Published in final edited form as:

Psychopharmacology (Berl). 2014 September ; 231(17): 3537–3556. doi:10.1007/s00213-014-3643-x.

Pregnenolone sulfate as a modulator of synaptic plasticity

Conor C. Smith,

Laboratory of Molecular Neurobiology, Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, 72 East Concord St., Boston, MA 02118, USA

Terrell T. Gibbs, and

Laboratory of Molecular Neurobiology, Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, 72 East Concord St., Boston, MA 02118, USA

David H. Farb

Laboratory of Molecular Neurobiology, Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, 72 East Concord St., Boston, MA 02118, USA

David H. Farb: dfarb@bu.edu

Abstract

Rationale—The neurosteroid pregnenolone sulfate (PregS) acts as a cognitive enhancer and modulator of neurotransmission, yet aligning its pharmacological and physiological effects with reliable measurements of endogenous local concentrations and pharmacological and therapeutic targets has remained elusive for over 20 years.

Objectives—New basic and clinical research concerning neurosteroid modulation of the central nervous system (CNS) function has emerged over the past 5 years, including important data involving pregnenolone and various neurosteroid precursors of PregS that point to a need for a critical status update.

Results—Highly specific actions of PregS affecting excitatory *N*-methyl-D-aspartate receptor (NMDAR)-mediated synaptic transmission and the pharmacological effects of PregS on various receptors and ion channels are discussed. The discovery of a high potency (nanomolar) signal transduction pathway for PregS-induced NMDAR trafficking to the cell surface via a Ca^{2+} - and G protein-coupled receptor (GPCR)-dependent mechanism and a potent ($\text{EC}_{50} \sim 2 \text{ pM}$) direct enhancement of intracellular Ca^{2+} levels is discussed in terms of its agonist effects on long-term potentiation (LTP) and memory. Lastly, preclinical and clinical studies assessing the promnesic effects of PregS and pregnenolone toward cognitive dysfunction in schizophrenia, and altered serum levels in epilepsy and alcohol dependence, are reviewed.

Conclusions—PregS is present in human and rodent brain at physiologically relevant concentrations and meets most of the criteria for an endogenous neurotransmitter/neuromodulator. PregS likely plays a significant role in modulation of glutamatergic excitatory synaptic

transmission underlying learning and memory, yet the molecular target(s) for its action awaits identification.

Keywords

Neurosteroid; NMDA receptor; Schizophrenia; GABA receptor

Introduction

Recent studies provide additional evidence that pregnenolone sulfate (PregS) may function as an endogenous neurotransmitter or neuromodulator, creating renewed interest in the identification of novel neuroactive steroid targets for pharmacological intervention (Kostakis et al. 2013; Marx et al. 2009; Rustichelli et al. 2013), including upstream precursors that act at micromolar concentrations and may exert actions in vivo via downstream metabolites including PregS (Paul et al. 2013; Vallée et al. 2014). The goal of this review is to highlight recent significant progress on the function of neuroactive steroids in the central nervous system (CNS) and possible new directions in cognitive neuroscience research.

Thirty years have elapsed since the first suggestion that PregS is an endogenous neurosteroid (Corpéchet et al. 1983). The term neurosteroid was coined by Etienne Baulieu to describe steroids that are synthesized from cholesterol in the CNS independently of the endocrine system (Baulieu 1997; Baulieu et al. 2001). Steroids are capable of affecting a diverse range of cellular functions by binding to cytosolic and nuclear receptors that bind to DNA and induce changes in gene transcription (Kawata et al. 2008; Marques et al. 2009). However, neurosteroids are also capable of pharmacologically modulating neurotransmission by non-genomic mechanisms via specific interactions with neurotransmitter receptors (Gibbs et al. 2006; Schumacher et al. 2008). Subsequent studies identified neuromodulatory effects of PregS on N-methyl-D-aspartate (NMDA) (Wu et al. 1991; Malayev et al. 2002), γ -aminobutyric acid type A (GABA_A) (Majewska et al. 1988; Jo et al. 1989), and glycine receptors (Park-Chung et al. 1997) and acts as an agonist at calcium-permeable transient receptor potential (TRP) channels (Wagner et al. 2008) (all with micromolar (10^{-6} M) potencies). PregS also exhibits higher potencies in the nanomolar to picomolar range (10^{-9} to 10^{-12} M) for inducing dopamine overflow and release from rat nigrostriatal terminals both in vivo and ex vivo (Fig. 1) (Sadri-Vakili et al. 2008; Whittaker et al. 2008). Two highly potent actions of PregS involve the direct increase of intracellular Ca^{2+} levels and cAMP responsive element binding protein (CREB) activation via a synaptic but not extrasynaptic N-methyl-D-aspartate receptor (NMDAR)-dependent pathway at picomolar PregS ($\text{EC}_{50} \sim 2$ pM), coordinating the non-genomic with genomic actions of PregS (Smith 2014). PregS also stimulates the trafficking of functional NMDA receptors to cell surface at relatively low nanomolar concentrations, resulting in delayed onset potentiation of the response to NMDA (Kostakis et al. 2013), consistent with its overall role as a potentiator of excitatory synaptic activity.

This can be seen in the ex vivo hippocampal slice preparation where PregS increases long-term potentiation (LTP) (Fig. 2) and enhances spatial memory in rat behavioral tests (Flood et al. 1995; Sliwinski et al. 2004), and inhibition of steroid sulfatase ameliorates deficits in

spatial memory induced by septal–hippocampal lesions (Babalola et al. 2012). Conversion of the precursor pregnenolone (PREG) to PregS may in part underlie PREG efficacy in ameliorating cognitive deficits in schizophrenia (Marx et al. 2009).

PregS also modulates synaptic transmission by both presynaptic and postsynaptic mechanisms across multiple neurotransmitter systems (reviewed in Zheng 2009). There are now many lines of evidence, both clinical and preclinical, linking neuroactive steroids to CNS disorders such as fetal alcohol spectrum disorder (Zimmerberg et al. 1995), epilepsy (Budziszewska et al. 1998; Hill et al. 2010; Pieribone et al. 2007), anxiety (Crawley et al.; Ströhle et al. 2002), depression, and schizophrenia (Uzunov et al. 1996; Wolkowitz et al. 1999; Khisti et al. 2000; Marx et al. 2006; Girdler et al. 2012; Wong et al. 2012; Zorumski et al. 2013). Multiple potential targets for pharmacologic intervention by neuroactive steroids have been reviewed previously as well (Valenzuela et al. 2008; Zheng 2009; Reddy 2010; Marx et al. 2011).

Recent preclinical research highlighted below provides new insight into neuroactive steroid modulation of synaptic function. Additionally, clinical studies discussed in this review indicate the therapeutic potential for neuroactive steroids in schizophrenia and cognitive dysfunction. In light of these new findings, we discuss whether PregS is a putative neurotransmitter or neuromodulator by asking whether it fulfills the criteria for neurotransmitter action, including presence in the CNS at pharmacologically relevant concentrations, an elicited neurophysiological response, pharmacological antagonism of the induced physiological response, and a mechanism for inactivation.

Evidence for meeting the criteria for neurotransmitter action

Synthesis of PregS in vivo

The rate-limiting step for neurosteroid production is the transport of cholesterol from the cytoplasm to the mitochondrial matrix (Stocco 2001) by the steroid acute regulatory (StAR) protein (Clark et al. 1994). Following cholesterol translocation, the enzymatic synthesis of PregS occurs in two enzymatic steps, first by cleavage of the C20–22 bond by cytochrome P450 side-chain cleavage (P450_{scc}) enzyme to yield PREG. PREG is lipid soluble and may remain in a subcellular fraction or aggregate in membranes following its conversion from cholesterol in the mitochondrial matrix.

In the second step, PregS is produced from PREG by sulfation of the C3 hydroxyl group by sulfotransferase (SULT) enzymes (Mellon et al. 2001). Messenger RNA (mRNA) for StAR (Furukawa et al. 1998) and P450_{scc} (Mellon and Deschepper 1993) are present and co-localize in rat brain (Furukawa et al. 1998). RNA for P450_{scc} was detected in postmortem human brain samples at equal levels in temporal lobe cortex, subcortical white matter, and hippocampus and at higher levels in women than men (Watzka et al. 1999). The human SULT gene family is divided into three groups according to sequence homology and substrate specificity (Falany et al. 2006). The SULT2B1 isoforms are stereoselective for β -hydroxysteroids (Meloche and Falany 2001), and mRNA for the SULT2B1a isoform is present in rat brain. PREG is a substrate for SULT2B1a (Kohjitani et al. 2006); and sulfotransferase protein (Fig. 3), StAR, and P450_{scc} were localized to rat CA1 pyramidal

neurons by immunohisto-chemistry (Kimoto et al. 2001). SULT2B isoform mRNA, protein, or activity was not detected in surgically resected human temporal lobe (Steckelbroeck et al. 2004). However, this analysis was limited to the temporal lobe, and it is known that SULT2B activity is lost upon cellular extraction (Falany et al. 2006). The SULT4A1 isoform is highly expressed in human and rat brain tissue and localizes to neurons (Liyou et al. 2003); however, the physiological substrate for SULT4 is unknown. Finally, an oligodendrocyte cell line derived from a human glioma expresses mRNA and protein peripheral benzodiazepine receptor (PBR) (a transporter protein that interacts with StAR) and P450scc and is capable of synthesizing PregS de novo (Brown et al. 2000).

In summary, evidence supports the presence of the enzymes necessary to synthesize PregS in rodent and human brain, but functional metabolic evidence demonstrating that SULT produces PregS from PREG in human brain and that this PregS pool may be used as a neurotransmitter remains to be determined.

Presence of PregS and PREG in nervous tissue

As the immediate precursor to PregS, PREG is present in the CNS. Bulk concentrations of PREG are consistent between human and rat brains. Utilizing liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), Caruso et al. (2013) determined PREG in male rat cerebral spinal fluid, plasma, hippocampus, and cortex at concentrations of 3, 1, 20, and 17 ng/g, respectively. Porcu et al. (2009), using GC-MS, found PREG plasma levels of 0.3 ng/mL in rats and 0.8 ng/mL in humans. Using the same method, Marx et al. (2006) found hippocampal PREG levels of 3 ng/g in rats, lower than those found by Caruso et al. (2013). A determination of whole rat brain by Ebner et al. (2006) found PREG at a concentration of 1.7 ng/g. In a study by Marx et al. (2006) comparing the prefrontal cortex of cognitively intact (human) subjects vs. subjects with Alzheimer's disease, PREG concentrations (in ng/g) were 8.6 and 21, respectively. Levels of PREG are similarly elevated in temporal cortex of cognitively intact subjects vs. subjects with Alzheimer's disease (10.2 and 22.2 ng/g, respectively) (Naylor et al. 2010b).

Reliable estimates of PregS bulk tissue levels in CNS have been methodologically difficult. Early PregS measurements were inflated by contamination by a lipoidal PREG derivative that co-purified with PregS (Liere et al. 2004, 2009). Revised methods designed to avoid this problem have generally yielded lower bulk concentrations of PregS in brain tissue (reviewed by Gibbs et al. 2006). Recent determinations of PregS concentration in bulk brain tissue are summarized in Table 1.

Using an enzyme-linked immunosorbent assay (ELISA) for PregS without solvolysis, Higashi et al. (2003) detected PregS in rat whole brain homogenate in eight of ten rats above the detection limit of 0.03 ng/g tissue (corresponding to a bulk concentration of 85 pM). Liere et al. (2004), using a modified solvolysis assay to eliminate contaminating lipoidal PREG derivatives, detected PregS above the limit of detection of 0.15 ng/g tissue (378 pM) in two of five samples of anterior rat brain. Moreover, two postmortem human brain tissue samples contained PregS at 1.85 ng/g tissue (4.7 nM) in cerebellum and 1.11 ng/g tissue (2.8 nM) in frontal cortex, respectively (Liere et al. 2004). Two other reports failed to detect PregS in rat brain either by nanoscale liquid chromatography-electrospray mass

spectrometry with a detection limit of 0.3 ng/g tissue (757 pM) (Liu et al. 2003) or by anion exchange chromatography and gas chromatography mass spectrometry (GC-MS) with a detection limit of 0.05 ng/g tissue (126 pM) (Ebner et al. 2006).

More recently, Rustichelli et al. (2013), utilizing an improved liquid chromatography method coupled with electrospray tandem mass spectroscopy with removal of interfering phospholipids, obviating the need to make derivatives or hydrolysis of PregS, and with deuterated internal standards, detected average concentrations of 4.45 ng/g tissue (11.5 nM) of PregS in rat cortex and 10.25 ng/g tissue (26.5 nM) in hippocampus. Thus, while there is conclusive evidence that PregS is present in rat CNS as well as human, the exact concentration remains to be established upon across species and brain regions (Schumacher et al. 2008). Improvements in PregS tissue extraction and more sensitive quantification methods may be required before a definitive answer regarding PregS concentration in bulk tissue, subcellular fractions, and amounts released in response to stimuli can be determined.

Stimulus-dependent release of PregS and sulfated neurosteroids

Neurosteroid levels could attain concentrations much in excess of bulk tissue levels if localized stimulus-dependent release occurs. Measurements of neurosteroid concentrations at the synaptic level have not been made, but several findings provide indirect evidence for stimulus-dependent neurosteroid release.

Consistent with a possible role for stimulus-dependent release of endogenous neurosteroids, blocking aromatase production of estradiol inhibits high frequency stimulation-induced LTP in rat vestibular brain slices (Grassi et al. 2009). Similarly, inhibition of P450scc blocks α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-dependent LTP in rat dentate gyrus (DG), which can be restored by the addition of 500 nM PREG, dehydroepiandrosterone (DHEA), or dehydroepiandrosterone sulfate (DHEAS) (Tanaka and Sokabe 2012). As P450scc catalyzes the conversion of cholesterol to PREG (the precursor to all neurosteroids including PregS, DHEA and thus DHEAS), these results suggest that endogenous production of DHEA, DHEAS, or PregS is required for DG synaptic LTP induction.

As a unique negatively charged steroid, PregS would be well-suited to intracellular compartmentation and vesicular or specific transporter-mediated release. The existence of PregS-specific efflux transporters in steroidogenic cells (Fang et al. 2010) provides supporting evidence for on-demand, localized PregS release. Notably, PregS (25 μ M) increases excitatory postsynaptic potential (EPSP) frequency in rat CA1 pyramidal cells in early postnatal (<5 days) hippocampal slices via an NMDAR-dependent mechanism. Moreover, postsynaptic depolarization initiates similar increases in EPSP frequency, which could be eliminated using a PregS-specific antibody to scavenge extracellular PregS, consistent with a role of PregS as a retrograde messenger (Mameli et al. 2005).

PregS as a structurally specific and selective agonist at 10^{-12} to 10^{-9} M

Determining what the physiologically relevant concentration is for a neurotransmitter is a daunting challenge. Local concentrations will almost certainly be much higher than average bulk concentrations as a result of local synthesis and accumulation of transmitters, e.g., in

synaptic vesicles of PregS, a number of reports have identified PregS effects near or below reported bulk tissue levels. PregS enhances binding of ifenprodil (an NMDAR antagonist) to rat cortical membranes with a maximal modulatory effect at 13 nM (Johansson et al. 2005). PregS inhibits GABA spontaneous inhibitory postsynaptic currents (sIPSCs) with an IC_{50} of 26 nM (Mtchedlishvili and Kapur 2003). PregS increases NR1 trafficking to the cell surface in *Xenopus laevis* oocytes expressing NR1/NR2A via a G protein-coupled pathway dependent on intracellular Ca^{2+} and phospholipase C (Fig. 4, summarized in Fig. 5) (Kostakis et al. 2013). In hippocampal slices, 300 nM PregS enhances LTP (Sliwinski et al. 2004). PregS at concentrations as low as 25 pM stimulates [3H]DA release from striatal synaptosomes via an NMDAR-dependent pathway (Whittaker et al. 2008), while 10 nM PregS stimulates overflow of endogenous striatal dopamine release measured by in vivo reverse microdialysis (Sadri-Vakili et al. 2008). Based on reports of bulk tissue levels of PregS in the nanomolar range, the agonist effects of PregS in the picomolar to nanomolar range satisfy the requirement for a putative neurotransmitter, i.e., it is present at endogenous concentrations necessary to elicit a physiological response.

An obstacle for understanding the mechanism of action of neurosteroids in general, and the negatively charged PregS in particular, is relating bulk concentration of steroids to effective concentration at membrane targets. In the case of PregS, its hydrophobic nature combined with polar negatively charged moiety in the 3-position complicates interpretation of bulk levels. If PregS and other modulatory steroids are dynamically synthesized and/or degraded, then concentrations within the bilayer could change dramatically within the membrane on a time scale of minutes, which may not be reflected in aqueous phase concentrations. In addition, charged steroids such as PregS may also redistribute within the membrane as a result of membrane potential shifts (Caruso et al 2013). Thus, caution must be exercised when attempting to draw conclusions concerning the action of endogenous hydrophobic modulators particularly in comparing concentration dependence over different time scales.

Transport or uptake and removal mechanisms for PregS

In contrast to synthesis in the CNS, PregS also can access the brain parenchyma from blood by transport across the blood–brain barrier (BBB). Although PregS is negatively charged and would not be expected to cross cell membranes rapidly, multiple isoforms of organic anion transporters (OATP) are expressed in human choroid plexus (Steckelbroeck et al. 2004) and serve as PregS transporters. Sulfated steroids including estradiol sulfate (Wood et al. 2003) and PregS (Wang et al. 1997) are known to gain access to the brain from serum.

Could PregS actions at neuronal membrane receptors be terminated via a combination of reuptake and enzymatic degradation by sulfatases? The presence of mRNA and protein for an organic solute transporter OST α -OST β (with high substrate specificity for PregS) expressed in cerebellar Purkinje cells (which are postsynaptic to glutamatergic cerebellar granule cells) and hippocampal CA1, CA2, and CA3 and dentate gyrus cells in mouse would be consistent with mechanism for transmitter removal (Fang et al. 2010).

PregS exhibits selective pharmacological modulation of glutamate receptors (NMDARs and AMPARs)

Ligand-gated glutamate receptors, consisting of NMDA, AMPA, and kainate receptors, mediate the vast majority of excitatory transmission in the brain and spinal cord. The NMDAR contains an obligatory NR1 subunit and one of four NR2 subunits (NR2A–D) and is under magnesium block during resting membrane potential. NMDARs are modulated by protons, zinc, and polyamines and regulated by phosphorylation of amino acid residues and the reduction/oxidation (redox) state of the cell (Dingledine et al. 1999). PregS modulation of NMDARs is subtype specific; PregS potentiates the response of NMDARs containing NR2A and NR2B subunits and inhibits the response of receptors containing NR2C and NR2D subunits (Yaghoubi et al. (1998); Malayev et al. 2002). A steroid modulatory domain in the fourth transmembrane region of the NMDAR NR2B subunit mediates potentiation by PregS (Jang et al. 2004). Rapid solution exchange experiments indicate that PregS also acts at a separate inhibitory site (Horak et al. 2006), possibly the same as that mediating inhibition by inhibitory neuroactive steroids such as pregnanolone sulfate (Park-Chung et al. 1997; (PAS, also referred to as $3\alpha,5\beta$ S)), with the net positive (NR1/NR2A or NR1/NR2B) or negative (NR1/NR2C or NR1/NR2D) modulatory effect of PregS determined by its relative potency for the positive and negative modulatory sites (reviewed by Gibbs et al. 2006; Korinek et al. 2011).

In order to model PregS binding to NMDARs and AMPA receptors (AMPARs), Cameron et al. (2012) expressed and purified the NMDAR GluN2B and AMPAR GluA2B domains. PregS binding to S1S2 region on GluN2B and the amino terminal domain of GluA2B was demonstrated (Cameron et al. 2012). PregS binding to the AMPAR amino terminal domain is a novel finding and supports allosteric modulation by PregS of both AMPAR and NMDAR signaling (Cameron et al. 2012). Interestingly, structure–activity studies show that PregS modulation of NR1/NR2A-containing receptors has both a pH-dependent and independent component. PregS potentiation of NMDA-induced currents in oocytes expressing these receptors is reduced at high pH where tonic proton inhibition of the receptor is negligible (Kostakis et al. 2011). Introducing a point mutation known to decrease proton sensitivity of NR2A-containing NMDARs does not affect PregS potentiation dependence on pH, while addition of an NR1 amino acid exon-5 insert enhances sensitivity to both proton inhibition and to potentiation by PregS. In contrast, PregS modulation of NMDARs containing NR2B is minimally sensitive to proton inhibition and is not affected by the exon-5 insert (Kostakis et al. 2011). PregS potentiation of NMDA-induced currents is diminished following membrane patch excision and by kinase inhibitors staurosporine (serine/threonine kinase inhibitor) and H89 (protein kinase A (PKA) inhibitor), indicating that PregS modulation of NMDARs is also phosphorylation state-dependent (Petrovic et al. 2009).

Ifenprodil is a non-competitive antagonist selective for NR2B-containing NMDARs (Williams 1993; Grimwood et al. 2000). PregS at 30 nM can alter the kinetics of [3 H]ifenprodil binding to rat frontal cortex membranes, suggesting that nanomolar concentrations of PregS might induce a conformational change in NR2B-containing NMDARs (Johansson et al. 2005). Pregnanolone sulfate decreases (while PregS increases)

[³H]ifenprodil binding to rat frontal cortex membranes (Johansson et al. 2005). This result indicates that the effect is structurally specific because PregS has a carbon 4–5 double bond while PAS does not. Consequently, PAS is planar and PregS is in a bent configuration, which changes the spatial location of the 3-sulfate group in relation to the plane of the cholesterol backbone. In order to further study the mechanism of PregS modulation of NR2B-containing NMDARs, Johansson et al. (2008) studied PregS displacement of [³H]ifenprodil from membranes derived from CHO-E2 cells expressing NR1/NR2B receptors (under the control of a heat-sensitive promoter). They determined that PregS increases [³H]ifenprodil-specific binding to the membranes in a bell-shaped concentration dependence with a peak at 1 nM PregS, while 100 pM PregS alters the kinetics of [³H]ifenprodil dissociation from CHO-E2 homogenates (Johansson et al. 2008). PAS decreased specific binding of [³H]ifenprodil in a similar manner with maximal inhibition at 10 nM. The modulatory effects of PregS but not PAS on [³H]ifenprodil binding were sensitive to glutamate, indicating separate binding sites for the neurosteroids on NR2B-containing NMDARs. Moreover, functional calcium assays demonstrated that 100 pM PregS enhances the inhibitory effect of ifenprodil on glutamate-induced calcium influx, while 1 nM PAS has the opposite effect (Johansson et al. 2008). In a companion study, nanomolar concentrations of PregS and PAS (100 pM and 1 nM, respectively) increased the number of binding sites (from one to two sites) for [³H]-MK-801 binding to rat hippocampal membranes in the presence of ifenprodil (Elfverson et al. 2008). These results indicate that these neurosteroids may allosterically modulate NR2B-containing NMDARs in such a way that a high-affinity binding site for [³H]-MK-801 is revealed in the presence of ifenprodil (Elfverson et al. 2008).

Long-term treatment of rats with morphine resulted in an increase in specific binding of [³H]ifenprodil in frontal cortex and not the hippocampus or hypothalamus, suggesting a biologically relevant specific upregulation or unblocking of NR2B receptors (Johansson et al. 2010). The effects of PregS and DHEAS on [³H]ifenprodil binding to membrane homogenates were unaffected by morphine treatment, while the PAS binding profile in cortex was altered, suggesting a change in NR2B receptors affecting PAS but not PregS or DHEAS allosteric modulation of the receptor (Johansson et al. 2010). Overall, the results provide compelling evidence that picomolar to nanomolar concentrations of PregS interact with the NMDAR via an indirect circuitry level pathway or direct modulatory mechanism. Given that endogenous bulk levels of PregS are low nanomolar or greater, endogenous PregS may well play a physiological role as a neuromodulatory of neurotransmitter.

PregS and synaptic activity

Glutamatergic transmission

PregS at micromolar concentrations induces an increase in AMPA receptor-mediated miniature excitatory postsynaptic current (mEPSC) frequency (which is blocked by the AMPAR antagonist GYK 53655) and, to a lesser extent, increases in GABA_AR-mediated miniature postsynaptic currents in acute rat cerebellar slices (Zamudio-Bulcock and Valenzuela 2011). The effect of PregS on mEPSC frequency is more robust in P4–10 rats than P12 rats and is not blocked by antagonists targeting NMDA, glycine, voltage-gated

Ca²⁺ channels, $\sigma 1$, or $\alpha 7$ nACh receptors. Bath application of a membrane permeable Ca²⁺ chelator blocks the PregS effect on mEPSC, while dialyzing the chelator via patch pipet has no effect on PregS potentiation, supporting the conclusion that PregS acts presynaptically. Finally, lanthanide³⁺, a non-selective TRP channel blocker, eliminates the PregS effect (Zamudio-Bulcock and Valenzuela 2011), further supporting transient receptor potential melastatin 3 (TRPM3) as a target for PregS-induced glutamate release. A model proposed by Valenzuela et al. (2008) posits that PregS induces presynaptic Ca²⁺ influx, increasing presynaptic glutamate release, and promoting insertion of AMPARs to the postsynaptic membrane, thereby activating silent synapses. Such a model would be consistent with the recent discovery of delayed onset potentiation of the NMDA response reported by Kostakis et al. (2013).

Activation of glutamatergic signaling may also exhibit feedback regulation of neurosteroid synthesis and/or degradation. Incubation of rat C6 glioma cells with glutamate (1 mM) and prevention of AMPA receptor desensitization attenuate sulfotransferase 2B (SULT2B1a) mRNA expression (Kohjitani et al. 2008). The decrease in SULT2B1a mRNA expression is inhibited by an AMPAR antagonist and attenuated by a neuronal nitric oxide synthase (nNOS) inhibitor. Nitric oxide (NO) donors also attenuate SULT2B1a RNA expression. The results suggest that AMPAR activation could lead to inhibition of SULT2B1a expression via an NO-mediated pathway.

Synaptic plasticity

PregS potentiates both short-term (Schiess et al. 2006; Chen et al. 2007) and long-term potentiation (LTP) (Sabeti et al. 2007; Sliwinski et al. 2004; Chen et al. 2009). PregS (5 μ M) potentiates LTP at hippocampal CA1 synapses (following 100 Hz stimulation) and PregS surmounts D-AP5 inhibition of LTP (Sabeti et al. 2007). Chronic intermittent ethanol-treated rats exhibit a D-AP5-insensitive form of LTP, and this form of LTP is resistant to potentiation by PregS (Sabeti and Gruol 2008), though the significance of this result is unclear.

In the medial prefrontal cortex (mPFC), PregS inhibits LTP in a manner that depends on an $\alpha 2$ -adrenoreceptor, G_i protein-coupled, adenylate cyclase to PKA signaling pathway (Wang et al. 2008b). PregS potentiation of LTP in the dentate gyrus correlates with increased phosphorylation of NR2B-containing NMDARs, ERK and CREB (Chen et al. 2007). Also in the dentate gyrus, PregS induces a leftward shift in a hippocampal LTP/LTD frequency curve (Chen et al. 2009). These studies used a voltage-sensitive dye to measure changes in EPSP slope and amplitude following stimuli of varying frequency in rat hippocampal slice granule cells. Maximal PregS potentiation of LTP occurs ~20 Hz and correlates with increased ERK phosphorylation. The effect also requires L-type voltage-gated Ca²⁺ channel activation but is insensitive to NMDAR inhibition by D-AP5 or σ R block by NE100.

In the hippocampal trisynaptic pathway (DG, CA3, CA1), the change in population spike amplitude is stimulation frequency-dependent. Over a stimulation frequency range of 2–77 Hz, the dentate field acts as a low-pass filter, while the CA3 and CA1 fields act as a bandpass filter (with CA1 having a narrower range than CA3). PregS (1 μ M) has no effect on dentate filtering properties, while it increases the gain (population spike amplitude at

select stimulation frequencies) in the CA3 (θ , 4–8 Hz range) and CA1 (γ , 30–100 Hz range), supporting a role for PregS in modulating hippocampal filtering properties in the CA1 and CA3 regions (Scullin and Partridge 2012).

We recently reported that PregS, at nanomolar to low micromolar concentrations, stimulates NMDAR trafficking to the surface of cortical neurons and *Xenopus* oocytes expressing NR1/NR2A or NR1/NR2B receptors via a non-canonical (not requiring ion conductance through the ion channel target) mechanism that requires NMDARs but does not involve activation of the NMDAR ion channel (Kostakis et al. 2013). Pharmacological evidence indicates that PregS-induced release of intracellular Ca^{+} ($[\text{Ca}^{+}]_i$) is mediated by G protein-coupled activation of phospholipase C. It is unclear whether the increase in neuronal $[\text{Ca}^{+}]_i$ elicited by PregS is related to that finding. In addition to the large difference in potency, the increase in $[\text{Ca}^{2+}]_i$ produced by pM PregS is dependent upon the involvement of L-type voltage-gated Ca^{2+} channels, suggesting entry of extracellular Ca^{2+} .

Whether the recognition site at which picomolar PregS acts to increase $[\text{Ca}^{+}]_i$ and trafficking of receptor to the cell surface is different from the site that micromolar PregS acts to potentiate the NMDA response (Jang et al. 2004) remains a provocative question, although the fast modulation and delayed onset potentiation phenomena are clearly separable using receptor chimeras (Kostakis et al. 2013). Inhibition by AP5, ifenprodil, and Ro 25–6981 strongly suggests involvement of NMDARs. The steroid structure–activity relationship for the PregS-induced $[\text{Ca}^{+}]_i$ increase is consistent with that previously described for rapid allosteric modulation of NMDA-induced $[\text{Ca}^{+}]_i$ increase by PregS (Weaver et al 2000), but the EC_{50} for PregS is lower (higher potency), which argues for a distinct binding site. On the other hand, PregS is lipophilic (Caruso et al. 2013) and charged, so the observed potency could be substantially enhanced if PregS accesses its recognition site from within or at the lipid bilayer. This leaves the intriguing possibility that PregS may trigger downstream signaling events by coupling to NMDARs, GPCRs, and Ca^{+} channels with long-term changes in gene expression and memory consolidation via the activation of protein kinases leading to ERK and CREB phosphorylation, possibly coordinating the non-genomic and genomic actions of neurosteroids via synaptic but not extrasynaptic glutamatergic synaptic regulation.

PregS in therapeutics and disease

Animal models of therapeutics and disease—translation from cell culture

At a concentration of 500 nM, PREG protects against 20 μM $\text{A}\beta$ peptide toxicity after 72 h exposure in PC12 cells. In the same assay, 500 nM PregS has no protective effect on cell death but decreases cell viability during $\text{A}\beta$ -exposure. At higher concentrations, both 50 μM PREG and PregS potentiate $\text{A}\beta$ -induced toxicity over a 72-h treatment (Akan et al. 2009). In contrast, PregS has been shown to enhance the survival of DG cells and to potentiate EPSPs in the DG of mouse hippocampal slices (Yang et al. 2010). Treatment with PregS (20 mg/kg subcutaneously, 100 μL) rescues deficits in spatial memory performance and cell death caused by a single (icv) injection of aggregated $\text{A}\beta_{(25-35)}$ (3 nmol/mouse), an effect which is mediated by $\sigma 1$ Rs, $\alpha 7$ nAChRs, and correlates with changes in phosphorylated ERK (Yang et al. 2012). Sustained increases in EPSPs (60 min post-PregS) are blocked by $\alpha 7$ nAChR,

σ 1R, and NMDAR antagonists; however, only NMDAR antagonists are capable of reversing the PregS effect. The NMDAR antagonist D-AP5 blocks PregS enhancement of DG neuron survival (Yang et al. 2012), consistent with a role for the NMDAR in PregS-induced neuroprotection. In addition to neuroprotection from exogenously applied A β , PregS also protects transgenic mice (APP/PS1) that express amyloid plaques. In this model, PregS enhances neurite outgrowth, neuronal survival, and measures of spatial memory in behavioral tests (Xu et al. 2012).

Behavioral studies that examine the effects of PregS on measures of learning memory have yielded mixed results. PregS and its synthetic enantiomer (*ent*-PregS) injected into the lateral ventricle in mice have promnesic effects, in that mice are more able to remember which of two arms of a maze they have previously visited (termed retention performance). Surprisingly, the PregS effect, but not *ent*-PregS, is sensitive to forebrain NR1 knockout (Fig. 6) (Petit et al. 2011), again suggesting that the NMDAR is the target for PregS memory enhancement. PregS (10 mg/kg; 1 mL/kg; 2 h prior to evaluation) increases the number of correct responses while decreasing reference memory areas in tasks measuring spatial orientation-acquisition and simple and complex visual object discrimination. The same study demonstrated that PregS increases the spontaneous activity, frequency, and maximum firing rates of neurons in CA3 and perirhinal cortex (required for object recognition) brain regions (Fig. 7) (Plescia et al. 2014). In contrast, PregS (5 ng) injected bilaterally into the hippocampal CA1 area of rats immediately after a passive avoidance training session impairs 24-h passive avoidance retention, whereas allopregnanolone (AlloP) has no effect (Martín-García and Pallarés 2008). A concentration-dependent effect of PregS has also been reported; a 1.2 μ M injection of PregS into the lateral septum impairs novel object recognition, while injections of 0.12 and 12 μ M (for both PregS and Preg) have no effect (Nanfaro et al. 2010).

In a study by Darbra et al. (2012), bilateral intrahippocampal CA1 infusions of AlloP at 0.2 mg were chosen such that systemic subcutaneous administration of 8 mg/kg of AlloP results in a cortical concentration (0.4 mg/g) known to cause anxiolytic effects. Doses of PregS were then chosen such that 5 ng of PregS, diluted in a 0.5 μ L infusion volume, results in a concentration (24 μ M) known to modulate GABA_A and NMDA receptors. PregS does not affect prepulse inhibition (PPI), while AlloP enhances PPI startle inhibition. The same CA1 infusions of AlloP (0.2 mg) or PregS (5 ng) decrease locomotor activity. Neonatal exposure to AlloP potentiates intrahippocampal PregS-induced decreases in locomotor activity, suggesting a possible increase in the GABA_AR response to neurosteroids. However, neonatal exposure to AlloP or finasteride (5 α -reductase inhibitor) has no effect on subsequent PregS or AlloP modulation of open field behavior, a measure of anxiety (Darbra and Pallarès 2011). The effects of PregS and AlloP would be expected based on their respective activity at NMDARs and GABA_ARs and indicate that predictions of PregS effects on behavior may, with restraint, be made based on its effects on ion channels.

A recent study determined that PREG is increased in the frontal cortex, nucleus accumbens, striatum, and other brain regions following administration of THC (the main active component of marijuana) to rats, showing that the increase in PREG was blocked by a type-1 cannabinoid receptor (CB₁R) antagonist (Vallée et al. 2014). Notably, PREG

administration to rats blocks the cannabinoid “tetrad” (hypocomotion, hypothermia, catalepsy, analgesia), considered to be a behavioral correlate to cannabinoid intoxication. These results suggest that PREG is an endogenous negative modulator of CB₁R-mediated signaling. Also consistent with this hypothesis is the finding that 1 μ M PREG inhibits THC-induced ERK1/2 phosphorylation in CHO cells expressing the human CB₁R (hCB₁R), and 100 nM PREG attenuates ERK1/2 phosphorylation induced by THC in HEK293 cells transfected with hCB₁Rs. The effects of THC on direct metabolites of PREG including PregS, progesterone, and 17-OH PREG (Do Rego et al. 2009) were not determined, and their contribution to the observed effect remains an open question.

Given the expected ratio of conversion of PregS from PREG in brain (Wang et al. 1997; PregS/Preg=0.025), an increase of PREG from 1.27 ± 0.21 to 17.27 ± 1.2 ng/g in the nucleus accumbens 30 min after THC injection (Vallée et al. 2014) would be expected to elevate levels of PregS from 0.03 ± 0.01 ng/g (0.08 ± 0.01 nM) to 0.43 ± 0.03 ng/g (1.1 ± 0.08 nM). Additionally, the highest dose (6 mg/kg) of PREG administered during behavioral studies raised levels of PREG in the nucleus accumbens to ~90 ng/g which, according to the conversion rate from PREG to PregS, would result in levels of PregS ~2.3 ng/g (~6 nM). These increased levels of PregS over existing baseline levels are reasonably within the concentration range at which effects of PregS have been observed on LTP (Sliwinski et al. 2004), NMDAR conformational changes (Johansson et al. 2008), dopamine overflow from nigrostriatal terminals measured by in vivo microdialysis (Sadri-Vakili et al. 2008), dopamine release from striatal nerve terminals (Whittaker et al. 2008), and NMDA receptor trafficking to the cell surface (Kostakis et al. 2013).

The relevance of PregS as an endogenous neurosteroid transmitter (as contrasted with a neuroactive steroid) has been the subject of considerable controversy because a functional role at physiologically relevant concentrations has to be demonstrated. The recent article concerning PREG actions on CB₁ receptors reports PREG potency was in the micromolar range (Vallée et al. 2014). Our recent results demonstrate that PregS at physiological (low pM) concentrations, without added glutamate or NMDA, is a surprisingly potent positive modulator of intracellular calcium signaling that involves but does not require a modulatory action at NMDARs (Smith CC et al., unpublished). What is currently known about PregS and NMDARs does not include this novel signaling pathway that depends solely on the presence of ongoing excitatory synaptic transmission via NR2B receptor containing synapses. Our results do not indicate that there is a role of high potency actions of PregS working at the NMDA receptor to mediate this response but rather that its effects are dependent upon synapses that contain specific NMDARs.

At the concentrations of PREG used in functional experiments in the 100 nM range for PREG as compared with a potency of 2 pM PregS in stimulating calcium increases, the possibility of rapid conversion of even 1/1,000 parts of Preg to PregS could lead to significant physiological effects. The novel role of PREG modulation of cannabinoid receptor signaling as well as the questions raised by the recent findings of high-affinity PregS actions increasing $[Ca^{2+}]_i$ highlights the need for continued research into better methods for accurately determining the bulk and more importantly local concentrations of PregS and related neurosteroids.

In the mouse tail suspension test (TST), used as an animal model of depression, 40 mg/kg subcutaneous PregS and 10 and 40 mg/kg (but not 20 mg/kg) DHEAS were found to decrease immobility time; these responses were blocked by pretreatment with putative σ 1R antagonists BD1047, progesterone and rimcazole (Dhir and Kulkarni 2008). The 3β -hydroxysteroid dehydrogenase (3β -HSD) inhibitor trilostane would be expected to halt conversion of PREG to progesterone and 17-OH PREG to 17-OH progesterone. Trilostane has antidepressant-like properties in that it decreases immobility time in a forced swim test in mice and is equally active in control and stressed mice. Trilostane also has anxiolytic effects in behavioral tests, although the dose–response is bell-shaped, indicating a narrow window of efficacious concentrations (Espallargues et al. 2009). The effects of β -HSD inhibition on specific neurosteroid concentrations in the brain are unknown, making it difficult to correlate the behavioral effects with changes in neurosteroid levels. Neonatal administration of finasteride has an anxiogenic effect in adult rats that is not rescued by hippocampal CA1 infusion of AlloP but exacerbated by hippocampal PregS (Martín-García et al. 2008). AlloP has anxiolytic effects when administered bilaterally in the hippocampal CA1 region. While PregS had no effect on behavioral measures of anxiety, it enhanced postacquisition measure of memory, consistent with actions at both NMDAR- and acetylcholine-mediated pathways (Mòdol et al. 2011).

In summary, PregS has NMDAR-dependent promnestic and neuroprotective effects and early results suggest the promnestic effect of PregS likely depends on the mode of administration and behavioral model being tested. The promnestic effect of PregS in parallel to a neuroprotective effect in the presence of A β toxicity, as assayed in culture, suggests that PregS may be a potential therapeutic in Alzheimer's disease; however, no recent clinical trials have been completed to date using PregS as an adjunct therapeutic in the disease. The PregS-induced reduction of depression-like symptoms in mice may also carry over into the clinic, as indicated below. Additionally, several promising clinical trials indicate a potential role for the PregS precursor PREG in ameliorating symptoms of schizophrenia, as well as a correlation between serum PregS as a biomarker for treatments for alcohol addiction and epilepsy.

PregS as an endogenous cognitive enhancer

The development of a more sensitive methodology for the measurement of steroid levels and their localization within cells is of major importance to investigating the functional roles of neurosteroids in general and PregS in particular. Pharmacological evidence demonstrates that PregS is a useful platform for development of high-affinity NMDAR-positive modulators as receptor subtype selective cognitive enhancers. The effect of micromolar concentrations of PregS on NMDARs is subtype-specific (Park-Chung et al. 1997; Malayev et al. 2002). We previously documented picomolar (10^{-12} M) to nanomolar (10^{-9} M) NMDAR-dependent modulatory effects of PregS on dopamine overflow and release in the striatum (Sadri-Vakili et al. 2008; Whittaker et al. 2008).

PregS at 100 nM stimulates delayed onset potentiation of NMDAR receptor subunit trafficking to the cell surface (Kostakis et al. 2013). Delayed onset potentiation occurs with greater potency than potentiation of fast (msec) NMDAR-mediated responses. A PregS-like

small molecule or a prodrug like PREG, which has been shown to elevate brain PregS levels (Wang et al. 1997), would have great promise in the treatment of multiple neurological disorders such as Alzheimer's disease, depression, and schizophrenia toward the symptoms of cognitive dysfunction.

PregS and PREG in human clinical trials

The use of peripheral levels of neurosteroids can be used in the diagnosis of CNS disorders (Meloun et al. 2009; Kancheva et al. 2010). Two recent clinical trials investigated the effect of medications for alcohol dependency or epilepsy on serum levels of neuroactive steroids. In one study focusing on the hypothalamic–pituitary–adrenal axis (HPA), PregS serum levels are responsive to HPA hormone levels. Deoxycorticosterone levels are increased by naloxone therapy in alcohol-dependent patients, while ovine corticotropin-releasing hormone (oCRH)-induced deoxycorticosterone levels are blunted in alcohol-dependent patients and decrease following treatment with dexamethasone. PregS serum levels are increased by cosyntrophin and decreased by dexamethasone but unaffected by naloxone or oCRH (Porcu et al. 2008). In a study of 28 female patients with epilepsy, treatment with carbamazepine or primidone decreases serum levels of DHEAS in patients in the follicular and luteal phase of menstruation, while carbamazepine decreases levels of PregS in the luteal phase (Hill et al. 2010).

Levels of circulating steroid hormones are predictive of schizophrenia symptoms, as indicated in a study of 13 male patients with schizophrenia (22 control subjects) and 18 female patients with schizophrenia (25 control subjects) (Bicikova et al. 2013). In support of the potential for a therapeutic application of neurosteroids in schizophrenia, a clinical trial demonstrated that PREG reduces negative symptoms in patients with schizophrenia while increasing serum levels of PREG, PregS, AlloP, DHEAS, and progesterone (Marx et al. 2009). More recently, it was demonstrated that an 8-week dose of PREG reduces positive symptoms of schizophrenia and improves memory and attention in patients concurrently taking antipsychotic medication (Ritsner et al. 2010, 2014; Kreinin et al. 2014). In a clinical trial comparing PREG to placebo (19 PREG subjects, 18 placebo subjects), PREG supplementation resulted in a significantly greater decrease in depressive symptoms in patients with mono- and bipolar depression with a history of substance abuse (Osuji et al. 2010). One critical limitation to these studies is the small sample size; while encouraging, larger clinical trials are needed to verify the efficacy of adjunctive PREG or PregS in schizophrenia.

Other targets

GABA_A receptors

PregS at mid-micromolar concentrations acts as a negative allosteric modulator (Mienville and Vicini 1989), while pregnane steroids at nanomolar concentrations positively modulate (Harrison et al. 1987) GABA_AR responses via distinct sites (Park-Chung et al. 1999). The reduced metabolites of PREG neurosteroids act as endogenous GABA_AR-positive allosteric modulators with high potency (Belelli and Lambert 2005), based upon structure-activity studies. The GABA_AR is the primary mediator of inhibitory synaptic transmission in the

vertebrate CNS, and its therapeutic potential as a drug target is evidenced by involvement in the mechanism for action of a large number of drug classes such as barbiturates, anesthetics, and benzodiazepines (Sieghart 1995).

Progress has been made on identifying structural determinants of steroid action at GABA_ARs. A recent study demonstrated that 50 μ M PregS increases the apparent rate of desensitization of GABA_A receptors and found that novel amino acids in a membrane spanning domain (M1) of the alpha subunit of the GABA_A receptor specifically interact with potentiating neurosteroids such as AlloP (Akk et al. 2008). Additionally, PregS increases the off rate of GABA site activation, while 3,20(r/s)-pregnandiols (5 α -pregnan-3 β , 20(S)-diol and 5 β -pregnan-3 β , 20(S)-diol decrease the fast offset rate in a manner distinct from that of PregS in recombinant rat GABA_ARs ($\alpha_1\beta_2\gamma_{2L}$), consistent with distinct binding sites for positive and negative neurosteroid modulation of GABA_ARs (Wang et al. 2008a). Using chimeric receptors expressed in oocytes (Baker et al. 2010), it was shown that residues rendering *Caenorhabditis elegans* receptors insensitive to PregS actually increased sensitivity to PregS and 5 α ,3 α -pregnane-3 α , 21-diol-20-one (THDOC) in human or rat GABA_ARs. In addition, a potential endogenous role for PregS inhibition of spontaneously active $\alpha_6\beta_2\gamma$ receptors expressed in developing cerebellar granule cells has been described (Baker et al. 2010).

Additionally, dipicrylamine, a hydrophobic anion structurally unrelated to PregS, exhibits many properties similar to PregS inhibition of GABA_ARs including concentration-dependent inhibition, enhanced receptor desensitization, and sensitivity to the mutation of a residue in the receptor that abolishes inhibition by PregS and not picrotoxin (Chisari et al. 2011). It is thought that dipicrylamine and PregS may interact with GABA_ARs via a membrane-sensitive site rather than a traditional lock and key site on the oligomeric protein complex. The abundance of structure–function studies of neurosteroid interactions with GABA_ARs lays the foundation for a structural model of neurosteroid modulation that can recapitulate the previous datasets and provide a platform to test hypotheses of neurosteroid binding and receptor gating.

In addition to direct modulation of GABA_ARs, neurosteroids also influence expression of other GABA_AR modulators such as Acyl-CoA binding protein. The acyl-CoA binding protein is proteolytically cleaved to peptides TTN and ODN (termed endozepines) that can bind to and reduce the affinity of GABA_ARs for GABA. These peptides also compete with benzodiazepine binding to GABA_ARs and to the PBR. Both the acyl-CoA binding protein and PBR are highly expressed in astrocytes but not neurons. Nanomolar concentrations of neurosteroids (cortisol, 5 nM; PregS, 10 nM; PREG, 50 nM) induce the release of endozepines from mouse primary astrocytes within 5 min, indicating a non-genomic mechanism (Loomis et al. 2010). The effect of cortisol is blocked by a protein kinase A inhibitor but is insensitive to brefeldin A blockade of secretion pathways or pertussis toxin inhibition of downstream G protein-coupled receptor activity. This suggests a secondary mode of action for indirect neurosteroid regulation of GABA_AR signaling and an additional potential therapeutic target for neurosteroid modulation of GABA_ARs.

Sigma receptors

Previous studies in the literature also suggest that there is a role for sigma receptor (σ R)-mediated neurosteroid action (Sabeti and Gruol 2008; Mtchedlishvili and Kapur 2003; Yang et al. 2012). Nandrolone, an anabolic steroid that increases aggressive behavior and down-regulates NR2B-containing NMDARs, is hypothesized to modulate PregS targets including the NMDAR and σ R (Elfverson et al. 2011). Chronic treatment of rats with nandrolone was shown to decrease high-affinity binding of neurosteroids PregS, PAS, and DHEAS in the presence of ifenprodil to rat brain extracts (Elfverson et al. 2011). However, the treatment did not alter neurosteroid binding to NR2B-containing NMDARs, suggesting a selective down-regulation of σ Rs and subsequent PregS binding (Elfverson et al. 2011). It has also been reported that PREG (10 mg/kg) increases σ 1R immunoreactivity in rat retina and decreases intraocular pressure in a rat model of ocular hypertension (Sun et al. 2012).

Voltage-gated channels

Neurosteroid-specific responses have also been reported for members of a subfamily of inwardly rectifying K^+ channel (Kir). The Kir2.3 channel, which is expressed in mammalian forebrain, is potentiated by PregS but not by other neurosteroids including PREG, DHEA, DHEAS, progesterone, AlloP, or THDOC. PregS potentiation of Kir2.3 has an EC_{50} of 16 μ M and is selective for Kir2.3 channels (Kir 1.1, 2.1, 2.2, and 3.1/3.2 channels were also tested) (Kobayashi et al. 2009). These results indicate that at micromolar concentrations, PregS may induce hyperpolarization in cells expressing the Kir2.3 channel. Blockade of L-type voltage-gated Ca^{2+} channels by verapamil inhibits PregS-induced upregulation of a CRE-reporter gene in pancreatic beta islet cells, while PregS is inactive in a cell line that expresses L-type voltage-gated Ca^{2+} channels and not TRPM3 (Müller et al. 2011). PregS, and with lower potency, DHEA, and DHEAS (but not PREG) decrease current through $Na_v1.2$, a voltage-gated sodium channel (Horishita et al. 2012).

Taken together, PregS modulation of neurotransmitter receptor function highlights the need for structure-function studies of PregS-specific interactions at GABA_ARs and NMDARs. PregS modulation of GABA_ARs, NMDARs, TRP, and voltage-gated channels may provide novel targets for neurosteroid-based therapeutics. Further structure-function studies may also lead to refined neurosteroid pharmacophores specific to its respective target to reduce the potential for off-target effects. Additionally neurosteroid modulation of neurotransmitter receptor function determines the role neurosteroids play in regulation of CNS function. Further elucidation of PregS targets and its mechanism of action at the molecular level will lead to a better understanding of PregS modulation of synaptic activity.

TRP channels

PregS, acting at micromolar concentrations, is an agonist at TRPM3 channels (Wagner et al. 2008), which are highly expressed in brain tissue (Lee et al. 2003; Fonfria et al. 2006; Hoffmann et al. 2010). PregS stimulates entry of Zn^{2+} through the TRPM3 channels in mouse pancreatic beta islet cells (Wagner et al. 2010), yet another novel candidate target for mediating PregS modulation of CNS function, but of low affinity and not selective toward PregS (reviewed in: Harteneck 2013).

PregS increases the frequency (with no effect on amplitude) of glutamatergic spontaneous excitatory postsynaptic currents (EPSCs) with an EC_{50} of 65 μ M in mechanically isolated rat hippocampal hilar neurons with intact proximal dendrites and cell-free presynaptic nerve terminals (Lee et al. 2010). At first glance, this may seem inconsistent with the results of the sections above, however, not necessarily, as at this concentration and cell type, PregS action does not involve NMDAR-mediated synaptic transmission and could reflect enhanced presynaptic Zn^{2+} entry, increasing the frequency of neurotransmitter release. The effect of PregS is dependent on external Ca^{2+} and blocked by an intracellular Ca^{2+} chelator, reduced by CPA (a sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase [SERCA] antagonist), and blocked by non-specific TRP channel blockers but is not blocked by antagonists of NMDARs (D-AP5), $\sigma 1$ receptors (BD1063), $\alpha 7$ nicotinic receptors (MLA), voltage-dependent Na^+ channels (TTX), adenylyl cyclase (Sq22536), or PKA (KT5720) (Lee et al. 2010). This phenomenon is thus quite different mechanistically than the classical NMDAR synapse-dependent modulatory effect.

However, there is support for TRPM3 modulation of AMPAR-dependent glutamatergic synaptic activity as mefenamic acid, which blocks TRPM3 channels and inhibits the PregS (25 μ M) induced increases in AMPA-miniature EPSC (mEPSC) frequency (Zamudio-Bulcock et al. 2011). TRPM3 channels are expressed in the developing rat cerebellar cortex, and TRPM3-like channels are expressed at glutamatergic synapses in neonatal Purkinje cells. PregS (2.5 μ M), epipregnanolone sulfate (25 μ M), and nifedipine (which acts as an agonist at TRPM3 channels) were also sufficient to induce an increase in mEPSC frequency. Progesterone, which does not activate TRPM3 channels, does not increase mEPSC frequency (Zamudio-Bulcock et al. 2011).

In non-neuronal tissues, TRPM3 channels are required for the PregS-induced upregulation of c-Jun, c-Fos promoter activity, and transcription of a CRE-regulated reporter gene in an insulinoma cell line and in primary cultured mouse pancreatic β -cell islets (Müller et al. 2011). PregS induces Ca^{2+} increases in fibroblast-like synoviocyte primary cells derived from patients with rheumatoid arthritis and inhibited secretion of hyaluronan (Ciurtin et al. 2010). PregS activation of TRPM3 in human vascular smooth muscle cells results in increased intracellular Ca^{2+} and disinhibition of IL-6 and hyaluronan secretion, while PregS-induced contractile responses in mouse aorta were inhibited by a TRPM3 inhibitor but insensitive to voltage-gated Ca^{2+} channel inhibition (Naylor et al. 2010a).

Dopamine

The diverse effects of PregS on neuronal function continue into the regulation of dopaminergic transmission, where PregS (25 pM, 25 nM, and 50 μ M) has been shown to induce dopamine (DA), but not GABA or glutamate, release from rat striatal nerve terminals. DA release induced by 25 nM PregS is blocked by D-AP5 (NMDARs) but not by CNQX (AMPA antagonist) or a mixture of antagonists (receptors blocked: $GABA_A$ R, 5-HT₃R, nAChR, D₁R, D₂R, and AMPA/KaR). PREG has no effect on DA release in the same assay (Whittaker et al. 2008), suggesting that sulfation is required for the response. The effect of PregS on DA release from terminals *ex vivo* is supported by an *in vivo* microdialysis study in which PregS (10, 25, 50 nM) increases and a synthetic structural

analog, PREG hemisuccinate (50 and 300 nM), increases rat striatal DA levels. The concentration–effect curve for PregS-induced DA increase is bell-shaped, in that 5 nM, 300 nM, and 100 μ M PregS are without effect, suggesting competing targets. Fifty nanomolar PregS-induced DA release is blocked by D-AP5 but not by the σ 1R antagonist BD1063 (while PREG, pregnanolone, and progesterone are without effect on striatal DA levels) (Sadri-Vakili et al. 2008).

Overall, PregS has an excitatory effect on neurotransmission including an increase in glutamate, glycine and DA release, potentiation of the NMDAR, NMDA-induced cGMP production, and potentiation of LTP (summarized in Table 2). However, the effect of PregS on synaptic transmission is not always clear-cut. For instance, in rat hippocampus, PregS potentiates LTP (Sabeti and Gruol 2008), while in the rat mPFC, PregS inhibits LTP (Wang et al. 2008b). Not surprisingly, the effect of PregS on behavioral tests of memory is also complex, as the effects of PregS seem to depend on the experimental measure of memory as well as the site of PregS injection (see for example; Petit et al. 2011 vs. Nanfaro et al. 2010). The existence of multiple pre- and postsynaptic targets of PregS at a given synapse would complicate the overall effect of PregS on synaptic transmission. Therefore, any working model of the effect of PregS on a given synapse would have to take into account multiple potential sites of action for PregS, each with unique affinity and maximal effect contributing to the overall response that PregS would have on synaptic function.

Additional complexity resulting from neural network effects of PregS on different neuronal pathways may reduce the predictive value of therapeutic design based on its modulation of a given target, necessitating preclinical and clinical studies that verify the concentration-dependent effects of PregS on complicated biological functions. Recent results of preclinical cell-based models and behavioral tests are consistent with PregS potentiation of the NMDAR and excitatory neurotransmission and may provide valuable insight into the role of PregS in neuronal function and its potential use in the development of novel therapeutics.

Conclusions

The development of cognitive enhancers is urgently needed for the treatment of neurological disorders such as Alzheimer's disease, depression, and schizophrenia that have component symptoms associated with deficits in learning and memory (Lee and Silva 2009). For example, the negative symptoms of schizophrenia such as cognitive deficits are currently not managed by antipsychotic treatments and are correlated with poor clinical outcomes (Milev et al. 2005). Several lines of study point to positive modulation of NMDARs as a potential mechanism for cognitive enhancer action/discovery in these disease states (Bibb et al. 2010). NMDAR antagonists reproduce symptoms resembling schizophrenia in humans (Gaspar et al. 2009), while the NMDAR has been shown to be a validated therapeutic target for glutamatergic dysfunction in depression, schizophrenia, and Alzheimer's disease (Buchanan et al. 2007; Butterfield and Pocernich 2003; Gaspar et al. 2009; Pilc et al. 2013; Collingridge et al. 2013). Recent research on the effects of PregS at the molecular, synaptic, and behavioral level all indicate that PregS is a promising candidate as a cognitive enhancer. Novel structure–function studies may result in the design of a PregS-based molecule specific

for the NMDAR, while within the last 5 years, novel PregS targets such as the TRPM3 channel and the Kir2.3 channel have been discovered.

Additionally, the excitatory effect of PregS on synaptic function is consistent with the potential for cognitive enhancement, including potentiation of glutamatergic signaling and long-term synaptic potentiation. Preclinical results for PregS, such as its promnesic effects and ability to provide neuroprotection from A β toxicity, are promising, particularly in light of new data for its precursor, PREG, in clinical trials for schizophrenia (Marx et al. 2009) and depression (Osuji et al. 2010). Overall, the current body of evidence strongly supports further research into PregS and related small molecules as cognitive enhancers for a range of neurological disorders.

Acknowledgments

The authors gratefully acknowledge the very helpful input from Shelley Russek, PhD. This work was supported in part by the National Institutes of Health, the National Institute of Mental Health [Grant R01MH049469], and the National Institutes of Health, National Institute of General Medical Sciences grants [T32GM008541] to D.H.F.

References

- Akan P, Kizildag S, Ormen M, Genc S, Oktem MA, Fadiloglu M. Pregnenolone protects the PC-12 cell line against amyloid beta peptide toxicity but its sulfate ester does not. *Chem Biol Interact.* 2009; 177:65–70. [PubMed: 18926803]
- Akk G, Li P, Bracamontes J, Reichert DE, Covey DF, Steinbach JH. Mutations of the GABA-A receptor $\alpha 1$ subunit M1 domain reveal unexpected complexity for modulation by neuroactive steroids. *Mol Pharmacol.* 2008; 74:614–627. [PubMed: 18544665]
- Babalola PA, Fitz NF, Gibbs RB, Flaherty P, Li PK, Johnson DA. The effect of the steroid sulfatase inhibitor (p-O-sulfamoyl)-tetradecanoyl tyramine (DU-14) on learning and memory in rats with selective lesion of septal-hippocampal cholinergic tract. *Neurobiol Learn Mem.* 2012; 98:303–310. [PubMed: 23022361]
- Baker C, Sturt BL, Bamber BA. Multiple roles for the first transmembrane domain of GABAA receptor subunits in neurosteroid modulation and spontaneous channel activity. *Neurosci Lett.* 2010; 473:242–247. [PubMed: 20193738]
- Baulieu EE. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. *Recent Prog Horm Res.* 1997; 52:1–32. [PubMed: 9238846]
- Baulieu EE, Robel P, Schumacher M. Neurosteroids: beginning of the story. *Int Rev Neurobiol.* 2001; 46:1–32. [PubMed: 11599297]
- Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci.* 2005; 6(7):565–575. [PubMed: 15959466]
- Bibb JA, Mayford MR, Tsien JZ, Alberini CM. Cognition enhancement strategies. *J Neurosci.* 2010; 30:14987–14992. [PubMed: 21068302]
- Bicikova M, Hill M, Ripova D, Mohr P, Hampl R. Determination of steroid metabolome as a possible tool for laboratory diagnosis of schizophrenia. *J Steroid Biochem Mol Biol.* 2013; 133:77–83. [PubMed: 22944140]
- Brown RC, Cascio C, Papadopoulos V. Pathways of neurosteroid biosynthesis in cell lines from human brain: regulation of dehydroepiandrosterone formation by oxidative stress and beta-amyloid peptide. *J Neurochem.* 2000; 74:847–859. [PubMed: 10646538]
- Buchanan RW, Javitt DC, Marder SR, Schooler NR, Gold JM, McMahon RP, Heresco-Levy U, Carpenter WT. The cognitive and negative symptoms in schizophrenia trial (CONSIST): the efficacy of glutamatergic agents for negative symptoms and cognitive impairments. *Am J Psychiatry.* 2007; 164:1593–1602. [PubMed: 17898352]

- Budziszewska B, Siwanowicz J, Le kiewicz M, Jaworska-Feil L, Laso W. Protective effects of neurosteroids against NMDA-induced seizures and lethality in mice. *Eur Neuropsychopharmacol.* 1998; 8:7–12. [PubMed: 9452934]
- Butterfield DA, Pocernich CB. The glutamatergic system and Alzheimer's disease: therapeutic implications. *CNS Drugs.* 2003; 17:641–652. [PubMed: 12828500]
- Cameron K, Bartle E, Roark R, Fanelli D, Pham M, Pollard B, et al. Neurosteroid binding to the amino terminal and glutamate binding domains of ionotropic glutamate receptors. *Steroids.* 2012; 77:774–779. [PubMed: 22504555]
- Caruso D, Pesaresi M, Abbiati F, Calabrese D, Giatti S, Garcia-Segura LM, et al. Comparison of plasma and cerebrospinal fluid levels of neuroactive steroids with their brain, spinal cord and peripheral nerve levels in male and female rats. *Psychoneuroendocrinology.* 2013; 38:2278–2290. [PubMed: 23706961]
- Cauli O, González-Usano A, Agustí A, Felipo V. Differential modulation of the glutamate-nitric oxide-cyclic GMP pathway by distinct neurosteroids in cerebellum in vivo. *Neuroscience.* 2011; 190:27–36. [PubMed: 21703332]
- Chen L, Miyamoto Y, Furuya K, Mori N, Sokabe M. PREGS induces LTP in the hippocampal dentate gyrus of adult rats via the tyrosine phosphorylation of NR2B coupled to ERK/CREB [corrected] signaling. *J Neurophysiol.* 2007; 98:1538–1548. [PubMed: 17625058]
- Chen L, Cai W, Chen L, Zhou R, Furuya K, Sokabe M. Modulatory metaplasticity induced by pregnenolone sulfate in the rat hippocampus: a leftward shift in LTP/LTD-frequency curve. *Hippocampus.* 2009; 20:499–512. [PubMed: 19475651]
- Chisari M, Wu K, Zorumski CF, Mennerick S. Hydrophobic anions potently and uncompetitively antagonize GABA(A) receptor function in the absence of a conventional binding site. *Br J Pharmacol.* 2011; 164:667–680. [PubMed: 21457224]
- Ciurtin C, Majeed Y, Naylor J, Sukumar P, English AA, Emery P, et al. TRPM3 channel stimulated by pregnenolone sulphate in synovial fibroblasts and negatively coupled to hyaluronan. *BMC Musculoskelet Disord.* 2010; 11:111. [PubMed: 20525329]
- Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). *J Biol Chem.* 1994; 269:28314–28322. [PubMed: 7961770]
- Collingridge GL, Volianskis A, Bannister N, France G, Hanna L, Mercier M, et al. The NMDA receptor as a target for cognitive enhancement. *Neuropharmacology.* 2013; 64:13–26. [PubMed: 22796429]
- Corpéchet C, Synguelakis M, Talha S, Axelson M, Sjövall J, Vihko R, et al. Pregnenolone and its sulfate ester in the rat brain. *Brain Res.* 1983; 270:119–125. [PubMed: 6223687]
- Crawley JN, Glowa JR, Majewska MD, Paul SM. Anxiolytic activity of an endogenous adrenal steroid. *Brain Res.* 1986; 398:382–385. [PubMed: 2879610]
- Darbra S, Pallarès M. Interaction between early postnatal neurosteroid manipulations and adult infusion of neurosteroids into CA1 hippocampal region on the open field behaviour. *Behav Brain Res.* 2011; 216:705–711. [PubMed: 20920532]
- Darbra S, Mòdol L, Pallarès M. Allopregnanolone infused into the dorsal (CA1) hippocampus increases prepulse inhibition of startle response in Wistar rats. *Psychoneuroendocrinology.* 2012; 37:581–585. [PubMed: 21871738]
- Dhir A, Kulkarni S. Involvement of sigma (sigma1) receptors in modulating the anti-depressant effect of neurosteroids (dehydroepiandrosterone or pregnenolone) in mouse tail-suspension test. *J Psychopharmacol.* 2008; 22:691–696. [PubMed: 18308813]
- Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev.* 1999; 51:7–61. [PubMed: 10049997]
- Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, Tsutsui K, et al. Neurosteroid biosynthesis: Enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. *Front Neuroendocrinol.* 2009; 30(3):259–301. [PubMed: 19505496]

- Ebner MJ, Corol DI, Havlíková H, Honour JW, Fry JP. Identification of neuroactive steroids and their precursors and metabolites in adult male rat brain. *Endocrinology*. 2006; 147:179–190. [PubMed: 16223859]
- Elfverson M, Linde A-M, Le Grevès P, Zhou Q, Nyberg F, Johansson T. Neurosteroids allosterically modulate the ion pore of the NMDA receptor consisting of NR1/NR2B but not NR1/NR2A. *Biochem Biophys Res Commun*. 2008; 372:305–308. [PubMed: 18498760]
- Elfverson M, Johansson T, Zhou Q, Le Grevès P, Nyberg F. Chronic administration of the anabolic androgenic steroid nandrolone alters neurosteroid action at the sigma-1 receptor but not at the sigma-2 or NMDA receptors. *Neuropharmacology*. 2011; 61:1172–1181. [PubMed: 21251916]
- Espallergues J, Givalois L, Temsamani J, Laruelle C, Maurice T. The 3beta-hydroxysteroid dehydrogenase inhibitor trilostane shows antidepressant properties in mice. *Psychoneuroendocrinology*. 2009; 34:644–659. [PubMed: 19117688]
- Falany CN, He D, Dumas N, Frost AR, Falany JL. Human cytosolic sulfotransferase 2B1: isoform expression, tissue specificity and subcellular localization. *J Steroid Biochem Mol Biol*. 2006; 102:214–221. [PubMed: 17055258]
- Fang F, Christian WV, Gorman SG, Cui M, Huang J, Tieu K, et al. Neurosteroid transport by the organic solute transporter OST α -OST β . *J Neurochem*. 2010; 115:220–233. [PubMed: 20649839]
- Flood JF, Morley JE, Roberts E. Pregnenolone sulfate enhances post-training memory processes when injected in very low doses into limbic system structures: the amygdala is by far the most sensitive. *Proc Natl Acad Sci U S A*. 1995; 92:10806–10810. [PubMed: 7479888]
- Fonfria E, Murdock PR, Cusdin RS, Benham CD, Kelsell RE, McNulty S. Tissue distribution profiles of the human TRPM cation channel family. *J Recept Signal Transduct Res*. 2006; 26:159–178. [PubMed: 16777713]
- Furukawa A, Miyatake A, Ohnishi T, Ichikawa Y. Steroidogenic acute regulatory protein (StAR) transcripts constitutively expressed in the adult rat central nervous system: colocalization of StAR, cytochrome P-450SCC (CYP XIA1), and 3beta-hydroxysteroid dehydrogenase in the rat brain. *J Neurochem*. 1998; 71:2231–2238. [PubMed: 9832120]
- Gaspar PA, Bustamante ML, Silva H, Aboitiz F. Molecular mechanisms underlying glutamatergic dysfunction in schizophrenia: therapeutic implications. *J Neurochem*. 2009; 111:891–900. [PubMed: 19686383]
- Gibbs TT, Russek SJ, Farb DH. Sulfated steroids as endogenous neuromodulators. *Pharmacol Biochem Behav*. 2006; 84:555–567. [PubMed: 17023038]
- Girdler SS, Lindgren M, Porcu P, Rubinow DR, Johnson JL, Morrow AL. A history of depression in women is associated with an altered GABAergic neuroactive steroid profile. *Psychoneuroendocrinology*. 2012; 37:543–553. [PubMed: 21890277]
- González-Usano A, Cauli O, Agustí A, Felipe V. Hyperammonemia alters the modulation by different neurosteroids of the glutamate-nitric oxide-cyclic GMP pathway through NMDA-GABAA – or sigma receptors in cerebellum in vivo. *J Neurochem*. 2013; 125:133–143. [PubMed: 23227932]
- Gonzalez-Usano A, Cauli O, Agusti A, Felipe V. Pregnenolone sulfate restores the glutamate-nitric-oxide-cGMP pathway and extracellular GABA in cerebellum and learning and motor coordination in hyperammonemic rats. *ACS Chem Neurosci*. 2014; 5:100–105. [PubMed: 24256194]
- Grassi S, Frondaroli A, Dieni C, Scarduzio M, Pettorossi VE. Long-term potentiation in the rat medial vestibular nuclei depends on locally synthesized 17beta-estradiol. *J Neurosci*. 2009; 29:10779–10783. [PubMed: 19710328]
- Grimwood S, Richards P, Murray F, Harrison N, Wingrove PB, Hutson PH. Characterisation of N-methyl-D-aspartate receptor-specific [3 H]ifenprodil binding to recombinant human NR1a/NR2B receptors compared with native receptors in rodent brain membranes. *J Neurochem*. 2000; 75:2455–2463. [PubMed: 11080197]
- Harrison NL, Majewska MD, Harrington JW, Barker JL. Structure-activity relationships for steroid interaction with the gamma-aminobutyric acidA receptor complex. *J Pharmacol Exp Ther*. 1987; 241:346–353. [PubMed: 3033209]
- Harteneck C. Pregnenolone sulfate: from steroid metabolite to TRP channel ligand. *Molecules*. 2013; 18:12012–12028. [PubMed: 24084011]

- Higashi T, Sugitani H, Yagi T, Shimada K. Studies on neurosteroids XVI. Levels of pregnenolone sulfate in rat brains determined by enzyme-linked immunosorbent assay not requiring solvolysis. *Biol Pharm Bull.* 2003; 26:709–711. [PubMed: 12736517]
- Hill M, Vrbíková J, Zárubová J, Vceláková H, Dusková M, Kancheva R, et al. Sulphates of 3beta-hydroxy-5-ene steroids in women with epilepsy. *Prague Med Rep.* 2010; 111:111–126.
- Hoffmann A, Grimm C, Kraft R, Goldbaum O, Wrede A, Nolte C, et al. TRPM3 is expressed in sphingosine-responsive myelinating oligodendrocytes. *J Neurochem.* 2010; 114:654–665. [PubMed: 20163522]
- Hong JS, Cho JH, Choi IS, Lee MG, Jang IS. Pregnenolone sulfate modulates glycinergic transmission in rat medullary dorsal horn neurons. *Eur J Pharmacol.* 2013; 712:30–38. [PubMed: 23665111]
- Horak M, Vlcek K, Chodounska H, Vyklický L. Subtype-dependence of N-methyl-D-aspartate receptor modulation by pregnenolone sulfate. *Neuroscience.* 2006; 137:93–102. [PubMed: 16257494]
- Horishita T, Ueno S, Yanagihara N, Sudo Y, Uezono Y, Okura D, et al. Inhibition by pregnenolone sulphate, a metabolite of the neurosteroid pregnenolone, of voltage-gated sodium channels expressed in *Xenopus* oocytes. *J Pharmacol Sci.* 2012; 120:54–58. [PubMed: 22878600]
- Jang MK, Mierke DF, Russek SJ, Farb DH. A steroid modulatory domain on NR2B controls N-methyl-D-aspartate receptor proton sensitivity. *Proc Natl Acad Sci U S A.* 2004; 101:8198–8203. [PubMed: 15150412]
- Jääntti SE, Tammimäki A, Raattamaa H, Piepponen P, Kostianen R, Ketola RA. Determination of steroids and their intact glucuronide conjugates in mouse brain by capillary liquid chromatography-tandem mass spectrometry. *Anal Chem.* 2010; 82:3168–3175. [PubMed: 20345173]
- Jo DH, Abdallah MA, Young J, Baulieu EE, Robel P. Pregnenolone, dehydropiandrosterone, and their sulfate and fatty acid esters in the rat brain. *Steroids.* 1989; 54:287–297. [PubMed: 2531481]
- Johansson T, Frändberg PA, Nyberg F, Le Grevès P. Low concentrations of neuroactive steroids alter kinetics of [³H]ifenprodil binding to the NMDA receptor in rat frontal cortex. *Br J Pharmacol.* 2005; 146:894–902. [PubMed: 16170326]
- Johansson T, Frändberg P-A, Nyberg F, Le Grevès P. Molecular mechanisms for nanomolar concentrations of neurosteroids at NR1/NR2B receptors. *J Pharmacol Exp Ther.* 2008; 324:759–768. [PubMed: 18006693]
- Johansson T, Elfverson M, Zhou Q, Nyberg F. Allosteric modulation of the NMDA receptor by neurosteroids in rat brain and the impact of long term morphine administration. *Biochem Biophys Res Commun.* 2010; 401:504–508. [PubMed: 20869946]
- Kancheva R, Hill M, Novák Z, Chrastina J, Velíková M, Kancheva L, et al. Peripheral neuroactive steroids may be as good as the steroids in the cerebrospinal fluid for the diagnostics of CNS disturbances. *J Steroid Biochem Mol Biol.* 2010; 119:35–44. [PubMed: 20036740]
- Kawata M, Nishi M, Matsuda K, Sakamoto H, Kaku N, Masugi-Tokita M, et al. Steroid receptor signalling in the brain—lessons learned from molecular imaging. *J Neuroendocrinol.* 2008; 20:673–676. [PubMed: 18601688]
- Khisti RT, Chopde CT, Jain SP. Antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice forced swim test. *Pharmacol Biochem Behav.* 2000; 67:137–143. [PubMed: 11113493]
- Kimoto T, Tsurugizawa T, Ohta Y, Makino J, Tamura Ho Hojo Y, et al. Neurosteroid synthesis by cytochrome p450-containing systems localized in the rat brain hippocampal neurons: N-methyl-D-aspartate and calcium-dependent synthesis. *Endocrinology.* 2001; 142:3578–3589. [PubMed: 11459806]
- Kobayashi T, Washiyama K, Ikeda K. Pregnenolone sulfate potentiates the inwardly rectifying K channel Kir2.3. *PLoS One.* 2009; 4:e6311. [PubMed: 19621089]
- Kohjitani A, Fuda H, Hanyu O, Strott CA. Cloning, characterization and tissue expression of rat SULT2B1a and SULT2B1b steroid/sterol sulfotransferase isoforms: divergence of the rat SULT2B1 gene structure from orthologous human and mouse genes. *Gene.* 2006; 367:66–73. [PubMed: 16368200]

- Kohjitani A, Fuda H, Hanyu O, Strott CA. Regulation of SULT2B1a (pregnenolone sulfotransferase) expression in rat C6 glioma cells: relevance of AMPA receptor-mediated NO signaling. *Neurosci Lett*. 2008; 430:75–80. [PubMed: 18054434]
- Korinek M, Kapras V, Vyklicky V, Adamusova E, Borovska J, Vales K, Stuchlik A, Horak M, Choudounska H, Vyklicky L. Neurosteroid modulation of N-methyl-D-aspartate receptors: molecular mechanism and behavioral effects. *Steroids*. 2011; 76:1409–1418. [PubMed: 21925193]
- Kostakis E, Jang MK, Russek SJ, Gibbs TT, Farb DH. A steroid modulatory domain in NR2A collaborates with NR1 exon-5 to control NMDAR modulation by pregnenolone sulfate and protons. *J Neurochem*. 2011; 119:486–496. [PubMed: 21883211]
- Kostakis E, Smith C, Jang MK, Martin SC, Richards KG, Russek SJ, et al. The neuroactive steroid pregnenolone sulfate stimulates trafficking of functional N-methyl-D-aspartate receptors to the cell surface via a noncanonical G-protein and Ca²⁺-dependent mechanism. *Mol Pharmacol*. 2013; 84:261–274. [PubMed: 23716622]
- Kreinin A, Bawakny N, Ritsner MS. Adjunctive pregnenolone ameliorates the cognitive deficits in recent-onset schizophrenia. *Clin Schizophr Relat Psychoses*. 2014;1–31. [PubMed: 24496044]
- Lanthier A, Patwardhan VV. Sex steroids and 5-en-3 beta-hydroxysteroids in specific regions of the human brain and cranial nerves. *J Steroid Biochem*. 1986; 25:445–449. [PubMed: 2945971]
- Lee YS, Silva AJ. The molecular and cellular biology of enhanced cognition. *Nat Rev Neurosci*. 2009; 10:126–140. [PubMed: 19153576]
- Lee N, Chen J, Sun L, Wu S, Gray KR, Rich A, et al. Expression and characterization of human transient receptor potential melastatin 3 (hTRPM3). *J Biol Chem*. 2003; 278:20890–20897. [PubMed: 12672827]
- Lee KH, Cho JH, Choi IS, Park HM, Lee MG, Choi BJ, et al. Pregnenolone sulfate enhances spontaneous glutamate release by inducing presynaptic Ca²⁺-induced Ca²⁺ release. *Neuroscience*. 2010; 171:106–116. [PubMed: 20816925]
- Liere P, Pianos A, Eychenne B, Cambourg A, Liu S, Griffiths W, Schumacher M, Sjövall J, Baulieu EE. Novel lipoidal derivatives of pregnenolone and dehydroepiandrosterone and absence of their sulfated counterparts in rodent brain. *J Lipid Res*. 2004; 45:2287–2302. [PubMed: 15342680]
- Liere P, Pianos A, Eychenne B, Cambourg A, Bodin K, Griffiths W, et al. Analysis of pregnenolone and dehydroepiandrosterone in rodent brain: cholesterol autooxidation is the key. *J Lipid Res*. 2009; 50:2430–2444. [PubMed: 19506304]
- Liu S, Sjövall J, Griffiths WJ. Neurosteroids in rat brain: extraction, isolation, and analysis by nanoscale liquid chromatography-electrospray mass spectrometry. *Anal Chem*. 2003; 75:5835–5846. [PubMed: 14588024]
- Liyou NE, Buller KM, Tresillian MJ, Elvin CM, Scott HL, Dodd PR, et al. Localization of a brain sulfotransferase, SULT4A1, in the human and rat brain: an immunohistochemical study. *J Histochem Cytochem*. 2003; 51:1655–1664. [PubMed: 14623933]
- Loomis WF, Behrens MM, Williams ME, Anjard C. Pregnenolone sulfate and cortisol induce secretion of acyl-CoA-binding protein and its conversion into endozepines from astrocytes. *J Biol Chem*. 2010; 285:21359–21365. [PubMed: 20452969]
- Majewska MD, Mienville JM, Viccini S. Neurosteroid pregnenolone sulfate antagonizes electrophysiological responses to GABA in neurons. *Neurosci Lett*. 1988; 90:279–284. [PubMed: 3138576]
- Malayev A, Gibbs TT, Farb DH. Inhibition of the NMDA response by pregnenolone sulphate reveals subtype selective modulation of NMDA receptors by sulphated steroids. *Br J Pharmacol*. 2002; 135:901–909. [PubMed: 11861317]
- Mameli M, Carta M, Partridge LD, Valenzuela CF. Neurosteroid-Induced plasticity of immature synapses via retrograde modulation of presynaptic NMDA receptors. *J Neurosci*. 2005; 25:2285–2294. [PubMed: 15745954]
- Marques AH, Silverman MN, Sternberg EM. Glucocorticoid dysregulations and their clinical correlates. From receptors to therapeutics. *Ann N Y Acad Sci*. 2009; 1179:1–18.
- Martín-García E, Pallarés M. A post-training intrahippocampal anxiogenic dose of the neurosteroid pregnenolone sulfate impairs passive avoidance retention. *Exp Brain Res*. 2008; 191:123–131. [PubMed: 18665356]

- Martín-García E, Darbra S, Pallarés M. Neonatal finasteride induces anxiogenic-like profile and deteriorates passive avoidance in adulthood after intrahippocampal neurosteroid administration. *Neuroscience*. 2008; 154:1497–1505. [PubMed: 18539400]
- Marx CE, Trost WT, Shampine LJ, Stevens RD, Hulette CM, Steffens DC, et al. The neurosteroid allopregnanolone is reduced in prefrontal cortex in Alzheimer's disease. *Biol Psychiatry*. 2006; 60(12):1287–1294. [PubMed: 16997284]
- Marx CE, Keefe RS, Buchanan RW, Hamer RM, Kilts JD, Bradford DW, Strauss JL, Naylor JC, Payne VM, Lieberman JA, Savitz AJ, Leimone LA, Dunn L, Porcu P, Morrow AL, Shampine LJ. Proof-of-concept trial with the neurosteroid pregnenolone targeting cognitive and negative symptoms in schizophrenia. *Neuropsychopharmacology*. 2009; 34:1885–1903. [PubMed: 19339966]
- Marx CE, Bradford DW, Hamer RM, Naylor JC, Allen TB, Lieberman JA, Strauss JL, Kilts JD. Pregnenolone as a novel therapeutic candidate in schizophrenia: emerging preclinical and clinical evidence. *Neuroscience*. 2011; 191:78–90. [PubMed: 21756978]
- Mellon SH, Deschepper CF. Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain. *Brain Res*. 1993; 629:283–292. [PubMed: 8111631]
- Mellon SH, Griffin LD, Compagnone NA. Biosynthesis and action of neurosteroids. *Brain Res Brain Res Rev*. 2001; 37:3–12. [PubMed: 11744070]
- Meloche CA, Falany CN. Expression and characterization of the human 3 beta-hydroxysteroid sulfotransferases (SULT2B1a and SULT2B1b). *J Steroid Biochem Mol Biol*. 2001; 77:261–269. [PubMed: 11457664]
- Meloun M, Hill M, Vceláková-Havlíková H. Minimizing the effects of multicollinearity in the polynomial regression of age relationships and sex differences in serum levels of pregnenolone sulfate in healthy subjects. *Clin Chem Lab Med*. 2009; 47:464–470. [PubMed: 19278363]
- Mienville JM, Vicini S. Pregnenolone sulfate antagonizes GABAA receptor-mediated currents via a reduction of channel opening frequency. *Brain Res*. 1989; 489:190–194. [PubMed: 2472854]
- Milev P, Ho BC, Arndt S, Andreasen NC. Predictive values of neurocognition and negative symptoms on functional outcome in schizophrenia: a longitudinal first-episode study with 7-year follow-up. *Am J Psychiatry*. 2005; 162:495–506. [PubMed: 15741466]
- Mòdol L, Darbra S, Pallarés M. Neurosteroids infusion into the CA1 hippocampal region on exploration, anxiety-like behaviour and aversive learning. *Behav Brain Res*. 2011; 222:223–229. [PubMed: 21463656]
- Mtchedlishvili Z, Kapur J. A presynaptic action of the neurosteroid pregnenolone sulfate on GABAergic synaptic transmission. *Mol Pharmacol*. 2003; 64:857–864. [PubMed: 14500742]
- Müller I, Rössler OG, Thiel G. Pregnenolone sulfate activates basic region leucine zipper transcription factors in insulinoma cells: role of voltage-gated Ca²⁺ channels and transient receptor potential melastatin 3 channels. *Mol Pharmacol*. 2011; 80:1179–1189. [PubMed: 21948387]
- Nanfaro F, Cabrera R, Bazzocchini V, Laconi M, Yunes R. Pregnenolone sulfate infused in lateral septum of male rats impairs novel object recognition memory. *Pharmacol Rep*. 2010; 62:265–272. [PubMed: 20508281]
- Naylor J, Li J, Milligan CJ, Zeng F, Sukumar P, Hou B, et al. Pregnenolone sulphate- and cholesterol-regulated TRPM3 channels coupled to vascular smooth muscle secretion and contraction. *Circ Res*. 2010a; 106:1507–1515. [PubMed: 20360246]
- Naylor JC, Kilts JD, Hulette CM, Steffens DC, Blazer DG, Ervin JF, et al. Allopregnanolone levels are reduced in temporal cortex in patients with Alzheimer's disease compared to cognitively intact control subjects. *Biochim Biophys Acta*. 2010b; 1801(8):951–959. [PubMed: 20488256]
- Osuji JJ, Vera-Bolaños E, Carmody TJ, Brown ES. Pregnenolone for cognition and mood in dual diagnosis patients. *Psychiatry Res*. 2010; 178:309–312. [PubMed: 20493557]
- Park-Chung M, Wu FS, Purdy RH, Malayev AA, Gibbs TT, Farb DH. Distinct sites for inverse modulation of N-methyl-D-aspartate receptors by sulfated steroids. *Mol Pharmacol*. 1997; 52:1113–1123. [PubMed: 9396781]
- Park-Chung M, Malayev A, Purdy RH, Gibbs TT, Farb DH. Sulfated and unsulfated steroids modulate gamma-aminobutyric acidA receptor function through distinct sites. *Brain Res*. 1999; 830:72–87. [PubMed: 10350561]

- Paul SM, Doherty JJ, Robichaud AJ, Belfort GM, Chow BY, Hammond RS, Crawford DC, Linsenbardt AJ, Shu HJ, Izumi Y, Mennerick SJ, Zorumski CF. The major brain cholesterol metabolite 24(S)-hydroxycholesterol is a potent allosteric modulator of N-methyl-D-aspartate receptors. *J Neurosci*. 2013; 33:17290–17300. [PubMed: 24174662]
- Petit GH, Tobin C, Krishnan K, Moricard Y, Covey DF, Rondi-Reig L, et al. Pregnenolone sulfate and its enantiomer: differential modulation of memory in a spatial discrimination task using forebrain NMDA receptor deficient mice. *Eur Neuropsychopharmacol*. 2011; 21:211–215. [PubMed: 21036556]
- Petrovic M, Sedlacek M, Cais O, Horak M, Chodounska H, Vyklicky L. Pregnenolone sulfate modulation of N-methyl-D-aspartate receptors is phosphorylation dependent. *Neuroscience*. 2009; 160:616–628. [PubMed: 19272423]
- Pieribone VA, Tsai J, Soufflet C, Rey E, Shaw K, Giller E, et al. Clinical evaluation of ganaxolone in pediatric and adolescent patients with refractory epilepsy. *Epilepsia*. 2007; 48:1870–1874. [PubMed: 17634060]
- Pilc A, Wieroska JM, Skolnick P. Glutamate-based anti-depressants: preclinical psychopharmacology. *Biol Psychiatry*. 2013; 73:1125–1132. [PubMed: 23453290]
- Plescia F, Sardo P, Rizzo V, Cacace S, Marino RA, Brancato A, et al. Pregnenolone sulphate enhances spatial orientation and object discrimination in adult male rats: evidence from a behavioural and electrophysiological study. *Behav Brain Res*. 2014; 258:193–201. [PubMed: 24149069]
- Porcu P, O'Buckley TK, Leslie Morrow A, Adinoff B. Differential hypothalamic-pituitary-adrenal activation of the neuroactive steroids pregnenolone sulfate and deoxycorticosterone in healthy controls and alcohol-dependent subjects. *Psychoneuroendocrinology*. 2008; 33:214–226. [PubMed: 18096321]
- Porcu P, O'Buckley TK, Alward SE, Marx CE, Shampine LJ, Girdler SS, Morrow AL. Simultaneous quantification of GABAergic 3 α ,5 α /3 α ,5 β neuroactive steroids in human and rat serum. *Steroids*. 2009; 74:463–473. [PubMed: 19171160]
- Reddy DS. Neurosteroids: endogenous role in the human brain and therapeutic potentials. *Prog Brain Res*. 2010; 186:113–137. [PubMed: 21094889]
- Ritsner MS, Gibel A, Shleifer T, Boguslavsky I, Zayed A, Maayan R, et al. Pregnenolone and dehydroepiandrosterone as an adjunctive treatment in schizophrenia and schizoaffective disorder: an 8-week, double-blind, randomized, controlled, 2-center, parallel-group trial. *J Clin Psychiatry*. 2010; 71:1351–1362. [PubMed: 20584515]
- Ritsner MS, Bawakny H, Kreinin A. Pregnenolone treatment reduces severity of negative symptoms in recent-onset schizophrenia: an 8-week, double-blind, randomized add-on two-center trial. *Psychiatry Clin Neurosci*. 2014
- Rustichelli C, Pinetti D, Lucchi C, Ravazzini F, Puia G. Simultaneous determination of pregnenolone sulphate, dehydroepiandrosterone and allopregnanolone in rat brain areas by liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci*. 2013; 930:62–69.
- Sabeti J, Gruol DL. Emergence of NMDAR-independent long-term potentiation at hippocampal CA1 synapses following early adolescent exposure to chronic intermittent ethanol: role for sigma-receptors. *Hippocampus*. 2008; 18:148–168. [PubMed: 17960647]
- Sabeti J, Nelson TE, Purdy RH, Gruol DL. Steroid pregnenolone sulfate enhances NMDA-receptor-independent long-term potentiation at hippocampal CA1 synapses: role for L-type calcium channels and sigma-receptors. *Hippocampus*. 2007; 17:349–369. [PubMed: 17330865]
- Sadri-Vakili G, Janis GC, Pierce RC, Gibbs TT, Farb DH. Nanomolar concentrations of pregnenolone sulfate enhance striatal dopamine overflow in vivo. *J Pharmacol Exp Ther*. 2008; 327:840–845. [PubMed: 18772319]
- Schiess AR, Scullin CS, Partridge LD. Neurosteroid-Induced enhancement of short-term facilitation involves a component downstream from presynaptic calcium in hippocampal slices. *J Physiol*. 2006; 576:833–847. [PubMed: 16931546]
- Schumacher M, Liere P, Akwa Y, Rajkowski K, Griffiths W, Bodin K, Sjövall J, Baulieu EE. Pregnenolone sulfate in the brain: a controversial neurosteroid. *Neurochem Int*. 2008; 52:522–540. [PubMed: 18068870]

- Scullin CS, Partridge LD. Modulation by pregnenolone sulfate of filtering properties in the hippocampal trisynaptic circuit. *Hippocampus*. 2012; 22:2184–2198. [PubMed: 22648992]
- Sieghart W. Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. *Pharmacol Rev*. 1995; 47:181–234. [PubMed: 7568326]
- Sliwinski A, Monnet FP, Schumacher M, Morin-Surun MP. Pregnenolone sulfate enhances long-term potentiation in CA1 in rat hippocampus slices through the modulation of N-methyl-D-aspartate receptors. *J Neurosci Res*. 2004; 78:691–701. [PubMed: 15505794]
- Smith, CS. Dissertation. Boston University School of Medicine; 2014. Non-canonical cell signaling actions of pregnenolone sulfate, a neurosteroid that increases intracellular calcium, activates CREB phosphorylation and stimulates trafficking of NMDA receptors to the surface of neurons.
- Steckelbroeck S, Nassen A, Ugele B, Ludwig M, Watzka M, Reissinger A, et al. Steroid sulfatase (STS) expression in the human temporal lobe: enzyme activity, mRNA expression and immunohistochemistry study. *J Neurochem*. 2004; 89:403–417. [PubMed: 15056284]
- Stocco DM. StAR protein and the regulation of steroid hormone biosynthesis. *Annu Rev Physiol*. 2001; 63:193–213. [PubMed: 11181954]
- Ströhle A, Romeo E, di Michele F, Pasini A, Yassouridis A, Holsboer F, et al. GABA(A) receptor-modulating neuroactive steroid composition in patients with panic disorder before and during par-oxetine treatment. *Am J Psychiatry*. 2002; 159:145–147. [PubMed: 11772707]
- Sun X, Cheng F, Meng B, Yang B, Song W, Yuan H. Pregnenolone sulfate decreases intraocular pressure and changes expression of sigma receptor in a model of chronic ocular hypertension. *Mol Biol Rep*. 2012; 39:6607–6614. [PubMed: 22311017]
- Tanaka M, Sokabe M. Continuous de novo synthesis of neurosteroids is required for normal synaptic transmission and plasticity in the dentate gyrus of the rat hippocampus. *Neuropharmacology*. 2012; 62:2373–2387. [PubMed: 22365983]
- Uzunov DP, Cooper TB, Costa E, Guidotti A. Fluoxetine-elicited changes in brain neurosteroid content measured by negative ion mass fragmentography. *Proc Natl Acad Sci U S A*. 1996; 93:12599–12604. [PubMed: 8901628]
- Valenzuela CF, Partridge LD, Mameli M, Meyer DA. Modulation of glutamatergic transmission by sulfated steroids: role in fetal alcohol spectrum disorder. *Brain Res Rev*. 2008; 57:506–519. [PubMed: 17597219]
- Vallée M, Vitiello S, Bellocchio L, Hébert-Chatelain E, Monlezun S, Martin-Garcia E, et al. Pregnenolone can protect the brain from cannabis intoxication. *Science*. 2014; 343:94–98. [PubMed: 24385629]
- Wagner TF, Loch S, Lambert S, Straub I, Mannebach S, Mathar I, et al. Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells. *Nat Cell Biol*. 2008; 10:1421–1430. [PubMed: 18978782]
- Wagner TF, Drews A, Loch S, Mohr F, Philipp SE, Lambert S, et al. TRPM3 channels provide a regulated influx pathway for zinc in pancreatic beta cells. *Pflugers Arch*. 2010; 460:755–765. [PubMed: 20401728]
- Wang MD, Wahlström G, Bäckström T. The regional brain distribution of the neurosteroids pregnenolone and pregnenolone sulfate following intravenous infusion. *J Steroid Biochem Mol Biol*. 1997; 62:299–306. [PubMed: 9408083]
- Wang MD, Borra VB, Strömberg J, Lundgren P, Haage D, Bäckström T. Neurosteroids 3beta, 20 (R/S)-pregnandiol decrease offset rate of the GABA-site activation at the recombinant GABA A receptor. *Eur J Pharmacol*. 2008a; 586:67–73. [PubMed: 18374329]
- Wang ZM, Qi YJ, Wu PY, Zhu Y, Dong YL, Cheng ZX, Zhu YH, Dong Y, Ma L, Zheng P. Neuroactive steroid pregnenolone sulphate inhibits long-term potentiation via activation of alpha2-adrenoreceptors at excitatory synapses in rat medial prefrontal cortex. *Int J Neuropsychopharmacol*. 2008b; 11:611–624. [PubMed: 18184443]
- Watzka M, Bidlingmaier F, Schramm J, Klingmüller D, Stoffel-Wagner B. Sex- and age-specific differences in human brain CYP11A1 mRNA expression. *J Neuroendocrinol*. 1999; 11:901–905. [PubMed: 10583724]
- Weaver CE, Land MB, Purdy RH, Richards KG, Gibbs TT, Farb DH. Geometry and charge determine pharmacological effects of steroids on n-methyl-d-aspartate receptor-induced Ca^{2+}

accumulation and cell death. *J Pharmacol Exp Ther.* 2000; 293(3):747–754. [PubMed: 10869372]

Weill-Engerer S, David JP, Sazdovitch V, Liere P, Eychenne B, Pianos A, et al. Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. *J Clin Endocrinol Metab.* 2002; 87:5138–5143. [PubMed: 12414884]

Whittaker MT, Gibbs TT, Farb DH. Pregnenolone sulfate induces NMDA receptor dependent release of dopamine from synaptic terminals in the striatum. *J Neurochem.* 2008; 107:510–521. [PubMed: 18710414]

Williams K. Ifenprodil discriminates subtypes of the N-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors. *Mol Pharmacol.* 1993; 44:851–859. [PubMed: 7901753]

Wolkowitz OM, Reus VI, Keebler A, Nelson N, Friedland M, Brizendine L, et al. Double-blind treatment of major depression with dehydroepiandrosterone. *Am J Psychiatry.* 1999; 156:646–649. [PubMed: 10200751]

Wong P, Chang CC, Marx CE, Caron MG, Wetsel WC, Zhang X. Pregnenolone rescues schizophrenia-like behavior in dopamine transporter knockout mice. *PLoS One.* 2012; 7:e51455. [PubMed: 23240026]

Wood CE, Gridley KE, Keller-Wood M. Biological activity of 17beta-estradiol-3 -sulfate in ovine fetal plasma and uptake in fetal brain. *Endocrinology.* 2003; 144:599–604. [PubMed: 12538622]

Wu FS, Gibbs TT, Farb DH. Pregnenolone sulfate: a positive allosteric modulator at the N-methyl-D-aspartate receptor. *Mol Pharmacol.* 1991; 40:333–336. [PubMed: 1654510]

Xu B, Yang R, Chang F, Chen L, Xie G, Sokabe M, Chen L. Neurosteroid PREGS protects neurite growth and survival of newborn neurons in the hippocampal dentate gyrus of APPswe/PS1dE9 mice. *Curr Alzheimer Res.* 2012; 9:361–372. [PubMed: 22272612]

Yaghoubi N, Malayev A, Russek SJ, Gibbs TT, Farb DH. Neurosteroid modulation of recombinant ionotropic glutamate receptors. *Brain Res.* 1998; 803(1–2):153–60. [PubMed: 9729352]

Yang R, Zhou R, Chen L, Cai W, Tomimoto H, Sokabe M, et al. Pregnenolone sulfate enhances survival of adult-generated hippocampal granule cells via sustained presynaptic potentiation. *Neuropharmacology.* 2010; 60:529–541. [PubMed: 21111750]

Yang R, Chen L, Wang H, Xu B, Tomimoto H, Chen L. Anti-amnesic effect of neurosteroid PREGS in Aβ25–35-injected mice through σ1 receptor- and α7nAChR-mediated neuroprotection. *Neuropharmacology.* 2012; 63:1042–1050. [PubMed: 22884465]

Zamudio-Bulcock PA, Valenzuela CF. Pregnenolone sulfate increases glutamate release at neonatal climbing fiber-to-purkinje cell synapses. *Neuroscience.* 2011; 175:24–36. [PubMed: 21130844]

Zamudio-Bulcock PA, Everett J, Harteneck C, Valenzuela CF. Activation of steroid-sensitive TRPM3 channels potentiates glutamatergic transmission at cerebellar purkinje neurons from developing rats. *J Neurochem.* 2011; 119:474–485. [PubMed: 21955047]

Zheng P. Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance. *Prog Neurobiol.* 2009; 89:134–152. [PubMed: 19595736]

Zimmerberg B, Drucker PC, Weider JM. Differential behavioral effects of the neuroactive steroid allopregnanolone on neonatal rats prenatally exposed to alcohol. *Pharmacol Biochem Behav.* 1995; 51:463–468. [PubMed: 7667369]

Zorumski CF, Paul SM, Izumi Y, Covey DF, Mennerick S. Neurosteroids, stress and depression: potential therapeutic opportunities. *Neurosci Biobehav Rev.* 2013; 37:109–122. [PubMed: 23085210]

Fig. 1.

Nanomolar PregS induces dopamine (DA) overflow and release in the rat striatum. **a** The PregS-induced DA increase is inhibited by D-AP5. Bars indicate mean DA content of dialysate collected while perfusing PregS and/or D-AP5 through the dialysis probe for 20 min, expressed as percentage of baseline. There was a significant main effect on extracellular DA levels [$F(3, 25)=9.948$; $p<0.0002$]. Asterisk denotes significant differences from baseline values (Bonferroni, $p<0.05$). Number sign denotes significant difference from 50 nM PregS (Bonferroni, $p<0.002$). Number of animals per group is given in parentheses. **b** Dose–response relationship for PregS-induced increase in [³H]DA release reveals a potency in the picomolar range. PregS (25 fM) did not increase [³H]DA release relative to control. These experiments were carried out using superfusion buffer that contained the thiol-containing antioxidant compound *n*-acetylcysteine (NAC, 500 µM). The presence of NAC reduced background [³H]DA release and did not significantly influence the 25 nM PregS effect on [³H]DA release (+NAC 31.6 ± 4.5 %, $n=4$; –NAC 35.9 ± 5.4 %, $n=10$). * $p<0.05$; ** $p<0.01$ compared to buffer-treated control (paired *t* test). Values above error bars indicate the number of replicate experiments carried out for each condition. Reprinted with permission from Sadri-Vakili et al. (2008) and Whittaker et al. (2008)

Fig. 2.

PregS enhances LTP. **a** The PregS effects on representative recordings of the somatic field excitatory postsynaptic potentials (fEPSPs) during LTP paradigm under baseline conditions (**a**) and during potentiation (**b**) and maintenance (**c**; 60 min) phases (vertical scale 1 mV, horizontal scale 10 ms). **b** Normalized fEPSPs slopes following tetanic stimulation (3 × 300 Hz/1 s; arrow) in control and PregS-exposed slices (10 min, horizontal bar). Note the significant enhancement of LTP in slices exposed to PregS (300 nM; ANOVA, $p = 0.025$, vs. control LTP, $n=12$ in each group). **c** Concentration responses of normalized fEPSP slopes during LTP paradigm from slices exposed to different PregS concentrations [control ($n=12$), 100 nM ($n=6$), 300 nM ($n=12$), 600 nM ($n=6$), or 3,000 nM ($n=6$)]. **d** Normalized fEPSPs slopes produced by tetanic stimulation (3 × 300 Hz/1 s; arrow) in slices exposed to PREG (300 nM horizontal black bar, 10 min, $n=8$) compared with control exposure ($n=12$). Data are mean ± SEM of responses, taken every minute. Reprinted with permission from Sliwinski et al. (2004)

Fig. 3. Immunohistochemical staining of the hydroxysteroid sulfotransferase in hippocampal slices of an adult male rat. **a** Low magnification image of the whole hippocampus, stained with antibodies against rat hydroxysteroid sulfotransferase. **b** The hippocampal CA1 region, stained with antibodies against rat sulfotransferase. **c** Staining with anti-sulfotransferase IgG, preincubated with a saturating concentration of purified hydroxysteroid sulfotransferase in the CA1 region. **d** Fluorescence dual staining of the sulfotransferase (green) and NeuN (red). **e** Fluorescence dual staining of the sulfotransferase (green) and GFAP (red). **f** Fluorescence dual staining of the sulfotransferase (green) and MBP (red). A superimposed region of green and red fluorescence is represented in yellow. **b–f** are at the same magnification. *so* stratum oriens; *pcl* pyramidal cell layer; *sr* stratum radiatum. **a–c** Immunoreactive cells were visualized by diaminobenzidine-nickel staining. Scale bar, 800

μm (**a**), 120 μm (**b**, **c**), and 100 μm (**d–f**). *Reprinted with permission from Kimoto et al. (2001)*

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 4.

PregS induces delayed onset, non-canonical, potentiation of the NMDA response in neurons. **a–c** Potentiation of the NMDA response by PregS (100 μ M) in primary embryonic spinal cord neurons increases upon continued exposure to PregS and typically levels off after about 85 s. Initial control application of NMDA is followed by 2-min perfusion, PregS application, wash (4.5 min), and final control NMDA response. **d–f** PregS and NMDA are applied by bath perfusion to cells (*Xenopus* oocytes) expressing NR1/2A subunits while recording in two-electrode whole-cell voltage clamp mode. **d** Black bars indicate successive applications of NMDA (300 μ M). Breaks between traces are 1.3–1.8 min. PregS (100 μ M) was added to the perfusion buffer at $t=0$ and included for 7 min (overlying black bar). **e** PregS (100 nM) initiates substantial delayed onset potentiation without classical rapid positive allosteric modulation. Typical responses to NMDA (10 μ M) alone, 100 nM PregS + NMDA (10 μ M) (applied simultaneously), and 100 nM PregS + NMDA (10 μ M) after 10-min preincubation with 100 nM PregS. **f** Averaged values of normalized peak current responses for rapid and delayed increase in NR1/2A receptors ($n=8–10$). PregS (100 nM) did not potentiate NMDA responses, whereas after 10-min preincubation with 100 nM PregS, the response to NMDA was enhanced by $45 \pm 3\%$ Error bars represent SEM ($n=8–10$). Asterisk indicates a significant difference between rapid and delayed potentiation, $p < 0.0005$. Reprinted with permission from Kostakis et al. (2013)

Fig. 5. Model of signal transduction pathway for delayed onset potentiation of the NMDA response by PregS. *Diagram* illustrates the proposed intracellular pathways that participate in the PregS-stimulated trafficking of functional NMDARs to the cell surface via a non-canonical G protein, phospholipase C (PLC), and Ca²⁺- and PKC-dependent mechanism. *Reprinted with permission from Kostakis et al. (2013)*

Fig. 6.

Effects of PregS and ent-PregS on retention performances of control and NR1-KO mice in the Y-maze test. Data were expressed as mean $\pm$ SEM. For the percentage of time in the novel arm (panel **a**), two-way ANOVA indicated a significant interaction between genotype and treatment ($p<0.05$). For the amount of time in the novel arm (panel **b**), two-way ANOVA showed significant effects of both treatment ($p<0.001$) and genotype ($p<0.05$). The amount of time in the familiar arms (panel **c**) was not statistically different according to treatment or genotype (two-way ANOVA; $p>0.05$). * $p<0.05$; ** $p<0.01$; Student–Newman–Keuls post hoc test. Control: VEH, $n=14$; PregS, $n=13$; ent-PregS, $n=13$; NR1-KO: VEH, $n=11$; PregS, $n=14$; ent-PregS, $n=12$. VEH: Vehicle. Reprinted from Petit et al. (2011)

Fig. 7.

PregS increases cognitive performance. PregS increases simple object discrimination as determined by correct responses (**a**) and decreases in reference memory errors (**b**). *White square* Tween, *black square* PregS. **c** Histogram showing more spontaneously active CA3 hippocampal and perirhinal cortical (*Cprh*) neurons in PregS-treated animals. **a, b** Each value represents the mean $\pm$ SEM of 16 rats. *White columns* represent data from control animals, whereas *black columns* describe results from PregS-treated ones. *** p <0.001, * p <0.05 vs. Tween. *Figure adapted from Plescia et al. (2014)*

Table 1

PregS bulk brain tissue concentration

Species	Tissue	Method notes	Brain bulk concentration (pM conversion)	References
Human (<i>n</i> =1)	Cerebellum	Indirect detection, GC-MS (solvolysis with HFBA acetylation)	4,700	Liere et al. (2004)
Human (<i>n</i> =1)	Frontal cortex	After lipoidal conjugates removed by C18 SPE column	2,800	
Human (control)	Hippocampus	Indirect detection, HPLC-GC-MS sulfated steroids separated with C18	5,000±500	Weill-Engerer et al. (2002)
Human (control)	Cortex		6,000±1,000	
Human (Alzheimer)	Hippocampus		4,000±1,000	
Human (Alzheimer)	Cortex		2,000±500	
Human	Frontal cortex	Indirect detection, ELISA, solvolysis followed by detection with PREG antisera	9,400 ± 2,100	Lanthier and Patwardhan (1986)
Rat (male, 6–8 weeks)	Hippocampus	Direct detection HPLC-MS/MS in negative ion mode electrospray MS, endogenous phospholipids removed	25,900±3,600	Rustichelli et al. (2013)
Rat (male, 6–8 weeks)	Cortex		11,200±900	
Rat (adult male)	Anterior brain	Indirect detection, GC-MS (solvolysis with HFBA acetylation) after lipoidal conjugates removed by C18 SPE column	<380 (LOD, 3/5)	
Rat (male, 7 weeks)	Whole brain	Direct detection, ELISA, Oasis HLB sorbent used to separate unconjugated steroids and sulfates	>85 (8/10 rats) <85 pM (LOD, 2/10)	Higashi et al. (2003)
Rat	Whole brain	Indirect detection, GC-MS (solvolysis with HFBA acetylation) after lipoidal conjugates removed using isoctane partition and HLB cartridge	<126	Ebner et al. (2006)
Rat (male and female)	Whole brain, amygdala, hippocampus	Direct detection, nano-LC-ES, steroid sulfates isolated by anion exchange column followed by C18 column SPE	<760	Liu et al. (2003)
Mouse	Hippocampus, hypothalamus	Direct detection, capillary LC-ESI MS after SPE column to reduce interfering phospholipids	<50	Jäntti et al. (2010)

HIPP hippocampus, *HPLC* high-performance liquid chromatography, *MS* mass spectrometry, *ESI* electrospray ionization, *ELISA* enzyme-linked immunosorbent assay, *GC* gas chromatography, *LC-ES* liquid chromatography electrospray ionisation, *SPE* solid phase extraction, *HLB* hydrophilic lipophilic based, *HFBA* heptafluorobutyric acid anhydride, *LOD* limit of detection

Table 2

Effect of PregS on synaptic transmission

Effect	Experimental system	Notes (PregS conc.)	References
sEPSC frequency (+)	Mechanically isolated rat hilar neurons	Ca ²⁺ and SERCA dependent (EC50=25μM)	Lee et al. (2010)
Paired-pulse ratio (+)	Climbing fiber–purkinje cell synapse	TRPM3 dependent (25μM)	Zamudio-Bulcock and Valenzuela (2011)
AMPA-mEPSC frequency (+)	Rat cerebellar brain slice		Zamudio-Bulcock et al. (2011)
LTP (+)	Rat hippocampal brain slice	Ca ²⁺ _L and sigmaR dependent (5μM)	Sabeti et al. (2007)
LTP (–)	Rat medial prefrontal cortex brain slice	αAR, GPCR, PKA dependent (1μM)	Wang et al. (2008b)
LTP (+)	Rat hippocampal brain slice	Ca ²⁺ _L dependent (50μM)	Chen et al. (2009)
cGMP (+)	In vivo microdialysis in rat cerebellum	NMDAR potentiation (100nM)	Cauli et al. (2011), Gonzalez-Usano et al. (2013), Gonzalez-Usano et al. (2014)
mIPSC frequency (+)	Mechanically dissociated rat medullary dorsal horn neuron	Ca ²⁺ dependent (100μM)	Hong et al. (2013)
DA release (+)	In vivo microdialysis	NMDAR-dependent (10nM)	Sadri-Vakili et al. (2008)
[³ H]DA release (+)	Rat striatum synaptosome	NMDAR-dependent (25pM)	Whittaker et al. (2008)

TRPM3 transient receptor potential M3 channel, Ca²⁺_L L-type voltage-gated Ca²⁺ channel, σR sigma receptor, α2AR alpha 2 adrenoreceptor, GPCR G protein-coupled receptor, PKA protein kinase A, mIPSC miniature inhibitory postsynaptic current, sEPSC spontaneous excitatory postsynaptic current, mEPSC miniature excitatory postsynaptic current, SERCA sarcoplasmic/endoplasmic reticulum Ca²⁺ ATP ase (+) increase, (–) decrease