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The effects of DHEA, 3β-hydroxy-5α-androstane-6,17-dione, and 7-amino-DHEA analogues on short term and long term memory in the mouse

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ABSTRACT

Neurosteroids have been reported to modulate memory processes in rodents. Three analogues of dehydroepiandrosterone (DHEA), two of them previously described (7β-aminoDHEA and 7β-amino-17-ethylenedioxy-DHEA), and a new one (3β-hydroxy-5α-androstane-6,17-dione) were synthesized, and their effects were evaluated on memory. This study examined their effects on long term and short term memory in male (6 weeks old) NMRI mice in comparison with the reference drug. Long term memory was assessed using the passive avoidance task and short term memory (spatial working memory) using the spontaneous alternation task in a Y maze. Moreover, the effects of DHEA and its analogues on spontaneous locomotion were measured. In all tests, DHEA and analogues were injected at three equimolar doses (0.300–1.350–6.075 μM/kg). DHEA and its three analogues administered immediately post-training at the highest doses (6.075 μM/kg, s.c.) improved retention in passive avoidance test. Without effect *per se* in the spatial working memory task, the four compounds failed to reverse scopolamine (1 mg/kg, i.p.)-induced deficit in spontaneous alternation. These data suggested an action of DHEA and analogues in consolidation of long term memory particularly when emotional components are implied. Moreover, data indicated that pharmacological modulation of DHEA as performed in this study provides derivatives giving the same mnemonic profile than reference molecule.

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1. Introduction

Steroid hormones exert important functions in the control of growth, maturation and differentiation of the central and peripheral nervous systems. These actions have long been attributed exclusively to steroid hormones secreted by endocrine glands, *i.e.* adrenal, ovary, and testis. However, during the last decade, it has been shown that nerve cells (both neurons and glial cells) are capable of synthesizing bioactive steroids, now called neurosteroids [1]. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) were the first steroids identified in large concentrations in the rat brain [2]. P450c17, the enzyme that is required for synthesis of DHEA from pregnenolone, is found in specific neurons of embryonic rodent brains [3]. DHEA and DHEAS were the most abundant neurosteroids which were both synthesized and accumulated in the nervous system to levels at least in part independent of peripheral steroidogenesis [4]. It has been reported that DHEA and DHEAS exhibit a broad spectrum of biological actions, from interactions with brain development to complex processes such as learning, enhancing memory and cognitive performances during development and into adulthood [5–10]. Vallée et al. [11] have reviewed the role of dehydroepiandrosterone and its sulfate ester on learning and memory in cognitive aging. DHEA and DHEAS increase the effects of the excitatory neurotransmitter glutamate [12], and decrease the effects of the inhibitory neurotransmitter GABA [13].

DHEA and DHEAS also have neuroprotective effects. They protect hippocampal neurons against glutamate excitotoxicity [14]. Other authors showed that neuroprotection by DHEA, but not DHEAS, was mediated through inhibition of nitric oxide (NO) production and inhibition of calcium-sensitive NO synthase (NOS) activity, caused by NMDA stimulation [15].

Neurosteroids, which are involved in the regulation of stress responses, anxiety, sleep, neurodegenerative processes, aggressive behavior, and cognitive activities, were considered as key factors of chemical neurotransmission [16].

In addition, many studies showed clearly the beneficial role of DHEA administration on Alzheimer's disease [17], immune system [18], aging [19], obesity [20], diabetes [21], cardiovascular disease [22], cancer [23–24], metabolic changes [25], acquired immune deficiency syndrome [26], and depression [27]. Svec and Porter have

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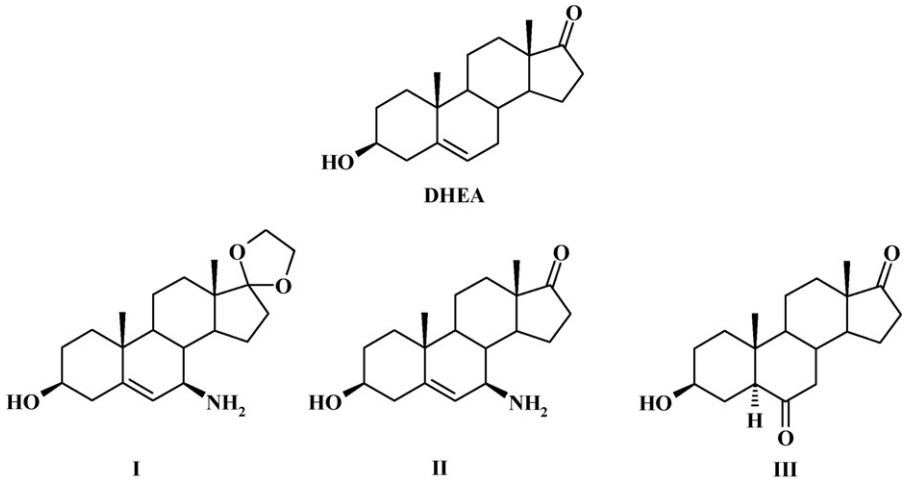


Fig. 1. DHEA and DHEA analogues.

reviewed the actions of exogenous DHEA in animal experimental models and in humans [28]. Although DHEA is purported to have many beneficial effects, there is only limited evidence to support its use in humans and there have been few clinical trials that clearly substantiate the efficacy and safety for use of DHEA supplements.

It has been well established that a significant portion of circulating DHEA is further metabolized to its 7-oxygenated derivatives (7-oxo-DHEA, 7α- and 7β-hydroxy-DHEA) in several different tissues, including liver and brain [29–31]. These DHEA metabolites are widely studied and seem to have even a wider activity than the parent steroids [32–35]. However, it is not well clarified what are the physiological functions of DHEA and its metabolites. Moreover, the broad effects of DHEA limit its therapeutic use. On the other hand, few DHEA analogues have been developed to our knowledge. Several monohydroxylated derivatives of DHEA (4α, 5α, 7α, 7β, 11β and 16α) and androst-5-ene-3β,17β-diol have been synthesized and evaluated for the induction of the thermogenic enzymes. Only 7-oxygenated derivatives induced thermogenic enzymes [36]. Other authors have demonstrated that the reduction of the C-5–C-6 double bond of DHEA, C-7 hydroxylation or oxidation of DHEA (such as DHEA metabolites) reduces the derivatives' ability to activate NMDA receptors. The hydroxylation on C-17 position of pregnenolone also reduces this ability [37]. Since DHEA has a broad spectrum of activities, chemical modification on C-7, C-17, C-5, and/or C-6 position of the steroid core might lead to changes related to one of these activities.

In this pathway, and in our studies related to the synthesis of amino and polyaminosteroids [38–44], we now report the synthesis of 3β-hydroxy-5α-androstane-6,17-dione and we examine its effect and that of two other DHEA analogues on memory in mice (Fig. 1). With this goal, we have first tested the capacity of new derivatives to improve retention of passive avoidance (long term memory), and second, if they would reverse amnesia induced by scopolamine in spontaneous alternation test (short term memory).

2. Experimental

2.1. Chemistry

2.1.1. General remarks

All solvents were distilled and dried prior to use. Reagents and materials were obtained from commercial suppliers and were used without further purification. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick). The spots were detected using a UV lamp (254 nm) and by spraying with sulfuric acid/ethanol (2:8) on TLC and subsequent heating. Column chromatography was carried out using silica gel 60 (0.063–0.2 mm) (Merck). Melting points were determined on a Kofler block. IR spectra were recorded on a PerkinElmer BX FT-IR spectrometer. EI mass spectra were recorded on a Jeol-GCmate (GC–MS system) spectrometer with ionisation energy from 30 to 40 eV. ^{1}H NMR and ^{13}C NMR spectra were recorded using $CDCl_3$ respectively at 400 MHz (Jeol Lambda 400 spectrometer) and at 100 MHz. Chemical shifts are reported relative to TMS; *J* values are given in Hz. ^{13}C NMR spectra are ^{1}H-decoupled. Elemental analyses were performed at the "Institut de Recherche en Chimie Organique Fine" (Rouen, France).

2.1.2. Chemical synthesis

*2.1.2.1. 7β-Amino-17,17-ethylenedioxy-androst-5-en-3β-ol (**I**).* Compound **I** has been prepared according to a reported procedure [44].

*2.1.2.2. 7β-Amino-3β-hydroxy-androst-5-en-17-one (**II**).* Compound **II** has been prepared according to a reported procedure [44].

*2.1.2.3. 3β-Acetoxy-androst-5-en-17-one (**1**).* Acetic anhydride (26 mL, 277 mmol) was added dropwise to a solution of dehydroepiandrosterone (10 g, 35 mmol) in pyridine (25 mL). The solution was stirred at room temperature and under argon for 12 h. Ice water (20 mL) was poured into the mixture. The white precipitate formed was dissolved in CH_2Cl_2 (200 mL). The organic layer was washed successively with 1 M HCl (3× 20 mL), 5% $NaHCO_3$ (1× 30 mL), brine (1× 30 mL) and water (1× 30 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was recrystallized from acetone to give 3β-acetoxy-androst-5-en-17-one (**1**) (10.9 g, 95%) as a white powder [44].

*2.1.2.4. 3β-Acetoxy-6-nitro-androst-5-en-17-one (**2**).* Fuming nitric acid (7.7 mL, 158.6 mmol) was added dropwise to a solution of 3β-acetoxy-androst-5-en-17-one (**1**) (10.0 g, 30.3 mmol) in acetic acid (25 mL). The solution was stirred at room temperature under room atmosphere for 2 h. The precipitate formed was dissolved in CH_2Cl_2 (300 mL). The organic layer was washed successively with 0.5N NaOH (2× 100 mL), brine (1× 50 mL) and water (1× 50 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was recrystallized from diethyl ether/ethyl acetate (10:1) to give 3β-acetoxy-6-nitro-androst-5-en-17-one (**2**) (9.4 g, 83%) as a white powder. Mp 219 °C. IR (KBr) ν (cm^{-1}): 2940–2864 (C–H alkane), 1736 (C=O ester), 1725 (C=O ketone), 1516, 1364, 1245

(N–O), 1029. ^{1}H NMR ($CDCl_3$): 0.63 (s, 3H, Me_{18}), 1.13 (s, 3H, Me_{19}), 2.02 (s, 3H, –OCOCH_3), 4.61–4.70 (m, 1H, H_3). ^{13}C NMR ($CDCl_3$): 13.7 (C18), 19.9 (C19), 20.5 (–OCOCH_3), 21.4, 21.9, 27.1, 31.2, 31.3, 31.4, 32.4, 35.8, 36.3, 38.1, 47.5, 49.2, 51.3, 71.9 (C3), 138.0, 146.1, 170.2 (–O$COCH_3$), 219.8 (C17). MS (EI, 30 eV) m/z (%): 315 (6) [$M^{+\bullet}$–AcOH], 300 (9) [$M^{+\bullet}$–(AcOH+$Me^{\bullet}$)], 286 (100), 271 (49), 258 (24), 231 (24), 163 (19). Anal. calcd. for $C_{21}H_{29}NO_5$: C, 67.18; H, 7.79; N, 3.73, found: C, 66.84; H, 7.51; N, 3.43.

*2.1.2.5. 3β-Acetoxy-5α-androstane-6,17-dione (**3**).* A suspension of zinc dust (22.3 g, 341.3 mmol) in acetic acid (150 mL) was refluxed for 20 min. After cooling to room temperature, 3β-acetoxy-6-nitro-androst-5-en-17-one (**2**) (8.0 g, 21.3 mmol) dissolved in acetic acid (15 mL) was added. The mixture was heated at 80 °C for 6 h. Zinc was filtered and the solution was diluted with water and extracted with CH_2Cl_2 (4× 100 mL) and the organic layer was washed with aqueous 0.5N NaOH (2× 50 mL) and then with water (1× 100 mL), dried over $MgSO_4$ and $CaCl_2$, and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, cyclohexane/ethyl acetate (9:1)) to give 3β-acetoxy-5α-androstane-6,17-dione (**3**) (6.3 g, 86%) as a white powder. Mp 188 °C. IR (KBr) ν (cm^{-1}): 2938–2860 (C–H alkane), 1734 (3× C=O), 1698, 1242, 1033. ^{1}H NMR ($CDCl_3$): 0.81 (s, 3H, Me_{18}), 0.88 (s, 3H, Me_{19}), 2.04 (s, 3H, –OCOCH_3), 4.64–4.73 (m, 1H, H_3). ^{13}C NMR ($CDCl_3$): 13.0 (C18), 13.8 (C19), 20.7 (–OCOCH_3), 21.3, 21.6, 26.1, 26.8, 31.1, 35.6, 36.3, 37.4, 40.9, 45.3, 48.1, 51.6, 53.9, 56.5, 72.6, 170.6 (–O$COCH_3$), 209.3 (C6), 219.8 (C17). MS (EI, 30 eV) m/z (%): 346 (1) [$M^{+\bullet}$], 286 (2) [$M^{+\bullet}$–AcOH], 271 (1) [$M^{+\bullet}$–(AcOH+$Me^{\bullet}$)], 117 (2), 87 (10), 85 (66), 83 (100). Anal. calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73, found: C, 73.02; H, 8.68.

*2.1.2.6. 3β-Hydroxy-5α-androstane-6,17-dione (**III**).* Aqueous 10% KOH (30 mL) was added to a solution of 3β-acetoxy-5α-androstane-6,17-dione (**3**) (5.0 g, 14.4 mmol) in ethanol (20 mL) and the mixture was then refluxed for 4 h. Ethanol was evaporated under reduced pressure and the aqueous solution was acidified with 2N HCl (pH 4). The solution was extracted with ethyl acetate (2× 100 mL) and the organic layer was washed with water (1× 50 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, cyclohexane/ethyl acetate (7:3)) to give 3β-hydroxy-5α-androstane-6,17-dione (**III**) (1.4 g, 32%) as a pale yellow powder. Mp 184 °C. IR (KBr) ν (cm^{-1}): 3420 (br, O–H), 2943–2857 (C–H alkane), 1735 and 1706 (2× C=O ketone), 1452, 1376, 1261, 1058. ^{1}H NMR ($CDCl_3$): 0.79 (s, 3H, Me_{18}), 0.87 (s, 3H, Me_{19}), 3.53–3.62 (m, 1H, H_3). ^{13}C NMR ($CDCl_3$): 13.0 (C19), 13.6 (C18), 20.6, 21.5, 29.7, 30.4, 31.0, 35.5, 36.5, 37.2, 40.7, 45.2, 48.0, 51.5, 53.7, 56.7, 70.1 (C3), 210.0 (C6), 219.9 (C17). MS (EI, 30 eV) m/z (%): 304 (100) [$M^{+\bullet}$], 289 (25) [$M^{+\bullet}$–$Me^{\bullet}$], 248 (29), 233 (21), 139 (89), 95 (77). Anal. calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27, found: C, 75.12; H, 9.45.

2.2. Behavioral study

2.2.1. Animals

Experiments were performed on male NMRI mice (6 weeks old) obtained from Centre d'Elevage René Janvier (Le Genest, France). They were maintained in a regulated environment (22 ± 2 °C; 55 ± 10% humidity) under a reversed 12–12 h light/dark cycle (light on between 20:00 and 8:00) with food and water available *ad libitum*. All experiments were complied with the European Directives and the French law on animal experimentation.

2.2.2. Drug administration

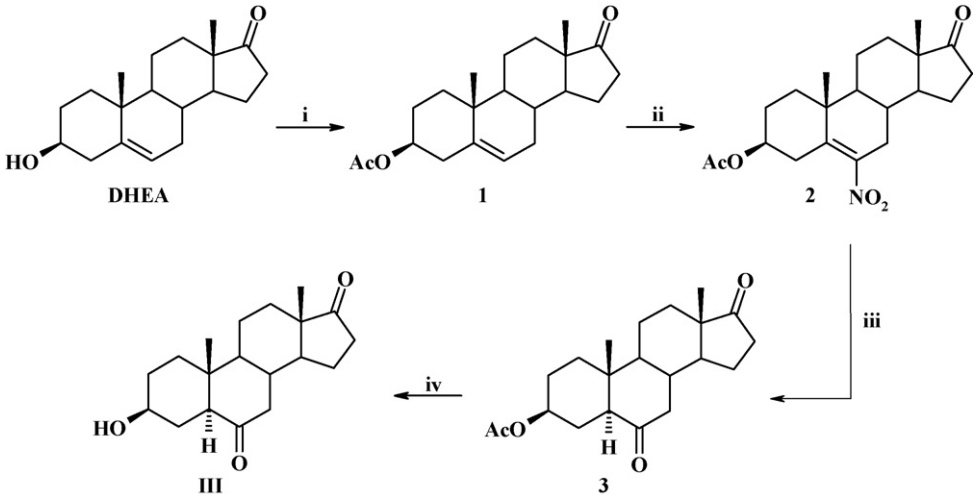
Scopolamine hydrochloride, from Sigma–Aldrich (Lyon, France) was dissolved in physiological saline (1 mg/kg) before use and prepared daily. DHEA and derivatives **I–III** were solubilized in dimethylsulfoxide (DMSO, Sigma–Aldrich) and then in saline solution, final vehicle being 5% DMSO in saline. All drugs were administered in a volume less than 0.1 mL/10 g body weight. In all tests, DHEA and analogues **I–III** were s.c. injected at 3 equimolar doses: 0.300–1.350–6.075 μmol/kg. In the spontaneous alternation task, scopolamine and DHEA/derivatives were all administered 30 min before the test; DHEA/derivatives were injected sub-cutaneously and scopolamine (1 mg/kg) was injected intra-peritoneally immediately after. The drug doses and administration routes were selected according to previous studies [35,45]. For clarification, the terms D1, D2 and D3 were attributed to the three increasing doses: 0.300, 1.350 and 6.075 μmol/kg, respectively in the results part (corresponding table and figures).

2.2.2.1. Locomotor activity. Spontaneous activity was recorded with a photoelectric activity meter [46] (APELAB) composed with Plexiglas enclosures (25.5 cm × 20.5 cm × 9 cm) equipped with two crisscross photo-cells in a dark enclosure. The number of interruptions of the photoelectric beams by each animal (n = 10 per group) was counted during a 5 min test 24 h after treatments.

2.2.2.2. Long term memory. A step-through type passive avoidance box (LETICA LE 872), divided into a wide, white and illuminated compartment (22 cm × 21 cm × 30 cm) and a small, black and dark compartment (7.3 cm × 7.5 cm × 14 cm) with a grid floor delivering electric foot shock, was used [47,48]. A guillotine door separated the two compartments. During a first training trial ("Session 1"), each mouse (n = 13 per group) was placed in the white compartment. As soon as the mouse had entered into the dark compartment (the step-through maximal latency was fixed at 50 s), the door was closed and the mouse received a unique inescapable electric shock (0.4 mA, 2 s). Twenty-four hours later, the mouse was tested for retention ("Session 2"); mouse was placed again into the white compartment and the time until it re-entered into the dark compartment was measured (the step-through maximal latency was fixed at 300 s; no electric shock was delivered). Drug was administered just after the session 1.

2.2.2.3. Short term memory. Immediate spatial working memory performances were assessed by recording spontaneous alternation behavior in a single-session Y-maze test [49,50]. The maze consisted of three equally spaced arms (22 cm long, 6.5 cm wide, walls of 10 cm high) made of black-painted wood. Thirty minutes after vehicle or drug injection, each mouse (n = 10 per group) was placed at the end of one arm and allowed to explore the maze freely during a 5 min session. The number and the sequence of arm entries were recorded by the observer. An arm entry was scored when all four feet crossed into the arm. Alternation behavior is defined as consecutive entries into all three arms. The percentage of alternation was calculated as a memory index by the (number of alternation/maximal theoretical number of alternation) × 100. DHEA and derivatives **I–III** were administered 30 min before the test. In the case of co-administration with scopolamine, neurosteroid derivatives were administered just after scopolamine which was injected 30 min before the test.

2.2.2.4. Statistical analyses. All experimental series were analyzed separately as independent series with their own control groups. In all studies, results were expressed as means ± SD. p-Values less than 0.05 were considered to be significant. For the spontaneous alternation and locomotor activity experiments, data were analyzed through a one-way analysis of variance (ANOVA) with "dose of drug" (for the dose–response studies) or "pharmacological treatment" (for the combined treatment studies) as an independent factor, followed, when appropriate, by a post hoc multiple comparison test (PLSD of Fischer) to locate the principal effect. Passive



Scheme 1. Synthesis of 3β-hydroxy-5α-androstane-6,17-dione (**III**). Reagents and reaction conditions: (i) Ac_2O, Py, r.t., 12 h; (ii) HNO_3, AcOH, r.t., 2 h; (iii) Zn, AcOH, 80 °C, 6 h; (iv) aqueous 10% KOH, EtOH, reflux, 4 h.

Table 1
Spontaneous locomotor activity expressed as the mean (±SD) number of beam interruptions in the activity box over a single 5 min session in mice 24 h after administration of either DMSO (5% in saline: control) or DHEA, **I**, **II**, and **III** at three different doses (D1, D2, and D3, see details in Section 2). ANOVA analysis did not reveal any effect of the four drugs ($n = 10$ in each group).

Control: 116.8 ± 60.6	Control: 132.0 ± 67.6	Control: 107.0 ± 47.7	Control: 97.5 ± 47.4
DHEA D1: 139.4 ± 52.4	**I** D1: 147.3 ± 79.4	**II** D1: 94.5 ± 57.7	**III** D1: 104.4 ± 57.7
DHEA D2: 114.6 ± 55.6	**I** D2: 121.8 ± 43.0	**II** D2: 110.4 ± 51.1	**III** D2: 82.9 ± 60.1
DHEA D3: 110.9 ± 54.9	**I** D3: 140.9 ± 30.2	**II** D3: 102.6 ± 56.4	**III** D3: 74.9 ± 52.3

avoidance learning was analyzed by a repeated measure ANOVA with interdependent "session" effect and an independent "group" effect. In the case of a significant principal effect and an interaction, a one-way ANOVA was undertaken and followed, if appropriate, by a PLSD of Fischer to locate the principal effect. Statistical analysis was performed with STATVIEW® software.

3. Results

3.1. Chemical study

7β-Amino-17,17-ethylenedioxy-androst-5-en-3β-ol (**I**) and 7β-amino-3β-hydroxy-androst-5-en-17-one (**II**) have been prepared according to a reported procedure [44].

3β-Hydroxy-5α-androstane-6,17-dione (**III**) was prepared as shown in Scheme 1. 3β-Acetoxy-androst-5-en-17-one **(1)** was treated with high density nitric acid ($d = 1.51$) in anhydrous acetic acid to give the 6-nitro derivative **2** in 83% yield. The nitro derivative **2** was then treated with zinc in acetic acid to give ketone **3**. The latter ketone was saponified by ethanolic potassium hydroxide to give the corresponding deacetylated ketone **III**.

3.2. Behavioral studies in mice

3.2.1. Locomotor activity

Results concerning horizontal locomotor activities registered 24 h after s.c. injection of DHEA and compounds **I**–**III** are indicated in Table 1. None of these derivatives altered spontaneous activ-

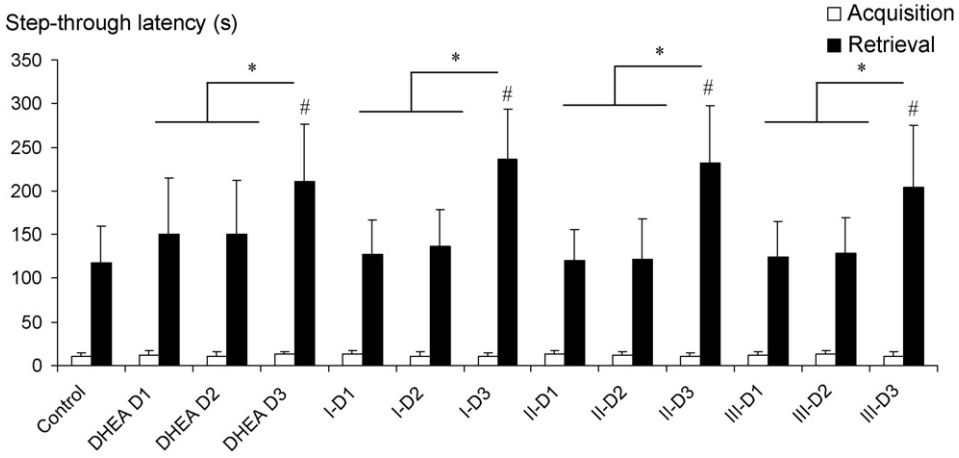


Fig. 2. Passive avoidance performances in control, DHEA, **I**, **II**, and **III** groups expressed by the step-through latency in seconds (mean ± SD). Each drug was administrated at three different doses (D1, D2, and D3, see details in Section 2). All the four drugs increased the step-through latency at the retrieval session at the highest doses. #Indicates a significant difference with control group; *indicates differences with the two doses D1 and D2.

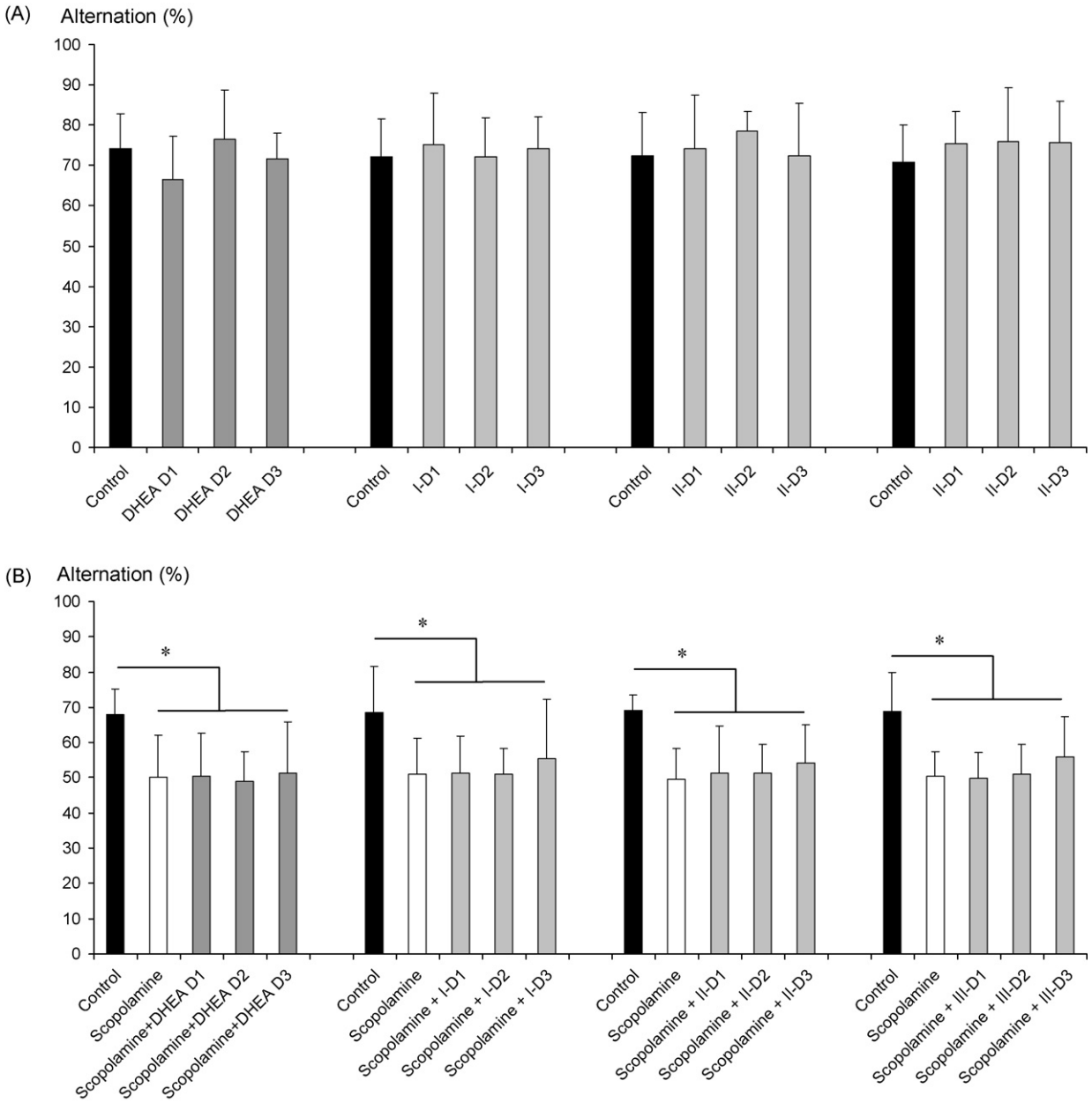


Fig. 3. Spontaneous alternation performances (mean percentages ± SD). (A) Effect of administration of either saline (control) or DHEA, **I**, **II**, and **III** at three different doses (D1, D2, and D3, see details in Section 2) on spontaneous alternation percentages in the Y-maze in mice. ANOVA did not reveal any effect of the four drugs. (B) Effect of scopolamine and DHEA, **I**, **II**, and **III** at three different doses on spontaneous alternation. ANOVA.

ity [ANOVA $F(3.36) = 0.529$; $p = 0.6651$ for DHEA; $F(3.36) = 0.814$, $p = 0.49$ for **I**; $F(3.36) = 0.165$, $p = 0.49$ for **II**, $F(3.36) = 0.0.606$, $p = 0.62$ for **III**)]. These data suggest that like DHEA, its derivatives also have no effect on spontaneous locomotor activity.

3.2.2. Long term memory (passive avoidance test)

At the acquisition session, there was no difference between groups; all mice entered the dark compartment within 50 s. At the retention session, there was a global difference between groups (ANOVA $F(12.56) = 9.105$; $p < 0.0001$), a global difference between the sessions showing that the retention session was significantly different from the acquisition session in all groups (ANOVA $F(1.12) = 1310.4$; $p < 0.0001$) and an interaction group × session (ANOVA $F(12.1) = 9.241$; $p < 0.0001$). Fisher's PLSD revealed that the highest doses (D3) of the three drugs induced an increase in step-through latency compared to controls (see Fig. 2). Indeed, in the DHEA injected groups, only DHEA D3 differed from control ($p < 0.0001$); the same holds true for the mice injected with **I**, **II**, or **III**, in which the highest doses induced an increase in the latency to enter the dark compartment compared to controls ($p < 0.0001$ for the three synthetic DHEA analogues at the dose D3). Accordingly, for each drug the dose D3 significantly differed from D1 and D2 ($p < 0.05$). These results suggest that at the maximal dose tested (6.075 μmol/kg) the four derivatives increase the retention performances of passive avoidance.

3.2.3. Short term memory

Working memory was assessed through spontaneous alternation in the Y-maze. The results (Fig. 3A) did not reveal any deleterious effect of the drugs, neither for the DHEA itself nor for its analogues, administered alone. Indeed, mice treated with the three different doses of DHEA or with the three analogues displayed

similar spontaneous alternation performances as controls (about 70%) and this was not modified whatsoever by the doses administered [ANOVA $F(3.36) = 1.921$; $p = 0.1437$ for DHEA; $F(3.36) = 0.256$, $p = 0.8564$ for **I**; $F(3.36) = 0.666$, $p = 0.5782$ for **II**, $F(3.36) = 0.569$, $p = 0.6389$ for **III**]. Fig. 3B shows the results obtained in condition of scopolamine-induced amnesia. Scopolamine was efficient in all experiments to induce deficits in spontaneous alternation shifting spontaneous alternation percentages from 70% to 50%. Indeed in all tested groups, there was a global group effect [ANOVA $F(4.45) = 5.072$, $p = 0.0018$ for DHEA + scopolamine; $F(4.45) = 3.820$, $p = 0.0093$ for **I** + scopolamine; $F(4.45) = 7.056$, $p = 0.0002$ for **II**; $F(4.45) = 7.306$, $p = 0.0001$ for **III** + scopolamine]. The difference was significant between control and scopolamine groups but not between mice administered by scopolamine and animals treated with DHEA or DHEA analogues [Fischer's PLSD: control *vs.* scopolamine: $p < 0.05$ (in all experiments); DHEA (D1, D2, D3) *vs.* scopolamine: $p > 0.05$; **I** (D1, D2, D3) *vs.* scopolamine: $p > 0.05$; **II** (D1, D2, D3) *vs.* scopolamine: $p > 0.05$; **III** (D1, D2, D3) *vs.* scopolamine: $p > 0.05$]. These results suggest that DHEA or its analogues were not able to reverse the deficits induced by scopolamine at any dose tested.

4. Discussion

4.1. Chemical study

In a previous paper [44] we described the synthesis of 7β-amino-17,17-ethylenedioxy-androst-5-en-3β-ol (**I**), 7β-amino-3β-hydroxy-androst-5-en-17-one (**II**) and 7α-amino-3β-hydroxy-androst-5-en-17-one. In that study, we only tested the β epimer (**II**), which was synthesized in greatest yield.

Compound **III** was prepared by the oxidation of DHEA acetate via nitration reaction on C-6 position of DHEA acetate. The ketone on C-6 was obtained with *trans* junction between A and B rings. It can be noticed that nitric acid with a density of 1.51 was needed in the nitration reaction. No reaction was observed with a lower density nitric acid (*e.g.*, $d = 1.49$).

4.2. Behavioral study

This study demonstrated the synthesis and action of new DHEA derivatives which had the same pharmacologic profile of the chemical reference in terms of mnemonic activity. Indeed, DHEA and compounds **I–III** improved consolidation of passive avoidance at equimolecular doses. These data are in agreement with studies indicating that immediate post-training administration of DHEA or DHEA sulfate improved retention of active and passive avoidance in mice [5,7,51–53]. Interestingly, DHEA and also derivatives **I–III** were only active at the highest dose suggesting similar dose–response scheme for the four compounds in this task. The mechanism of action of DHEA is complex and particularly involves an interaction between several receptors of neurotransmitters (GABA, acetylcholine, glutamate) implied in modulation of mnesic processes. The effect observed with the highest dose at the passive avoidance test suggests that a high level of occupancy (blockade of GABA receptors and parallel stimulation of cholinergic and glutamatergic receptors) of these targets is necessary to induce a promnesic action. Among these neurotransmitter systems, relationships between glutamate modifications and effects of DHEA have been highlighted. Lhullier et al. [54] reported that DHEA increased glutamate release *in vitro* and *in vivo* and improved performances in an avoidance task. On the same line, Wen et al. [55] showed that 5 days administration of DHEA increased the number of NMDA binding sites. Interestingly, DHEA could even reverse deficits induced by dozicilpine (a NMDA receptor antagonist) in passive avoidance test [56]. Therefore, DHEA and possibly its derivatives tested here could improve memory performances through an action potentiating glutamate transmission. Moreover, the effects of neurosteroids in the passive avoidance task seemed memory specific since in the present study, at the active dose none of these compounds altered spontaneous locomotor activity measured 24 h after s.c. injection. In addition, the active dose (20 mg/kg for DHEA) is the same that reported as improving retention deficit of spatial reference memory in aging mice [35]. In the spontaneous alternation task, the four neurosteroids, without *per se* effects on immediate working memory performances, failed to improve scopolamine-induced memory deficit. These results (especially, those concerning DHEA) did not fully agree with those of Urani et al. [57] who demonstrated that DHEA sulfate at 20 mg/kg (but not at 5 and 10 mg/kg) produced a significant attenuation of scopolamine (2 mg/kg, s.c.)-induced alternation deficit. Such discrepancies could be attributable to differences in experimental conditions (strain: Swiss mice in Urani et al. study, NMRI in our case; light/dark cycle: light on at 8 h for Urani et al., light on at 20 h for us; dimensions of the Y maze and protocol used: maze exploration during 8 min for Urani et al., 5 min herein). Concerning comparison of potential differences in mnemonic effects of the four compounds, these results confirm that they had similar profiles: compounds **I–III** as well as DHEA improved consolidation of long term memory but failed to improve scopolamine-induced working memory deficit. Moreover, these data suggested a particular action of DHEA in emotionally loaded memory [52]. In conclusion, these results seemed particularly innovative because the new DHEA analogues synthesized and tested are not biosynthetic products of DHEA.

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