Brain Research Bulletin 87 (2012) 280–285

Contents lists available at SciVerse ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Allopregnanolone prevents memory impairment: Effect on mRNA expression and enzymatic activity of hippocampal 3-α hydroxysteroid oxide-reductase

Carla Escudero[a,1], Sebastián Casas[a,1], Fernando Giuliani[a], Vanesa Bazzocchini[a], Sebastián García[a,b], Roberto Yunes[a,b], Ricardo Cabrera[a,*]

[a] Instituto de Investigaciones Biomédicas (INBIOMED), Universidad de Mendoza (IMBECU-CONICET), Paseo Dr. Emilio Descote 720, 5500 Mendoza, Argentina
[b] Área de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Av. Libertador 80, Centro Universitario, 5500 Mendoza, Argentina

ARTICLE INFO

Article history:
Received 24 June 2011
Received in revised form 3 November 2011
Accepted 25 November 2011
Available online 6 December 2011

Keywords:
Allopregnanolone
Oestrogen
Progesterone
Memory
3α-HOR
3α-HSD

ABSTRACT

In this work we investigated how the neurosteroid allopregnanolone can modulate learning and memory processes. For this purpose, we used ovariectomized (OVX) rats subcutaneously injected with oestradiol benzoate (E) alone or E and progesterone (P). Then, rats were injected in dorsal hippocampus with allopregnanolone or vehicle. Animals were tested in inhibitory avoidance task (IA task). After behavioural test hippocampal mRNA expression and enzymatic activity of 3α-HOR, the enzyme responsible of allopregnanolone synthesis, were analysed. In IA task OVX-EP rats spent less time on platform, compared to those OVX or OVX-E. Regression analyses revealed that there was a significant negative relationship between E-P infusion and performance in this task. Pre-training allopregnanolone administration to OVX-EP rats increased the time spent on the platform. Interestingly, when enzymatic activity of 3α-HOR was tested, OVX-EP rats showed a significant decrease in the enzymatic activity, compared with OVX and OVX-E rats. In addition, OVX-EP group showed a significant increase in the enzymatic activity after intrahippocampal infusion of allopregnanolone. On the other hand, when mRNA expression of 3α-HOR was analysed no differences were observed when the hippocampal allopregnanolone injection was done. These results suggest that E and P have amnesic effects on female rats, being reversed by allopregnanolone through its modulation on hippocampal 3α-HOR activity.

1. Introduction

Under diverse conditions, neuroactive steroids and neurosteroids could affect a broad spectrum of behavioural functions, such as sexual and feeding behaviour, responses to stress, emotion, memory and cognition [27,22,38].

Allopregnanolone is a neurosteroid derived from progesterone. The serum levels of this neurosteroid are low during diestrous and increase following the peak in E levels during oestrus [13]. It is synthetized by the enzyme 3α-hydroxysteroid oxide-reductase (3α-HOR) which belongs to aldo-keto reductase superfamily [21] and catalyses the interconversion of dihydroprogesterone (5α-DHP), into 3α,5α-reduced metabolite allopregnanolone. These enzymes involved in steroidogenesis in peripheral glands were identified also in the nervous system. They are typically expressed in brain at levels 2–5 fold lower than in adrenal or gonadal tissues [8,15]. Enzyme 3α-HOR has been characterized in different regions of human and in rodent brains. There are four isoforms of 3α-HOR identified in human brain and only one in rat brain [17,32,19,33]. In striatum of rat 3α-HOR mRNA has been found in medium spiny GABAergic neurons [1]. In cortex, hippocampus, amygdale, thalamus and olfactory bulb, 3α-HOR has been found in principal output glutamatergic neurons but is not expressed in GABAergic interneurons [1].

The hippocampus is a centre of learning and memory processes, and is known to be a target for neuromodulatory actions of sex hormones and neurosteroids. The hippocampus has a pivotal role in learning about spatial relationships or in the formation of short- and long-term complex associations, e.g., spatial learning and memory in rodents [31,28] and declarative or episodic memory in man [35,31]. Inhibitory avoidance task (IA task) involves both an explicit, associative component, and an operant-like conditioning component. The last component is a type of implicit memory in the one-trial version of IA [34]. Thus, in the IA task the animal learns that a specific place should be avoided since it is associated with an aversive event. Additionally, there are different experimental approach

Abbreviations: 3α-HOR, 3α-hydroxysteroid oxide reductase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3α, 5α-THP, tetrahydroprogesterone; 5α-DHP, dihydroprogesterone; DH, dorsal hippocampus; E, oestrogen; IA task, inhibitory avoidance task; OVX, ovariectomized rats; OVX-E, ovariectomized plus oestrogen rats; OVX-EP, ovariectomized plus oestrogen and progesterone rats; P, progesterone.

* Corresponding author. Tel.: +54 261 4230127; fax: +54 261 42001100.
E-mail addresses: ricardo.cabrera@um.edu.ar, rcabrera@mendoza-conicet.gob.ar (R. Cabrera).

[1] Both authors contributed equally to this work.

0361-9230/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2011.11.019

which use inhibitory passive avoidance learning in the rat to asses hippocampus dependent learning [2,40,3]. It is known that this kind of learning triggers biochemical events in the hippocampus that are necessary for the retention of this task [20].

Fluctuations in hormonal steroids and neurosteroids levels, across the oestrous cycle alter several behavioural processes among rodents, including cognitive performance. Administration of oestrogens and/or progestins post-training to ovariectomized (OVX) rats produces increased levels of these steroids in prefrontal cortex and hippocampus concomitant with enhanced performance in object recognition task [41]. On the other hand, rats with high circulating levels of E either following cyclic injections of E and P or during proestrous, the phase of the oestrous cycle in which E and P levels are high, performed more poorly than did rats at nadirs in their hormone cycle or with no hormone treatment [24]. This performance deficiency induced by hormone treatment is supported by other reports demonstrating that swim task performance is impaired by increase in E plus P levels across the oestrous cycle [12,42]. Allopregnanolone induces several effects on CNS including anxiolytic, antiseizure and neurogenesis. Administration of this neurosteroid immediately post-training, in similar concentrations as observed in behavioural oestrous rats, enhances object recognition performance [41]. The magnitude to which these endogeneous fluctuations in oestrogens, progestins and allopregnanolone affect hippocampus dependent learning remains unclear.

The aim of this work was to investigate the role of allopregnanolone in the hippocampus for memory performance and its relation with endogenous fluctuations of ovarian hormones. For this purpose we investigate: (1) the effects of allopregnanolone on IA task performance in OVX rats under different concentrations of ovarian hormones and (2) the potential mechanism of action of allopregnanolone related to the expression and activity of 3α-HOR in hippocampus.

2. Materials and methods

2.1. Animals

Adult Sprague-Dawley female rats (146 rats; 60–90 days old; 240–280 g) were housed in groups of four per cage until surgeries day (see Section 2.3). 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 of the National Research Council (National Academies, USA, 8th edition, 2011).

2.2. Drugs

The reagents used were allopregnanolone, oestrogen and progesterone (SIGMA, St. Louis, MO, USA). Penicillin G benzathine (Richet, Argentina) and chloral hydrate (Anedra, Argentina). Allopregnanolone was initially dissolved in propylene glycol to a concentration of 600 μM. The dose of allopregnanolone used in the experiments (6 μM) was obtained by successive dilution in sterile saline. The 6 μM concentration of allopregnanolone was chosen to mimic its maximal circulating level during sexual behaviour [25]. Control animals were injected with sterile saline with 1% propylene glycol.

2.3. Surgical procedures

Rats were anaesthetized with chloral hydrate 8% (5 ml/kg, ip) and fixed in a stereotaxic frame (David Kopf, USA). They were bilaterally implanted in CA1 dorsal hippocampus (DH) region, according to the coordinates of Paxinos and Watson's atlas: AP −3.5 mm, L ±2 mm, DV −2.5 mm. When the cannulae were implanted they were 1 mm above the structure in order to minimize damage. Guide cannulae were made from stainless steel (0.80 mm × 38 mm) and were fixed to the skull with dental cement and a stainless steel screw. At the end of the surgery, cannulae were sealed with a stainless steel wire to protect them from obstruction. In the same surgery were bilaterally ovariectomized. To prevent infections, all animals received an intramuscular (im) injection of 1,200,000 U penicillin G benzathine.

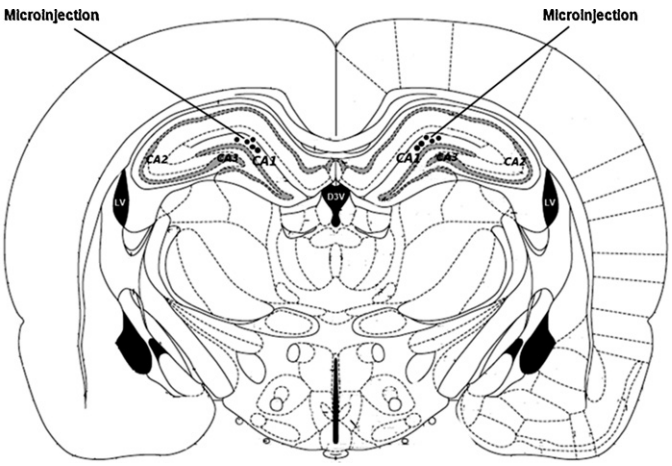

Fig. 1. Schematic representation of a brain coronary section showing the dorsal hippocampus and the place where illustrative microinjections were performed. Abbreviations: LV, lateral ventricle; D3V, third ventricle.

2.4. Histology and implant localization

After the behavioural test, animals were injected through the guide cannulae with 1 μl of blue ink to confirm the location of the guide cannulae into DH and then decapitated. The site of the microinjection was assessed with a magnifying lens (Zeiss West Germany, 4× magnification) by two investigators blind to treatment, to determine placements of cannulae. Improper placement of cannulae mandated exclusion of those animals from the experiments reported here. Brain examination of 50 cannulated rats revealed improper placement of cannulae in two animals (96% of the animals with correct placement). Only the behavioural data from animals with the cannulae located in the intended site were included in the final analysis. A schematic drawing of the brain shows some representatives points of microinjections (Fig. 1).

2.5. Experimental procedures-1

In this work, we wanted to study the influence of E and P on learning and memory processes in relationship with allopregnanolone modulatory effect on neuronal hippocampal circuits that regulate this cognitive function. For this reason, we ovariectomized rats and infused them with E or E/P in order to have homogeneous hormonal profiles in all experimental groups before IA task. After surgeries the animals were left to recover for 7–10 days. They were then divided in three groups: (1) OVX rats without hormonal replacement, (2) OVX-E rats injected subcutaneously (s.c.) with oil 0.1 mg/kg E (48 h before training) and (3) OVX-EP rats injected s.c. with 0.1 mg/kg E and 4 mg/kg P (48 h and 5 h before training, respectively). This experimental protocol has been used in different experimental approaches [37,6,16]. For all experiments, the animals were injected in DH (1 μl/per site) with allopregnanolone (6 μM) or saline as control (n = 8 per group). Drug administration was performed with a needle (0.5 mm outer diameter), connected to a 10 μl syringe (Hamilton, Reno, NV, USA), introduced through the guide cannula until its tip was 1.5 mm below the end of the cannula. The syringe was gently and slowly depressed for 1 min, and left in situ for a further minute to allow diffusion from the needle into DH before the experimenter removed the needle. Thirty minutes after drug administration, animals were put into the arena to perform behavioural session as described below. A schematic representation of experimental procedures is shown in Fig. 2.

2.5.1. Behavioural testing

To exclude the eventual drugs effect on locomotor and exploratory activities, we assessed each experimental group (n = 8 per group) with open field test before step down inhibitory avoidance task (IA task). Immediately after, the different groups of animals were evaluated on IA task. Behavioural testing was performed by 1 of 3 independent observers (98% concordance), who were blind to experimental conditions.

2.5.2. Open field

A commercial photoelectric device known as Opto-Varimex (OVM; Columbus Instruments, USA), designed to measure photobeam interruptions in individually tracked photocells, was used to assess the locomotor and exploratory activity of the animals. The OVM consisted of a plexiglas transparent cage (30 cm × 42 cm × 42 cm) with a homogenous black plastic floor. The walls housed infrared emisors and detectors in order to automatically register several measures: (1) *horizontal activity*: all movements performed on the horizontal axis; (2) *ambulatory activity*: all movements detected as displacement; (3) *non-ambulatory activity*: all movements performed by the animal while remains in the same place; (4) *number of movements*: number of episodic or consecutive movements performed by the animal; and (5)

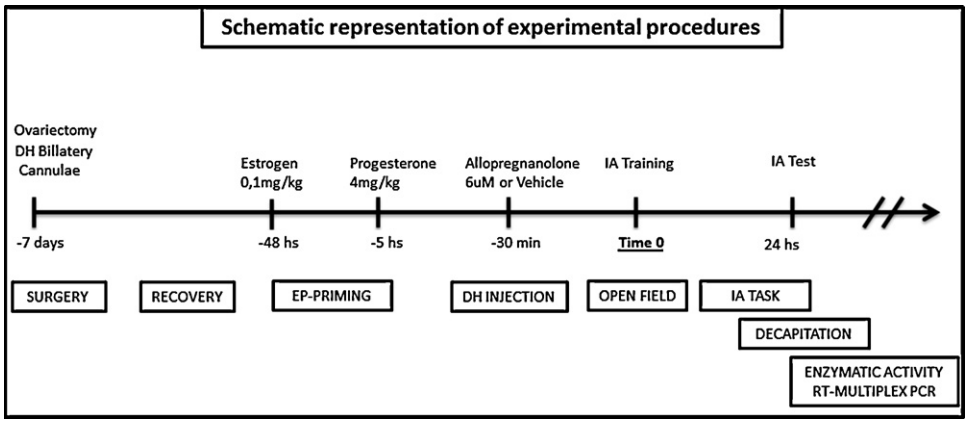

Fig. 2. Schematic representation of experimental procedures. Time 0: training-day on IA task, thus all the times were relative at this day. Abbreviations: DH: dorsal hippocampus, EP-priming: subcutaneous administration of oestrogen and or progesterone, IATask: inhibitory avoidance task.

vertical activity: number of times the subject rises on their rear feet in the air or against the walls during at least 2-s. Regarding any measure other than discrete ones (for example vertical activity), the measures are referred to as total counts/5 min, scoring a count as an interruption of the photobeam per second [30]. The numbers of horizontal, vertical and total movements were recorded during a five-minute test period.

2.5.3. Step down inhibitory avoidance task

Animals were trained in a one-trial step down inhibitory avoidance task (IA), a learning task in which stepping-down from a platform present in a given context is associated with an electric foot shock, resulting in an increase in step-down latency when tested for retention 24 h later. The training apparatus consisted of a 50 cm × 25 cm × 25 cm Plexiglas box with a 4-cm-high, 8.5-cm-wide, and 17-cm-long platform on the left end of a series of bronze bars that constitute the floor of the box. During training, animals were gently placed on the platform facing the left rear corner of the training box [7]. When they stepped down and placed their four paws on the grid, they received a 3-s, 0.5-mA scrambled foot shock and were immediately withdrawn from the training box. Memory retention was evaluated in test sessions carried out 24 h after training. At test, trained animals were put back on the training box platform until they eventually stepped down to the grid. The latency to step down was taken as an indicator of memory retention. A 180-s ceiling was imposed on step-down latency during test sessions. Additionally, to exclude the possible effect of drugs administrations on locomotor and exploratory activity, and since these drugs were administered before training, the step-down latency during training sessions were reported as additional control.

2.6. Experimental procedures-2

Immediately after IA test (24 h after IA training), the animals were decapitated and dorsal hippocampi were removed and stored at −20 °C until determination of enzyme activity assay (n = 8 per group) and RNA expression of 3α-HOR by multiplex RT-PCR (n = 8 per group).

2.6.1. Enzymatic activity assay of 3α-HOR

Isolated DH was homogenized in 2 ml of ice-cold 10 mM phosphate buffer (pH 6.5) containing 0.154 M KCl, 1 mM dithiothreitol, 0.5 mM EDTA, and 1 μM PMSF. The homogenate was centrifuged at 105,000 × g for 60 min at 4 °C in an ultracentrifuge with a T40.2 rotor, Beckman Model L (Palo Alto, CA). The supernatant fraction (cytosolic fraction) was stored at −80 °C until enzymatic activity assay and quantitative analysis. Enzyme activity assay was development as described [36] with minor modification. Briefly, 3α-HOR activity was determined spectrophotometrically by measuring the oxidation rate of NADPH at 340 nm and 37 °C in a 1.0-cm-pathlength cuvet with a Metrolab 1600 DR (USA) spectrophotometer. The reductase activity was measured in 100 mM phosphate buffer (pH 6.5) containing 0.1 mM NADPH, 0.08 mM 5α-DHP (substrate), and enzyme solution (100 μl, cytosolic fraction) in a total volume of 1.0 ml. The reaction was initiated by addition of cofactor to the assay mixture. A blank without substrate was included. Protein concentration was determined by the Lowry method using BSA as a standard.

2.6.2. RNA isolation and multiplex RT-PCR analysis of 3α-HOR

Total RNA was isolated using TRIZOL reagent (Invitrogen Life Technologies), according to the manufacturer's instructions. Gel electrophoresis and ethidium bromide staining confirmed the integrity of the samples. Quantification of RNA was based on spectrophotometric analysis at 260 nm. Two micrograms of total RNA were reverse transcribed with 200 U of MMLV Reverse Transcriptase (Promega Inc.) using random primer hexamers in a 50-μl reaction mixture following manufacturer's instructions. Fragments coding for 3α-HOR and rat ciclophiline (as endogenous control) were amplified by multiplex PCR with specific primers for 3α-HOR (5′-CAAGTGCCTTTGAATGCTGA-3′ and 5′-CCTGGAGCTCTGGTTCTTGG-3′) and rat ciclophiline (5′-CAAGACTGAGTGGGTGGATGG-3′ and 5′-ACTTGAAGGGGAATGAGGAAA-3′), in a reaction mixture contained 5X Go Taq reaction buffer, 0.2 mM deoxynucleoside triphosphates, 0.6 μl 3α-HOR (5 μM) and 0.3 μl (5 μM) ciclophiline primers, respectively and 0.3 μl Go Taq DNA polymerase (Promega Inc.) and 7 μl RT-generated cDNA in a 25-μl final reaction volume. The predicted sizes of the PCR-amplified products were 379 pb for 3α-HOR and 293 pb for ciclophiline. PCR products were electrophoresed on 2% agarose gels, visualized with ethidium bromide (5.5 mg/ml), and examined by ultra-violet transillumination. Band intensities of PCR products were quantified using Image J (Image Prodessing and Analysis from http://rsb.info.nih.gov/ij) and expressed as arbitrary units relative to ciclophiline levels.

2.7. Statistical analysis

All variables were analysed with two-way ANOVA in a 3 × 2 factorial design, where hormonal profile (OVX, OVX-E, and OVX-EP) and experimental conditions (control and allopregnanolone) were the factors. Each analysis was followed by multiple comparisons using Tukey–Kramer HSD post hoc test. The significance level was set at $p < 0.05$ for all statistical tests. Data analyses were performed by of Statistica (StatSoft, Cracow, Poland).

3. Results

3.1. Effect of E, EP and allopregnanolone on locomotor and exploratory activity

To exclude the eventual effect of confounding variables related to locomotor and exploratory activity of the animals, we performed open field test. Statistical analysis showed that neither of the parameters evaluated were modified by allopregnanolone infusions (Table 1). Additionally, since the drugs were administered before training and to exclude the eventual confounding effect, the step-down latency during training sessions was reported for each group and no difference was found (Table 2).

3.2. Effect of E, EP and allopregnanolone on inhibitory avoidance task

On inhibitory avoidance task, OVX-EP control rats spent significantly less time on the platform in comparison with OVX or OVX-E control rats ($F_{2,30} = 98.72$; $p < 0.001$ and $p < 0.001$, respectively). The allopregnanolone injection 30 min before training reversed de amnesic effect observed in OVX-EP group ($F_{1,30} = 129.99$; $p < 0.001$). Non-significant differences were observed in OVX and OVX-E after allopregnanolone injection (Fig. 3).

Table 1
Effect of allopregnanolone on locomotor and exploratory activity. Results are expressed as mean ± SEM of several types of movements recorded in open field, from OVX, OVX-E and OVX-EP-primed rats infused with saline or allopregnanolone into DH (for each group $n = 8$). Data were analysed using a two way analysis of variance (ANOVA).

Type of movements	OVX Control vs Allo	OVX-E Control vs Allo	OVX-EP Control vs Allo	Statistics
Horizontal	4839 ± 398.0 vs 4680 ± 415.3	4777 ± 454 vs 4350 ± 427.3	4595 ± 407 vs 4439 ± 439.6	$F_{1,42} = 0.049$; $p > 0.05$
Vertical	36.2 ± 4.5 vs 38.1 ± 3.6	36.9 ± 3.41 vs 37.6 ± 2.9	38 ± 3.25 vs 37.2 ± 2.88	$F_{1,42} = 0.55$; $p > 0.05$
Ambulatory	4122.3 ± 369 vs 4003 ± 325	3995 ± 346 vs 4120.6 ± 315	3996 ± 375 vs 4258 ± 350	$F_{1,42} = 0.79$; $p > 0.05$
Non Ambulatory	899 ± 63 vs 911.3 ± 66	906.8 ± 57.6 vs 896.3 ± 61.3	915 ± 65 vs 868.2 ± 59.3	$F_{1,42} = 0.70$; $p > 0.05$
Number of movements	136.9 ± 6.9 vs 137.6 ± 6	133.6 ± 9.8 vs 135.1 ± 7.3	134.5 ± 9.3 vs 138.3 ± 5.32	$F_{1,42} = 0.82$; $p > 0.05$

Table 2
Training latencies on IA task. Results are expressed as mean ±SEM of latency in step down in seconds of OVX, OVX-E and OVX-EP-primed rats infused with saline or allopregnanolone into DH (for each group $n = 8$). Data were analysed using a two way analysis of variance (ANOVA).

	OVX Control vs Allo	OVX-E Control vs Allo	OVX-EP Control vs Allo	Statistics
Mean ± SEM	2.83 ± 0.477 vs 2.67 ± 0.333	2.33 ± 0.421 vs 2.66 ± 0.421	3.17 ± 0.307 vs 2.66 ± 0.333	$F_{1,30} = 0.12$; $p > 0.05$

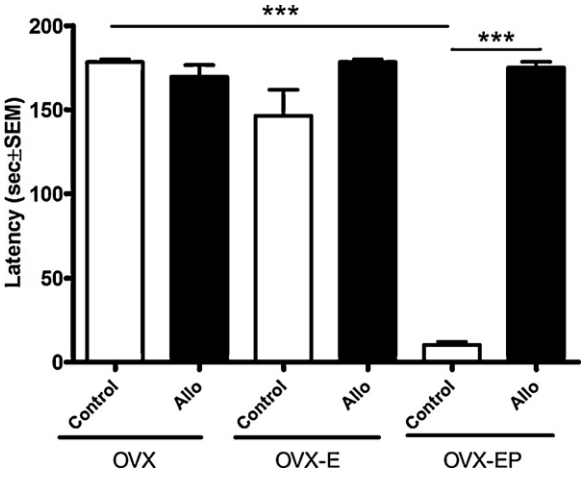

Fig. 3. Effect of E, P and allopregnanolone on learning and memory (step down inhibitory avoidance task). Results are expressed as mean ± SEM of latency in step down of OVX, OVX-E and OVX-EP-primed rats infused with saline (control group, white) or allopregnanolone (black) into DH (for each group $n = 8$). $^{***}p < 0.001$ for two-way ANOVA.

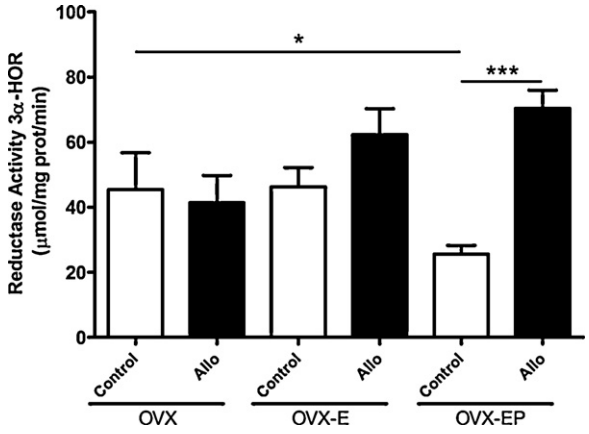

Fig. 4. Effect of E, P and allopregnanolone on enzymatic activity of 3α-HOR. Results are expressed as mean ± SEM of μmol/mg prot/min of OVX, OVX-E and OVX-EP-primed rats infused with saline (control group, white) or allopregnanolone (black) into DH (for each group $n = 8$). $^{*}p < 0.05$, $^{***}p < 0.001$ for two-way ANOVA.

3.3. Effect of E, EP and allopregnanolone on enzymatic activity of 3α-HOR in hippocampus

After saline injection, OVX-EP rats showed a significantly lower enzymatic activity of 3α-HOR compared to OVX or OVX-E ($F_{2,26} = 1.85$; $p < 0.05$ and $p < 0.05$, respectively). Intrahippocampal allopregnanolone administration reversed the significant decrease in 3α-HOR enzymatic activity observed in OVX-EP group ($F_{1,26} = 9.38$; $p < 0.001$). No changes in OVX and OVX-E groups were observed compared with controls (Fig. 4).

3.4. Effect of E, EP and allopregnanolone on hippocampal 3α-HOR expression

Analysing mRNA 3α-HOR expression on hippocampal samples, obtained after IA task, none of control groups showed significant changes ($F_{2,45} = 0.96$; $p > 0.05$). Intrahippocampal allopregnanolone administration reversed the effect observed only in OVX compared to OVX control rats ($F_{1,45} = 3.78$; $p < 0.05$) (Fig. 5).

4. Discussion

Learning and memory processes may be influenced by fluctuations of ovarian steroid hormones and neurosteroids such as E, P and allopregnanolone. In this study, we have used an animal model to investigate the effects of systemic administration of ovarian steroids to OVX rats in combination with intrahippocampal infusion of allopregnanolone in learning and memory processes using the IA task. Because central neurosteroidogenesis is an active neuromodulator mechanism, we found very interesting to study the relationship between ovarian hormonal variables and its effects over key enzyme that are involved in allopregnanolone synthesis, as modifiers of learning and memory processes.

Our data showed that EP priming to OVX rats caused an amnesic effect of in IA task. Surprisingly when allopregnanolone was administered into dorsal hippocampus to OVX-EP rats 30 min before training, an increase in time over the platform was observed indicating a reversal of amnesic effect in this group. It is known that endogenous fluctuations in progestins may influence cognitive performance mediated by the hippocampus. Young rats, which have higher allopregnanolone levels in the cortex (proestrous and late pregnancy), have better performance in the object recognition task than diestrous rats or rats in early stage of sexual development [14]. Other studies have shown that administration of P, 5α-DHP, or allopregnanolone to OVX rats enhances the performance in the object recognition and Y-maze tasks, as well as conditioned and passive avoidance tasks [9,11,39,41]). Our results revealed a significant negative relationship between E-P priming to OVX rats and performance on IA task; as well as a novel strong effect of allopregnanolone reversing this memory deficit. In accordance to these results, there are reports of disorganizing effects of progestins that

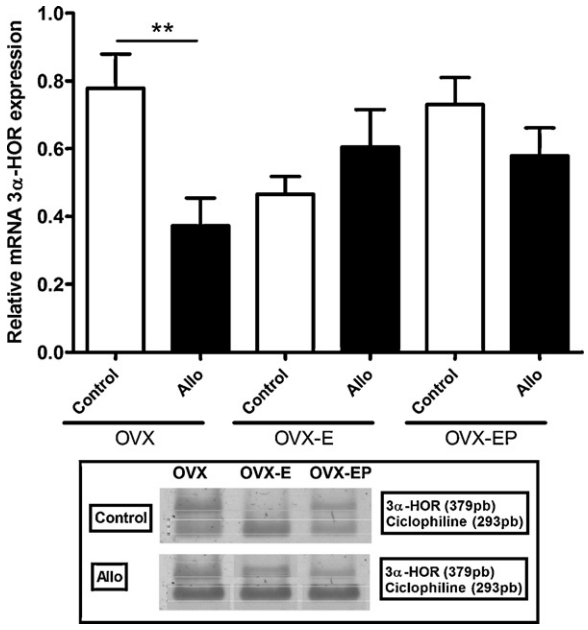

Fig. 5. Effect of E, P and allopregnanolone on mRNA expression of 3α-HOR. Results are expressed as mean ± SEM of relative units mRNA expression of OVX, OVX-E and OVX-EP-primed rats infused with saline (control group, white) or allopregnanolone (black) into DH (for each group $n = 8$). $^{*}p < 0.05$ for two-way ANOVA. Ethidium bromide fluorescence photograph of the gel electrophoresis of the amplification products. The gel photographs were quantified using Image J software and expressed as arbitrary units relative to ciclophiline.

need to be taken into consideration. For example, studies in which OVX improved learning performance or progestin administration failed to enhance performance in OVX rats may be due in part to disruptive effects of high progestin levels [4,5,22,43], this could be a reason for the decrease of optimal levels of learning/memory in our subjects. Thus, high levels of allopregnanolone may improve cognitive performance of rodents and, conversely decline in allopregnanolone may contribute to cognitive functions deficits.

The nervous system expresses all the enzymes required for neurosteroid biosynthesis [23]. Interestingly, in this work when enzymatic activity of 3α-HOR was tested, OVX-EP rats showed a significant lower enzymatic activity. This effect was reversed after infusion of allopregnanolone in hippocampus. Neurosteroids like allopregnanolone can have immediate, non-genomics rapid-signalling effects. This neurosteroids is a positive allosteric modulator of γ-aminobutyric acid type A (GABAA) receptors, potentiating GABAergic neurotransmission in a manner that is 20-fold more potent than benzodiazepines and 200-fold more potent than barbiturates [26,29]. In hippocampus, 3α-HOR co-localizes with 5α-reductase type I which are the rate-limiting-step enzymes of allopregnanolone biosynthesis, in principal glutamatergic output neurons [1] but they are not detectable in GABAergic interneurons. Recent findings suggest that the activity of key steroidogenic enzymes is finely tuned by various neurotransmitters strongly suggesting that some of the central effects of these neuromodulators may be mediated via the regulation of neurosteroid production [10]. Taking in consideration that our results have shown that allopregnanolone plays an important role in learning and memory processes through regulation of the enzyme responsible of its synthesis, we propose that allopregnanolone would be acting through presynaptic GABAA receptors located in hippocampal GABAergic neurons [18]. Then, allopregnanolone would inhibit GABA release and in this way promote memory processes through glutamate transmission and consequently its own synthesis in glutamatergic neurons. Therefore, allopregnanolone actions through non-genomic mechanisms in hippocampal neuronal circuits may be involved in the recovery of learning and memory deficit observed in our experiments.

We cannot ignore the possibility of more complex mechanisms regarding the putative reverting effects of allopregnanolone. Thus, after allopregnanolone injection only OVX group decreased its 3α-HOR mRNA expression. It must be noticed that there are effects of ovarian steroids in hippocampal mRNA expression in OVX rats, and allopregnanolone only acts without hormone replacement. Because little is known about the relationship between ovarian steroids hormone and hippocampal mRNA expression of 3α-HOR, more studies are needed in order to understand this condition. Here we showed a differential regulation on the mRNA expression of this enzyme by allopregnanolone, although these actions would not directly relate with the effect observed over the spent time on platform showed by OVX-EP group, but suggest dependence on the hippocampal plasticity in learning and memory processes; as well as rapid genomic activations mediated by neurosteroids in this cognitive phenomena.

5. Conclusions

Taken together, these results suggest that E and P priming to OVX rats have amnesic effects and allopregnanolone can reverse this action. These effects might be exercised through the following not mutually exclusive mechanisms: (1) ovarian steroid hormones influence through genomic mechanisms, that could be mediated by classics receptors for these steroids and (2) allopregnanolone may have a non-genomic neuromodulatory effect on learning and memory processes, regarding its synthesis on hippocampus. Finally, allopregnanolone has beneficial effects to enhance cognitive performance on hippocampus, then some changes in activity and expression of 3α-HOR in this brain region could modify the neuronal network functions and to control learning and memory processes.

Conflict of interest statement

The authors declare that there is no conflict of interest that would prejudice the impartiality of scientific work.

Acknowledgements

We wish to thank Mr. N. Persia (IMBECU-UM) by his technical assistance. This work was partly supported by a grant from the National Research Council of Argentina (CONICET, PIP N° 11220100100126/11) and a grant from the Universidad of Mendoza (113/10) to RC and RY.

References

[1] R. Agís-Balboa, G. Pinna, A. Zhubi, E. Maloku, M. Veldic, E. Costa, A. Guidotti, Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 14602–21407.
[2] M. Alonso, H. Viola, I. Izquierdo, H. Medina, Aversive experiences are associated with a rapid and transient activation of ERKs in the rat hippocampus, Neurobiol. Learn. Mem. 77 (2002) 119–124.
[3] P. Bekinschtein, C. Katche, L. Slipczuk, C. Gonzalez, G. Dorman, M. Cammarota, I. Izquierdo, J.H. Medina, Persistence of long-term memory storage: new insights into its molecular signatures in the hippocampus and related structures, Neurotox. Res. 18 (2010) 377–385.
[4] H.A. Bimonte-Nelson, R.S. Singleton, C.L. Hunter, K.L. Price, A.B. Moore, A.C. Granholm, Ovarian hormones and cognition in the aged female rat. I. Long-term, but not short-term, ovariectomy enhances spatial performance, Behav. Neurosci. 117 (2003) 1395–1406.
[5] H.A. Bimonte-Nelson, R.S. Singleton, B.J. Williams, A.C. Granholm, Ovarian hormones and cognition in the aged female rat. II. Progesterone supplementation reverses the cognitive enhancing effects of ovariectomy, Behav. Neurosci. 118 (2004) 707–714.

[6] L. Caligaris, J.J. Astrada, S. Taleisnik, Effect of oestrogen and progesterone on the release of luteinizing hormone in pseudopregnant rats, Acta Endocrinol. (Copenh.) 70 (1972) 163–166.
[7] M. Cammarota, L.R. Bevilaqua, J.I. Rossato, R.H. Lima, J.H. Medina, I. Izquierdo, Parallel memory processing by the CA1 region of the dorsal hippocampus and the basolateral amygdala, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 10279–10284.
[8] N.A. Compagnone, S.H. Mellon, Neurosteroids: biosynthesis and function of these novel neuromodulators, Front. Neuroendocrinol. 21 (2000) 1–56.
[9] G. Díaz-Véliz, F. Urresta, N. Dussaubat, S. Mora, Progesterone effects on the acquisition of conditioned avoidance responses an other motoric behaviors in intact and ovariectomized rats, Psychoneuroendocrinology 19 (1994) 387–394.
[10] J.L. Do Rego, J.Y. Seong, D. Burel, J. Leprince, V. Luu-The, K. Tsutsui, M.C. Tonon, G. Pelletier, H. Vaudry, Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides, Front. Neuroendocrinol. 30 (2009) 259–301.
[11] D.L. Ebner, R. Richardson, D.C. Riccio, Ovarian hormones and retention of learned fear in rats, Behav. Neural Biol. 33 (1981) 45–58.
[12] C.A. Frye, Estrus-associated decrements in a water maze task are limited to acquisition, Physiol. Behav. 57 (1995) 5–14.
[13] C.A. Frye, L.E. Bayon, Mating stimuli influence endogenous variations in the neurosteroids 3alpha, 5alpha-THP and 3alpha-diol, J. Neuroendocrinol. 11 (1999) 839–847.
[14] C.A. Frye, Neurosteroids' effects and mechanisms for social, cognitive, emotional, and physical functions, Psychoneuroendocrinology 34 (2009) 143–161.
[15] A. Furukawa, A. Miyatake, T. Ohnishi, Y. Ichikawa, Steroidogenic acute regulatory protein (StAR) transcripts constitutively expressed in the adult rat central nervous system: colocalization of StAR, cytochrome P-450SCC (CYP XIA1), and 3beta-hydroxysteroid dehydrogenase in the rat brain, J. Neurochem. 71 (1998) 2231–2238.
[16] F.A. Giuliani, R. Yunes, C.E. Mohn, M. Laconi, V. Rettori, R. Cabrera, Allopregnanolone induces LHRH and glutamate release through NMDA receptor modulation, Endocrine 40 (2011) 21–26.
[17] A. Hara, Y. Inoue, M. Nakagawa, F. Naganeo, H.J. Sawada, Purification and characterization of NADP$^+$-dependent 3 alpha-hydroxysteroid dehydrogenase from mouse liver cytosol, J. Biochem. 103 (1988) 1027–1034.
[18] M.B. Herd, D. Belelli, J.J. Lambert, Neurosteroid modulation of synaptic and extrasynaptic GABAA receptors, Pharmacol. Ther. 116 (2007) 20–34.
[19] S.S. Hoog, J.E. Pawlowski, P.M. Alzari, T.M. Penning, M. Lewis, Three-dimensional structure of rat liver 3 alpha-hydroxysteroid/dihydrodiol dehydrogenase: a member of the aldo-keto reductase superfamily, Proc. Natl. Acad. Sci. U.S.A 91 (1994) 2517–2521.
[20] I. Izquierdo, J.H. Medina, Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures, Neurobiol. Learn. Mem. 68 (1997) 285–316.
[21] J.M. Jez, T.M. Penning, The aldo-keto reductase (AKR) superfamily: an update, Chem. Biol. Interact. 130-132 (2001) 499–525.
[22] I.M. Johansson, V. Birzniece, C. Lindblad, T. Olsson, T. Bäckström, Allopregnanolone inhibits learning in the Morris water maze, Brain Res. 934 (2002) 125–131.
[23] H.J. Karavolas, D.R. Hodges, Neuroendocrine metabolism of progesterone and related progestins, Ciba Found. Symp. 153 (1990) 22–44, discussion 44–55.
[24] D.L. Korol, K. Unick, K. Goosens, C. Crane, P.E. Gold, T.C. Foster, Estrogen effects on spatial performance and hippocampal physiology in female rats, Soc. Neurosci. Abstr. 20 (1994) 1436.
[25] M.R. Laconi, P.C. Reggiani, A. Penissi, R. Yunes, R.J. Cabrera, Allopregnanolone modulates striatal dopaminergic activity of rats under different gonadal hormones conditions, Neurol. Res. 29 (2007) 622–627.
[26] M.D. Majewska, N.L. Harrison, R.D. Schwartz, J.L. Barker, S.M. Paul, Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor, Science 23 (1986) 1004–1007.
[27] R.C. Melcangi, A.G. Mensah-Nyagan, Neurosteroids: measurement and pathophysiologic relevance, Neurochem. Int. 52 (2008) 503–505.
[28] R.G. Morris, P. Garrud, J.N. Rawlins, J. O'Keefe, Place navigation impaired in rats with hippocampal lesions, Nature 297 (1982) 681–683.
[29] A.L. Morrow, P.D. Suzdak, S.M. Paul, Steroid hormone metabolites potentiate GABA receptor-mediated chloride ion flux with nanomolar potency, Eur. J. Pharmacol. 142 (1987) 483–485.
[30] F. Nanfaro, R. Cabrera, V. Bazzocchini, M. Laconi, R. Yunes, Pregnenolone sulfate infused in lateral septum of male rats impairs novel object recognition memory, Pharmacol. Rep. 62 (2010) 265–272.
[31] J. O'Keefe, L. Nadel, The Hippocampus as a Cognitive Map, University Press, Oxford, 1978.
[32] J.E. Pawlowski, M. Huizinga, T.M. Penning, Cloning and sequencing of the cDNA for rat liver 3 alpha-hydroxysteroid/dihydrodiol dehydrogenase, J. Biol. Chem. 266 (1991) 8820–8825.
[33] T.M. Penning, Y. Jin, V.V. Heredia, M. Lewis, Structure-function relationships in 3alpha-hydroxysteroid dehydrogenases: a comparison of the rat and human isoforms, J. Steroid Biochem. Mol. Biol. 85 (2003) 247–255.
[34] J.A. Quillfeldt, Behavioral Methods to Study Learning and Memory in Rats, vol. 21, 2006.
[35] W.B. Scoville, B. Milner, Loss of recent memory after bilateral hippocampal lesions, J. Neurochem. 20 (1957) 11–21.
[36] M. Takahashi, N. Iwata, S. Hara, T. Mukai, M. Takayama, T. Endo, Cyclic change in 3α-hydroxysteroid dehydrogenase in rat ovary during the estrous cycle, Biol. Reprod. 53 (1995) 1265–1270.
[37] S. Taleisnik, L. Caligaris, J.J. Astrada, Effect of copulation on the release of pituitary gonadotropins in male and female rats, Endocrinology 79 (1966) 49–54.
[38] M. Vallée, W. Mayo, M. Darnaudéry, C. Corpéchot, J. Young, M. Koehl, M. Le Moal, E.E. Baulieu, P. Robel, H. Simon, Neurosteroids: deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 14865–21470.
[39] T.B. Van Wimersma Greidanus, Pregnene-type steroids and impairment of passive avoidance behavior in rats, Horm. Behav. 9 (1977) 49–56.
[40] M.R. Vianna, L. Muller Igaz, A.S. Coitinho, J.H. Medina, I. Izquierdo, Memory extinction requires gene expression in rat hippocampus, Neurobiol. Learn. Mem. 79 (2003) 199–203.
[41] A.A. Walf, M.E. Rhodes, C.A. Frye, Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats, Neurobiol. Learn. Mem. 86 (2006) 35–46.
[42] S.G. Warren, J.M. Juraska, Spatial and nonspatial learning across the rat estrous cycle, Behav. Neurosci. 111 (1997) 259–266.
[43] L.B. Zou, K. Yamada, M. Sasa, Y. Nakata, T. Nabeshima, Effects of s1 receptor agonist SA4503 and neuroactive steroids on performance in a radial arm maze task in rats, Neuropharmacology 39 (2000) 1617–1627.