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Pregnenolone sulfate infused in lateral septum of male rats impairs novel object recognition memory

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Abstract:
In the present paper we show for the first time that pregnenolone sulfate (Preg-S) impairs rats' memory for novel object recognition when injected in lateral septum (1.2 μM). The effect of Preg-S is clearly related to the moment the reagent is administered: if administered shortly after the training phase, or prior to the test phase of the experiment, there is no amnesic effect. It is only amnesic when administered 30 min before training. Accordingly, Preg-S does not appear to affect the storage of new memories or their retrieval but rather the acquisition itself. Based on the described afferences and efferences of lateral septum, we suggest a possible stimulatory effect of Preg-S regarding glutamate receptors and/or an inhibitory effect of GABA receptors located in local interneurons or recurrent axon collaterals, both of which have been reported to exist in the aforementioned nucleus.

Key words:
neuroactive steroids, memory, pregnenolone sulfate, lateral septum, novel object recognition, male rats (Sprague-Dawley)

Introduction

Steroid hormones are able to exert their effects *via* at least two well-studied kind of events by: 1) signaling their intracellular receptors, and 2) modulating cell membrane receptors. The second one involves the action of steroid molecules collectively known as neuroactive steroids [17], being the so-called neurosteroids the subclass of neuroactive steroids synthesized strictly in the central nervous system (CNS) independently of any peripheral source [3]. Pregnenolone sulfate (Preg-S) is considered an excitatory neuroactive steroid since it negatively modulates the main inhibitory receptor in the nervous system, the $GABA_A$ receptor and additionally because it positively modulates excitatory glutamatergic NMDA receptors [7].

Neuroactive steroids and neurosteroids among them influence cognitive functions, particularly memory processes [14]. On the one hand, systemic or intracerebral administration of neuroactive steroids, like pregnenolone (Preg) or Preg-S, enhance memory in both young and old rats [15]. Also, several studies performed in rodents have demonstrated the promnesic effect of Preg-S in a passive avoidance test and on spatial memory [13, 16, 27], while allopregnanolone showed opposite effects by deteriorating memory in the Morris water maze when a sort of "human episodic like" memory was evaluated [14, 10]. However, there is an increasing controversy regarding the effect of sulfated steroids on memory, suggesting a more complex modulatory scene. Vallee et al. [26] revised evidence showing that dehydroepiandrosterone and its sulfate derivative are both able of presenting any kind of effect in humans, ranging from memory improvements to memory dysfunctions. In addition, it has been reported that post-training injected Preg-S impairs passive avoidance retention in rats [12]. Lateral septum forms part of the medial temporal system, which is crucial for memory and learning. It connects directly to the hippocampus through the septohippocampal formation, receiving heavy glutamatergic inputs through it [9, 23]. It also receives projections from the entorhinal cortex [11] and other subcortical structures. Injection of β-amyloid peptide into the entorhinal cortex induced selective cognitive deficits in tasks regarding recognition and spatial memory [22]. Little is known about lateral septum complex and the role of neuroactive steroids regarding cognitive functions. Some information related to the presence of Preg-S in the nervous system was considered to some extent controversial [21], although now is accepted that while sulfated steroids are present in the CNS of human beings they would not be natural endogenous modulator in rodents [21]. In this study we have evaluated the pharmacological effects of Preg and Preg-S injected intracerebroventricularly (*icv*) and in lateral septum (LS) on a novel object recognition task. This task is a paradigm based on appetitive dispositions instead of aversive behavioral responses (step through, step down, context conditioning, among others). It is possible that modulation of cognitive-memory processes are differently coordinated when the task to deal with for the subject is an appetitive one instead of its aversive counterpart. This could be particularly relevant for neuroactive steroids, whose subtle modulatory actions – particularly at the low doses utilized in the present study – could be to a great extent different from those attributed to classical neurotransmitters, i.e., modulatory actions quite dependent on context, age, previous state, gender, just to mention a few. We selected the appetitive task in order to avoid a possible confounding factor – the emotional component – by appealing to a more natural behavior, the attraction for novelty in the wild. From all this background we hope to get a better understanding of the role of neuroactive steroids in cognitive processes, in order to eventually attain new insights for future therapeutic applications regarding the important and growing field of neurodegenerative diseases.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (60–70 days old; 280–320 g) were housed in groups of four per cage until surgery. After surgery the rats were housed alone. Room temperature was maintained at 22 ± 1°C with lights on from 7.00 a.m to 7.00 p.m. Food and water were freely available throughout the experiments. Animals for these experiments were kept and handled according to the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, USA, 1996. All efforts were made to minimize animal suffering.

Reagents

The reagents utilized were Preg-S and Preg (SIGMA, St. Louis, MO, USA), penicillin G benzathine (Richet, Argentina) and chloral hydrate (Anedra, Argentina). Stocks of Preg and Preg-S were initially dissolved in propylene glycol to a concentration of 0.6 mM and 0.4 mM, respectively. The different doses of Preg-S and Preg used in the experiments were obtained by dilution in sterile saline in order to make negligible the final amount of propylene glycol. Notwithstanding, control animals were injected with sterile saline containing propylene glycol in equivalent concentrations to that used in experimental groups.