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Research report

Pregnenolone sulphate enhances spatial orientation and object discrimination in adult male rats: Evidence from a behavioural and electrophysiological study

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HIGHLIGHTS

- Pregnenolone sulphate facilitates the acquisition of spatial information.
- Pregnenolone sulphate enhances simple and complex object discrimination.
- Pregnenolone sulphate increases hippocampal and perirhinal neuronal firing.

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ABSTRACT

Neurosteroids can alter neuronal excitability interacting with specific neurotransmitter receptors, thus affecting several functions such as cognition and emotionality. In this study we investigated, in adult male rats, the effects of the acute administration of pregnenolone-sulfate (PREGS) (10 mg/kg, s.c.) on cognitive processes using the Can test, a non aversive spatial/visual task which allows the assessment of both spatial orientation–acquisition and object discrimination in a simple and in a complex version of the visual task. Electrophysiological recordings were also performed *in vivo*, after acute PREGS systemic administration in order to investigate on the neuronal activation in the hippocampus and the perirhinal cortex. Our results indicate that, PREGS induces an improvement in spatial orientation–acquisition and in object discrimination in the simple and in the complex visual task; the behavioural responses were also confirmed by electrophysiological recordings showing a potentiation in the neuronal activity of the hippocampus and the perirhinal cortex. In conclusion, this study demonstrates that PREGS systemic administration in rats exerts cognitive enhancing properties which involve both the acquisition and utilization of spatial information, and object discrimination memory, and also correlates the behavioural potentiation observed to an increase in the neuronal firing of discrete cerebral areas critical for spatial learning and object recognition. This provides further evidence in support of the role of PREGS in exerting a protective and enhancing role on human memory.

1. Introduction

Neurosteroids may be synthesized in the brain itself and act through both genomic and non genomic mechanisms. They reveal the capability to exert a relevant role as endogenous modulators of neuronal functions and behavioural processes, through different signalling pathways involving classical and non-classical steroid receptors. Among them pregnenolone sulfate (PREGS) facilitates neurotransmitter release at excitatory synapses: at the molecular level, it is thought to allosterically modulate ligand-gated ion

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channels [1], by attenuating γ-aminobutyric acid (GABA) A [2,3] and glycine receptor currents [4], and enhancing *N*-methyl-D-aspartate (NMDA) receptor currents [5–9].

Alterations in neurosteroid concentrations may contribute to the pathophysiology of several neuronal disorders [1,10–16]. Decreased concentrations of PREGS have been detected in patients suffering from Alzheimer's disease and multinfarct dementia [17,18], as well as in sleep and affective disorders [19–27], indicating that PREGS most prominent and valuable functions deal with the positive modulation of mood and memory in humans and in animals [6,28–31]. Learning and memory consist mainly on acquisition of information, their storage and retrieval, functions that reflect a complex set of neural processes whose activation depends on the learning and memory tasks requested: processing spatial information or object discrimination. The hippocampus undoubtedly plays a very important role in orientation in space and in the construction of cognitive maps [32–35]; the perirhinal cortex, on the other hand, is mainly required for the recognition of object information *per se* [36]. On the basis of these reports the present study was aimed at elucidating the potential effect of PREGS as cognitive enhancer, through the assessment of different learning and memory processes: acquisition and object discrimination.

To do that we employed the Can test, a validated non-aversive reward-facilitated task which enables the study of the spatial and visual abilities of the rats, assessing both acquisition, and working- and reference-memory in the same task [37–41]. Finally, in order to correlate the behavioural effects exerted by the systemic administration of PREGS on spatial orientation and object discrimination to the activation of discrete neural processes, electrophysiological recordings were performed on the hippocampus and the perirhinal cortex.

2. Materials and methods

2.1. Animals

Experiments were carried out on adult male Wistar rats (initial weight 230–250, Harlan, Udine, Italy). The animals were housed in a standard plastic cages, two per cage, in a temperature (22 ± 2 °C) – and humidity (55 ± 10%) – controlled room. Normally, food and water were available *ad libitum*, and the colony was maintained on a 12 h light–dark cycle (8.00–20.00 h).

2.2. Pharmacological treatment

Rats were injected with PREGS (Sigma-Aldrich SRL, Milan Italy) (10 mg/kg; $n = 16$) or vehicle (0.1% Tween 80; $n = 16$) on the day of behavioural testing. PREGS was dissolved in 0.1% Tween 80, and injected (1 ml/kg subcutaneously) 2 h before each experimental session, in order to observe a minor inter-individual variability of the effects. Control rats received the same of the vehicle volume (1 ml/kg), at the same time. In this study we used PREGS at 10 mg/kg, single dose, because it was reported to induce the most prominent effects on different behavioral parameters [23,42].

2.3. Behavioural experiments

On the test days, the animals were brought into the laboratory and allowed to acclimatise for at least 60 min prior of the experimental sessions. The experiments were performed in sound-isolated chambers between 8:00 and 14:00. Animal performance was recorded on a videotape placed in an adjacent room. An experimenter, unaware of the different treatments, scored the parameters from the videotape. The devices were thoroughly cleaned before the introduction of each animal to ensure that a particular rat's behaviour was not affected by the detection of another rat's scent. All the experiments were carried out in accordance with the current Italian legislation [D.L. 116, 1992] that allows experimentation on laboratory animals only after submission and approval of a research project to the Ministry of Health (Rome, Italy), and in strict accordance with European Council directives on the matter (No. 2010/63/UE). All possible efforts were made to minimize animal pain and discomfort and to reduce the number of experimental subjects.

2.3.1. The Can test

For the evaluation of learning and memory functions, animals were trained in the Can-test, a novel motivated non-aversive spatial-object discrimination task, developed by Popoviç et al. in 2001 [43] and further employed in our previous studies [44–46]. The behavioural protocol consisted of four separate parts: shaping period; spatial orientation–acquisition; simple visual and complex visual task. The experiments were performed under 100 lx light intensity. The tops of seven soft-drink cans were cut off. Cans were painted in white or left in their imprinted colours, according to the task administered. The cans were put upside down in a square plexiglas compartment (100 cm × 100 cm × 43 cm). This allowed their indented bottoms to hold water. The cans were placed on a solid pedestal, which elevated the upper edge of the can to a height of 14 cm. The cans were placed in a fan shaped pattern, in which the distance from each can to a start point was 70 cm and the distance between the cans was 7 cm. In the task, rats were trained to identify a single rewarded can among a set of seven cans. The reward consisted of 0.3 ml of tap water, using 23 h water deprivation schedule for motivation. When the rat stood on its hind paws and brought its nose up to the level of the top edge of the can, this was considered a "visit". The parameters measured were: (1)"Activity", the number of trials on which rats visited at least one can (up to 10 during each experimental session); (2) "Correct responses", the number of trials in which the rat visited the rewarded can first, divided by activity score (up to 1 per each experimental session); (3) "Reference memory errors", the first visits to a non-rewarded can on each trial, divided by activity score (up to 6 per each experimental session); (4) "Working memory errors", repeated visits to the same non-rewarded can on the same trial divided by the number of activity. Rats were allowed to drink freely for 20 min at the end of the experimental sessions.

2.3.2. Learning paradigm

2.3.2.1. Shaping period. This period took two days. During this session the animals were drug-free, and they started habituation by familiarizing with the environment. On the first day, rats were put in the compartment with seven cans. The bottom of each can was filled with 0.3 ml of tap water. The rats were allowed to explore the compartment and take water from the cans for 20 min. Animals were then removed and placed in their home cages. On the second day, two cans randomly plus the middle one were rewarded with water. The rats had up to 10 min to visit and drink water. After a 15 s interval the procedure was repeated again. To avoid possible differences in motivation to perform the Can test, rats that failed to approach and drink from a can over 50% of the trials were excluded. From this step the number of animals from each experimental group was 16.

2.3.2.2. Spatial orientation–acquisition task. Twenty four hours after the shaping period, two hours following PREGS administration, on four consecutive days and along 10 trials per day, rats were placed in the compartment where all the cans were painted in white and the single rewarded can was always placed in the middle position among the non-rewarded cans. Rats could spend up to 3 min per trial in order to visit and obtain water; once the reward was received, the animal was immediately removed from