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Mnemonic effects of progesterone to mice require formation of 3 α ,5 α -THP

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Abstract

Ovarian hormones organize and activate neural circuits for reproduction and may also mediate cognition. Research has focused on estradiol's mnemonic effects, albeit progesterone co-varies with estradiol and its mechanisms for cognition require attention. Studies tested the hypothesis that cognitive effects of progesterone occur subsequent to its metabolism to 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), which does not bind progesterin receptors. Cognitive performance and progesterone levels in plasma, hippocampus, and cortex, were determined in ovariectomized mice administered vehicle, or progestins that differentially form 3 α ,5 α -THP and bind progesterin receptors (progesterone, 3 α ,5 α -THP, and/or medroxyprogesterone acetate). Only treatments that increased 3 α ,5 α -THP levels during memory consolidation (progesterone, 3 α ,5 α -THP, 3 α -5 α -THP+medroxyprogesterone acetate, but not progesterone+medroxyprogesterone acetate) improved cognitive performance. Thus, formation of 3 α ,5 α -THP may be required for progesterone's cognitive-enhancing effects.

Keywords

cognition; learning; memory; neurosteroids; hormone replacement therapy

Introduction

Ovarian hormones, such as estradiol (E₂) and progesterone (P₄), are known for their role in organizing and activating neural circuits related to reproduction, but there is emerging evidence for their role for cognitive processes across the lifespan. For example, women are twice as likely as are men to develop Alzheimer's Disease [1]. Among younger individuals, differences in cognitive performance have been noted with variations in hormonal milieu (e.g. pregnancy) [2,3]. There has been considerable interest in the effects of aging-related decline in these hormones for cognition, and whether deficits can be ameliorated with their replacement. Despite evidence from animal models that ovarian hormones can benefit cognitive performance [4,5,6,7], the data on whether hormone therapies reduce risk of cognitive decline with aging are controversial. Synthetic E₂/progestin (medroxyprogesterone acetate; MPA) therapies (e.g. PremPro[®]) may not be beneficial [8] and increase risk for Alzheimer's Disease and stroke [9,10]. However, PremPro[®] improved memory and attention in a younger population of women in another study [11]. Women receiving synthetic E₂ and micronized P₄ had better encoding, consolidation and retrieval in verbal memory tasks [12]. It must be noted that the focus in these studies has been on E₂, but P₄ naturally co-varies with E₂ and its mechanisms for cognition are largely unknown. Understanding how P₄ influences cognitive performance is important for gaining insight into the neurobiology of learning and memory.

A question that needs to be addressed to further understand the role of hormones for cognition is why there is heterogeneity in the responses of individuals to progestins for cognitive performance. Differences in responses may be related to the divergent mechanisms of action of progestins. Although P₄ and MPA bind progestin receptors, they differ in their actions at androgen [13] and glucocorticoid [14] receptors. The effects of P₄ to improve cognitive performance may not rely upon actions at cognate progestin receptors. When administered P₄, both progestin receptor-replete mice, and those with lifelong depletion of progestin receptors, have improved performance across several cognitive tasks, compared to that seen with vehicle/placebo [15]. P₄ and MPA also differ in their ability to be metabolized to 3 α ,5 α -THP, a progestin that does not bind progestin receptors. MPA inhibits the enzyme 3 α -hydroxysteroid dehydrogenase, and decreases formation of 3 α ,5 α -THP [16]. P₄ is a precursor for 3 α ,5 α -THP, and can augment E₂'s effects to enhance formation of 3 α ,5 α -THP. Thus, some cognitive effects of P₄ may occur subsequent to metabolism to 3 α ,5 α -THP, and its actions independent of progestin receptors.

To address mechanisms by which progestins exert mnemonic effects, we investigated acute effects on cognitive performance of female mice administered progestins that differentially form 3 α ,5 α -THP and bind progestin receptors. We compared the effects of P₄, 3 α ,5 α -THP, and/or MPA on cognitive performance of ovariectomized mice, and measured levels of progestins in plasma and putative brain targets (cortex, hippocampus). We hypothesized that mnemonic effects of P₄ occur subsequent to its metabolism to 3 α ,5 α -THP, which does not bind progestin receptors.

Methods

Methods were approved by the IACUC at University at Albany and carried out using measures to minimize pain or discomfort (NIH Guide for the Care and Use of Laboratory Animals, #80-23, 1996).

Animals and housing

Female mice (C57BL/6UA; N=71) were obtained from our breeding colony at University at Albany. All mice were ovariectomized under sodium pentobarbital anesthesia (80 mg/kg; intraperitoneal injection) as adults (8 weeks old) and started in the testing protocol one week later. Mice were group-housed (4–5/cage) on a reversed 12/12 h light/dark cycle (lights on 0800 h) with continuous access to rodent chow and tap water.

Hormone treatments

Mice were randomly-assigned to treatment conditions, and grouping was counter-balanced across cages and two cohorts. In Experiment 1, the role of 3 α ,5 α -THP was determined by comparing effects of subcutaneous administration of vehicle (vegetable oil, 20% DMSO), P₄ (4 mg/kg; Steraloids, Newport, RI), and 3 α ,5 α -THP (4 mg/kg; Sigma, St. Louis, MO). In Experiment 2, the effects of MPA, and whether MPA can block effects of P₄ and 3 α ,5 α -THP, were determined by comparing vehicle, MPA (10 mg/kg; Sigma), P₄+MPA, and 3 α ,5 α -THP+MPA. The dosing utilized was based upon previous and pilot studies [4,17]. All treatments were administered immediately post-training, during consolidation [4], and levels of progestogens at this time were verified.

Procedure

Mice were screened for normative motor and species-typical behavior (grooming, nest building, huddling with cagemates) and sensory responses and visually inspected for good general health (fur, whiskers, posture, gait, muscle tone) before inclusion in the study [4].

Mice were trained and tested in each of the tasks described below once per week. Behavioral data was collected by an observer uninformed of the coded treatment conditions of mice, using automated tracking systems (Any-Maze for object recognition/placement, water maze; San Diego Instruments Freeze Monitor for conditioned fear).

Object Recognition and Object Placement

Mice were trained with two novel objects placed in an open field (46×57×35 cm) in a single 3-min trial [17]. Four hours later, mice were tested for object recognition and object placement memory. For testing, the percentage of time mice spent exploring a novel (object recognition), or displaced (object placement), object as a function of total time exploring both objects during the 3-min trial was calculated. Mice were tested in these tasks with different objects to minimize potential for test decay effects [17].

Water Maze

Mice were habituated to the water maze (San Diego Instruments; 97 cm diameter, 23–25 °C water made opaque with white tempera paint) for 60 s. Mice were given 12, 1-min trials, which were organized into three blocks of 4 trials. Mice were randomly placed in one of the 4 starting positions in the maze during these trial blocks. Mice were tested in a 60-sec probe trial 1 hour later, where the time spent in the quadrant where the platform had been located during training was recorded as an index of memory in this task [4].

Conditioned Contextual/Cued Fear

Mice were habituated to the conditioning chamber for 4 min. During training, a 10-sec tone was sounded, immediately followed by a footshock (2 s, 0.35 mA). Mice received tone-shock pairings in 3 consecutive training trials. Twenty-four hours later, mice were tested for contextual and cued conditioning as determined by time spent freezing to these stimuli over 8, 1-min trials [4].

Tissue collection/radioimmunoassay

Some mice were re-administered treatments and trunk blood and whole brains were collected after cervical sublaxation/decapitation so that progesterone levels during consolidation could be determined by radioimmunoassay of plasma and dissected out cortex and hippocampus [5,17].

Data Analyses

One-way analyses of variance (ANOVAs), with Fisher's *post hoc* tests, were utilized, with an α level of $p < 0.05$ for significance, and $p < 0.10$ for a trend.

Results

Experiment 1: P₄ and 3 α ,5 α -THP enhance cognitive performance, and increase plasma, cortical, and hippocampal 3 α ,5 α -THP levels

Post-training, subcutaneous administration of P₄ or 3 α ,5 α -THP, but not vehicle, significantly increased the percentage of time mice spent exploring the novel object in the object recognition task ($F(2,52)=4.19$, $P<0.02$; Figure 1, top left). However, in the object placement task, there were no differences across groups in the percentage of time spent exploring the displaced object ($p=0.83$; vehicle: 57.5 ± 3.4 , P₄: 55.7 ± 3.3 , 3 α ,5 α -THP: 59.2 ± 4.8). In the water maze, P₄ or 3 α ,5 α -THP, compared to vehicle, significantly decreased latencies to reach the platform quadrant ($F(2,26)=4.93$, $P<0.01$; Figure 1, bottom left). In the conditioned fear paradigm, P₄ and 3 α ,5 α -THP, compared to vehicle, tended to,

and significantly increased, time spent freezing in the context and cued tests, respectively ($F(2,26)=2.85$, $P<0.08$; $F(2,26)=4.52$, $P<0.02$; Figure 1, right).

P_4 , but neither $3\alpha,5\alpha$ -THP, nor vehicle, significantly increased plasma ($F(2,18)=15.37$, $P<0.01$), hippocampus ($F(2,20)=8.64$, $P<0.01$), and cortical ($F(2,20)=10.13$, $P<0.01$) levels of P_4 . Plasma ($F(2,18)=18.06$, $P<0.01$), hippocampus, ($F(2,20)=39.71$, $P<0.01$), and cortex ($F(2,20)=16.08$, $P<0.01$) levels of $3\alpha,5\alpha$ -THP were significantly increased by P_4 and $3\alpha,5\alpha$ -THP, compared to vehicle. See Table 1.

Experiment 2: MPA alone, or with P_4 , fails to enhance cognitive performance or increase plasma, cortical, and hippocampal $3\alpha,5\alpha$ -THP levels

Post-training $3\alpha,5\alpha$ -THP+MPA significantly increased the percentage of time spent exploring the novel object in the object recognition task, compared to vehicle, MPA, or P_4 +MPA ($F(3,71)=2.66$, $P<0.05$; Figure 2, top left). Yet, in the object placement task, there were no differences among groups in the percentage of time spent exploring the displaced object ($p=0.55$; vehicle: 49.8 ± 4.8 , MPA: 53.8 ± 5.0 , MPA+ P_4 : 52.6 ± 6.3 , MPA+ $3\alpha,5\alpha$ -THP: 61.6 ± 7.3). In the water maze, post-training $3\alpha,5\alpha$ -THP+MPA significantly decreased latencies to reach the platform quadrant compared to vehicle or MPA ($F(3,38)=4.02$, $P<0.01$; Figure 2, bottom left). In the conditioned fear paradigm, $3\alpha,5\alpha$ -THP+MPA, compared to vehicle, MPA, or P_4 +MPA tended to, and significantly increased, time spent freezing in the context and cued tests, respectively, ($F(3,38)=2.49$, $P<0.07$; $F(3,38)=3.32$, $P<0.03$; Figure 2, right).

Administration of P_4 +MPA, but not vehicle, MPA, or $3\alpha,5\alpha$ -THP+MPA, increased P_4 levels in plasma ($F(3,21)=2.92$, $P<0.05$), hippocampus ($F(3,25)=5.29$, $P<0.01$), and cortex ($F(3,25)=12.60$, $P<0.01$). Plasma ($F(3,21)=2.67$, $P<0.07$) levels of $3\alpha,5\alpha$ -THP tended to be increased, and hippocampus ($F(3,25)=10.42$, $P<0.01$) and cortex ($F(3,25)=8.27$, $P<0.01$) levels of $3\alpha,5\alpha$ -THP were significantly increased, only with $3\alpha,5\alpha$ -THP+MPA. See Table 1.

Discussion

Results support our hypothesis that P_4 's cognitive-enhancing effects may be due to actions of $3\alpha,5\alpha$ -THP in the cortex and hippocampus. Treatments that increased $3\alpha,5\alpha$ -THP levels during memory consolidation (P_4 , $3\alpha,5\alpha$ -THP, or $3\alpha,5\alpha$ -THP+MPA) improved performance in the water maze, object recognition, and conditioned fear tasks, compared to vehicle. Thus, formation of $3\alpha,5\alpha$ -THP in the hippocampus and cortex may be essential for some of P_4 's mnemonic effects.

The present results contribute to the growing literature on the influence of ovarian hormones for the neurobiology of learning and memory. These results show that P_4 , but not MPA, improve cognitive performance when administered post-training. These data suggest that different mechanisms of action of progestins, may underlie their mnemonic effects. P_4 and MPA both bind progestin receptors; whereas, MPA also binds androgen and glucocorticoid receptors. Interestingly, P_4 , but not MPA, protects hippocampal neurons in culture against glutamate toxicity [18], and can prevent neuronal loss in the hilus of rats injected with kainic acid [19]. P_4 , but not MPA, reduces spatial learning deficits following cerebral edema of male rats [20]. P_4 alone reduces brain inflammation and enhances functional recovery of mice following ischemia [21]. These data substantiate further investigation of whether there are actions at divergent receptor targets underlying the beneficial effects of progestins in the central nervous system.

The present results suggest that progestins may improve cognitive performance, in part, subsequent to conversion to 3 α ,5 α -THP. First, similar effects were found in mice administered P₄ and 3 α ,5 α -THP (which both increased 3 α ,5 α -THP levels), to enhance performance in the object recognition, water maze, and conditioned contextual/cued fear task. Second, MPA, alone or in conjunction with P₄, neither improved cognitive performance nor increased 3 α ,5 α -THP levels. We have previously demonstrated in other mouse strains that reduced production of 3 α ,5 α -THP in the hippocampus negatively effects cognitive performance [5,17]. Age-related decrements in cognitive performance coincides with reduced hippocampal 3 α ,5 α -THP levels among wildtype mice and those with Alzheimer's Disease-related pathologies [17]. Third, in this study, P₄, 3 α ,5 α -THP, or 3 α ,5 α -THP+MPA, enhanced cognitive performance and increased 3 α ,5 α -THP levels in brain areas associated with cognitive tasks. Notably, P₄ and MPA, can bind progestin receptors, but 3 α ,5 α -THP does not. Indeed, regimen that enabled 3 α ,5 α -THP formation enhanced cognitive performance, irrespective of their capacity to bind progestin receptors. 3 α ,5 α -THP can have rapid actions at neurotransmitter targets, such as glutamate [22,23,24], that may underlie these effects. Thus, cognitive effects may be independent of progestogens' acute actions at progestin receptors and, instead, involve formation of 3 α ,5 α -THP, and rapid, downstream actions at neurotransmitter targets to initiate signal transduction cascades.

Short conclusion

Post-training administration of the synthetic progestin, MPA, unlike P₄ or 3 α ,5 α -THP, was ineffective in enhancing cognitive performance in young mice or increasing cortical and hippocampal 3 α ,5 α -THP levels during memory consolidation. MPA and P₄ bind progestin receptors, but 3 α ,5 α -THP does not. The beneficial effects of progestins may be independent of intracellular progestin receptors, and subsequent to production of 3 α ,5 α -THP, to improve cognitive function. Further investigation of the mechanisms of progestins in the cortex and hippocampus are important for elucidating neurobiological factors underlying sex/gender differences, and age-related decline, in cognitive processes.

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Figure 1. Effects of vehicle, progesterone (P₄), and 3α,5α-THP for performance in the object recognition, water maze, conditioned contextual and cued fear tasks. * p < 0.05.

Figure 2. Effects of vehicle, medroxyprogesterone acetate (MPA), MPA+progesterone (P₄) and MPA+3α,5α-THP for performance in the object recognition, water maze, conditioned contextual and cued fear tasks. * $p < 0.05$. # $p < 0.10$.

Table 1

Progestogen levels in Experiments 1 and 2.

	Progesterone (P ₄)			3 α -5 α -THP		
	Plasma (ng/ml)	Hippo (ng/mg)	Cortex (ng/mg)	Plasma (ng/ml)	Hippo (ng/mg)	Cortex (ng/mg)
Experiment 1						
Vehicle	2.1 ($\pm$ 0.01)	3.4 ($\pm$ 1.6)	3.1 ($\pm$ 0.9)	3.6 ($\pm$ 1.2)	3.4 ($\pm$ 0.6)	3.3 ($\pm$ 0.9)
P ₄	18.5 ($\pm$ 2.7) *	18.6 ($\pm$ 3.6) *	14.5 ($\pm$ 2.4) *	17.6 ($\pm$ 2.1) *	18.3 ($\pm$ 0.6) *	18.8 ($\pm$ 1.1) *
3 α ,5 α -THP	4.2 ($\pm$ 1.2)	7.4 ($\pm$ 1.2)	5.8 ($\pm$ 1.1)	20.6 ($\pm$ 1.0) *	20.6 ($\pm$ 1.8) *	16.0 ($\pm$ 2.4) *
Experiment 2						
Vehicle	2.1 ($\pm$ 0.1)	1.9 ($\pm$ 0.6)	2.6 ($\pm$ 1.0)	1.9 ($\pm$ 0.3)	3.6 ($\pm$ 0.8)	3.2 ($\pm$ 1.2)
MPA	5.1 ($\pm$ 1.9)	5.3 ($\pm$ 0.5)	4.4 ($\pm$ 0.9)	4.7 ($\pm$ 3.1)	7.3 ($\pm$ 2.5)	5.9 ($\pm$ 2.9)
MPA + P ₄	14.7 ($\pm$ 4.8) *	16.3 ($\pm$ 3.3) *	14.8 ($\pm$ 2.5) *	13.0 ($\pm$ 2.9) #	9.3 ($\pm$ 3.0)	6.3 ($\pm$ 2.4)
MPA + 3 α ,5 α -THP	5.6 ($\pm$ 1.9)	8.0 ($\pm$ 2.7)	4.1 ($\pm$ 0.1)	6.4 ($\pm$ 3.0)	21.3 ($\pm$ 1.5) *	18.1 ($\pm$ 1.7) *

* p < 0.05.

p < 0.10.