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Modulation of Glutamatergic Transmission by Sulfated Steroids: Role in Fetal Alcohol Spectrum Disorder

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Abstract

It is well established that sulfated steroids regulate synaptic transmission by altering the function of postsynaptic neurotransmitter receptors. In recent years, evidence from several laboratories indicates that these agents also regulate glutamatergic synaptic transmission at the presynaptic level in an age-dependent manner. In developing neurons, pregnenolone sulfate (PREGS) increases the probability of glutamate release, as evidenced by an increase in the frequency of AMPA receptor-mediated miniature excitatory postsynaptic currents and a decrease in paired-pulse facilitation. In hippocampal slices from postnatal day 3–5 rats, this effect is mediated by an increase in Ca^{2+} levels in the axonal terminal that depends on presynaptic NMDA receptors. This is followed by delayed potentiation of postsynaptic AMPA receptor currents. Importantly, depolarization of postsynaptic neurons, inhibition of hydroxysteroid sulfatase activity and acute exposure to ethanol mimics the effect of exogenous PREGS application. This developmental form of synaptic plasticity cannot be observed in slices from rats older than postnatal day 6, when presynaptic NMDA receptors are no longer expressed in CA1 hippocampal region. Both in the CA1 hippocampal region and the dentate gyrus of more mature rats, PREGS, dehydroepiandrosterone sulfate and hydroxysteroid sulfatase inhibitors increase paired-pulse facilitation, without affecting basal glutamate release probability. This effect depends on activation of σ 1-like receptors and $\text{G}_{i/o}$ and involves a target in the release machinery that is downstream of residual Ca^{2+} . These presynaptic actions of sulfated steroids could play important roles in physiological processes ranging from synapse maturation to learning and memory, as well as pathophysiological conditions such as fetal alcohol spectrum disorder.

Keywords

steroid; neurotransmitter; release; channel; presynaptic; plasticity

1. Introduction

Neurosteroids, produced in the brain independently of peripheral endocrine glands and acting locally in the nervous system, play significant roles in mental health related disorders and have great potential for neuropsychopharmacological applications. In this context, the actions of sulfated steroids have been the focus of intense research for the last 25 years (reviewed in [20]). Initial interest in the actions of these agents was sparked by reports indicating that

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pregnenolone sulfate (PREGS) and dehydroepiandrosterone sulfate (DHEAS) were produced in the rodent brain independently of peripheral endocrine organs (for instance see: [12]). Subsequent studies by several laboratories focused on characterizing the targets of these sulfated steroids. It was demonstrated that PREGS modulates several ionotropic neurotransmitter receptors, including NMDA, AMPA, kainate, and glycine receptors [20]. Metabotropic receptors, such as σ_1 receptors and adrenergic receptors, as well as voltage-gated Ca^{2+} and K^+ channels, have also been identified as targets of PREGS [6,17,18,23,28,32,33,36,37,40,46].

One of the best characterized neuronal targets of PREGS is the NMDA subtype of glutamate ionotropic receptors (NMDAR). Using diverse experimental approaches, it has been shown that PREGS potentiates the function of this receptor [20]. We initially became interested in studying NMDAR modulation by PREGS because of its potential role in the pathophysiology of fetal alcohol spectrum disorder (FASD), an umbrella term that is used to describe the wide range of disabilities associated with prenatal ethanol exposure [21]. Studies have implicated alterations in neuroactive steroid function in the pathophysiology of FASD. Ethanol exposure affects peripherally produced steroids with profound effects in the pathophysiology of FASD [52] and, importantly, several lines of evidence suggest that steroids produced locally in the brain also play a role in this disorder.

2. Neurosteroids and FASD

Zimmerberg et al [54] studied the behavioral effects of allopregnanolone on neonatal rats exposed to ethanol *in utero* and found that these animals exhibited a dose-dependent shift to the right in the anxiolytic effect of intracerebroventricular injection of this agent (i.e. a decrease in the production of ultrasonic vocalizations in response to brief maternal separation). In a follow up study, these investigators assessed the effect of prenatal ethanol exposure on the anxiogenic actions of PREGS [55]. These studies revealed that intracerebroventricular injection of high doses of PREGS increased maternal separation-induced ultrasonic vocalizations in the control animals, but not in those exposed to ethanol *in utero*. More recently, Zimmerberg and Brown [53] reported that chronic prenatal ethanol exposure, given in combination with early maternal separation-induced stress, increases allopregnanolone levels in both the prefrontal cortex and hippocampus of adult offspring stressed via the forced swim test. Interestingly, Allan et al [1] reported that chronic prenatal ethanol exposure diminished the GABA_A receptor inhibitory actions of PREGS in the medial prefrontal cortex but not the hippocampus of adult offspring. Bailey et al [3] found that chronic prenatal ethanol exposure did not affect allopregnanolone-induced potentiation of [^3H]-muscimol or [^3H]-flunitrazepam binding in cerebral cortical cell membranes from guinea pigs euthanized at different developmental time points; however, it decreased the efficacy of allopregnanolone potentiation of [^3H]-flunitrazepam binding in hippocampal membranes from adult guinea pigs [24] (Table 1).

Collectively, these studies suggest that developmental exposure to ethanol affects endogenous neurosteroid levels as well as the sensitivity to these agents both at the behavioral and cellular levels. We initially focused on investigating whether the fetal ethanol-induced decrease in neonatal behavioral sensitivity to PREGS reported by Zimmerberg and McDonald [55] could be attributable to a decrease in sensitivity of ligand-gated ion channels to this neurosteroid [13]. Sprague-Dawley rats were administered throughout pregnancy an ethanol-containing liquid diet that produces blood alcohol levels near the legal intoxication limit (0.08 g/dl = 17 mM), a liquid diet containing 0% ethanol (isocaloric to the ethanol-containing diet), or Purina breeder block chow and water *ad libitum*. Primary hippocampal cultures from the offspring of these rats were prepared at postnatal day (P) 3–4 and PREGS modulation of NMDA-evoked currents was assessed after 6–7 days in culture. Unexpectedly, neither PREGS nor

pregnenolone hemisuccinate produced a potentiation of NMDA-evoked currents in neurons from neonates chronically exposed to ethanol *in utero*. The actions of 5 β -pregnan-3 α -ol-20-one sulfate or hemisuccinate, which inhibit NMDA receptor function, were not affected by prenatal ethanol exposure. PREGS-induced inhibition of non-NMDA- and GABA_A receptor-mediated responses was not affected by ethanol exposure in these neurons. We concluded that the ethanol-induced decrease in the stress-enhancing actions of PREGS could be, in part, mediated by a reduction in the sensitivity of NMDARs to the modulatory actions of PREGS (Table 1).

In our initial attempts to determine the mechanism of this effect, we could detect no changes in NMDAR subunit composition or function [13]. However, studies had shown that ligand-gated ion channels develop tolerance to the modulatory actions of neurosteroids after long-term exposure to these agents [48–50]. We therefore assessed whether chronic prenatal ethanol exposure induced changes in PREGS levels [8] (Table 1). Brain steroids were extracted with methanol-containing 1% acetic acid, fractionated using C18 minicolumns with [³H]-DHEAS added as an internal standard to determine recovery of sulfated steroids, and conjugated steroids were eluted with methanol-water (40:60 v/v). Radioimmunoassays were then performed using antiserum that recognizes either pregnenolone (PREG)-related steroids or DHEAS. It was found that prenatal ethanol exposure increases levels of conjugated PREG-like neurosteroids, but not DHEAS, in brains from embryonic day (E) 14 to P 5 animals. This increase was detected in fetal brain but not placenta, fetal liver, maternal brain, or maternal blood. In the fetal brain, we also measured levels of other steroids at E 20 and found that pregnenolone levels were elevated, but not those of DHEAS or allopregnanolone. In a control experiment, we determined that exposure of neonatal slices to DU-14, an inhibitor of 3- β -hydroxysteroid sulfatase, increased levels of conjugated PREG-like neurosteroids in our radioimmunoassay, suggesting that we measured levels of a sulfated steroid. The findings of this study and those discussed above prompted us to perform a more detailed characterization of the actions of PREGS on synaptic transmission and this revealed novel effects of this steroid on glutamate release.

3. Modulation of glutamatergic transmission in developing neurons

3.1. Studies with cultured hippocampal neurons

We observed in cultured hippocampal neurons that exogenous application of PREGS produces an increase in the frequency, but not the amplitude, of AMPA receptor (AMPA)-mediated miniature excitatory postsynaptic currents (mEPSCs) [30] (Table 2). Using an internal solution containing CsCH₃SO₃ (a salt that has been shown to be the least disruptive to intracellular structures and Ca²⁺ homeostasis [51]) and 10 mM of the Ca²⁺ chelator EGTA, this effect was reversible upon washout. The minimum concentration of PREGS that produced a significant effect on mEPSC frequency was 10 μ M. We also examined the effect of PREGS on paired-pulse facilitation (PPF) and found that it reduced this presynaptic form of neuronal plasticity. These effects are consistent with a PREGS-induced increase in glutamate release probability.

Further investigation of the mechanism of action of PREGS in cultured hippocampal neurons [30] revealed that: 1) the effect of PREGS is not affected by the NMDAR antagonist, 2-amino-5-phosphonopivalic acid (APV); 2) the presynaptic effect of PREGS is prevented by the σ_1 receptor antagonists, haloperidol and BD-1063, and mimicked by the σ_1 receptor agonists, DHEAS and (+)-pentazocine; and 3) treatment with pertussis toxin blocks the PREGS-induced increase of mEPSC frequency. The presynaptic effect of PREGS involved Ca²⁺ influx independent of voltage-gated Ca²⁺ channels since it was not observed in presence of the membrane-permeable Ca²⁺ chelator, BAPTA-AM, but was eliminated by removal of extracellular Ca²⁺ (Fig 1) although not by 100 μ M Cd²⁺ or Ni²⁺ (not shown). Inhibition of phospholipase C (not shown), sarco-endoplasmic reticulum Ca²⁺ pumps, or inositol 1, 4, 5-trisphosphate receptors (Fig 2) did not affect the action of PREGS, suggesting that intracellular

Ca^{2+} stores are not involved in this process. Ni^{2+} , at concentrations known to block channels mediating store-operated Ca^{2+} influx (2 mM) significantly reduced the effect of PREGS (not shown). We postulate that stimulation of metabotropic σ_1 -like receptors by PREGS acts through an unconventional mechanism involving $\text{G}_{i/o}$ to activate channels mediating store-operated Ca^{2+} influx. However, it should be kept in mind when interpreting these results that the cultured neurons used for these studies were a mixture of those from all hippocampal subfields and that maturation of synaptic connections between these neurons *in vitro* is not necessarily equivalent to that observed *in vivo*. Accordingly, we undertook follow-up studies with hippocampal slices, and observed that PREGS can affect glutamate release via a different mechanism.

3.2. Studies with hippocampal slices

In CA1 pyramidal neurons in acute hippocampal slices of P3–4 Sprague-Dawley rats, PREGS induced a robust increase in the frequency, but not the amplitude, of AMPAR-mediated mEPSCs [26]. Although the magnitude and dose-response characteristics of the effect detected in the slices were in general agreement with those observed in the mixed hippocampal cultures, in the slices, the effect was not reversible upon PREGS washout. Rather, mEPSC frequency continued to augment after removal of PREGS, suggesting a long-lasting increase in glutamate release probability. To test this possibility, we characterized the effect of PREGS on PPF of AMPAR-mediated EPSCs evoked by stimulating the Schaffer collaterals. Consistent with a presynaptic site of action for PREGS, we found that this steroid reversibly decreases PPF. However, the amplitude of evoked EPSCs continued to increase after washout. This delayed increase in AMPA EPSC amplitude is the result of a postsynaptic effect, as indicated by our finding that PREGS induces a delayed enhancement of currents evoked by bath- or pressure-applied AMPA [26]. Thus, PREGS induces a transient increase in glutamate release probability that is followed by delayed potentiation of postsynaptic AMPARs.

To further characterize this delayed postsynaptic effect of PREGS, either BAPTA (10 mM) or MK-801 (5 mM) were dialyzed into the postsynaptic neuron via the patch pipette [26]. In the presence of these agents, the PREGS-induced increase of mEPSC frequency reversed upon washout. Bath application of ifenprodil produced a similar effect. Taken together, these results indicate that an increase in postsynaptic $[\text{Ca}^{2+}]_i$ involving NR2B-containing NMDARs is required for the late-phase of the PREGS-induced plasticity.

An important finding of this study was that the effect of PREGS is age-dependent; the magnitude of the PREGS-induced increase of mEPSC frequency was less robust in slices from P5 rats than in those from P3–4 rats, and the effect could no longer be observed in slices from animals older than P6 [26]. It has been subsequently verified that PREGS does not affect basal glutamate release probability in hippocampal slices from mature rats (see Section 4). Age-dependent effects of PREGS have also been documented in the large presynaptic terminal of the calyx of Held in the brainstem, where PREGS enhances evoked EPSCs in 7–9 day-old rats, but by 13–14 days, this effect has largely disappeared [23]. At least in these two brain regions, the presynaptic effects of sulfated steroids are significantly affected by important presynaptic changes at glutamatergic synapses that occur during early brain development (Table 2).

In the hippocampus, Mameli et al [26] found that neither the early phase nor the late phase of the PREGS-induced increase of mEPSC frequency were observed in slices treated with a membrane permeable Ca^{2+} chelator (BAPTA-AM), nonselective NMDAR antagonists (APV and 7-chlorokynurenate), or a compound that preferentially antagonizes NR2D-containing NMDARs (1-(phenanthrene-2-carbonyl) piperazine-2,3-dicarboxylic acid; PPDA). The early phase of the presynaptic effect of PREGS was mimicked by bath application of the NMDAR agonist homoquinolinic acid in the presence of MK-801 in the internal solution and this agonist

was ineffective in slices from rats older than P6. These data suggest that PREGS increases the probability of glutamate release via an increase in presynaptic $[Ca^{2+}]_i$ that requires the activation of presynaptic NMDARs that might contain NR2D subunits. To test whether developing hippocampal neurons could release an endogenous PREGS-like neurosteroid, CA1 pyramidal neurons were depolarized for 15 s to mimic early network-driven bursts of activity that are commonly observed in these neurons in slices from animals younger than P5 [19]. This manipulation increased mEPSC frequency in slices from P3–4 but not P6–10 rats, and this effect could not be observed in presence of an antibody scavenger that binds PREG-related neurosteroids or the NR2D antagonist, PPDA. Consequently, immature CA1 pyramidal neurons appear to release a PREGS-like neurosteroid retrograde messenger in an activity-dependent manner.

As mentioned above, PREGS application induces a delayed increase in the amplitude of AMPA-evoked currents without affecting mEPSC amplitude or decay [26]. These effects were observed in active neurons that exhibited AMPAR mEPSC events at rest. If PREGS induced potentiation of the function of AMPARs present at active synapses or insertion of receptors into these synapses, the mEPSC amplitude or decay should have changed. Therefore, our current working model is that the late phase of the PREGS-induced plasticity can be explained by an increase in AMPAR insertion into postsynaptically silent synapses (i.e. lacking postsynaptic AMPARs) (see model in Fig 3). To initially test this possibility, we recorded from CA1 pyramidal neurons in hippocampal slices from P3–4 rats, about 50% of which do not exhibit AMPAR mEPSCs and are considered to be silent at rest with respect to glutamatergic synaptic transmission. As shown in Fig 4, brief application of PREGS, but not vehicle, elicited the persistent appearance of AMPAR mEPSCs in silent neurons. These findings generally support the model shown in Fig 3 and we are currently performing additional studies to characterize the postsynaptic mechanism of the PREGS-induced plasticity.

3.3. Ethanol and PREGS modulation of glutamatergic transmission in developing neurons

In light of the findings that fetal ethanol exposure affects the sensitivity of NMDARs to PREGS [13], we investigated whether the presynaptic actions of this steroid could also be altered by prenatal ethanol exposure [9]. Sprague-Dawley rats were exposed to ethanol during pregnancy, hippocampal neuronal cultures were prepared as in the study of Costa et al [13], and AMPAR-mediated mEPSCs were recorded as described by Meyer et al [30]. We found that chronic prenatal ethanol exposure did not affect basal mEPSC frequency or amplitude [9]. Moreover, exposure to ethanol did not affect the PREGS-induced increase of mEPSC frequency in these neurons. Taken together with the findings of Costa et al [13], these results indicate that *in utero* ethanol exposure affects PREGS sensitivity of postsynaptic NMDARs, but not presynaptic $G_{i/o}$ -coupled σ_1 receptors in cultured hippocampal neurons. As will be discussed in Section 4, we have found that PREGS increases PPF of NMDAR and non-NMDAR-mediated synaptic responses in CA1 pyramidal neurons in hippocampal slices of mature rats. Carta et al [9] also evaluated whether chronic prenatal ethanol exposure produced long-lasting alterations in these modulatory actions of PREGS and no differences were detected in the PREGS-induced enhancement of PPF in slices from control and *in utero* ethanol-exposed adult offspring (Table 1).

Mameli et al. [27] investigated whether acute ethanol exposure of developing hippocampal neurons results in alterations in glutamatergic synaptic transmission in a neurosteroid-dependent manner. AMPAR EPSCs, mediated by a few synapses in CA1 pyramidal neurons, were evoked by minimal stimulation of Schaffer collaterals. Under those conditions, stimulation failed to elicit synaptic responses in 50–90% of the trials. In agreement with the findings of Mameli et al [26], bath application of PREGS or DU-14, a hydroxysteroid sulfatase inhibitor, persistently reduced the EPSC failure rate in slices from P3–4 but not P6–10 rats and

bath application of ethanol produced a similar effect in a dose-dependent manner. Importantly, the minimum ethanol concentration that produced a significant effect was 15 mM, which as mentioned above, is near the legal intoxication limit in the U.S.A. The effect of ethanol was prevented by the antibody scavenger that binds PREG-related steroids, as well as aminogluthetimide, an inhibitor of P450_{sc}. Moreover, pre-application of exogenous PREGS occluded the effect of ethanol. These findings indicate that acute ethanol exposure, during a developmental period that is equivalent to a portion of the third trimester-equivalent of human pregnancy, strengthens AMPAR-mediated synaptic transmission via an increase in the synthesis and/or release of a PREGS-like neurosteroid. During this developmental period, the brain is particularly vulnerable to environmental insults because synapses are undergoing profound remodeling. We hypothesize that this neurosteroid-dependent effect of ethanol results in abnormal “AMPAfication” of glutamatergic synapses, which could induce premature stabilization of synapses, thereby preventing axons that normally arrive at later stages of development from making appropriate connections with target neurons [45]. This effect of ethanol could be a molecular underpinning of the hippocampal abnormalities observed in individuals suffering from FASD. The mechanism by which ethanol increases synaptic levels of PREGS-like neurosteroids is presently unknown. It could involve an increase in the delivery of cholesterol to the inner mitochondrial membrane by the steroidogenic acute regulatory protein or the translocator protein (formerly known as the peripheral benzodiazepine receptor). We are currently investigating these and other possible mechanisms of actions.

4. Modulation of glutamatergic transmission in mature neurons

While there is substantial evidence for the action of sulfated steroids on postsynaptic receptors in more mature synapses (e.g. [47]), there is considerably less evidence for presynaptic sites of action for sulfated neurosteroids in these neurons [20]. For example, microdialysis of PREGS into the nucleus accumbens dose-dependently increases dopamine release [4], intracerebroventricular injection of PREGS increases acetylcholine levels in rat hippocampus [14,44] and DHEAS potentiates and PREGS inhibits NMDA-evoked release of norepinephrine from preloaded hippocampal slices from adult rats [31]. While each of these examples demonstrates sulfated steroid modulation of neurotransmitter release, none of them excludes the possibility that the site of action is upstream from the presynaptic terminal itself. The strongest evidence for a presynaptic site of action for sulfated steroids in mature brains, however, comes from studies of glutamatergic transmission.

Studies of the presynaptic effects of neurosteroids on glutamatergic transmission in mature neurons have been focused primarily on two brain regions, the hippocampal formation and the prefrontal cortex. Figs 5A and B show the enhancement of PPF by PREGS in the adult hippocampus. In most synapses, PPF results from presynaptic residual Ca^{2+} ($[\text{Ca}^{2+}]_{\text{res}}$) and thus modulation of PPF is a good indication of a presynaptic site of action [56]. In CA1, this effect was found for both NMDA and AMPA transmission [34] further supporting a presynaptic effect on glutamate release. Interestingly, this enhancement of PPF by PREGS appears to be a selective effect on facilitated release with little effect on basal release (Fig 5D). In prefrontal cortex slices (P20–30 rats) at 20 μM , both DHEAS [16] and PREGS [17] were shown to increase the frequency of mEPSCs while 1 μM PREGS was found to have no effect on mEPSC frequency. Furthermore, in slices from this same brain region, 1 μM PREGS increased short-term facilitation by decreasing the amplitude of the first of a pair of evoked EPSCs [42]. In the CA1 region of the hippocampus [34,38] and in the dentate [43], PREGS at < 1 μM was shown to enhance facilitated glutamate release without affecting basal release (see Fig 5). In addition, chronic administration of DHEAS to adult rats enhanced LTP in CA1 without affecting basal or facilitated glutamate release [10]. However, optical recordings in the dentate with voltage-sensitive dyes have shown an enhancement by 50 μM PREGS of evoked glutamate release in P40–50 rats [11].

It is noteworthy that a recent study did not detect effects of PREGS on PPF of field excitatory postsynaptic potentials (fEPSPs) in the CA1 region of the hippocampus of male Wistar rats (160–260 g) [37]. However, a PREGS-induced increase in glutamate release (i.e. decrease in the paired-pulse ratio) was detected during the maintenance phase of NMDAR-independent LTP. The reasons for the discrepancies between the findings of this study and those of our experiments are unknown, but these might reflect differences in experimental conditions. For example, PPF was measured at a shorter 40 ms interpulse interval, where feedforward paired pulse inhibition may have a more significant contribution [43], and PREGS was sonicated for 20 – 30 minutes in distilled water rather than being dissolved in DMSO. Nonetheless, the findings of Sabeti et al [37] indicate that the presynaptic actions of PREGS can play a role in the modulatory actions of this neurosteroid on hippocampal synaptic plasticity.

4.1. Differential modulation of basal vs. facilitated glutamate release

There is an important distinction between effects of sulfated steroids on evoked release recorded under basal and facilitated conditions in preparations from mature brains. Basal release is frequently determined by assessing changes in mEPSC frequency, although direct measurements of basal evoked release are also possible under conditions that eliminate contamination from postsynaptic effects. Both DHEAS [16] and PREGS [17] at 20 μ M caused an increase in mEPSC frequency in prelimbic cortex. Interestingly, however, the effect was not detectable when the concentration of PREGS was reduced to 10 μ M [17]. Furthermore, 1 μ M PREGS was found to have no effect on mEPSC frequency in layers V – VI of the prelimbic cortex [42]. As mentioned above, the effect of 25 μ M PREGS to increase mEPSC frequency in the CA1 region of the hippocampus was absent in brains from animals older than P5 [26]. No change in evoked release in the CA1 region of the hippocampus was found using optical signals following chronic administration of DHEAS to rats [10]. A similar absence of an effect was observed in CA1 EPSPs for concentrations of PREGS up to 10 μ M, however at 100 μ M, there was a significant increase in EPSP amplitude [34]. More direct measurements of the probability of evoked glutamate release were carried out by determining the timecourse of decay of NMDA EPSCs in the presence of MK-801 [22]. In this assay, an increase in glutamate release probability is expected to enhance use-dependent block of NMDARs by MK-801. For PREGS at ≤ 1 μ M, there was no change in release probability in either the dentate [43] or in CA1 [38] (Fig 5D) indicating that PREGS does not affect basal (i.e. as opposed to facilitated) evoked release. On the other hand, 50 μ M PREGS was found to enhance evoked EPSPs in the dentate [11] while 1 μ M PREGS depressed evoked EPSPs in the prelimbic cortex [42]. Thus, it appears that the effects of sulfated steroids in different brain regions are highly dependent on the experimental conditions, including the steroid concentrations used (Table 2; for a discussion about endogenous levels of sulfated steroids, see Section 5 and [20]).

We have shown that PREGS at < 1 μ M enhanced PPF in CA1 and dentate without altering the amplitude of the first postsynaptic response [34,35]. This is an interesting observation because it differs from the usually described mechanism in which PPF is increased with decreasing initial release and often this inverse relationship is used as an indicator of the basal level of release. Consistent with this traditional expectation, there is one recent report that a low concentration of PREGS does increase PPF in prelimbic cortex indirectly through a reduction in the initial release [42]. The former independent effect of PREGS on facilitated release, however, was observed at all interpulse intervals between 50 and 300 ms (Fig 5C) and occurred independently of the amplitude of the first evoked response [34,43]. Endogenous sulfated neurosteroids are capable of this same enhancement of facilitated release, since application of the hydroxysteroid sulfatase inhibitor, DU-14, mimicked the effect of exogenously applied PREGS [43].

4.2. Role of sigma receptors

Although it has been shown that sulfated steroids act through presynaptic NMDARs in neonates [26], the mechanisms by which they act in the mature brain is not well understood. One report shows that PREGS acts presynaptically in the dentate with an $EC_{50} = 11.2 \mu\text{M}$ through a mechanism that involves $\alpha 7$ nAChRs and L-type calcium channels to depress PPF [11] while another report, based on studies of prefrontal cortex, indicates that $20 \mu\text{M}$ PREGS acts at presynaptic $\alpha 1$ adrenergic receptors to cause Ca^{2+} release from intracellular stores [17].

Considerable *in vivo* evidence suggests that sigma receptors specifically bind steroids [41] and they have been shown to be involved in rapid non-genomic effects of DHEAS and PREGS in several brain areas [28,29]. At the subcellular level, however, cloned sigma receptors have been localized primarily to endoplasmic reticulum membranes, somas, and dendrites [29,32] and to presynaptic terminals of mossy fibers in CA3 [15] while their unequivocal localization in presynaptic terminals of the CA1 region is less clear. It remains a possibility that sulfated neurosteroids interact with $\sigma 1$ receptors located elsewhere than the presynaptic terminal and that an additional messenger acts to cause the presynaptic effects.

Schiess et al. [38] showed that the $\sigma 1$ receptor agonist, PRE-084, enhances PPF by increasing the amplitude of the second of paired EPSPs without significantly altering the amplitude of the first. This effect occurs with the same input-output relationship and over the same timecourse as does the identical effect of PREGS. Furthermore, the $\sigma 1$ receptor antagonists BD-1063 and IPAG block this PREGS-induced enhancement of PPF. Finally, a 12 hour treatment in pertussis toxin prevents the PREGS-induced enhancement of PPF. One mechanism that is consistent with these observations is that PREGS acts through a $\sigma 1$ receptor and a $G_{i/o}$ pathway to increase PPF by specifically enhancing the process by which the second of a pair of release events is facilitated.

Several additional studies further indicate an involvement of $\sigma 1$ receptors in the presynaptic actions of sulfated steroids. In both prefrontal cortex and hippocampus, $20 \mu\text{M}$ DHEAS acts at least partially through a $\sigma 1$ receptor and a PKA pathway to increase the frequency of mEPSCs [16]. Although similar studies in the prefrontal cortex with $20 \mu\text{M}$ PREGS did not indicate a robust role for the $\sigma 1$ receptor, this receptor was implicated in the hippocampus in the effect of PREGS on mEPSCs. [17].

4.3. Involvement of residual calcium

PPF is generally attributed to result from $[\text{Ca}^{2+}]_{\text{res}}$ that remains in the presynaptic terminal for a few hundred milliseconds after an evoked release. [7,25]. One commonly accepted model is that this $[\text{Ca}^{2+}]_{\text{res}}$ acts to facilitate release through a site that is independent of the primary release site [2]. Furthermore, PREGS and DHEAS, acting through $\sigma 1$ receptors, are known to have effects on cytoplasmic Ca^{2+} homeostasis [29]. It was thus reasonable to determine the involvement of presynaptic $[\text{Ca}^{2+}]_{\text{i}}$ in the effect of PREGS on facilitated release.

One early suggestion of the involvement of presynaptic Ca^{2+} ($[\text{Ca}^{2+}]_{\text{pre}}$) mechanisms in the action of neurosteroids was the demonstration that PREGS, with an IC_{50} of 11 nM inhibited peak I_{Ca} in freshly dissociated pyramidal neurons from adult guinea pig hippocampus. This effect was blocked by pertussis toxin, GDP- βS , and the kinase inhibitors H-7 and staurosporine. These authors proposed that PREGS acted through a G-protein dependent pathway to modulate Ca^{2+} influx and thereby regulate neurotransmitter release [18].

We used simultaneous measurements of $[\text{Ca}^{2+}]_{\text{pre}}$ in presynaptic Schaffer collateral fibers that were filled with the fluorescent Ca^{2+} indicator Mg^{2+} -green-AM and postsynaptic fEPSPs in CA1 pyramidal neurons (Fig 6A) to demonstrate that PREGS acts presynaptically at a site downstream from $[\text{Ca}^{2+}]_{\text{pre}}$ to enhance facilitated glutamate release [39]. Several experimental

observations support this conclusion. First PREGS enhancement of fEPSP PPF occurs without a similar increase in $[Ca^{2+}]_{res}$ (Fig 6A–B). Rather than a possible increase in $[Ca^{2+}]_{res}$ in PREGS, there is a decrease in the $\Delta F/F_0$ of the $[Ca^{2+}]_{res}$ -subtracted second of paired responses as compared with that seen in ACSF (see [39] for details). Enhancement of PPF by PREGS occurs with no change in presynaptic Ca^{2+} buffering. Importantly, PREGS causes a leftward shift of the facilitated synaptic input-output relationship (the second fEPSP population spike as a function of simultaneously recorded $\Delta F/F_0$ from which $[Ca^{2+}]_{res}$ has been subtracted (Fig 6C). Finally, modeling studies suggest that the action of PREGS is to increase the fraction of vesicles influenced by $[Ca^{2+}]_{res}$. Our current working hypothesis for the mechanism of action of PREGS at mature CA1 presynaptic terminals is summarized in Fig 7.

4.4. Potential functional significance

An important consideration is the functional implication of the presynaptic modulatory effects of neurosteroids on glutamate release in mature neurons. As an extension of its effect on PPF, we have shown that PREGS enhances the postsynaptic responses to repetitive stimuli at the Schaffer collateral input to CA1 [35]. This effect is most pronounced between 10 and 50 Hz with little or no enhancement at higher or lower frequencies. Thus PREGS acts to produce a rather sharp bandpass filter characteristic for signals at this synapse. A similar frequency-dependent enhancement of glutamatergic transmission is observed in the dentate. However, at short interpulse intervals, enhanced presynaptic facilitation paradoxically increases paired-pulse inhibition by enhancing basket cell inhibitory input to granule cells. Thus, as in CA1, PREGS produces a bandpass filter characteristic on dentate transmission, but the high frequency cut-off of this filter is greatly sharpened by the enhanced short-interval inhibition [43]. Transmission through the trisynaptic circuit of the hippocampus would be subject to both of these frequency-dependent modulatory effects of PREGS along with any effects that PREGS may have in CA3. This tuning may have important implications in processing of spatial/relational information by the adult hippocampus.

5. Conclusion

Evidence from studies conducted at a number of laboratories indicates that sulfated steroids modulate glutamate release via complex mechanisms that are age- and brain region-dependent. These actions are likely to play important roles in physiological processes, such as synapse maturation, information processing and memory formation, as well as pathophysiological states such as FASD and perhaps other neurodevelopmental disorders. The presynaptic actions of sulfated steroids could also have neuropsychopharmacological applications in the future. It should be emphasized that recent studies have questioned whether PREGS and DHEAS are veritable neurosteroids; i.e. these studies have determined that brain levels of these sulfated steroids are substantially lower than was earlier estimated using more indirect techniques (reviewed in [20]). Thus, the chemical nature of the endogenous counterparts of these sulfated steroids is presently uncertain. We found, however, that the actions of exogenous PREGS (and DHEAS in some cases) are mimicked by a hydroxysteroid sulfatase inhibitor, and/or an endogenous agent that is released and/or produced in response to neuronal depolarization or developmental alcohol exposure. These results suggest that the brain produces sulfated neurosteroids that are chemically related to PREGS and/or DHEAS.

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References

1. Allan AM, Wu H, Paxton LL, Savage DD. Prenatal ethanol exposure alters the modulation of the gamma-aminobutyric acidA1 receptor-gated chloride ion channel in adult rat offspring. *J Pharmacol Exp Ther* 1998;284:250–257. [PubMed: 9435185]
2. Atluri PP, Regehr WG. Determinants of the time course of facilitation at the granule cell to Purkinje cell synapse. *J Neurosci* 1996;16:5661–5671. [PubMed: 8795622]
3. Bailey CD, Brien JF, Reynolds JN. Altered GABA(A)-benzodiazepine receptor number and pharmacology in the adult guinea pig cerebral cortex after chronic prenatal ethanol exposure. *Alcohol Clin Exp Res* 1999;23:1816–1824. [PubMed: 10591599]
4. Barrot M, Vallee M, Gingras MA, Le Moal M, Mayo W, Piazza PV. The neurosteroid pregnenolone sulphate increases dopamine release and the dopaminergic response to morphine in the rat nucleus accumbens. *Eur J Neurosci* 1999;11:3757–3760. [PubMed: 10564382]
5. Bouron A. Modulation of spontaneous quantal release of neurotransmitters in the hippocampus. *Prog Neurobiol* 2001;63:613–635. [PubMed: 11164998]
6. Bukusoglu C, Sarlak F. Pregnenolone sulfate increases intracellular Ca²⁺ levels in a pituitary cell line. *Eur J Pharmacol* 1996;298:79–85. [PubMed: 8867923]
7. Cabezas C, Buno W. Distinct transmitter release properties determine differences in short-term plasticity at functional and silent synapses. *J Neurophysiol* 2006;95:3024–3034. [PubMed: 16436482]
8. Caldeira JC, Wu Y, Mameli M, Purdy RH, Li PK, Akwa Y, Savage DD, Engen JR, Valenzuela CF. Fetal alcohol exposure alters neurosteroid levels in the developing rat brain. *J Neurochem* 2004;90:1530–1539. [PubMed: 15341536]
9. Carta M, Partridge LD, Savage DD, Valenzuela CF. Neurosteroid modulation of glutamate release in hippocampal neurons: lack of an effect of a chronic prenatal ethanol exposure paradigm. *Alcohol Clin Exp Res* 2003;27:1194–1198. [PubMed: 12878928]
10. Chen L, Dai XN, Sokabe M. Chronic administration of dehydroepiandrosterone sulfate (DHEAS) primes for facilitated induction of long-term potentiation via sigma 1 (sigma1) receptor: optical imaging study in rat hippocampal slices. *Neuropharmacology* 2006;50:380–392. [PubMed: 16364377]
11. Chen L, Sokabe M. Presynaptic modulation of synaptic transmission by pregnenolone sulfate as studied by optical recordings. *J Neurophysiol* 2005;94:4131–4144. [PubMed: 15972828]
12. Corpechot C, Synguelakis M, Talha S, Axelson M, Sjoval J, Vihko R, Baulieu EE, Robel P. Pregnenolone and its sulfate ester in the rat brain. *Brain Res* 1983;270:119–125. [PubMed: 6223687]
13. Costa ET, Olivera DS, Meyer DA, Ferreira VM, Soto EE, Frausto S, Savage DD, Browning MD, Valenzuela CF. Fetal alcohol exposure alters neurosteroid modulation of hippocampal N-methyl-D-aspartate receptors. *J Biol Chem* 2000;275:38268–38274. [PubMed: 10988286]
14. Darnaudery M, Koehl M, Piazza PV, Le Moal M, Mayo W. Pregnenolone sulfate increases hippocampal acetylcholine release and spatial recognition. *Brain Res* 2000;852:173–179. [PubMed: 10661509]
15. Debonnel G, Bergeron R, de Montigny C. Potentiation by dehydroepiandrosterone of the neuronal response to N-methyl-D-aspartate in the CA3 region of the rat dorsal hippocampus: an effect mediated via sigma receptors. *J Endocrinol* 1996;150:S33–S42. [PubMed: 8943785]
16. Dong LY, Cheng ZX, Fu YM, Wang ZM, Zhu YH, Sun JL, Dong Y, Zheng P. Neurosteroid dehydroepiandrosterone sulfate enhances spontaneous glutamate release in rat prelimbic cortex through activation of dopamine D1 and sigma-1 receptor. *Neuropharmacology* 2007;52:966–974. [PubMed: 17141281]
17. Dong Y, Fu YM, Sun JL, Zhu YH, Sun FY, Zheng P. Neurosteroid enhances glutamate release in rat prelimbic cortex via activation of alpha1-adrenergic and sigma1 receptors. *Cell Mol Life Sci* 2005;62:1003–1014. [PubMed: 15798892]
18. French-Mullen JM, Danks P, Spence KT. Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G-protein-coupled mechanism. *J Neurosci* 1994;14:1963–1977. [PubMed: 8158251]

19. Garaschuk O, Hanse E, Konnerth A. Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J Physiol* 1998;507(Pt1):219–236. [PubMed: 9490842]
20. Gibbs TT, Russek SJ, Farb DH. Sulfated steroids as endogenous neuromodulators. *Pharmacol Biochem Behav* 2006;84:555–567. [PubMed: 17023038]
21. Goodlett CR, Horn KH, Zhou FC. Alcohol teratogenesis: mechanisms of damage and strategies for intervention. *Exp Biol Med (Maywood)* 2005;230:394–406. [PubMed: 15956769]
22. Hessler NA, Shirke AM, Malinow R. The probability of transmitter release at a mammalian central synapse. *Nature* 1993;366:569–572. [PubMed: 7902955]
23. Hige T, Fujiyoshi Y, Takahashi T. Neurosteroid pregnenolone sulfate enhances glutamatergic synaptic transmission by facilitating presynaptic calcium currents at the calyx of Held of immature rats. *Eur J Neurosci* 2006;24:1955–1966. [PubMed: 17040476]
24. Iqbal U, Dringenberg HC, Brien JF, Reynolds JN. Chronic prenatal ethanol exposure alters hippocampal GABA(A) receptors and impairs spatial learning in the guinea pig. *Behav Brain Res* 2004;150:117–125. [PubMed: 15033285]
25. Katz B, Miledi R. The role of calcium in neuromuscular facilitation. *J Physiol* 1968;195:481–492. [PubMed: 4296699]
26. 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]
27. Mameli M, Valenzuela CF. Alcohol increases efficacy of immature synapses in a neurosteroid-dependent manner. *Eur J Neurosci* 2006;23:835–839. [PubMed: 16487164]
28. Maurice T. Neurosteroids and sigma1 receptors, biochemical and behavioral relevance. *Pharmacopsychiatry* 2004;37:S171–S182. [PubMed: 15547783]
29. Maurice T, Gregoire C, Espallergues J. Neuro(active)steroids actions at the neuromodulatory sigma1 (sigma1) receptor: biochemical and physiological evidences, consequences in neuroprotection. *Pharmacol Biochem Behav* 2006;84:581–597. [PubMed: 16945406]
30. Meyer DA, Carta M, Partridge LD, Covey DF, Valenzuela CF. Neurosteroids enhance spontaneous glutamate release in hippocampal neurons. Possible role of metabotropic sigma1-like receptors. *J Biol Chem* 2002;277:28725–28732. [PubMed: 12042305]
31. Monnet FP, Mahe V, Robel P, Baulieu EE. Neurosteroids, via sigma receptors, modulate the [3H] norepinephrine release evoked by N-methyl-D-aspartate in the rat hippocampus. *Proc Natl Acad Sci U S A* 1995;92:3774–3778. [PubMed: 7731982]
32. Monnet FP, Maurice T. The sigma1 protein as a target for the non-genomic effects of neuro(active) steroids: molecular, physiological, and behavioral aspects. *J Pharmacol Sci* 2006;100:93–118. [PubMed: 16474209]
34. Morin-Surun MP, Collin T, Denavit-Saubie M, Baulieu EE, Monnet FP. Intracellular sigma1 receptor modulates phospholipase C and protein kinase C activities in the brainstem. *Proc Natl Acad Sci U S A* 1999;96:8196–8199. [PubMed: 10393971]
34. Partridge LD, Valenzuela CF. Neurosteroid-induced enhancement of glutamate transmission in rat hippocampal slices. *Neurosci Lett* 2001;301:103–106. [PubMed: 11248433]
35. Partridge LD, Valenzuela CF. Neurosteroids enhance bandpass filter characteristics of the rat Schaffer collateral-to-CA1 synapse. *Neurosci Lett* 2002;326:1–4. [PubMed: 12052524]
36. Rupprecht R, Holsboer F. Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci* 1999;22:410–416. [PubMed: 10441302]
37. 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
38. Schiess AR, Partridge LD. Pregnenolone sulfate acts through a G-protein-coupled sigma1-like receptor to enhance short term facilitation in adult hippocampal neurons. *Eur J Pharmacol* 2005;518:22–29. [PubMed: 15996654]

39. 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]
40. Spence KT, Plata-Salaman CR, French-Mullen JM. The neurosteroids pregnenolone and pregnenolone-sulfate but not progesterone, block Ca^{2+} currents in acutely isolated hippocampal CA1 neurons. *Life Sci* 1991;49:PL235–PL239. [PubMed: 1660951]
41. Su TP. Sigma receptors. Putative links between nervous, endocrine and immune systems. *Eur J Biochem* 1991;200:633–642. [PubMed: 1655424]
42. Sun JL, Dong YL, Fu YM, Zhu YH, Dong Y, Zheng P. Neurosteroid pregnenolone sulfate inhibits stimulus-evoked EPSC via presynaptic inhibition of protein kinase A in rat prelimbic cortical neurons. *Neuropharmacology* 2005;49:389–399. [PubMed: 15993435]
43. Thomas MJ, Mameli M, Carta M, Valenzuela CF, Li PK, Partridge LD. Neurosteroid paradoxical enhancement of paired-pulse inhibition through paired-pulse facilitation of inhibitory circuits in dentate granule cells. *Neuropharmacology* 2005;48:584–596. [PubMed: 15755486]
44. Vallee M, Shen W, Heinrichs SC, Zorumski CF, Covey DF, Koob GF, Purdy RH. Steroid structure and pharmacological properties determine the anti-amnesic effects of pregnenolone sulphate in the passive avoidance task in rats. *Eur J Neurosci* 2001;14:2003–2010. [PubMed: 11860495]
45. Voigt T, Opitz T, de Lima AD. Activation of early silent synapses by spontaneous synchronous network activity limits the range of neocortical connections. *J Neurosci* 2005;25:4605–4615. [PubMed: 15872108]
46. Wang Q, Wang L, Wardwell-Swanson J. Modulation of cloned human neuronal voltage-gated potassium channels (hKv1.1 and hKv2.1) by neurosteroids. *Pflugers Arch* 1998;437:49–55. [PubMed: 9817785]
47. 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]
48. Yu R, Hay M, Ticku MK. Chronic neurosteroid treatment attenuates single cell GABAA response and its potentiation by modulators in cortical neurons. *Brain Res* 1996;706:160–162. [PubMed: 8720505]
49. Yu R, Ticku MK. Chronic neurosteroid treatment decreases the efficacy of benzodiazepine ligands and neurosteroids at the gamma-aminobutyric acidA receptor complex in mammalian cortical neurons. *J Pharmacol Exp Ther* 1995;275:784–789. [PubMed: 7473167]
50. Yu R, Ticku MK. Chronic neurosteroid treatment produces functional heterologous uncoupling at the gamma-aminobutyric acid type A/benzodiazepine receptor complex in mammalian cortical neurons. *Mol Pharmacol* 1995;47:603–610. [PubMed: 7700257]
51. Zhang L, Weiner JL, Valiante TA, Velumian AA, Watson PL, Jahromi SS, Schertzer S, Pennefather P, Carlen PL. Whole-cell recording of the Ca^{2+} -dependent slow afterhyperpolarization in hippocampal neurones: effects of internally applied anions. *Pflugers Arch* 1994;426:247–253. [PubMed: 8183635]
52. Zhang X, Sliwowska JH, Weinberg J. Prenatal alcohol exposure and fetal programming: effects on neuroendocrine and immune function. *Exp Biol Med (Maywood)* 2005;230:376–388. [PubMed: 15956767]
53. Zimmerberg B, Brown RC. Prenatal experience and postnatal stress modulate the adult neurosteroid and catecholaminergic stress responses. *Int J Dev Neurosci* 1998;16:217–228. [PubMed: 9785118]
54. 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]
55. Zimmerberg B, McDonald BC. Prenatal alcohol exposure influences the effects of neuroactive steroids on separation-induced ultrasonic vocalizations in rat pups. *Pharmacol Biochem Behav* 1996;55:541–547. [PubMed: 8981584]
56. Zucker RS. Short-term synaptic plasticity. *Annu Rev Neurosci* 1989;12:13–31. [PubMed: 2648947]

Figure 1. The PREGS-induced increase of mEPSC frequency depends on extracellular Ca²⁺ in cultured hippocampal neurons

Upper panel shows sample traces illustrating the effect of 20 μ M PREGS on mEPSC frequency recorded in the absence or presence of 2 mM external Ca²⁺. For this study, each neuron was sequentially exposed to Ca²⁺-free and 2 mM Ca²⁺-containing solutions. Scale bars = 16.4 pA and 655 ms. Lower panel shows a summary of the results obtained with 5 neurons. * $p < 0.014$ by t-test.

Figure 2. The PREGS-induced increase of mEPSC frequency does not depend on Ca^{2+} release from the endoplasmic reticulum in cultured hippocampal neurons

Upper panel shows sample traces illustrating the effect of 20 μM PREGS on mEPSC frequency recorded from neurons pre-incubated for 30–45 min at 37 °C with the sarco-endoplasmic reticulum Ca^{2+} ATPase inhibitor, thapsigargin (0.4 μM) or the inositol triphosphate receptor antagonist, xestospongine C (0.5 μM) [5]. Scale bars = 16.4 pA and 655 ms. Lower panel summarizes the effect of PREGS in control neurons and those pre-exposed to these agents (n = 5 cells for each group).

Fig. 3. Working model for the action of PREGS-like steroids at immature CA1 hippocampal glutamatergic synapses

A. Under control conditions, neonatal CA1 hippocampal pyramidal neurons could have a mixture of glutamatergic synapses containing both postsynaptic AMPARs (blue) and NMDARs (red) (active synapses) or only postsynaptic NMDARs (silent synapses at rest). A silent synapse is enlarged. Presynaptic NMDARs (orange) are also shown. **B.** Brief exposure to PREGS increases Ca^{2+} influx through presynaptic NMDARs, leading to higher glutamate release probability and activation of postsynaptic NMDARs. Pre- and postsynaptic NMDARs at these synapses might contain NR2D subunits, making them less sensitive to Mg^{2+} block. **C.** Glutamate release probability returns to baseline levels after PREGS washout. The postsynaptic increase in $[\text{Ca}^{2+}]$ shown in panel B elicits delayed insertion of AMPARs only in silent synapses.

Fig. 4. PREGS activates silent synapses

A. Sample traces illustrating a whole-cell patch-clamp recording from CA1 pyramidal neurons in hippocampal slices from a P3 rat. Note the lack of mEPSC activity during control (Ctrl) conditions and appearance of mEPSCs after 25 μ M PREGS exposure. This effect is irreversible upon washout. **B.** Time course corresponding to the recording shown in A. NBQX blocked the events indicating these are AMPAR mediated. **C.** Cumulative probability plot illustrating that mEPSC inter-event interval distribution does not differ at 10 vs 25 min after PREGS exposure. Average mEPSC traces further illustrating the lack of an effect of PREGS on mEPSC amplitude at these time points. **D.** Summary of the effect of PREGS or vehicle (DMSO) application (represented by the gray bar) on the number of events as a function of time ($n = 8-12$).

Figure 5. PREGS enhances facilitated release in adult Schaffer collateral presynaptic terminals in CA1 pyramidal neurons and perforant-path terminals in dentate granule neurons

A. Intracellularly recorded NMDA EPSPs in CA1 following paired Schaffer collateral stimuli in the presence of 20 μM bicuculline and 40 μM CNQX in 0 Mg^{2+} ACSF, after 5 min of 1 μM PREGS, and after 35 min of wash. **B.** Whole-cell patch-clamp recording of glutamatergic EPSCs from a dentate granule cell following paired stimuli to perforant pathway before and after 20 min of 300 nM PREGS exposure in ACSF. **C.** PPF timecourse in CA1 in ACSF (dotted, n=13) and following exposure to PREGS (solid, n=14). PREGS (100 μM) caused significant enhancement of PPF (p<0.005) at all interpulse intervals between 50 and 250 ms. **D.** Block of NMDA currents in 50 μM MK-801 in ACSF (■ solid line, n=7) or following exposure to 1 μM PREGS (● dotted line, n=4). There was no significant difference in the time constants of single exponential least squares regression fits. Modified with permission from [34,38,43]. © Neurosci Lett, Neuropharm and Eur J. Pharmacol

Figure 6. PREGS acts downstream from presynaptic $[\text{Ca}^{2+}]_{\text{res}}$ to enhance PPF

A. $\Delta F/F_0$ measured with Mg green (top) and simultaneously measured fEPSPs (bottom) in CA1 following paired stimuli to Schaffer collaterals (note different time scales). When compared with ACSF (A1), 1 μM PREGS (A2) enhances the second fEPSP without increasing $\Delta F/F_0$. **B.** fEPSP PPF was normalized to the maximum value in ACSF and plotted against simultaneously recorded $[\text{Ca}^{2+}]_{\text{res}}$. Data from ACSF (grey) and 1 μM PREGS (black) are fitted with least squares linear regressions ($n=6$). If PREGS enhanced PPF by increasing $[\text{Ca}^{2+}]_{\text{res}}$, it would be expected that the PREGS data (black) would fall to the right along the same line (grey) that fits the ACSF data. **C.** A typical synaptic input-output relationship for the second of paired pulses in ACSF ($\bullet$, grey) or in 1 μM PREGS ($\blacksquare$, black). In 6 similar experiments, there was a significant increase in y_{max} and decrease in EC_{50} for Hill functions fits. These findings are consistent with an increase in Ca^{2+} efficacy and potency, respectively, for glutamate release induction. Modified with permission from [39]. © J. Physiology.

Fig. 7. Working model for the action of PREGS-like steroids at mature hippocampal glutamatergic synapses

A1. Under control conditions, an axonal action potential activates voltage-gated Ca²⁺ channels, leading to glutamate release and activation of a few postsynaptic glutamatergic receptors (shown in red). Residual Ca²⁺ ([Ca²⁺]_{res}) following the first release interacts with the vesicle release machinery making it transiently more sensitive to subsequent Ca²⁺ influx. **A2.** Arrival of a second axonal action potential 50–300 ms after the first one elicits greater glutamate release and PPF due to the increased Ca²⁺ sensitivity of the release machinery. **B1.** PREGS does not affect basal glutamate release probability at t=0. **B2.** PREGS, via an effect dependent on σ₁-like receptors and G_{i/o}, increases the fraction of vesicles for which [Ca²⁺]_{res} increases the sensitivity to the second Ca²⁺ influx, thereby enhancing PPF. Note that [Ca²⁺]_{res} is slightly reduced in the presence of PREGS (see text for details).

Table 1

Chronological Summary of Studies on the Effect of Developmental Ethanol Exposure on Neurosteroid Levels and/or Modulatory Actions.^a

Neurosteroid	Assay	Region	Effect	Species and Offspring Age	EtOH Exposure Paradigm	Maternal Blood Alcohol Levels	Ref
Allopregnanolone	Maternal separation-induced ultrasonic vocalizations	Intracerebroventricular (injection)	Decrease in anxiolytic effect	Rat-P 6	Prenatal-liquid diet	Not reported	[54]
PREGS	Maternal separation-induced ultrasonic vocalizations	Intracerebroventricular (injection)	Decrease in low dose anxiolytic and high dose anxiogenic effects	Rat-P 7	Prenatal-liquid diet	Not reported	[55]
Allopregnanolone	Radioimmunoassay	Prefrontal cortex and hippocampus	Increase in levels in response to swim stress	Rat-Adult	Prenatal-liquid diet plus early maternal separation-induced stress	Not reported	[53]
PREGS	Modulation of ³⁶ Cl ⁻ flux into microsacs	Prefrontal cortex	Decrease inhibition of GABA _A receptor function	Rat-Adult	Prenatal-liquid diet	0.08 g/dl	[1]
Allopregnanolone	Modulation of [³ H]-flunitrazepam or [³ H]-muscimol binding	Cerebral cortex (membranes)	No effect	Guinea Pig-P 11, P 21, Adult	Prenatal-oral intubation	0.24 g/dl	[3]
Allopregnanolone	Modulation of [³ H]-flunitrazepam binding	Hippocampus (membranes)	Decreased in efficacy and potency	Guinea Pig-Adult	Prenatal-oral intubation	0.28 g/dl	[24]
PREGS	Whole-cell patch-clamp recordings	Hippocampus (cultured neurons)	Decreased potentiation of NMDARs. Inhibition of non-NMDARs and GABA _A -Rs was unaffected	Rat-P 3-4 plus 6-7 days in dispersed culture	Prenatal-liquid diet	0.08 g/dl	[13]
PREGS	Whole-cell patch-clamp and sharp electrode recordings	Hippocampus (cultured neurons and acute slices)	Presynaptic actions of PREGS were unaffected	Rat-P 3-4 plus 8-12 days in dispersed culture and slices from adult	Prenatal-liquid diet	0.08 g/dl	[9]
PREGS PREG DHEAS Allopregnanolone	Radioimmunoassay	Whole brain	Increase in PREGS and PREG levels only	Rat-E 14 to P 21	Prenatal-liquid diet	0.08 g/dl	[8]
PREGS	Whole-cell patch-clamp recordings	Hippocampus (acute slices)	Ethanol-induced potentiation of AMPAR-mediated neurotransmission that was PREGS dependent	Rat-P 3-4	Acute ethanol exposure of neonatal slices	Not applicable (in vitro concentration was near 0.08 g/dl)	[27]

^aFor an excellent recent review of the role of peripherally produced steroids on FASD, see [52]

Table 2

Summary of Studies on Modulation of Glutamate Release by Sulfated Steroids.

Steroid	Concentration	Region	Age	Spontaneous	Evoked	Facilitated	Target	Reference
PREGS	10 – 50 μ M	Hippocampal formation	P 3–4 plus 8–12 days in dispersed culture	Increased mEPSC freq	Increased EPSC ₁	Decreased PPF	G _{i/o} -coupled σ_1 -like receptor	[30]
PREGS	17 – 50 μ M	CA1	< P 5; no effect P 6–21	Increased mEPSC freq	Increased EPSC ₁	Decreased PPF	Presynaptic NMDAR	[26]
PREGS	1–100 μ M	CA1	P 50	Not determined	No effect on EPSC ₁	Enhanced PPF	G _{i/o} -coupled σ_1 -like receptor; not through enhancement of [Ca ²⁺] _{res}	[34,38,39]
PREGS	5 – 15 μ M	CA1	Adult	Not determined	No effect	Increased LTP; decreased paired-pulse ratio during LTP	LTP depended on σ_1 receptor and L-type Ca ²⁺ channels	[37]
DHEAS	20 mg/kg for 7 days	CA1	P 40 – 50	Not determined	No effect	No effect; enhanced LTP but not release in response to tetanus	σ_1 -like receptor	[10]
PREGS	0.3–1 μ M	Dentate	P 50	Not determined	No effect on EPSC ₁	Enhanced PPF	Not determined	[43]
PREGS	25–100 μ M	Dentate	P 40–50	Not determined	Potentiated EPSC ₁	Depressed PPF	α_7 nAChR and L-type Ca ²⁺ channels	[11]
PREGS	25–100 μ M	Calyx of Held	P7–9	Increased mEPSC freq	Potentiated EPSC	Not determined	P/Q- and N-type Ca ²⁺ channels	[23]
PREGS	20 μ M	Prelimbic cortex and hippocampus	P30	Increased mEPSC freq	Not determined	Not determined	σ_1 -like receptor (hippocampus) plus α_1 adrenergic, intracellular Ca ²⁺ PKC, adenylyl cyclase and PKA (cortex)	[17]
PREGS	1–20 μ M	Prelimbic cortex	P 20 – 30	No effect on mEPSCs	Decreased EPSC ₁	Increased PPF	PKA	[42]
DHEAS	15–40 μ M	Prelimbic cortex and hippocampus	P 30	Increased mEPSC freq	Not determined	Not determined	σ_1 receptor in both regions; D1 receptor, adenylyl cyclase and protein kinase A in the prelimbic cortex.	[16]