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Review

Neuroactive steroids: new biomarkers of cognitive aging☆

Monique Vallée[a,*], Robert H. Purdy[b], Willy Mayo[a], George F. Koob[b], Michel Le Moal[a]

[a] *INSERM U588, Institut F. Magendie, 1 rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France*

[b] *Department of Neuropharmacology, CVN-7, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, USA*

Abstract

Intensive studies in animals established that neuroactive steroids display neuronal actions and influence behavioral functions. We describe here investigations on the role of neuroactive steroids in learning and memory processes during aging and suggest their role as biomarkers of cognitive aging. Our work demonstrated the role of the steroid pregnenolone (PREG) sulfate as a factor underlying an individual's age-related cognitive decline in animals. As new perspectives of research we argue that knowing whether neuroactive steroids exist as endogenous neuromodulators and modulate physiologically behavioral functions is essential. To this end, a new approach using the sensitive, specific, and accurate quantitative determination of neuroactive steroids by mass spectrometry seems to have potential for examining the role of each steroid in discrete brain areas in learning and memory alterations, as observed during aging.

Keywords: Cognitive aging; Neuroactive steroids; Pregnenolone sulfate

Contents

1. Introduction

1.1. Neurosteroids: a new concept of brain–steroid interaction

Steroid hormones have profound influences on brain functions. Synthesized from adrenals and gonads, they can easily cross the blood–brain barrier and then accumulate within the brain. During the last two decades the investigation of hormone–brain interactions gained much greater attention due to the concept of neurosteroids. This concept derived from observations made in the 1980s by Baulieu and coworkers (reviewed in [8]). They demonstrated that some steroids, such as pregnenolone (PREG), dehydroepiandrosterone (DHEA), their sulfates (PREGS and DHEAS, respectively) and lipoidal esters are found in larger amounts in brain than in blood of rodents. Moreover, these steroids were still detectable within the brain 15 days after removing the peripheral glands responsible for steroidogenesis,

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* Corresponding author. Tel.: +33-5-5757-3666; fax: +33-5-5757-3669.
E-mail address: vallee@bordeaux.inserm.fr (M. Vallée).

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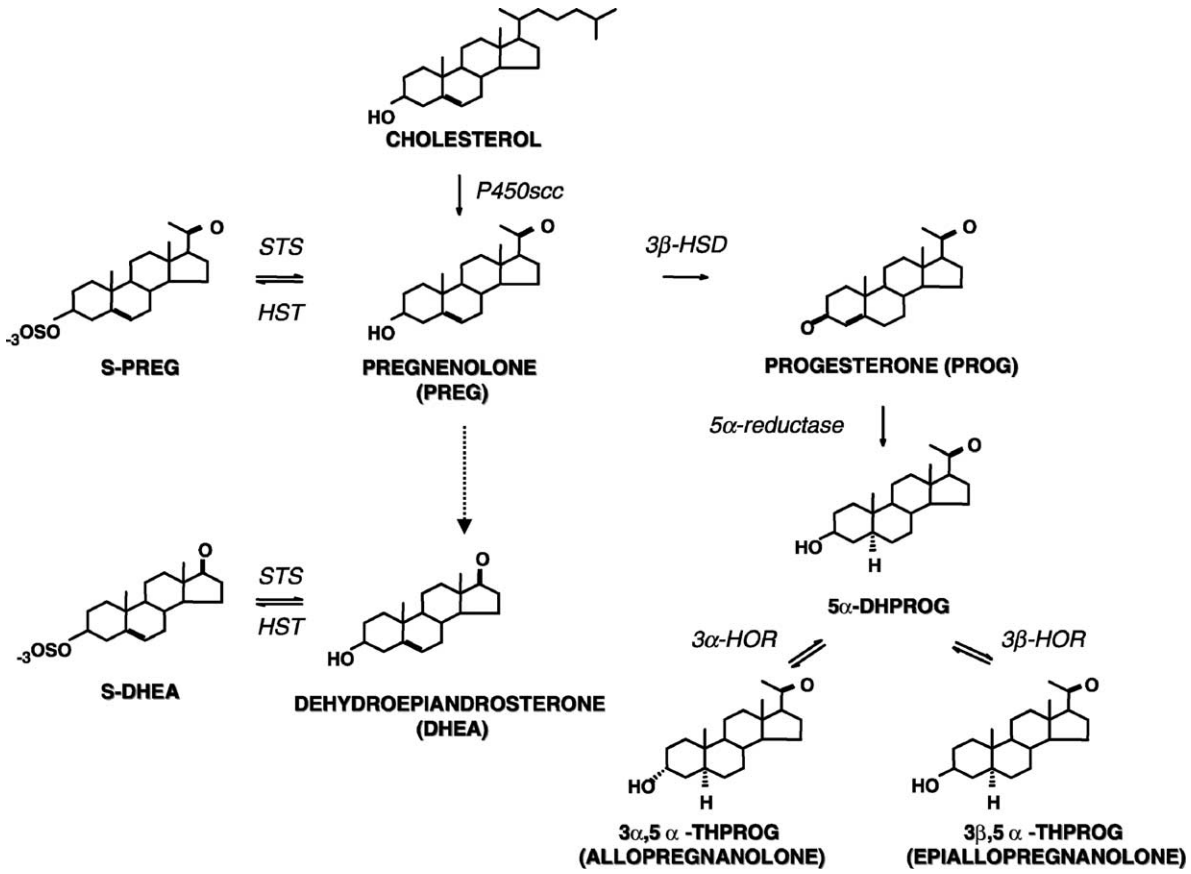

Fig. 1. Neurosteroid biosynthesis and metabolism in rat brain. Enzymes are presented in italics: *3α-HOR*: 3α-hydroxysteroid oxidoreductase; *3β-HOR*: 3β-hydroxysteroid oxidoreductase; *3β-HSD*: 3β-hydroxysteroid dehydrogenase; *HTS*: hydroxysteroid sulfotransferase; *P450scc*: cholesterol side chain cleavage cytochrome P450; *STS*: steroid sulfatase sulfohydrolase.

suggesting a biosynthesis of these steroids within the brain rather than as an accumulation from the periphery [7,14]. Such steroids that are biosynthesized from cholesterol in the central and peripheral nervous system are now referred to as neurosteroids (see Fig. 1). Animal studies have now firmly established the identification and localization of the enzymes required for neurosteroid synthesis in the nervous system during development and in adult rat brain (reviewed in [39,40]). Furthermore, the cerebral biosynthetic pathway of neurosteroids has been described in human brain as well (reviewed in [51]). In this review we designate a neurosteroid as any compound which has been shown to be biosynthesized from cholesterol in the nervous system, notwithstanding its formation in the adrenals and gonads. Because it is difficult to isolate specific neurosteroid effects from actions of metabolites of steroids that cross the blood–brain barrier, we are using here the general term of neuroactive steroids.

1.2. Neuroactive steroids and non-classical steroid receptors

Neuroactive steroids can rapidly modulate neurotransmission in an excitatory or inhibitory manner through binding to neurotransmitter-gated ion channels, rather than through the nuclear steroid receptors that promote the classical genomic action of steroids [44]. Among them, *N*-methyl-D-aspartate receptors (NMDARs) and γ-aminobutyric acid A receptors ($GABA_A$Rs) are the most potently affected. For example, it is clearly established that the neurosteroid PREGS enhances NMDA-activated currents and inhibits GABA-mediated currents in cultured rat hippocampal neurons [10,23,24,41,52]. In addition to ion channels, PREGS also has been shown to target metabotropic receptors, such as sigma (σ) receptors (reviewed in [32]). Moreover, Kimoto et al. [27] recently show a local neuronal synthesis of PREGS in hippocampal neurons of male adult rats, and the authors suggest the possibility of PREGS acting as a local mediator that contributes to glutamate-dependent neuronal excitability in the hippocampus, which is a brain area involved essentially in learning and memory processes [16].

2. Neuroactive steroids and cognitive aging

2.1. Neuroactive steroids as factors underlying an individual's age-related cognitive decline

Animal studies have shown that neuroactive steroids can affect multiple brain functions, such as behavioral

functions. In this regard, increasing evidence suggests that some neuroactive steroids play a critical role in learning and memory. For example, studies in rodents found that PREGS is one of the most effective memory-enhancing neuroactive steroids [18,35]. Knowing the importance of promnesic drugs in aging research, we investigated the physiological relevance of the action of PREGS on memory processes in aged rats (22–24-month-old). Two levels of investigations were carried on. First, the concentrations of PREGS were measured in memory deficient aged rats and in aged rats having memory capacities comparable to those of young animals. Second, we looked at the neuronal membrane targets that could be involved in the mechanisms underlying the memory action of PREGS.

2.2. The aged rat model

Our investigation on neuroactive steroid and cognitive aging was mainly motivated by the fact that memory deficit, the most prominent age-related functional decline in the central nervous system, exhibits inter-individual differences in humans [29] and in animals as well [13,45]. The heterogeneity as to the severity of the age-related cognitive deficits is of great interest for investigating the neurobiological factors underlying cognitive function, especially the neurobiological processes at the origin of age-related cognitive decline (reviewed in [9]). Aged rats serve as a useful model of human aging, especially of age-related memory dysfunctions [6,49]. Besides showing variability in age-related impairments of cognitive functioning, small rodents also possess other advantages for aging research: they have a relatively short lifespan (2–3 years) and their environment can be strictly controlled. Thus, studying the aged rat model allows the investigation of underlying substrates of cognitive processes and biomarkers of cognitive aging. Moreover, this model can be used to assess the effects of putative neuroprotective and/or cognition-enhancing compounds or treatments [3].

2.3. PREGS as biomarker of cognitive aging

Using the aged rat's model, we have shown that hippocampal administration of PREGS offsets the age-related memory deficits (Fig. 2) [54]. This pharmacological effect may have physiological relevance based on evidence that aged rats have decreased levels of PREGS, measured by radioimmunoassay, in the hippocampus compared to young animals [54]. Moreover, levels of PREGS in the hippocampus are positively correlated with the cognitive ability of aged rats (that is, animals that had lower PREGS levels displayed poorer learning and memory abilities) (see Fig. 3) [54]. However, there was no relationship between the performance and the PREGS content in other brain areas (such as the amygdala, frontal cortex, parietal cortex, and striatum) or in plasma. These results suggest a local action of PREGS in the hippocampus resulting in modulation of memory process.

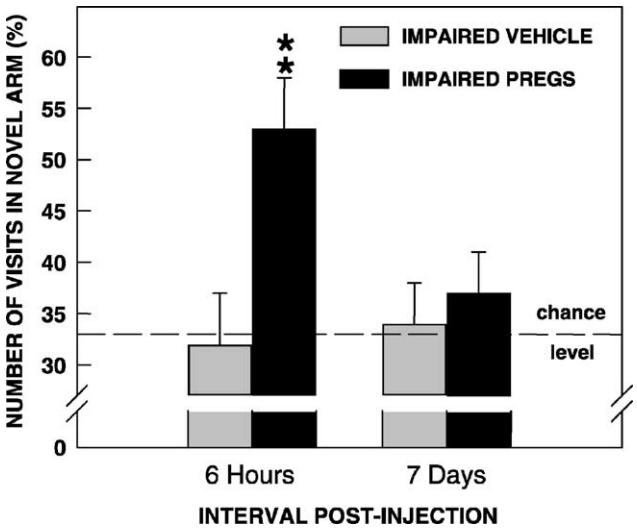

Fig. 2. The bilateral injection of PREGS (5 ng/0.5 μl) into the dorsal hippocampus restored memory-deficits of 22-month-old rats in the Y-maze discrimination arm task. The retention performance is expressed as the percentage of number of visits to the novel arm. The animals that were not previously able to discriminate between the novel and familiar arms of the maze could perform this discrimination after the injection of PREGS, while vehicle-treated animals still displayed impaired performance. $^{**}P < 0.01$, compared to chance level. The dotted line expresses the level of equivalent exploration of the three arms (chance level, 33%).

2.4. Mechanisms underlying the cognitive effect of PREGS

We investigated several mechanisms that could contribute to the promnestic actions of PREGS. One possibility is that PREGS enhances central cholinergic function, a major system involved in attention and memory processing (for review, see [17]). This concept is supported by the

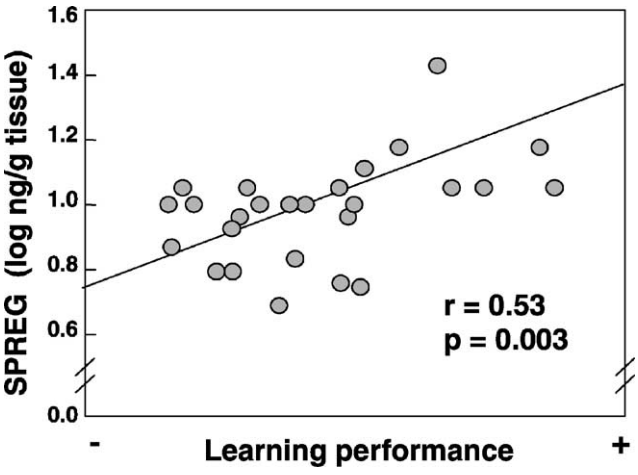

Fig. 3. Correlation between the levels of endogenous PREGS in the hippocampus and the learning performance in the water-maze task of individual 24-month-old rats. PREGS concentrations are expressed in log form (ng/g). Animals that exhibit worse performance had the lowest level of PREGS in the hippocampus.

observation that administration of PREGS in the nucleus basalis magnocellularis, the main source of cortical cholinergic innervation, improved memory performance of young rats [34]. Additionally, microdialysis studies showed that intracerebroventricular (i.c.v.) administration of PREGS increases extracellular levels of acetylcholine in the dorsal hippocampus of awake rats [15,54].

An alternative possibility is that the memory-enhancing actions of PREGS result from effects in neuronal membrane targets of PREGS (i.e. through NMDARs and $GABA_A$Rs). This is consistent with data from several studies showing that both NMDA and GABA systems participate in neuronal mechanisms underlying memory processes [25,37], including modulation of central cholinergic transmission [19–22,58]. In behavioral studies, PREGS reversed memory impairments induced either by competitive NMDAR antagonists [30,31], or reversed the effects of ethanol [38], a positive modulator of $GABA_A$Rs [1,46].

To investigate the possibility that the memory-enhancing properties of PREGS requires a direct action of PREGS on NMDARs and/or $GABA_A$Rs, we analyzed the memory effects of synthetic analogs of PREGS differing in their actions on NMDARs and $GABA_A$Rs [56]. Similar to PREGS, these compounds act on NMDARs and/or $GABA_A$Rs as allosteric modulators. However, small alterations in steroid levels have profound effects on receptor activity. The synthetic PREGS analogs tested were: 11-keto derivative of PREGS (an agent that is inactive at NMDARs and $GABA_A$Rs [42,43]) and 3β-hydroxy-5βpregnan-20-one sulphate (epipregnanolone sulphate (EpiS), an agent that, like PREGS, inhibits $GABA_A$Rs, but unlike PREGS, also inhibits NMDARs [42,43]). The ability of PREGS and its analogs to modulate memory function was analyzed in a passive avoidance paradigm assessing the effects of the steroids on basal learning and amnesia induced by the cholinergic drug scopolamine. The main findings are that intracerebroventricular (i.c.v.) administration of PREGS dose-dependently reverses the retention deficit induced by scopolamine. The effect of PREGS followed an inverted U-shape dose-response curve with a significant facilitative effect at 1 nmol and no effect at lower and higher doses (0.5 and 10 nmol). This type of dose-dependency is characteristic of PREGS, and has been described for other memory-enhancing drugs [5,48]. The results also show that: (i) the addition of a ketone group at C11 of PREGS (for 11-ketoPREGS) or (ii) the transformation from the pregn-5-ene configuration of PREGS to the 5β-pregnane configuration (for EpiS) eliminates the memory-enhancing properties of PREGS. The lack of effect of these analogs suggests strongly that the memory-enhancing effects of PREGS are structurally specific.

To further investigate structural requirements for steroid inhibition of scopolamine-induced amnesia, we examined *ent*-PREGS, the unnatural synthetic (−) PREGS enantiomer, that exhibits similar potency to biosynthetic (natural) PREGS in inhibiting and increasing $GABA_A$- and NMDA-mediated currents, respectively, in hippocampal neurons [41,56]. *Ent*-PREGS displayed a dose-related effect like PREGS, but was 10-fold less potent compared to endogenous PREGS in reversing scopolamine-induced amnesia in the passive avoidance task (see Fig. 4). The results indicate that the entire stereochemistry of the PREGS steroid is an important determinant of the behavioral action of PREGS, since the PREGS enantiomers displayed significantly different potencies with qualitatively similar behavioral activity and effectiveness. Accordingly, differential memory-enhancing properties for PREGS and *ent*-PREGS have been reported in adult rats and mice using a Y-maze two-trial recognition task; however, in this study *ent*-PREGS was more potent than the endogenous PREGS [2]. Thus, these results confirm the enantioselective behavioral properties of PREGS. Because the ratio of potency between PREGS enantiomers differs in these two studies, one can hypothesize that different systems are involved in mediating the memory effects of these compounds. Electrophysiological studies with cultured rat hippocampal neurons have shown that PREGS enantiomers have equal potency and efficacy in inhibiting GABA currents [41] and enhancing NMDA currents [56]. These data show that the interaction of PREGS with $GABA_A$R and NMDAR is not enantioselective in vitro and further suggest that PREGS may act on different targets than $GABA_A$Rs and NMDARs. In this regard, it was demonstrated a novel effect of PREGS on glutamate release, in cultured hippocampal neurons from neonatal rats, that depends of an activation of membrane sigma (σ) receptors [33]. This study reports that the interaction between metabotropic σ1-like receptors and PREGS is enantioselective, suggesting that the different interaction

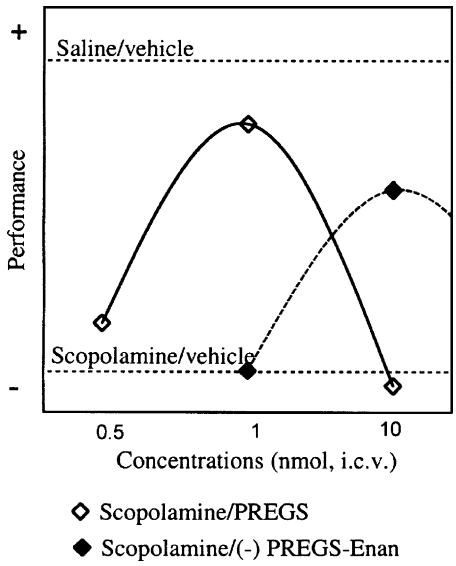

Fig. 4. Schematic representation of the dose effect curves of PREGS and its (−) enantiomer. The *y*-axis represents 24 h delay retention performance in a passive avoidance task. The upper and lower dotted lines represent the performance levels in i.c.v. vehicle-treated animals receiving either saline (1 ml/kg, s.c.) or scopolamine (1 mg/kg, s.c.).

of PREGS enantiomers with σ receptors could contribute to their differential effects in vivo.

3. Neuroactive steroids: new perspectives of research

3.1. Technical advances for measuring brain neurosteroid content

3.1.1. A new method of quantification of neuroactive steroids

Given that all neuroactive steroids are derived from PREG, and given the existence of some reversible metabolic pathways (for example, the interconversions between the non-sulfated and sulfated forms of PREG and DHEA, see Fig. 1), the possibility that several steroids might contribute to the effect observed following the administration of a single compound could not be ruled out. For example, several studies suggest that the effect of PREG or PREGS could be attributed to its conversion to allopregnanolone (3α,5α-TH PROG). This suggestion emerged from observations that administration of PREG and/or PREGS induced an increase of 3α,5α-TH PROG levels, and that the inhibition of 3α,5α-TH PROG synthesis abolishes the memory-enhancing effects of PREG or PREGS [12,47].

From these results emerged the need to assess the entire spectrum of neuroactive steroids in brain areas for studying the relation between steroid content and the behavioral-related steroid effects. Moreover, to know whether neurosteroids are synthesized in quantities sufficient to modulate neuronal activity, highly sensitive, selective, and structurally specific assays to measure small quantities of steroids in biological tissues and fluids are required.

To this end, given that the commonly used radioimmunoassay (RIA) method allows measurement of only one steroid at a time and because the insufficient specificity of antibodies used in RIA assay, new methods of quantification of steroids are necessary. Some methods combine RIA with high-performance liquid chromatography (HPLC) to improve the specificity of the analysis, but this combination does not offer a sufficient specificity for structural identification (reviewed in [4]).

3.1.2. Assay of neuroactive steroids using mass spectrometry

Several groups recently proposed new methods for the simultaneous quantification of traces of neuroactive steroids using mass spectrometry [11,26,28,50,53,55]. To date, the most sensitive, specific, and accurate method for the simultaneous analysis of several neuroactive steroids is the method of gas chromatography/negative chemical ionization mass spectrometry (GC/NCI-MS) (reviewed in [4]).

Using this method, we attempt to explore variations of steroid levels in specific brain areas during alterations of learning and memory processes, such as those which occur during aging. The first experiment investigated brain neurosteroid contents following stress, which is a factor that amplifies the aging process (reviewed in [36]). We analyzed contents of several neuroactive steroids in plasma and frontal cortex of male adult rats using GC/NCI-MS. Our most significant finding following a swim stress is a 10-fold increase of PREG in the cortex (to 7 ng/g) but no alteration of PREG in plasma. We also demonstrated an increase in plasma and cortex concentrations of allopregnanolone (to 1.5 ng/ml and 1.3 ng/g, respectively), while DHEA content was not altered. This type of analytical method can simultaneously quantify PREG and its metabolites in individual animals, allowing the demonstration that modifications of neurosteroid concentrations might be due to alterations of neurosteroid metabolic pathways induced by acute stress.

3.2. Applications to clinical studies

Animal studies strongly suggest that neuroactive steroids might be novel biomarkers of the aging process; therefore, measurement of cerebral steroid content could be a good indicator of the cognitive decline associated with normal aging and/or with pathological aging such as Alzheimer's disease. For instance, a recent study examined *post-mortem* content of neurosteroids using GC/MS in six brain regions of Alzheimer's and non-demented patients [57]. The main findings show that the concentrations of PREGS and DHEAS were the most altered steroids with a significant decrease in the striatum and cerebellum for PREGS and also in hypothalamus for DHEAS in Alzheimer's patients compared with the controls.

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