrasynaptic GABA_A receptors. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7132–7137.
- Agis-Balboa, R.C., Guidotti, A., Pinna, G., 2014. 5 α h-reductase type I expression is downregulated in the prefrontal cortex/Brodman's area 9 (BA9) of depressed patients. *Psychopharmacology (Berl.)* 231, 3569–3580.
- Baldwin, D.S., Aitchison, K., Bateson, A., Curran, H.V., Davies, S., Leonard, B., Nutt, D.J., Stephens, D.N., Wilson, S., 2013. Benzodiazepines: risks and benefits. A re-consideration. *J. Psychopharmacol.* 27, 967–971.
- Barletta, M., Alfaxalone: An old drug in a new formulation, 2019. *Today's Veterinary Practice*. online. <https://todaysveterinarypractice.com/alfaxalone-an-old-drug-in-a-new-formulation/>.
- Beckley, E.H., Finn, D.A., 2007. Inhibition of progesterone metabolism mimics the effect of progesterone withdrawal on forced swim test immobility. *Pharmacol. Biochem. Behav.* 87, 412–419.
- Barker, J.L., Harrison, N.L., Lange, G.D., Owen, D.G., 1987. Potentiation of gamma-aminobutyric-acid-activated chloride conductance by a steroid anaesthetic in cultured rat spinal neurones. *J. Physiol.* 386, 485–501.
- Belelli, D., Bolger, M.B., Gee, K.W., 1989. Anticonvulsant profile of the progesterone metabolite 5 α -pregnan-3 α -ol-20-one. *Eur. J. Pharmacol.* 166, 325–329.
- Belelli, D., Lan, N.C., Gee, K.W., 1990. Anticonvulsant steroids and the GABA/benzodiazepine receptor-chloride ionophore complex. *Neurosci. Biobehav. Rev.* 14, 315–322.
- Belelli, D., Lambert, J.J., 2005. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat. Rev. Neurosci.* 6, 565–575.
- Belelli, D., Harrison, N.L., Maguire, J., Macdonald, R.L., Walker, M.C., Cope, D.W., 2009. Extrasynaptic GABA_A receptors: form, pharmacology, and function. *J. Neurosci.* 29, 12757–12763.
- Belelli, D., Peden, D.R., Rosahl, T.W., Wafford, K.A., Lambert, J.J., 2005. Extrasynaptic GABA_A receptors of thalamocortical neurons: a molecular target for hypnotics. *J. Neurosci.* 25, 11513–11520.
- Boehm, S.L., 2nd, Homonics, G.E., Blednov, Y.A., Harris, R.A., 2006. delta-Subunit containing GABA_A receptor knockout mice are less sensitive to the actions of 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol. *Eur. J. Pharmacol.* 341, 158–162.
- Borowicz, K.K., Piskorska, B., Banach, M., Czuczwar, S.J., 2011. Neuroprotective actions of neurosteroids. *Front. Endocrinol. (Lausanne)* 2, 50.
- Brickley, S.G., Mody, I., 2012. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. *Neuron* 73, 23–34.
- Brinton, R.D., Hernandez, G.D., Kono, N., Lopez, C.M., Solinsky, C., Rodgers, K., Gahn, J., Aydogan, D., Shi, Y., Pawluczyk, S., Law, M., Mack, W., Schneider, L., 2018. Allopregnanolone regenerative therapeutic for mild cognitive impairment and mild Alzheimer's Disease: phase 1B/2A Outcomes Update. *J. Prev. Alzheimer's Dis.* 5 (Suppl. 1), 1.
- Brown, A.R., Herd, M.B., Belelli, D., Lambert, J.J., 2015. Developmentally regulated neurosteroid synthesis enhances GABAergic neurotransmission in mouse thalamocortical neurones. *J. Physiol.* 593, 267–284.
- Brown, N., Kerby, J., Bonnert, T.P., Whiting, P.J., Wafford, K.A., 2002. Pharmacological characterization of a novel cell line expressing human $\alpha(4)\beta(3)\delta$ GABA(A) receptors. *Br. J. Pharmacol.* 136, 965–974.
- Bruins Slot, L.A., Piazza, P.V., Colpaert, F.C., 1999. Neuroactive steroids and the constraint of memory. *Eur. J. Neurosci.* 11, 4081–4088.
- Callachan, H., Cottrell, G.A., Hather, N.Y., Lambert, J.J., Nooney, J.M., Peters, J.A., 1997. Modulation of the GABA_A receptor by progesterone metabolites. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 231 359–3.
- Caraiscos, V.B., Elliott, E.M., You-Ten, K.E., Cheng, V.Y., Belelli, D., Newell, J.G., Jackson, M.F., Lambert, J.J., Rosahl, T.W., Wafford, K.A., Macdonald, J.F., Orser, B.A., 2004. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by $\alpha 5$ subunit-containing gamma-aminobutyric acid type A receptors. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3662–3667.
- Carter, R.B., Wood, P.L., Wieland, S., Hawkinson, J.E., Belelli, D., Lambert, J.J., White, H.S., Wolf, H.H., Mirsadeghi, S., Tahir, S.H., Bolger, M.B., Lan, N.C., Gee, K.W., 1997. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one), a selective, high-affinity, steroid modulator of the gamma-aminobutyric acid(A) receptor. *J. Pharmacol. Exp. Ther.* 280, 1284–1295.
- Chandra, D., Jia, F., Liang, J., Peng, Z., Suryanarayanan, A., Werner, D.F., Spigelman, I., Houser, C.R., Olsen, R.W., Harrison, N.L., Homonics, G.E., 2006. GABA_A receptor $\alpha 4$ subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc. Natl. Acad. Sci. USA* 103, 15230–15235.
- Cheong, E., Lee, S., Choi, B.J., Sun, M., Lee, C.J., Shin, H.-S., 2008. Tuning thalamic firing modes via simultaneous modulation of T- and L-type Ca²⁺ channels controls pain sensory gating in the thalamus. *J. Neurosci.* 28, 13331–13340.
- Child, K.J., Currie, J.P., Davis, B., Dodds, M.G., Pearce, D.R., Twissell, D.J., 1971. The pharmacological properties in animals of CT1341-A new steroid anaesthetic agent. *Br. J. Anaesth.* 43, 2–13.
- Chisari, M., Eisenman, L.N., Covey, D.F., Mennerick, S., Zorumski, C.F., 2010. The sticky issue of neurosteroids and GABA(A) receptors. *Trends Neurosci.* 33, 299–306.
- Cope, D.W., Di Giovanni, G., Fyson, S.J., Orban, G., Errington, A.C., Lorincz, M.L., Gould, T.M., Carter, D.A., Crunelli, V., 2009. Enhanced tonic GABA_A inhibition in typical absence epilepsy. *Nat. Med.* 15, 1392–1398.
- Cottrell, G.A., Lambert, J.J., Peters, J.A., 1987. Modulation of GABA_A receptor activity by alfaxalone. *Br. J. Pharmacol.* 90, 491–500.
- Cox P. < <http://www.businesswire.com/news/home/20170912005509/en/Sage-Therapeutics-Reports-Top-Line-Results-Phase-3> > ; 2017 Accessed 21.11.17.
- Crowley, S.K., O'Buckley, T.K., Schiller, C.E., Stuebe, A., Morrow, A.L., Girdler, S.S., 2016. Blunted neuroactive steroid and HPA axis responses to stress are associated with reduced sleep quality and negative affect in pregnancy: a pilot study. *Psychopharmacology (Berl.)* 233, 1299–1310.
- Deligiannidis, K.M., Kroll-Desrosiers, A.R., Mo, S., Nguyen, H.P., Svenson, A., Jaitly, N., Hall, J.E., Barton, B.A., Rothschild, A.J., Shaffer, S.A., 2016. Peripartum neuroactive steroid and gamma-aminobutyric acid profiles in women at-risk for postpartum depression. *Psychoneuroendocrinology* 70, 98–107.
- Deligiannidis, K.M., Sikoglu, E.M., Shaffer, S.A., Frederick, B., Svenson, A.E., Kopoyan, A., Kosma, C.A., Rothschild, A.J., Moore, C.M., 2013. GABAergic neuroactive steroids and resting-state functional connectivity in postpartum depression: a preliminary study. *J. Psychiatr. Res.* 47, 816–828.
- Devaud, L.L., Purdy, R.H., Morrow, A.L., 1995. The neurosteroid, 3 α -hydroxy-5 α -pregnan-20-one, protects against bicuculline-induced seizures during ethanol withdrawal in rats. *Alcohol Clin. Exp. Res.* 19, 350–355.
- Edgar, D.M., Seidel, W.F., Gee, K.W., Lan, N.C., Field, G., Xia, H., Hawkinson, J.E., Wieland, S., Carter, R.B., Wood, P.L., 1997. CCD-3693: an orally bioavailable analog of the endogenous neuroactive steroid, pregnanolone, demonstrates potent sedative hypnotic actions in the rat. *J. Pharmacol. Exp. Ther.* 282, 420–429.
- Errington, A.C., Cope, D.W., Crunelli, V., 2011. Augmentation of tonic GABA(A) inhibition in absence epilepsy: therapeutic value of inverse agonists at extrasynaptic GABA(A) receptors. *Adv. Pharmacol. Sci.* 2011, 790590.
- Farrant, M., Nusser, Z., 2005. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat. Rev. Neurosci.* 6, 215–229.
- Fritschy, J.-M., Panzanelli, P., 2014. GABA_A receptors and plasticity of inhibitory neurotransmission in the central nervous system. *Eur. J. Neurosci.* 39, 1845–1865.
- Gasior, M., Carter, R.B., Witkin, J.M., 1999. Neuroactive steroids: potential therapeutic use in neurological and psychiatric disorders. *Trends Pharmacol. Sci.* 20, 107–112.
- Gee, K.W., Bolger, M.B., Brinton, R.E., Coirini, H., McEwen, B.S., 1988. Steroid

- modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. *J. Pharmacol. Exp. Ther.* 246, 803–812.
- Gee, K.W., McCauley, L.D., Lan, N.C., 1995. A putative receptor for neurosteroids on the GABA_A receptor complex: the pharmacological properties and therapeutic potential of epalons. *Crit. Rev. Neurobiol.* 9, 207–227.
- Gee, K.W., Tran, M.B., Hogenkamp, D.J., Johnstone, T.B., Bagnera, R.E., Yoshimura, R.F., Huang, J.-C., Belluzzi, J.D., Whittemore, E.R., 2010. Limiting activity at beta1-subunit-containing GABA_A receptor subtypes reduces ataxia. *J. Pharmacol. Exp. Ther.* 332, 1040–1053.
- Gilbert Evans, S.E., Ross, L.E., Sellers, E.M., Purdy, R.H., Romach, M.K., 2005. 3alpha-reduced neuroactive steroids and their precursors during pregnancy and the postpartum period. *Gynecol. Endocrinol.* 21, 268–279.
- Grognet, A., Hertz, F., DeFeudis, F.V., 1983. Comparison of the analgesic actions of THIP and morphine. *Gen. Pharmacol.* 14, 585–589.
- Guintivano, J., Manuck, T., Meltzer-Brody, S., 2018. Predictors of Postpartum Depression: a comprehensive review of the last decade of evidence. *Clin. Obstet. Gynecol.* 61, 591–603.
- Gunduz-Bruce, H., Silber, C., Kaul, I., Rothschild, A.J., Riesenberger, R., Sankoh, A.J., Li, H., Lasser, R., Zorumski, C.F., Rubinow, D.R., Paul, S.M., Jonas, J., Doherty, J.J., Kanes, S.J., 2019. Trial of SAGE-217 in patients with major depressive disorder. *N. Engl. J. Med.* 381, 903–911.
- Gunn, B.G., Cunningham, L., Cooper, M.A., Corteen, N.L., Seif, M., Swinny, J.D., Lambert, J.J., Belelli, D., 2013. Dysfunctional astrocytic and synaptic regulation of hypothalamic glutamatergic transmission in a mouse model of early-life adversity: relevance to neurosteroids and the programming of the stress response. *J. Neurosci.* 33 (50), 19534–19554.
- Gunn, B.G., Cunningham, L., Mitchell, S.G., Swinny, J.D., Lambert, J.J., Belelli, D., 2015. GABA_A receptor-acting neurosteroids: a role in the development and regulation of the stress response. *Front. Neuroendocrinol.* 36, 28–48.
- Gyermek, L., Iriarte, J., Crabbe, P., 1968. Steroids. CCCX. Structure-activity relationship of some steroidal hypnotic agents. *J. Med. Chem.* 11, 117–125.
- Harrison, N.L., Simmonds, M.A., 1984. Modulation of the GABA receptor complex by a steroid anaesthetic. *Brain Res.* 323 (2), 287–292.
- Hellgren, C., Akerud, H., Skalkidou, A., Backstrom, T., Sundstrom-Poromaa, I., 2014. Low serum allopregnanolone is associated with symptoms of depression in late pregnancy. *Neuropsychobiology* 69, 147–153.
- Hellgren, C., Comasco, E., Skalkidou, A., Sundstrom-Poromaa, I., 2017. Allopregnanolone levels and depressive symptoms during pregnancy in relation to single nucleotide polymorphisms in the allopregnanolone synthesis pathway. *Horm. Behav.* 94, 106–113.
- Herd, M.B., Brown, A.R., Lambert, J.J., Belelli, D., 2013. Extrasynaptic GABA(A) receptors couple presynaptic activity to postsynaptic inhibition in the somatosensory thalamus. *J. Neurosci.* 33, 14850–14868.
- Herd, M.B., Foister, N., Chandra, D., Peden, D.R., Homanics, G.E., Brown, V.J., Balfour, D.J.K., Lambert, J.J., Belelli, D., 2009. Inhibition of thalamic excitability by 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridine-3-ol: a selective role for delta-GABA(A) receptors. *Eur. J. Neurosci.* 29, 1177–1187.
- Herd, M.B., Haythornthwaite, A.R., Rosahl, T.W., Wafford, K.A., Homanics, G.E., Lambert, J.J., Belelli, D., 2008. The expression of GABA_A beta subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. *J. Physiol.* 586, 989–1004.
- Herd, M.B., Lambert, J.J., Belelli, D., 2014. The general anaesthetic etomidate inhibits the excitability of mouse thalamocortical relay neurons by modulating multiple modes of GABA_A receptor-mediated inhibition. *Eur. J. Neurosci.* 40, 2487–2501.
- Hill-Venning, C., Peters, J.A., Callachan, H., Lambert, J.J., Gemmill, D.K., Anderson, A., Byford, A., Hamilton, N., Hill, D.R., Marshall, R.J., Campbell, A.C., 1996. The anaesthetic action and modulation of GABA_A receptor activity by the novel water-soluble aminosteroid Org 20599. *Neuropharmacology* 35, 1209–1222.
- Hoestgaard-Jensen, K., Dalby, N.O., Wolinsky, T.D., Murphey, C., Jones, K.A., Rottlander, M., Frederiksen, K., Watson, W.P., Jensen, K., Ebert, B., 2010. Pharmacological characterization of a novel positive modulator at alpha 4 beta 3 delta-containing extrasynaptic GABA(A) receptors. *Neuropharmacology* 58, 702–711.
- Hogenkamp, D.J., Johnstone, T.B.C., Huang, J.-C., Li, W.-Y., Tran, M., Whittemore, E.R., Bagnera, R.E., Gee, K.W., 2007. Enaminone amides as novel orally active GABA_A receptor modulators. *J. Med. Chem.* 50, 3369–3379.
- Hogenkamp, D.J., Tran, M.B., Yoshimura, R.F., Johnstone, T.B., Kanner, R., Gee, K.W., 2014. Pharmacological profile of a 17beta-heteroaryl-substituted neuroactive steroid. *Psychopharmacology (Berl.)* 231, 3517–3524.
- Hosie, A.M., Wilkins, M.E., da Silva, H.M.A., Smart, T.G., 2006. Endogenous neurosteroids regulate GABA_A receptors through two discrete transmembrane sites. *Nature* 444, 486–489.
- Irwin, R.W., Solinsky, C.M., Brinton, R.D., 2014. Frontiers in therapeutic development of allopregnanolone for Alzheimer's disease and other neurological disorders. *Front. Cell. Neurosci.* 8, 203.
- Irwin, R.W., Solinsky, C.M., Loya, C.M., Salituro, F.G., Rodgers, K.E., Bauer, G., Rogawski, M.A., Brinton, R.D., 2015. Allopregnanolone preclinical acute pharmacokinetic and pharmacodynamic studies to predict tolerability and efficacy for Alzheimer's Disease. *PLoS One* 10, e0128313.
- Jacob, T.C., Moss, S.J., Jurd, R., 2008. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat. Rev. Neurosci.* 9, 331–343.
- Jensen, M.L., Wafford, K.A., Brown, A.R., Belelli, D., Lambert, J.J., Mirza, N.R., 2013. A study of subunit selectivity, mechanism and site of action of the delta selective compound 2 (DS2) at human recombinant and rodent native GABA(A) receptors. *Br. J. Pharmacol.* 168, 1118–1132.
- Jia, F., Pignataro, L., Harrison, N.L., 2007. GABA_A receptors in the thalamus: alpha4 subunit expression and alcohol sensitivity. *Alcohol* 41, 177–185.
- Johnson, R.A., Simmons, K.T., Fast, J.P., Schroeder, C.A., Pearce, R.A., Albrecht, R.M., Mecozzi, S., 2011. Histamine release associated with intravenous delivery of fluorocarbon-based sevoflurane emulsion in canines. *J. Pharm. Sci.* 100, 2685–2692.
- Johansson, I.M., Birzniece, V., Lindblad, C., Olsson, T., Backstrom, T., 2002. Allopregnanolone inhibits learning in the Morris water maze. *Brain Res.* 934, 125–131.
- Johnstone, T.B.C., McCarren, H.S., Spampinato, J., Dudek, F.E., McDonough, J.H., Hogenkamp, D., Gee, K.W., 2019a. Enaminone modulators of extrasynaptic alpha4-beta3delta gamma-aminobutyric acidA receptors reverse electrographic status epilepticus in the rat after acute organophosphorus poisoning. *Front. Pharmacol.* 10, 560.
- Johnstone, T.B.C., Xie, J.Y., Qu, C., Wasiak, D.J., Hogenkamp, D.J., Porreca, F., Gee, K.W., 2019b. Positive allosteric modulators of nonbenzodiazepine gamma-aminobutyric acidA receptor subtypes for the treatment of chronic pain. *Pain* 160, 198–209.
- Kaminski, R.M., Livingood, M.R., Rogawski, M.A., 2004. Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. *Epilepsia* 45, 864–867.
- Kavaliers, M., Wiebe, J.P., 1987. Analgesic effects of the progesterone metabolite, 3 alpha-hydroxy-5 alpha-pregnan-20-one, and possible modes of action in mice. *Brain Res.* 415, 393–398.
- Kharasch, E.D., Hollmann, M.W., 2015. Steroid anesthesia revisited: Again. *Anesth. Analg.* 120, 983–984.
- Kjaer, M., Nielsen, H., 1983. The analgesic effect of the GABA-agonist THIP in patients with chronic pain of malignant origin. A phase-1-2 study. *Br. J. Clin. Pharmacol.* 16, 477–485.
- Kokate, T.G., Cohen, A.L., Karp, E., Rogawski, M.A., 1996. Neuroactive steroids protect against picrocarpine- and kainic acid-induced limbic seizures and status epilepticus in mice. *Neuropharmacology* 35, 1049–1056.
- Kokate, T.G., Svensson, B.E., Rogawski, M.A., 1994. Anticonvulsant activity of neurosteroids: correlation with gamma-aminobutyric acid-evoked chloride current potentiation. *J. Pharmacol. Exp. Ther.* 270, 1223–1229.
- Krause, A.J., Simon, E.B., Mander, B.A., Greer, S.M., Saletin, J.M., Goldstein-Piekarski, A.N., Walker, M.P., 2017. The sleep-deprived human brain. *Nat. Rev. Neurosci.* 18 (7), 404–418.
- Licheri, V., Talani, G., Gorule, A.A., Mostallino, M.C., Biggio, G., Sanna, E., 2015. Plasticity of GABA_A receptors during pregnancy and postpartum period: from gene to function. *Neural Plast.* 2015, 11 170435.
- Lindeburg, T., Folsgard, S., Sillesen, H., Jacobsen, E., Kehlet, H., 1983. Analgesic, respiratory and endocrine responses in normal man to THIP, a GABA-agonist. *Acta Anaesthesiol. Scand.* 27, 10–12.
- Locci, A., Pinna, G., 2017. Neurosteroid biosynthesis down-regulation and changes in GABA_A receptor subunit composition: a biomarker axis in stress-induced cognitive and emotional impairment. *Br. J. Pharmacol.* 174, 3226–3241.
- Lodge, D., Anis, N.A., 1984. Effects of ketamine and three other anaesthetics on spinal reflexes and inhibitions in the cat. *Br. J. Anaesth.* 56, 1143–1151.
- Luisi, S., Petraglia, F., Benedetto, C., Nappi, R.E., Bernardi, F., Fadalti, M., Reis, F.M., Luisi, M., Genazzani, A.R., 2000. Serum allopregnanolone levels in pregnant women: changes during pregnancy, at delivery, and in hypertensive patients. *J. Clin. Endocrinol. Metab.* 85, 2429–2433.
- Luntz-Leybman, V., Freund, R.K., Collins, A.C., 1990. 5 alpha-pregnan-3 alpha-ol-20-one blocks nicotine-induced seizures and enhances paired-pulse inhibition. *Eur. J. Pharmacol.* 185, 239–242.
- Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat. Rev. Neurosci.* 10, 434–445.
- Luscher, B., Mohler, H., 2019. Brexanolone, a neurosteroid antidepressant, vindicates the GABAergic deficit hypothesis of depression and may foster resilience. *Fl1000 Research* 8.
- Lyden, P., Shin, C., Jackson-Friedman, C., Hassid, S., Chong, A., Macdonald, R.L., 2000. Effect of ganaxolone in a rodent model of cerebral hematoma. *Stroke* 31, 169–175 1.
- Macdonald, R.L., Kang, J.-Q., Gallagher, M.J., 2010. Mutations in GABA_A receptor subunits associated with genetic epilepsies. *J. Physiol.* 588, 1861–1869.
- Maguire, J., Mody, I., 2008. GABA(A)R plasticity during pregnancy: relevance to postpartum depression. *Neuron* 59, 207–213.
- Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 232 (4753), 1004–1007.
- Martinez Botella, G., Salituro, F.G., Harrison, B.L., Beresis, R.T., Bai, Z., Blanco, M.-J., Belfort, G.M., Dai, J., Loya, C.M., Ackley, M.A., Althaus, A.L., Grossman, S.J., Hoffmann, E., Doherty, J.J., Robichaud, A.J., 2017. Neuroactive Steroids. 2. 3alpha-Hydroxy-3beta-methyl-21-(4-cyano-1H-pyrazol-1-yl)-19-nor-5beta-pregnan-20-one (SAGE-217): a clinical next generation neuroactive steroid positive allosteric modulator of the gamma-aminobutyric acidA receptor. *J. Med. Chem.* 60, 7810–7819.
- Marx, C.E., Trost, W.T., Shampine, L.J., Stevens, R.D., Hulette, C.M., Steffens, D.C., Ervin, J.F., Butterfield, M.L., Blazer, D.G., Massing, M.W., Lieberman, J.A., 2006. The neurosteroid allopregnanolone is reduced in prefrontal cortex in Alzheimer's disease. *Biol. Psychol.* 60, 1287–1294.
- McCormick, D.A., 1992. Neurotransmitter actions in the thalamus and cerebral cortex. *J. Clin. Neurophysiol.* 9, 212–223.
- McNeil, R.G., Gee, K.W., Bolger, M.B., Lan, N.C., Wieland, S., Belelli, D., Purdy, R.H., Paul, S.M., 1992. Neuroactive steroids that act at GABA_A receptors: therapeutic implications. *Drug News Perspect.* 5, 145–152.
- Melcangi, R.C., Santi, D., Spezzano, R., Grimoldi, M., Tabacchi, T., Fusco, M.L., Diviccaro, S., Giatti, S., Carra, G., Caruso, D., Simoni, M., Cavaletti, G., 2017. Neuroactive steroid levels and psychiatric and andrological features in post-finasteride patients. *J. Steroid Biochem. Mol. Biol.* 171, 229–235.

- Meltzer-Brody, S., Colquhoun, H., Riesenberg, R., Epperson, C.N., Deligiannidis, K.M., Rubinow, D.R., Li, H., Sankoh, A.J., Clemson, C., Schacterle, A., Jonas, J., Kanes, S., 2018. Brexanolone injection in post-partum depression: two multicentre, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet (London, England)* 392, 1058–1070.
- Merryman, W., Boiman, R., Barnes, L., Rothchild, I., 1954. Progesterone anesthesia in human subjects. *J. Clin. Endocrinol. Metab.* 14, 1567–1569.
- Mihalek, R.M., Banerjee, P.K., Korpi, E.R., Quinlan, J.J., Firestone, L.L., Mi, Z.P., Lagenaur, C., Tretter, V., Sieghart, W., Anagnostaras, S.G., Sage, J.R., Fanselow, M.S., Guidotti, A., Spigelman, I., Li, Z., DeLorey, T.M., Olsen, R.W., Homanics, G.E., 1999. Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 96, 12905–12910.
- Modgil, A., Parakala, M.L., Ackley, M.A., Doherty, J.J., Moss, S.J., Davies, P.A., 2017. Endogenous and synthetic neuroactive steroids evoke sustained increases in the efficacy of GABAergic inhibition via a protein kinase C-dependent mechanism. *Neuropharmacology* 113, 314–322.
- Munari, C., Casaroli, D., Matteuzzi, G., Pacifico, L., 1979. The use of Althesin in drug-resistant status epilepticus. *Epilepsia* 20, 475–483.
- Nakamura, Y., Darnieder, L.M., Deeb, T.Z., Moss, S.J., 2015. Regulation of GABAARs by phosphorylation. *Adv. Pharmacol.* 72, 97–146.
- Neumann, S., Boothman-Burrell, L., Gowing, E.K., Jacobsen, T.A., Ahring, P.K., Young, S.L., Sandager-Nielsen, K., Clarkson, A.N., 2019. The delta-subunit selective GABA receptor modulator, DS2, improves stroke recovery via an anti-inflammatory mechanism. *Front. Neurosci.* 13, 1–13 10.3389.
- Newman, E.L., Gunner, G., Huynh, P., Gachette, D., Moss, S.J., Smart, T.G., Rudolph, U., DeBold, J.F., Miczek, K.A., 2016. Effects of Gabra2 point mutations on alcohol intake: increased binge-like and blunted chronic drinking by mice. *Alcohol Clin. Exp. Res.* 40, 2445–2455.
- Oliveras, J.L., Montagne-Clavel, J., 1994. The GABA receptor antagonist picrotoxin induces a 'pain-like' behavior when administered into the thalamic reticular nucleus of the behaving rat: a possible model for 'central' pain? *Neurosci. Lett.* 179, 21–24.
- Olsen, R.W., Sieghart, W., 2008. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. *Update. Pharmacol. Rev.* 60, 243–260.
- Olsen, R.W., Sieghart, W., 2009. GABA A receptors: subtypes provide diversity of function and pharmacology. *Neuropharmacology* 56, 141–148.
- Osborne, L.M., Gispen, F., Sanyal, A., Yenokyan, G., Meilman, S., Payne, J.L., 2017. Lower allopregnanolone during pregnancy predicts postpartum depression: an exploratory study. *Psychoneuroendocrinology* 79, 116–121.
- Pang, Y., Dong, J., Thomas, P., 2013. Characterization, neurosteroid binding and brain distribution of human membrane progesterone receptors delta and {epsilon} (mPRdelta and mPR{epsilon}) and mPRdelta involvement in neurosteroid inhibition of apoptosis. *Endocrinology* 154, 283–295.
- Paoletti, A.M., Romagnino, S., Contu, R., Orru, M.M., Marotto, M.F., Zedda, P., Lello, S., Biggio, G., Concas, A., Melis, G.B., 2006. Observational study on the stability of the psychological status during normal pregnancy and increased blood levels of neuroactive steroids with GABA-A receptor agonist activity. *Psychoneuroendocrinology* 31, 485–492.
- Parakala, M.L., Zhang, Y., Modgil, A., Chadchankar, J., Vien, T.N., Ackley, M.A., Doherty, J.J., Davies, P.A., Moss, S.J., 2019. Metabotropic, but not allosteric, effects of neurosteroids on GABAergic inhibition depend on the phosphorylation of GABA receptors. *J. Biol. Chem.* 294, 12220–12230.
- Patchev, V.K., Shoaib, M., Holsboer, F., Almeida, O.F., 1994. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. *Neuroscience* 62, 265–271.
- Peden, D.R., Petitjean, C.M., Herd, M.B., Durakoglugil, M.S., Rosahl, T.W., Wafford, K., Homanics, G.E., Belevi, D., Fritschy, J.-M., Lambert, J.J., 2008. Developmental maturation of synaptic and extrasynaptic GABA receptors in mouse thalamic ventrobasal neurons. *J. Physiol.* 586, 965–987.
- Peters, J.A., Kirkness, E.F., Callachan, H., Lambert, J.J., Turner, A.J., 1988. Modulation of the GABA receptor by depressant barbiturates and pregnane steroids. *Br. J. Pharmacol.* 94, 1257–1269.
- Purdy, R.H., Morrow, A.L., Moore, P.H.J., Paul, S.M., 1991. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 88, 4553–4557.
- Ramsay, M.A., Savege, T.M., Simpson, B.R., Goodwin, R., 1974. Controlled sedation with alphaxalone-alphadolone. *Br. Med. J.* 2, 656–659.
- Ratner, M.H., Kumaresan, V., Farb, D.H., 2019. Neurosteroid actions in memory and neurologic/neuropsychiatric disorders. *Front. Endocrinol. (Lausanne)* 10, 169.
- Reddy, D.S., Estes, W.A., 2016. Clinical potential of neurosteroids for CNS disorders. *Trends Pharmacol. Sci.* 37, 543–561.
- Reddy, D.S., Rogawski, M.A., 2012. In: Noebels, J.L., Avoli, M., Rogawski, M.A., Olsen, R.W., Delgado, E. (Eds.), *Neurosteroids - Endogenous Regulators of Seizure Susceptibility and Role in the Treatment of Epilepsy*.
- Reddy, D.S., Yoshimura, R.F., Ramanathan, G., Carver, C., Johnstone, T.B., Hogenkamp, D.J., Gee, K.W., 2018. Role of beta2/3-specific GABA-A receptor isoforms in the development of hippocampus kindling epileptogenesis. *Epilepsy Behav.* 82, 57–63.
- Rey, M., Coirini, H., 2015. Synthetic neurosteroids on brain protection. *Neural Regen. Res.* 10, 17–21.
- Reyes-Vazquez, C., Dafny, N., 1983. Microiontophoretically applied THIP effects upon nociceptive responses of neurons in medial thalamus. *Appl. Neurophysiol.* 46, 254–260.
- Reynolds, D.S., Rosahl, T.W., Cirone, J., O'Meara, G.F., Haythornthwaite, A., Newman, R.J., Myers, J., Sur, C., Howell, O., Rutter, A.R., Atack, J., Macaulay, A.J., Hadingham, K.L., Hutson, P.H., Belevi, D., Lambert, J.J., Dawson, G.R., McKernan, R., Whiting, P.J., Wafford, K.A., 2003. Sedation and anesthesia mediated by distinct GABA(A) receptor isoforms. *J. Neurosci.* 23, 8608–8617.
- Romeo, E., Strohle, A., Spalletta, G., di Michele, F., Hermann, B., Holsboer, F., Pasini, A., Rupprecht, R., 1998. Effects of antidepressant treatment on neuroactive steroids in major depression. *Am. J. Psychiatry* 155, 910–913.
- Rosenthal, E.S., Claassen, J., Wainwright, M.S., Husain, A.M., Vaitkevicius, H., Raines, S., Hoffmann, E., Colquhoun, H., Doherty, J.J., Kanes, S.J., 2017. Brexanolone as adjunctive therapy in super-refractory status epilepticus. *Ann. Neurol.* 82, 342–352.
- Rudolph, U., Knoflach, F., 2011. Beyond classical benzodiazepines: novel therapeutic potential of GABA receptor subtypes. *Nat. Rev. Drug Discov.* 10, 685–697.
- Scholfield, C.N., 1980. Potentiation of inhibition by general anaesthetics in neurones of the olfactory cortex in vitro. *Pflügers Arch. Eur. J. Physiol.* 383 (3), 249–255.
- Schule, C., Eser, D., Baghai, T.C., Nothdurfter, C., Kessler, J.S., Rupprecht, R., 2011. Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? *Neuroscience* 191, 55–77.
- Schule, C., Baghai, T.C., di Michele, F., Eser, D., Pasini, A., Schwarz, M., Rupprecht, R., Romeo, E., 2007. Effects of combination treatment with mood stabilizers and mirtazapine on plasma concentrations of neuroactive steroids in depressed patients. *Psychoneuroendocrinology* 32, 669–680.
- Schumacher, M., Mattern, C., Ghomari, i A., Oudinet, J.P., Liere, P., Labombarda, F., Sitruk-Ware, R., De Nicola, A.F., Guennoun, R., 2014. Revisiting the roles of progesterone and allopregnanolone in the nervous system: resurgence of the progesterone receptors. *Prog. Neurobiol.* 113, 6–39.
- Selye, H., 1941. Anesthetic effect of steroid hormones. *Proc. Soc. Exp. Biol. Med.* 46, 116–121.
- Sharp, S., Mitchell, S.J., Vallee, M., Kuzmanova, E., Cooper, M., Belevi, D., Lambert, J.J., Huang, J.T., 2018. Isotope dilution-based targeted and nontargeted carbonyl neurosteroid/steroid profiling. *Anal. Chem.* 90 (8), 5247–5255.
- Skolnick, B.E., Maas, A.L., Narayan, R.K., van der Hoop, R.G., MacAllister, T., Ward, J.D., Nelson, N.R., Stocchetti, N., 2014. A clinical trial of progesterone for severe traumatic brain injury. *N. Engl. J. Med.* 371, 2467–2476.
- Sneyd, J.R., Wright, P.M., Harris, D., Taylor, P.A., Vijn, P.C., Cross, M., Dale, H., Voortman, G., Boen, P., 1997. Computer controlled infusion of ORG 21465, a water soluble steroid i.v. anaesthetic agent, into human volunteers. *Br. J. Anaesth.* 79 (4), 433–439.
- Sperling, M.R., Klein, P., Tsai, J., 2017. Randomized, double-blind, placebo-controlled phase 2 study of ganaxolone as add-on therapy in adults with uncontrolled partial-onset seizures. *Epilepsia* 58, 558–564.
- Stell, B.M., Brickley, S.G., Tang, C.Y., Farrant, M., Mody, I., 2003. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABA receptors. *Proc. Natl. Acad. Sci. U.S.A.* 100, 14439–14444.
- Steriade, M., Deschenes, M., 1984. The thalamus as a neuronal oscillator. *Brain Res.* 320, 1–63.
- Stewart, G.O., Dobb, G.J., Craib, I.A., 1983. Clinical trial of continuous infusion of alphaxalone/alphadolone in intensive care patients. *Anaesth. Intensive Care* 11, 107–112.
- Svensson, E., Persson, J., FitzSimmonds, B., Yaksh, T.L., 2013. Intrathecal neurosteroids and a neurosteroid antagonist: effects on inflammation-evoked thermal hyperalgesia and tactile allodynia. *Neurosci. Lett.* 548, 27–32.
- Tomaselli, G., Vallee, M., 2019. Stress and drug-abuse-related disorders: the promising therapeutic value of neurosteroids focus on pregnenolone-progesterone-allopregnanolone pathway. *Neuroendocrinology* (in press).
- Towler, C.M., Garrett, R.T., Sear, J.W., 1982. Althesin infusion for maintenance of anaesthesia. *Anaesthesia* 37, 428–439.
- Turkmen, S., Backstrom, T., Wahlstrom, G., Andreen, L., Johansson, I.-M., 2011. Tolerance to allopregnanolone with focus on the GABA-A receptor. *Br. J. Pharmacol.* 162, 311–327.
- Uzunova, V., Sheline, Y., Davis, J.M., Rasmusson, A., Uzunov, D.P., Costa, E., Guidotti, A., 1998. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Proc. Natl. Acad. Sci. U.S.A.* 95, 3239–3244.
- Vaitkevicius, H., Husain, A.M., Rosenthal, E.S., Rosand, J., Bobb, W., Reddy, K., Rogawski, M.A., Cole, A.J., 2017. First-in-man allopregnanolone use in super-refractory status epilepticus. *Ann. Clin. Transl. Neurol.* 4, 411–414.
- Vanover, K.E., Hogenkamp, D.J., Lan, N.C., Gee, K.W., Carter, R.B., 2001. Behavioral characterization of Co 134444 (3alpha-hydroxy-21-(1'-imidazolyl)-3beta-methoxymethyl-5alpha-pregnan-20-one), a novel sedative-hypnotic neuroactive steroid. *Psychopharmacology* 155, 285–291.
- Vardya, I., Hoestgaard-Jensen, K., Nieto-Gonzalez, J.L., Dosa, Z., Boddum, K., Holm, M.M., Wolinsky, T.D., Jones, K.A., Dalby, N.O., Ebert, B., Jensen, K., 2012. Positive modulation of delta-subunit containing GABA(A) receptors in mouse neurons. *Neuropharmacology* 63, 469–479.
- Wafford, K.A., van Niel, M.B., Ma, Q.P., Horridge, E., Herd, M.B., Peden, D.R., Belevi, D., Lambert, J.J., 2009. Novel compounds selectively enhance delta subunit containing GABA A receptors and increase tonic currents in thalamus. *Neuropharmacology* 56, 182–189.
- Wafford, K.A., Ebert, B., 2008. Emerging anti-insomnia drugs: tackling sleeplessness and the quality of wake time. *Nat. Rev. Drug Discov.* 7, 530–540.
- Walf, A.A., Sumida, K., Frye, C.A., 2006. Inhibiting 5alpha-reductase in the amygdala attenuates anxiety and antidepressive behavior of naturally receptive and hormone-primed ovariectomized rats. *Psychopharmacology (Berl.)* 186, 302–311.
- Wang, J.M., Singh, C., Liu, L., Irwin, R.W., Chen, S., Chung, E.J., Thompson, R.F., Brinton, R.D., 2010. Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 107, 6498–6503.
- Weir, C.J., Mitchell, S.J., Lambert, J.J., 2017. Role of GABA receptor subtypes in the behavioural effects of intravenous general anaesthetics. *Br. J. Anaesth.* 119,

- i167–i175.
- Whissell, P.D., Lecker, I., Wang, D.-S., Yu, J., Orser, B.A., 2015. Altered expression of delta GABAA receptors in health and disease. *Neuropharmacology* 88, 24–35.
- Wlodarczyk, A.I., Sylantyev, S., Herd, M.B., Kersante, F., Lambert, J.J., Rusakov, D.A., Linthorst, A.C.E., Semyanov, A., Bellelli, D., Pavlov, I., Walker, M.C., 2013. GABA-independent GABAA receptor openings maintain tonic currents. *J. Neurosci.* 9, 3905–3914.
- Wright, D.W., Yeatts, S.D., Silbergleit, R., Palesch, Y.Y., Hertzberg, V.S., Frankel, M., Goldstein, F.C., Caveney, A.F., Howlett-Smith, H., Bengelink, E.M., Manley, G.T., Merck, L.H., Janis, L.S., Barsan, W.G., 2014. Very early administration of progesterone for acute traumatic brain injury. *N. Engl. J. Med.* 371, 2457–2466.
- Wu, Y.V., Burnham, W.M., 2018. Progesterone, 5 α -dihydroprogesterone and allopregnanolone's effects on seizures: a review of animal and clinical studies. *Seizure* 63, 26–36.
- Yoshimura, R.F., Tran, M.B., Hogenkamp, D.J., Johnstone, T.B., Xie, J.Y., Porreca, F., Gee, K.W., 2014. Limited central side effects of a beta-subunit subtype-selective GABAA receptor allosteric modulator. *J. Psychopharmacol.* 28, 472–478.