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Differential effect of dehydroepiandrosterone and its steroid precursor pregnenolone against the behavioural deficits in CO-exposed mice

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Abstract

The neuroactive steroids pregnenolone (3β-hydroxy-5-pregnen-20-one) and dehydroepiandrosterone (DHEA, 3α-hydroxy-5-androstene-17-one) are negative allosteric modulators of the $GABA_A$ receptors and positive modulators of acetylcholine, NMDA and σ_1 receptors. Pregnenolone was recently shown to potentiate the neuronal damage induced by excessive glutamate in cell culture models, whereas dehydroepiandrosterone was reported to present some neuroprotective activity. The in vivo relevance of these effects was investigated in mice submitted to an hypoxic insult, the repeated exposure to carbon monoxide (CO) gas, a model that leads to neurodegeneration in the CA_1 hippocampal area and learning deficits. Recording spontaneous alternation behaviour in the Y-maze assessed short-term memory and long-term memory was examined using a passive avoidance task. After exposure to CO, mice showed a progressive deterioration of their learning ability, reaching significance after 3 days and being maximal after 7 days. Pregnenolone administered before CO significantly facilitated the hypoxia-related deficits, which could be measured 1 day after CO and appeared maximal after 3 days. Dizocilpine blocked the deficits in vehicle- and pregnenolone-treated CO-exposed animals, showing that pregnenolone selectively facilitated the NMDA receptor-dependent excitotoxicity. Dehydroepiandrosterone blocked the appearance of the CO-induced deficits, even after 7 days. Interestingly, the σ_1 receptor antagonist *N,N*-dipropyl-2-(4-methoxy-3-(2-phenylethoxy) phenyl)ethylamine (NE-100) failed to affect the dehydroepiandrosterone-induced protection, showing the lack of involvement of σ_1 receptors. Cresyl violet-stained sections of the mouse hippocampal formation showed that the neurodegeneration observed in the CA_1 area after exposure to CO was augmented by pregnenolone and blocked by dehydroepiandrosterone. These results show that pregnenolone and dehydroepiandrosterone, although being similarly involved in modulating the excitatory/inhibitory balance in the brain, do not equally affect the extent of excitotoxic insults.

Keywords: CO-induced amnesia; Neuroactive steroid; Pregnenolone; Dehydroepiandrosterone; Learning; Memory; (Mouse)

1. Introduction

In 1981, the term ''neurosteroid'' was introduced to describe several steroid hormones which accumulate in the brain independently of endocrine sources and which can be synthesised from sterol precursors in nervous cells (Baulieu, 1981). These neurosteroids include pregnenolone (3β-hydroxy-5-pregnen-20-one), which can be converted into progesterone by the action of the 3β-hydroxysteroid deshydrogenase, and then into allopregnanolone (3α-hydroxy-5α-pregnane-20-one) in two steps involving a 5α-reductase and a 3α-hydroxysteroid oxidoreductase enzymes (Phan et al., 1999). The conversion from pregnenolone to dehydroepiandrosterone (DHEA, 3α-hydroxy-5-androstene-17-one) may involve a cytochrome $P450_{c17}$, as described for steroidogenic glands. Fifteen days after removing the sources of circulating steroids, by adrenalectomy and gonadectomy in male rats, no difference in the brain levels of these steroids could be measured as compared to non-operated animals (Corpéchot et al., 1981, 1983). Apart from their classical genomic effects (Rupprecht et al., 1996), neuroactive steroids are able to affect several neurotransmission systems, in an excitatory or inhibitory way. Dehydroepiandrosterone, pregnenolone and

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their sulphate esters act as excitatory neurosteroids, since they antagonise the activation of $GABA_A$ receptors (Majewska and Schwartz, 1987; Majewska et al., 1988, 1990), whereas they potentiate the activation of the NMDA-type of glutamate receptors (Wu et al., 1991; Irwin et al., 1992, 1994; Monnet et al., 1995; Bergeron et al., 1996). Some other neurosteroids including progesterone or allopregnanolone act as inhibitory neurosteroids, being very potent agonists of $GABA_A$ receptors with affinities comparable to those of benzodiazepines (Smith, 1991). Finally, pregnanolone (3α-hydroxy-5β-pregnan-20-one) and epipregnanolone (3β-hydroxy-5β-pregnan-20-one) act as negative modulators of the NMDA receptor function (Park-Chung et al., 1994, 1997). These neuromodulatory actions led in turn to major behavioural consequences. Neuroactive steroids are involved in several physiopathological responses, such as stress, depression, anxiety, sleep, epilepsy and memory formation (for recent reviews, see Schumacher et al., 1997; Maurice et al., 1999b).

The excessive NMDA receptor stimulation as been shown to contribute to the neurodegeneration associated with trauma (Faden and Simon, 1988; Gómez-Pinilla et al., 1989) or hypoxic/ischemic insults (Benveniste et al., 1984; Rothman and Olney, 1986). The important Ca^{2+} influx resulting from the hyper-activation of the NMDA receptor complex has been shown to be critically associated with delayed excitotoxic neuronal death (Choi, 1987). Several in vitro models of excitotoxicity, including administration of glutamate or NMDA to cortical or hippocampal neuronal cultures or to the isolated retina, were used to examine the effects of neuroactive steroids on the extent of the excitotoxic insults. Pregnenolone sulphate was found to potentiate the NMDA-induced neurodegeneration (Guarneri et al., 1998; Weaver et al., 1998), whereas other steroids, such as dehydroepiandrosterone or pregnanolone sulphate protected significantly against the excitatory amino-acid-induced damages (Weaver et al., 1997; Kimonides et al., 1998; Mao and Barger, 1998). However, very few studies addressed the efficiency of these steroids in vivo.

Repetitive exposure to carbon monoxide (CO) gas constitutes an in vivo model of hypoxia, that induces in mice a long-lasting but delayed amnesia measured 1 week after exposure (Nabeshima et al., 1991; Maurice et al., 1994, 1999a). The hippocampal cholinergic system appears markedly affected by the hypoxic toxicity (Nabeshima et al., 1991). Histological studies have shown that 7 days after exposure to CO, a moderate neuronal loss in the CA_1 region of the hippocampal formation could be observed, which was augmented by increasing the severity of the CO exposure (Ishimaru et al., 1991). This model involves the neurotoxicity of excitatory amino acids. Competitive or non-competitive NMDA receptor antagonists, acting through either the glycine or the phencyclidine modulatory site, efficiently prevented the CO-induced amnesia and the concomitant neurodegeneration that occurs in the hippocampus (Nabeshima et al., 1991; Ishimaru et al., 1992). In this study, we used the repetitive CO exposure model to determine whether the neuroactive steroids pregnenolone and dehydroepiandrosterone would affect the extent of the hypoxic insult, as regards the resulting neurodegeneration and the deleterious effects on cognitive functions. Our results show that pregnenolone accelerates, whereas dehydroepiandrosterone blocks the cell death in the CA_1 hippocampal area and the appearance of the learning deficits observed in CO-exposed mice using both a short-term and a long-term memory tests.

2. Material and methods

2.1. Animals

Male Swiss mice (Breeding centre of the Faculty of Pharmacy, Montpellier, France), aged 5–6 weeks and weighing 30–35 g, at the beginning of the experiments, were used. Animals were housed in plastic cages, with free access to laboratory chow and water, except during behavioural experiments, and kept in a regulated environment (23 ± 1°C, 50% humidity), under a 12-h light/dark cycle (lights on at 8:00 a.m.). Experiments were carried out between 10:00 a.m. and 6:00 p.m., in a soundproof and air-regulated experimental room. The use of laboratory animals and experimental protocols followed the guidelines approved by INSERM (Paris, France).

2.2. Drugs and administration procedures

Pregnenolone and dehydroepiandrosterone were from Sigma (Saint-Quentin Fallavier, France); dizocilpine ((+)-MK-801 maleate) was from Research Biochemicals (Natick, MA, US); *N,N*-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)ethylamine (NE-100) was from Taisho Pharmaceutical (Tokyo, Japan). Pregnenolone and DHEA were suspended in pure sesame oil (Sigma). Other drugs were solubilised in saline solution. Drugs were injected subcutaneously (s.c.) or intraperitoneally (i.p.), in a volume of 100 μl per 20 g of body weight. The drug doses and administration routes were selected according to our previous studies (Maurice et al., 1996, 1998, 1999a; Urani et al., 1998).

2.3. Exposure to CO

Exposure to CO was carried out as previously described (Ishimaru et al., 1991, 1992; Nabeshima et al., 1991; Maurice et al., 1994, 1999a). Mice were placed in a transparent plastic vessel (3-cm radius, 10 cm high), with a pipe feeding into it. CO gas was disseminated at the rate of 45 ml/min, and mice were exposed until they began gasping, i.e., between 70 and 90 s. Animals were exposed three times, with 1 h between each exposure. They were kept on a hot plate (Silab, Montpellier, France) immedi-

ately after the first exposure and up to 2 h after the third, in order to maintain their body temperature at 38°C and to avoid the hypothermia induced by CO, which lessens the damages induced by hypoxia (Ishimaru et al., 1991). The neurosteroids, dehydroepiandrosterone (20 mg/kg), pregnenolone (20 mg/kg) or the vehicle, was administered 20 min before each exposure to CO.

2.4. Spontaneous alternation performances

Recording spontaneous alternation behaviour in the Y-maze assessed spatial working memory performances (Maurice et al., 1994, 1998, 1999a; Urani et al., 1998). The maze was made of black painted wood. Each arm was 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converged at an equal angle. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries, including possible returns into the same arm, was recorded using an Apple IIe computer. An alternation was defined as entries into all three arms on consecutive occasions. The number of maximum alternations was therefore the total number of arm entries minus two and the percentage of alternation was calculated as (actual alternations/maximum alternations) $\times$ 100.

2.5. Step-down type passive avoidance test

Long-term memory was examined using the step-down type of passive avoidance task (Ishimaru et al., 1991; Nabeshima et al., 1991; Maurice et al., 1994, 1998, 1999a). The apparatus consisted of a transparent acrylic cage (30 $\times$ 30 $\times$ 40 cm, $L \times W \times H$) with a grid-floor, inserted in a soundproof outer box ($35 \times 35 \times 90$, $L \times W \times H$). The cage was illuminated with a 15-W lamp during the experimental period. A wooden platform ($4 \times 4 \times 4$ cm) was fixed at the centre of the grid-floor. Intermittent electric shocks (1 Hz, 500 ms, 45 V DC) were delivered to the grid-floor using an isolated pulse stimulator (Model 2100, AM System, Everett, MA). The test consisted of two training sessions, at 90-min time interval, and in a retention session, carried out 24 h after the first training. During training sessions, each mouse was placed on the platform. When it stepped down and placed its four paws on the grid-floor, shocks were delivered for 15 s. Step-down latency and the numbers of vocalisations and flinching reactions were measured. Shock sensitivity was evaluated by summing these two numbers. Mice that showed latencies ranging from 3 to 30 s, i.e., more than 95% of the animals, were used for the second training and retention test. Animals, which did not step down within 60 s during the second session, were considered as remembering the task and taken off, without receiving electric shocks any more. The retention test was performed in a similar manner as training, except that the shocks were not applied to the grid-floor. Each mouse was placed again on the platform, and the latency was recorded, with an upper cut-off time of 300 s. Two parametric measures of retention were analysed: the latency and the number of animals reaching the avoidance criterion, defined as reached if the latency measured during the retention test was greater than three-fold the latency showed by the animal during the second training session and, at least, greater than 60 s. Basically, median step-down latency could be considered as a qualitative index of memory capacities, whereas the percentage of animals to criterion could be considered as a quantitative index (Maurice et al., 1998, 1999a).

2.6. Histology

Each mouse was anaesthetised with sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 15 ml of phosphate buffered saline solution (PBS), pH = 7.2, followed by 50 ml of PBS containing 4% paraformaldehyde (w/v), and then by 50 ml of PBS containing 20% sucrose (v/v) and 4% paraformaldehyde. The brains were removed and kept overnight in the last fixative solution. Brain sections were embedded in paraffin, then cut in coronal sections (8 μm thickness) using a microtome. Serial sections were selected to include the hippocampal formation. Sections were stained with 0.2% Cresyl violet reagent (Sigma), and then dehydrated with graded alcohol, treated overnight with Xylene and mounted with DePeX medium (BDH Laboratory Supplies, England). The CA_1 length was measured and the number of pyramidal cells in the CA_1 area was counted with a microcomputer imaging device (SAMBA analyser software, Alcatel, France), capturing the image through a CCD camera connected to a light microscope (Axioskop, Zeiss, Germany). The mean number of CA_1 pyramidal cells per millimetre was calculated for each group of mice following the previously reported method (Nabeshima et al., 1991; Ishimaru et al., 1992).

2.7. Statistical analyses

Results are expressed as means $\pm$ S.E.M., excepting step-down latency, which are expressed in terms of medians and interquartile ranges. The latencies to convulse were analysed using a repeated measures parametric one-way Analysis of Variance (ANOVA, *F*-values), followed by a test for linear trend among exposures. Data from the spontaneous alternation test were analysed using the Student's *t*-test or the Dunnett's multiple comparison tests after a one-way ANOVA. Step-down latencies did not show a normal distribution, since cut-off times were set. They were analysed using the Mann–Whitney's test or the Kruskal–Wallis non-parametric ANOVA (KW values), group comparisons being made with the Dunn's non-parametric multiple comparisons test. The percentages of animals to criterion were analysed by using the chi-squared test. Data from morphometric analyses were analysed us-

ing the Dunnett's multiple comparison test after a one-way ANOVA. The levels for statistical significance were $P < 0.01$, $P < 0.05$.

3. Results

3.1. Lack of effect of pregnenolone or dehydroepiandrosterone on the severity of the hypoxic insult

During the repetitive exposures to CO, the latency to convulse was measured (Fig. 1A) and the percentage of animals that died after one of the exposures determined (Fig. 1B). For the vehicle-treated group, the latency to convulse were in the 80–90 s range and increased with a significant linear trend at each exposure ($F(2,143) = 26.46$, $P < 0.001$; Fig. 1A). However, after accounting the linear trend, the remaining variation among mean latencies was not significant ($F = 1.19$, $P > 0.05$). The pretreatments with pregnenolone or dehydroepiandrosterone failed to affect these latencies, regarding the absolute values or the tendency to increase among repetitive exposures ($F(2,122) = 27.11$, $P < 0.001$ and $F(2,122) = 61.53$, $P < 0.001$ for the pregnenolone-treated and dehydroepiandrosterone-treated groups, respectively; Fig. 1A). In terms of percentage of death, the repetitive exposure to CO led in our experimental conditions to a percentage about 25%, with no difference observed among treatment group ($F(2,12) = 0.13$, $P > 0.05$; Fig. 1B).

3.2. Effect of pregnenolone or dehydroepiandrosterone on the learning deficits in mice exposed to CO

Mice exposed to CO showed a progressive deterioration of working memory, which could be measured by recording the spontaneous alternation strategy in the Y-shaped maze. Untreated animals that were not exposed to CO showed an alternation percentage of $69.5 \pm 1.8\%$ ($n = 9$; white column, Fig. 2A) and performed 23 ± 2 arm entries during the 8 min session in the maze (Fig. 2A). Untreated mice exposed to CO showed a progressive diminution of spontaneous alternation, when the behaviour was recorded at day 1, 3 and 7 after the exposure to CO ($F(3,73) = 30.00$, $P < 0.001$; dark columns, Fig. 2A). Seven days after CO exposure, animals exhibited an alternation percentage of $54.0 \pm 1.7\%$, close to the chance level thus indicative of a marked working memory impairment (Fig. 2A). In parallel, animals showed a progressive increase in the number of arms entered during the session ($F(3,73) = 15.26$, $P < 0.001$; Fig. 2A), reflecting a marked hyperlocomotion induced by the hypoxic insult. Preadministration of pregnenolone (20 mg/kg, s.c.) before each exposure to CO also resulted in marked deterioration of alternation capacities ($F(3,81) = 14.77$, $P < 0.001$; grey columns, Fig. 2A). A facilitation of the appearance of alternation deficits was observed on days 1 and 3, since the pregnenolone-

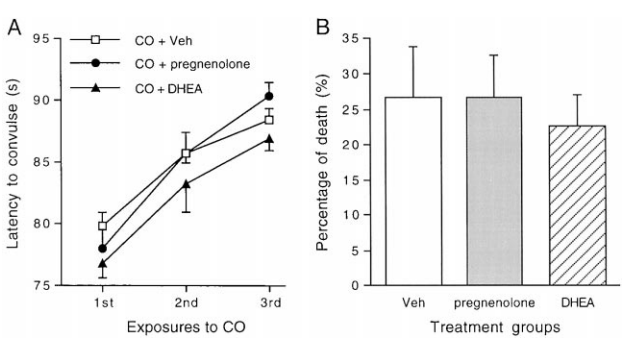

Fig. 1. Lack of effect of the pretreatments with the steroids pregnenolone or dehydroepiandrosterone on the severity of the hypoxic insults in mice exposed to CO: (A) latency to convulse; (B) percentage of death among groups. Mice were exposed three consecutive times to CO (45 ml/min, 70–90 s) at 38°C. The latency to convulse was measured during each exposure and the percentages of animals that died after one of the three exposures to CO were determined in five independent experiments. Pregnenolone (20 mg/kg s.c.), dehydroepiandrosterone (20 mg/kg s.c.) or the vehicle (Veh, sesame oil) was administered 20 min before each exposure.

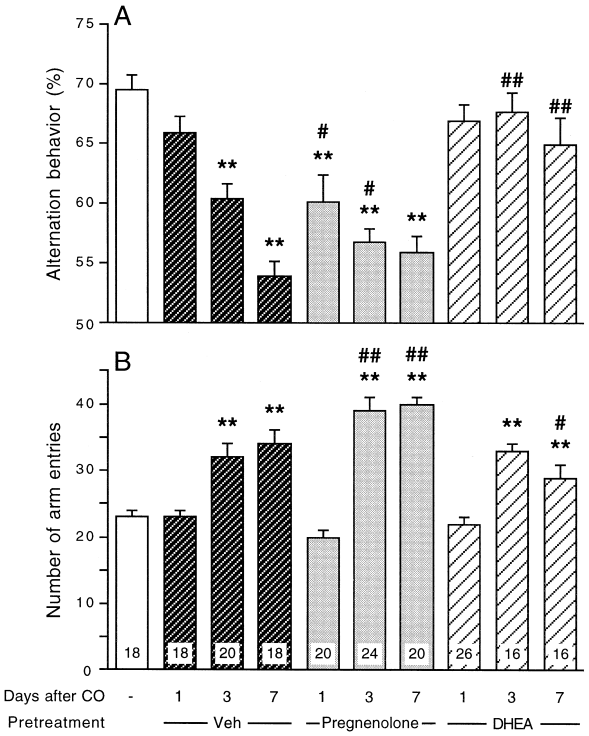

Fig. 2. Pretreatments with pregnenolone or dehydroepiandrosterone affect the appearance of behavioural deficits observed in mice after CO exposure in the Y-maze: (A) spontaneous alternation; (B) number of arm entries. Mice were exposed three consecutive times to CO (45 ml/min, 70–90 s) at 38°C, and alternation performances were examined 1, 3 and 7 days after exposure. Pregnenolone (20 mg/kg s.c.), dehydroepiandrosterone (20 mg/kg s.c.) or the vehicle (Veh, sesame oil) was administered 20 min before each exposure. The number of animals per group is indicated in (B). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control non-CO exposed group; $\#P < 0.05$, $\#\#P < 0.01$ vs. the CO-exposed group on the same day; Dunnett's test.

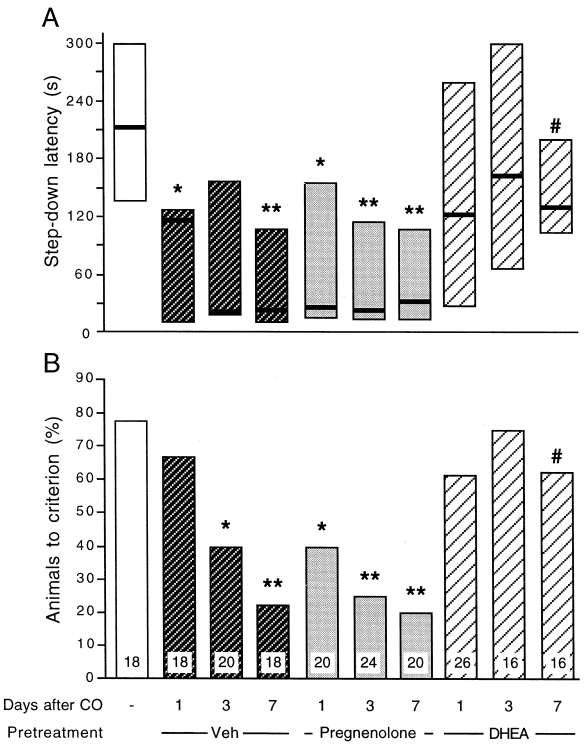

Fig. 3. Pretreatments with pregnenolone or dehydroepiandrosterone affect the passive avoidance deficits in CO-exposed mice: (A) step-down latency and (B) percentage of animals to criterion. Mice were exposed three consecutive times to CO (45 ml/min, 70–90 s) at 38°C, and animals were trained for passive avoidance behaviour 1, 3 or 7 days after exposure. Retention was examined 24 h after training. Pregnenolone (20 mg/kg s.c.), dehydroepiandrosterone (20 mg/kg s.c.) or the vehicle (Veh, sesame oil) was administered 20 min before each exposure. The number of animals per group is indicated in (B). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group; $\#P < 0.05$ vs. the CO-exposed group on the same day; Dunn's test in (A) and chi-squared test in (B).

treated CO exposed group showed a significantly decreased alternation percentage as compared to both the non-CO exposed group and the vehicle-treated CO-exposed group (Fig. 2A). In parallel, a significant potentiation of the hyperlocomotion was observed on days 3 and 7, as compared with the vehicle-treated CO-exposed group (Fig. 2B). On the contrary, preadministration of dehydroepiandrosterone led to an attenuation of the deficits induced after CO exposure. In terms of alternation percentages, no decrease was observed between day 1 and 7 after the CO exposure ($F(3,75) = 1.30$, $P > 0.05$; hatched columns, Fig. 2A). As a result, the deficits observed for the vehicle-treated CO-exposed groups on day 3 and 7 were significantly blocked (Fig. 2A). In parallel, the hyperlocomotion induced after CO was still observable ($F(3,75) = 21.89$, $P < 0.001$; Fig. 2B), but less pronounced since the number of arms entries showed on day 7 by the dehydroepiandrosterone-treated CO-exposed group was significant lower than the one showed by the vehicle-treated CO-exposed group (Fig. 2B).

The long-term memory deficits were measured after CO exposure using a passive avoidance test (Fig. 3). Untreated animals not exposed to CO showed a step-down latency of 214 [137–300] s (Fig. 3A) and a percentage of animals to criterion of 77.8% (Fig. 3B). Untreated mice exposed to CO showed a progressive diminution of their passive avoidance response from day 1 to 7 after CO, both in term of step-down latency ($KW = 15.13$, $P < 0.01$, Fig. 3A) and of percentage of animals to criterion (Fig. 3B). On day 7, both parameters were highly significantly diminished as compared with the control group. The preadministration of pregnenolone resulted in marked facilitation of the deficits ($KW = 16.85$, $P < 0.001$), already significant on day 1

Table 1
Effects of the different treatments on sensitivity to electric shocks during the first passive avoidance training session for CO-exposed mice
The shock sensitivity scores represent the sum of the numbers of vocalisations plus flinching reactions in response to electric shocks (1 Hz, 500 ms, 45 V, for 15 s) received during the first training session. Results are presented for the experiment presented in Fig. 3. Kruskal–Wallis ANOVA (KW values) for latencies; parametric ANOVA (F-values) for shock sensitivity.

Treatments	Day after CO	n	Latency (s) Median [I.R.]	Shock sensitivity Mean ± S.E.M.
Non-CO exposed mice				
+ Vehicle		18	4 [3–4]	12 ± 1
CO-exposed mice				
+ Vehicle	1	18	6 [4–7]	11 ± 1
	3	20	6 [4–9]	12 ± 1
	7	18	3 [3–9]	14 ± 1
			$KW = 4.80$, $P > 0.05$	$F(3,73) = 2.25$, $P > 0.05$
+ Pregnenolone	1	20	4 [3–7]	14 ± 1
	3	24	6 [5–11]	12 ± 1
	7	20	4 [4–10]	14 ± 1
			$KW = 10.99$, $P > 0.05$	$F(3,81) = 0.70$, $P > 0.05$
+ Dehydroepiandrosterone	1	26	6 [3–9]	13 ± 1
	3	16	7 [4–16]	15 ± 2
	7	16	7 [4–10]	14 ± 0
			$KW = 7.44$, $P > 0.05$	$F(3,75) = 1.67$, $P > 0.05$

and maximal on day 3 after CO, since no further augmentation of the deficits was observed on day 7 (Fig. 3A and B). On the contrary, the preadministration of dehydroepiandrosterone led to a blockade of the appearance of the deficits after exposure to CO, both in terms of step-down latency ($KW = 7.57$, $P > 0.05$, Fig. 3A) and of percentage of animals to criterion (Fig. 3B). None of the parameters appears different from the ones of control animals, whatever the day after CO. Furthermore, on day 7 significant differences were observed between the parameters showed by dehydroepiandrosterone-treated CO-exposed animals and vehicle-treated CO exposed animals (Fig. 3A and B).

These differences observed during the retention test could be interpreted in terms of learning impairments since, as detailed in Table 1, the different treatments (CO exposure or the subsequent administration of each steroid) failed to affect the latency during the first training session and the sensitivity to the shocks, in terms of number of flinching reactions and vocalisations.

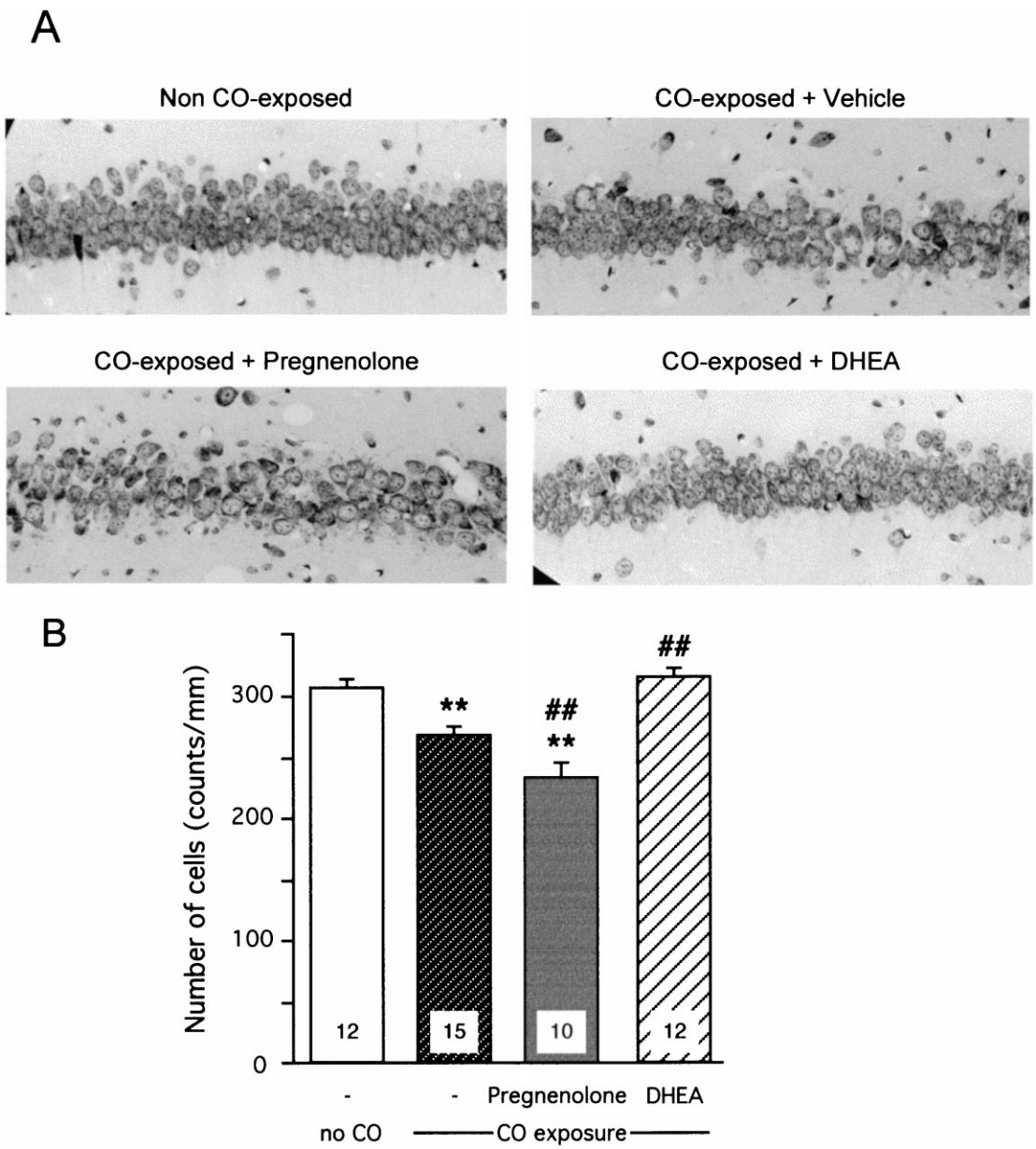

Fig. 4. Delayed death of CA_1 pyramidal cells in the hippocampus 7 days after CO exposure. (A) Representative microphotographs of 8 μm coronal sections of Cresyl violet-stained hippocampal CA_1 subfield. (B) Morphometric analysis of the number of hippocampal CA_1 pyramidal cells. The number of samples used is indicated within the columns; $F(3,48) = 19.89$, $P < 0.0001$. Treatment groups included vehicle-treated non CO-exposed animals (control group) and CO-exposed animals and pregnenolone (20 mg/kg)-treated and dehydroepiandrosterone (20 mg/kg)-treated CO-exposed animals. $^{**}P < 0.01$ vs. the control group; $\#\#P < 0.01$ vs. the CO-exposed group; Dunnett's test.

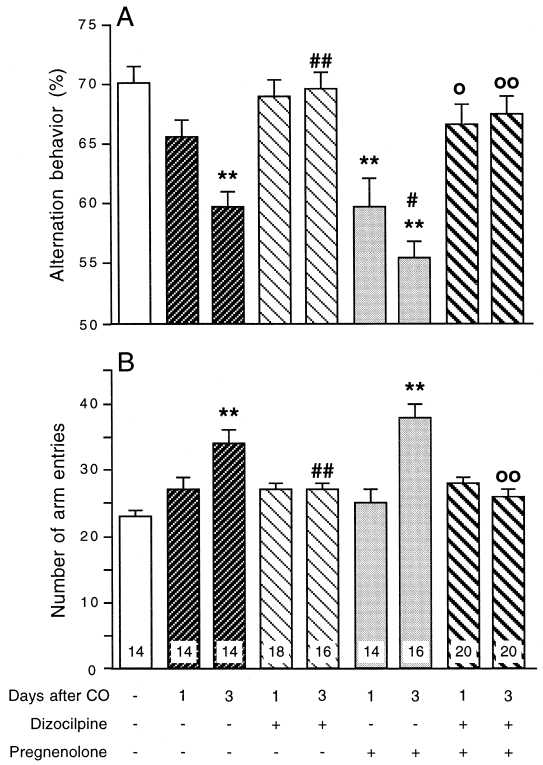

Fig. 5. Antagonism by dizocilpine of the facilitation by pregnenolone of the appearance of behavioural deficits observed in mice after CO exposure in the Y-maze: (A) spontaneous alternation; (B) number of arm entries. Mice were exposed three consecutive times to CO (45 ml/min, 70–90 s) at 38°C, and alternation performances were examined 1 and 3 days after exposure. Dizocilpine (0.1 mg/kg i.p.) was administered 10 min before pregnenolone (20 mg/kg s.c.), which was given 20 min before each exposure. The number of animals per group is indicated in (B). $F(8,145) = 10.86$, $P < 0.0001$ in (A) and $F(8,145) = 9.75$, $P < 0.0001$ in (B). $^{**}P < 0.01$ vs. the vehicle-treated non-CO exposed group; $\#P < 0.05$, $\#\#P < 0.01$ vs. the vehicle-treated CO-exposed group; $^{o}P < 0.05$, $^{oo}P < 0.01$ vs. the pregnenolone-treated CO-exposed group on the same day; Dunnett's test.

3.3. Morphometric analysis of the pregnenolone or dehydroepiandrosterone effects in the hippocampal formation of mice exposed to CO

Seven days after successive CO exposures, morphometric alterations were visualised as previously reported (Nabeshima et al., 1991; Ishimaru et al., 1992) in the CA_1 subfield of the mouse hippocampal formation, which appeared as the most vulnerable region to the hypoxic insult. The layer of the pyramidal cells in the CA_1 area appears thinner and damaged in CO-exposed animals as compared to control ones (Fig. 4A). Disappearance and atrophy of neural cells is observed. The cell density measured in the CA_1 hippocampal area of the control group was about 307 counts/mm (Fig. 4B). The successive CO exposures in the vehicle-treated group produced a significant 13% decrease in the CA_1 area (Fig. 4B). The treatment with pregnenolone (20 mg/kg) led to a significant augmentation of the neurodegeneration, since a 24% decrease in the number of cells was measured (Fig. 4A and B). On the contrary, the treatment with dehydroepiandrosterone (20 mg/kg) induced a complete protection against the neurodegeneration of the CA_1 hippocampal subfield (Fig. 4A and B).

3.4. Effect of dizocilpine on the facilitation of the learning deficits in mice exposed to CO by pregnenolone

The involvement of the NMDA receptor in the facilitation of the behavioural deficits observed after exposure to CO in mice, and in the facilitation induced by pregnenolone, was examined using the selective non-competitive NMDA receptor antagonist dizocilpine. Dizocilpine (0.1 mg/kg) was administered i.p. 10 min before pregnenolone, administered 20 min before each exposure to CO. The effect of the pre-treatment with dizocilpine was examined on the appearance of the learning deficits in the spontaneous alternation (Fig. 5) and passive avoidance

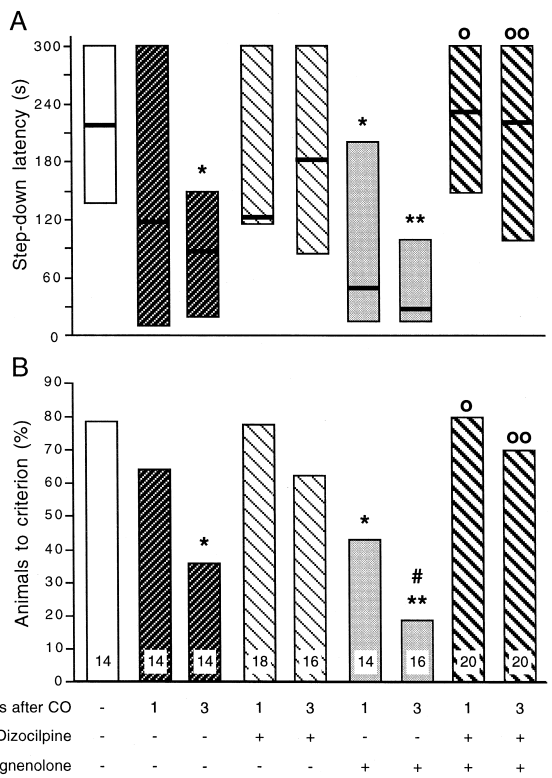

Fig. 6. Antagonism by dizocilpine of the facilitation by pregnenolone of the appearance of passive avoidance deficits in CO-exposed mice: (A) step-down latency and (B) percentage of animals to criterion. Mice were exposed three consecutive times to CO (45 ml/min, 70–90 s) at 38°C, and animals were trained for passive avoidance behaviour 1 or 3 days after exposure. Retention was examined 24 hr after training. Dizocilpine (0.1 mg/kg i.p.) was administered 10 min before pregnenolone (20 mg/kg s.c.), which was given 20 min before each exposure. The number of animals per group is indicated in (B). KW = 24.54, $P < 0.01$ in (A). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group; $\#P < 0.05$ vs. the CO-exposed group; $^{o}P < 0.05$, $^{oo}P < 0.01$ vs. the pregnenolone-treated CO-exposed group on the same day; Dunn's test in (A) and chi-squared test in (B).

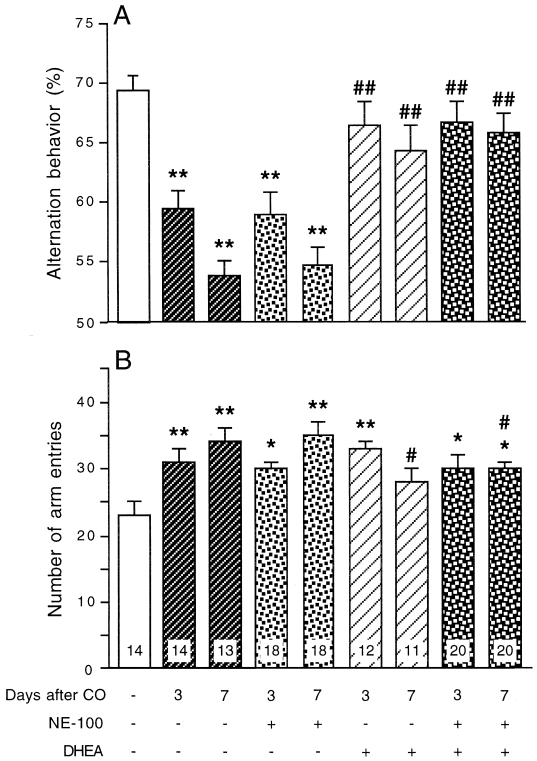

Fig. 7. Lack of antagonism by NE-100 on the prevention by dehydroepiandrosterone of the appearance of behavioural deficits observed in mice after CO exposure in the Y-maze: (A) spontaneous alternation; (B) number of arm entries. Mice were exposed three consecutive times to CO (45 ml/min, 70–90 s) at 38°C, and alternation performances were examined 3 and 7 days after exposure. NE-100 (1 mg/kg i.p.) was administered 10 min before dehydroepiandrosterone (20 mg/kg s.c.), which was given 20 min before each exposure. The number of animals per group is indicated in (B). $F(8,96) = 10.17$, $P < 0.0001$ in (A) and $F(8,96) = 4.63$, $P < 0.0001$ in (B). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the vehicle-treated non-CO exposed group; $\#P < 0.05$, $\#\#P < 0.01$ vs. the vehicle-treated CO-exposed group on the same day; Dunnett's test.

(Fig. 6) behaviours on day 1 and 3 after exposure to CO. In the Y-maze test, the pre-treatment with dizocilpine alone led to the blockade of the deficits observed on day 3 for CO-exposed mice, in terms of alternation percentage (Fig. 5A) and of number of arm entries (Fig. 5B). The treatment with pregnenolone resulted in a facilitation of the alternation deficits (Fig. 5A), since the decrease was significant on day 1 as compared to the control group, and since the alternation percentage was significantly lower for pregnenolone-treated CO-exposed animals as compared to the vehicle-treated CO-exposed ones (Fig. 5A). The pre-treatment with dizocilpine led to a complete blockade of both the alternation deficits observed on days 1 and 3 (Fig. 5A) and the hyperlocomotion observed on day 3 (Fig. 5B). Similar results were observed using the long-term memory test. The treatment with dizocilpine led to a blockade of the CO-induced deficits, both in terms of step-down latencies (Fig. 5A), and of percentage of animals to criterion (Fig. 5B). The treatment with pregnenolone led to a facilitation of the CO-induced deficits since the parameters appeared significantly decreased on day 1 and 3. Moreover, the percentage of animals to criterion on day 3 was significantly lower in pregnenolone-treated CO-exposed animals as compared to vehicle-treated CO-exposed ones (Fig. 5B). The dizocilpine pre-treatment led to a complete and significant prevention of the deficits on day 1 and 3 and both in terms of step-down latency (Fig. 6A) and percentage of animals to criterion (Fig. 6A).

3.5. Effect of NE-100 on the prevention of the learning deficits in mice exposed to CO by dehydroepiandrosterone

The putative involvement of the σ_1 receptor in the facilitation of the behavioural deficits observed after exposure to CO in mice was examined using the selective σ_1 receptor antagonist NE-100. The drug (1 mg/kg) was administered i.p. 10 min before the steroid, administered 20 min before each exposure to CO, and the appearance of the learning deficits in the spontaneous alternation (Fig. 7) and passive avoidance (Fig. 8) behaviours was measured on day 3 and 7 after exposure to CO. In the Y-maze test, the pre-treatment with NE-100 failed to affect the extent of

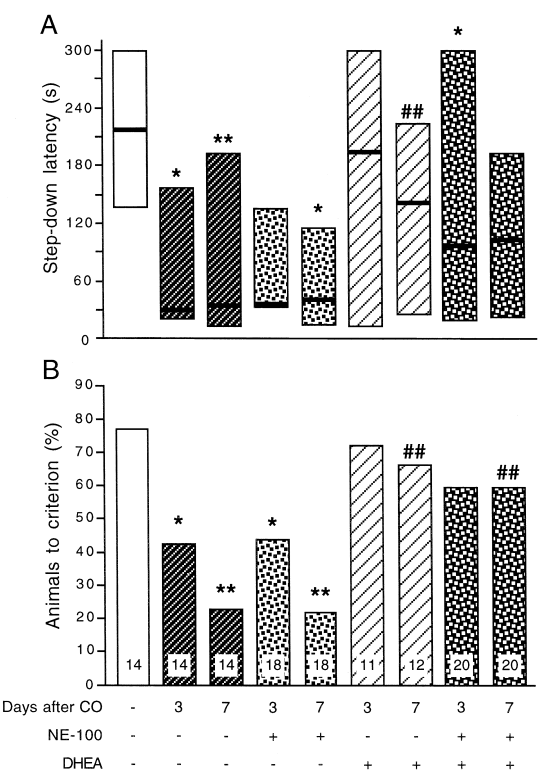

Fig. 8. Lack of antagonism by NE-100 on the prevention by dehydroepiandrosterone of the appearance of passive avoidance deficits in CO-exposed mice: (A) step-down latency and (B) percentage of animals to criterion. Mice were exposed three consecutive times to CO (45 ml/min, 70–90 s) at 38°C, and animals were trained for passive avoidance behaviour 3 or 7 days after exposure. Retention was examined 24 h after training. NE-100 (1 mg/kg i.p.) was administered 10 min before dehydroepiandrosterone (20 mg/kg s.c.), which was given 20 min before each exposure. The number of animals per group is indicated in (B). $KW = 19.49$, $P < 0.05$ in (A). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the control group; $\#P < 0.05$ vs. the CO-exposed group on the same day; Dunnett's in (A) and chi-squared test in (B).

the spontaneous alternation deficits and hyperlocomotion induced after exposure to CO (Fig. 7A and B). Moreover, the drug also failed to affect the prevention of these deficits observed after treatment with dehydroepiandrosterone, both in terms of spontaneous alternation on day 3 or 7 (Fig. 7A) or of number of arm entries (Fig. 7B). Similar results were observed in the long-term memory test (Fig. 8). The treatment with NE-100 failed to affect the deficits observed 3 and 7 days after exposure to CO, both in terms of step-down latency (Fig. 8A) and percentage of animals to criterion (Fig. 8B). Moreover, NE-100 failed to affect the prevention of these deficits observed after the dehydroepiandrosterone treatment, as shown for the step-down latencies (Fig. 8A) and, more clearly, for the percentage of animals to criterion (Fig. 8B).

4. Discussion

The experiments reported here show that the neuroactive steroids dehydroepiandrosterone and its precursor pregnenolone potently but differentially modulate the extent of an hypoxic insult in mice as measured through the neurodegeneration in the CA_1 hippocampal area and the behavioural consequences on learning. The animals were exposed three times to CO gas until they began gasping, following a procedure that was previously shown to induce marked neurodegeneration in the hippocampal formation (Nabeshima et al., 1991), correlated with the appearance of cognitive deficits (Nabeshima et al., 1991; Maurice et al., 1994, 1999a). This study was in accordance with these previously published observations. Indeed, marked neurodegeneration was visualised in the CA_1 pyramidal neuronal layer of the hippocampal formation 1 week after exposure to CO. Furthermore, both short-term and long-term memory deficits could be evidenced, developing gradually during seven days. One day after CO exposure, almost no measurable deficit could be observed, in terms of learning capacities in the Y-maze and passive avoidance tests or on the locomotion in the Y-maze. Deficits became significant three days after exposure and developed until 7 days after exposure, when they could be considered as maximal. These behavioural deficits and the neuronal death in the hippocampus are known to involve the excitotoxic hyper-activation of the NMDA receptors (Ishimaru et al., 1992) and led consequently to dysfunction of the cholinergic systems in the frontal cortex, striatum and hippocampus (Nabeshima et al., 1991). The systemic preadministration of pregnenolone, at a pharmacologically relevant dosage, led to clear augmentation of the neurodegeneration and to facilitation of the learning deficits that appeared maximal after only 3 days. A similar treatment with dehydroepiandrosterone led, however, to an almost complete protection against the CO-induced behavioural deficits, even seven days after exposure.

Pregnenolone is known to positively modulate the activation of the NMDA receptor complex. In particular, the steroid or its sulphate ester augmented the NMDA receptor-mediated increase in intracellular Ca^{2+} in hippocampal neuronal cultures (Irwin et al., 1991, Irwin et al., 1992; Bowlby, 1993; Weaver et al., 1998). At the behavioural level, pregnenolone sulphate potentiated the convulsing potency of NMDA (Maione et al., 1992) and antagonised the learning deficits induced in rodents by competitive or non-competitive NMDA receptor antagonists (Mathis et al., 1994; Cheney et al., 1995). The steroid acts through a specific, extracellularly directed modulatory site, located on the receptor complex, but distinct from either the spermine, glycine, phencyclidine, arachidonic acid, Mg^{2+} and redox sites (Park-Chung et al., 1997). Interestingly, some other steroids, like pregnanolone sulphate or epipregnanolone sulphate inhibited the NMDA response, through a similar direct action on the NMDA receptor, but that may involve a distinct site on the complex (Park-Chung et al., 1994, 1997).

Pregnenolone sulphate was also reported to facilitate the NMDA receptor-mediated excitotoxic cell death in several in vitro models of neurodegeneration. First, pregnenolone sulphate potentiated the increase in intracellular free calcium concentration ($[Ca^{2+}]_i$) induced by either acute or chronic NMDA exposure in primary cultures of rat hippocampal neurones and thereby exacerbated the resulting dizocilpine-sensitive neuronal death (Weaver et al., 1998). Second, pregnenolone sulphate potentiated the neurodegeneration observed in isolated and intact rat retina preparation exposed to NMDA (Guarneri et al., 1998). In these studies, competitive or non-competitive NMDA receptor antagonists, such as 3(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid (CPP) or dizocilpine, respectively, completely blocked the potentiating effect induced by the steroid, demonstrating the specificity of its modulatory action. In vivo, we also observed that a pre-treatment with dizocilpine allowed a complete protection against the hypoxic insult and against the pregnenolone-induced facilitation of the deficits. It can thus be concluded that pregnenolone induces a selective and efficient potentiation of the NMDA receptor activation that, in turn, presents major consequences in case of hyper-activation of this receptor. The steroid indeed potentiates the excitotoxic neurodegeneration and worsens the resulting behavioural deficits.

Dehydroepiandrosterone or its sulphate ester is also considered as an excitatory steroid. First, it acts as a negative allosteric modulator of the $GABA_A$ receptor (Majewska et al., 1990). Second, it potentiates several responses to NMDA in vitro and in vivo (Monnet et al., 1995; Bergeron et al., 1996). However, this last effect is unlikely to be related to the pregnenolone effect on NMDA receptors, since: (i) dehydroepiandrosterone/dehydroepiandrosterone sulphate does not interact directly with the NMDA receptor (Park-Chung et al., 1994; Kimonides et al., 1998), and (ii) the dehydroepiandrosterone/dehydroepiandrosterone sulphate-induced potentiation of NMDA responses rather involve an agonistic type effect at the σ_1

receptors (Monnet et al., 1995; Bergeron et al., 1996; Maurice and Privat, 1997; Maurice et al., 1998; Urani et al., 1998). However, contrarily to this excitatory neuromodulatory effect, dehydroepiandrosterone has been reported to attenuate the neurodegeneration in vitro. First, dehydroepiandrosterone/dehydroepiandrosterone sulphate prevented or reduced the neurotoxic effects in primary hippocampal cultures exposed to not only NMDA, but also α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainic acid (Kimonides et al., 1998). These authors also reported that s.c. implanted dehydroepiandrosterone pellets protected the CA_1 hippocampal neurones against the toxicity induced in vivo by unilateral infusions of NMDA. Second, Mao and Barger (1998) also observed a good neuroprotective efficacy of dehydroepiandrosterone sulphate, but not dehydroepiandrosterone, against the glutamate-induced neuronal death in primary cultures of rat hippocampal neurones. Interestingly, they observed that the differential neuroprotective activity could be related to the ability of each form of the steroid to elevate a κB-dependent transcription factor activity (Mao and Barger, 1998). Our results confirmed that dehydroepiandrosterone is a potent neuroprotective steroid, efficient against the hypoxia-related excitotoxicity in vivo. A first hypothesis to explain this differential effect, as compared with the other excitatory steroid pregnenolone, could be related to their different interaction with the NMDA receptor complex. Indeed, the direct and efficient interaction of pregnenolone is consistent with its ability to enhance the NMDA-induced toxicity. dehydroepiandrosterone or its sulphate ester, on the contrary, potentiates the NMDA-evoked responses through its interaction with the σ_1 receptor. Pregnenolone was reported to act as an inverse agonist on this σ_1 receptor in vitro (Monnet et al., 1995) or to be inefficient in vivo (Bergeron et al., 1996). This σ_1 receptor is known to mediate an apparently non-selective neuromodulation affecting several neurotransmission systems, and leading to major behavioural consequences in learning and memory processes, response to stress and depression, addiction, and neuroprotection (for reviews, see Maurice and Lockhart, 1997; Maurice et al., 1999b). Agonists at the σ_1 receptor exert a bell-shaped effect, since at low dosages they facilitate the NMDA receptor activation and at higher doses they become inefficient or inhibit the NMDA-mediated responses (Monnet et al., 1995; Bergeron et al., 1996; Maurice and Lockhart, 1997; Maurice et al., 1999b). In turn, several σ_1 receptor agonists showed a potent neuroprotective efficacy in several in vitro or in vivo models of excitotoxicity (reviewed in Maurice and Lockhart, 1997). Finally, this interaction of dehydroepiandrosterone/dehydroepiandrosterone sulphate with the σ_1 receptor could explain the neuroprotective efficacy of the steroid. In this study, we thus examined whether the selective σ_1 receptor antagonist NE-100 could block the dehydroepiandrosterone effect. NE-100 was used at a dose already reported to prevent all pharmacological or behavioural effects of numerous σ_1 receptor agonists or steroids (Bergeron et al., 1996; Maurice et al., 1998, 1999a,b). The drug failed to affect the dehydroepiandrosterone-mediated protection against the hypoxic-induced deficits, clearly indicating that this dehydroepiandrosterone effect does not involve its interaction with the σ_1 receptor. An alternate hypothesis could rely on a direct effect of the steroid on transcription factors as proposed by Mao and Barger (1998).

This study pointed out that dehydroepiandrosterone exerted a potent neuroprotective effect against the CO-induced behavioural and histological deficits, whereas its precursor steroid pregnenolone potentiated the toxicity. It appears thus that neurosteroidal hormones exert a complex modulation on the extent of hypoxia insults. The biosynthesis pathway by which pregnenolone is converted into dehydroepiandrosterone in the brain is not yet established, but it may constitute a limiting factor. Indeed, brain levels of dehydroepiandrosterone are very low in rodents (Corpéchot et al., 1981). Furthermore, the observation that exogenously administered pregnenolone was highly toxic (Guarneri et al., 1998; this study) confirmed that it is not quickly and quantitatively converted into dehydroepiandrosterone. Future studies must focus on this enzymatic step in the steroidal biosynthesis.

In conclusion, this study demonstrated that, on a reliable in vivo model of hypoxic insult and of the resulting behavioural consequences, the parent steroid pregnenolone markedly potentiated the NMDA-induced toxicity, whereas dehydroepiandrosterone was neuroprotective. The effect of pregnenolone is clearly due to its efficient potentiation of the activation of the NMDA receptor. However, the exact mechanism of the dehydroepiandrosterone-mediated neuroprotective effect remains to be determined.

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References

Baulieu, E.E., 1981. Steroid hormones in the brain: several mechanisms? In: Fuxe, K., Gustafson, J.A., Wettenberg, L. (Eds.), Steroid Hormone Regulation of the Brain. Pergamon, Oxford, pp. 3–14.

Benveniste, H., Drejer, J., Schousboue, A., Diemer, N.H., 1984. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. 43, 1369–1374.

Bergeron, R., de Montigny, C., Debonnel, G., 1996. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via σ receptors. J. Neurosci. 16, 1193–1202.

Bowlby, M.R., 1993. Pregnenolone sulphate potentiation of *N*-methyl-D-aspartate receptor channels in hippocampal neurons. Mol. Pharmacol. 43, 813–819.

Cheney, D.L., Uzunov, D., Guidotti, A., 1995. Pregnenolone sulphate

antagonizes dizocilpine amnesia: role for allopregnanolone. Neuroreport 6, 1697–1700.
Choi, D.W., 1987. Ionic dependence of glutamate neurotoxicity. J. Neurosci. 7, 369–379.
Corpéchot, C., Robel, P., Axelson, M., Sjövall, J., Baulieu, E.E., 1981. Characterization and measurement of dehydroepiandrosterone sulphate in rat brain. Proc. Natl. Acad. Sci. U. S. A. 78, 4704–4707.
Corpéchot, C., Synguelakis, M., Talha, S., Axelson, M., Sjövall, J., Vihko, R., Baulieu, E.E., Robel, P., 1983. Pregnenolone and its sulphate ester in the rat brain. Brain Res 270, 119–125.
Faden, A.I., Simon, R.P., 1988. A potential role for excitotoxins in the pathophysiology of spinal cord injury. Ann. Neurol. 23, 623–626.
Gómez-Pinilla, F., Tram, H., Cotman, C.W., Nieto-Saampedro, M., 1989. Neuroprotective effect of MK-801 and U-50488H after contusive spinal cord injury. Exp. Neurol. 104, 118–124.
Guarneri, P., Russo, D., Cascio, C., De Leo, G., Piccoli, T., Sciuto, V., Piccoli, F., Guarneri, R., 1998. Pregnenolone sulphate modulates NMDA receptors, inducing and potentiating acute excitotoxicity in isolated retina. J. Neurosci. Res. 54, 787–797.
Irwin, R.P., Lin, S.Z., Rogawski, M.A., Purdy, R.H., Paul, S.M., 1994. Steroid potentiation and inhibition of *N*-methyl-D-aspartate receptor-mediated intracellular Ca^{2+} responses: structure-activity studies. J. Pharmacol. Exp. Ther. 271, 677–682.
Irwin, R.P., Maragakis, N.J., Rogawski, M.A., Purdy, R.H., Farb, D.H., Paul, S.M., 1992. Pregnenolone sulphate augments NMDA receptor mediated increases in intracellular Ca^{2+} in cultured rat hippocampal neurons. Neurosci. Lett. 141, 30–34.
Ishimaru, H., Katoh, A., Suzuki, H., Fukuta, T., Kameyama, T., Nabeshima, T., 1992. Effect of *N*-methyl-D-aspartate receptor antagonists on carbon monoxide-induced brain damage in mice. J. Pharmacol. Exp. Ther. 261, 349–352.
Ishimaru, H., Nabeshima, T., Katoh, A., Suzuki, H., Fukuta, T., Kameyama, T., 1991. Effects of successive carbon monoxide exposures on delayed neuronal death in mice under the maintenance of normal body temperature. Biochem. Biophys. Res. Commun. 179, 836–840.
Kimonides, V.G., Khatibi, N.H., Svendsen, C.N., Sofroniew, M.V., Herbert, J., 1998. Dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc. Natl. Acad. Sci. U. S. A. 95, 1852–1857.
Maione, S., Berrino, L., Vitagliano, S., Leyva, J., Rossi, F., 1992. Pregnenolone sulphate increases the convulsant potency of *N*-methyl-D-aspartate in mice. Eur. J. Pharmacol. 219, 477–479.
Majewska, M.D., Demirgören, S., Spivak, C.E., London, E.D., 1990. The neurosteroid dehydroepiandrosterone sulphate is an allosteric antagonist of the $GABA_A$ receptor. Brain Res. 526, 143–146.
Majewska, M.D., Mienville, J.M., Vicini, S., 1988. Neurosteroid pregnenolone sulphate antagonizes electrophysiological responses to GABA in neurons. Neurosci. Lett. 90, 279–284.
Majewska, M.D., Schwartz, R.D., 1987. Pregnenolone sulphate: an endogenous antagonist of the γ-aminobutyric acid receptor complex in brain? Brain Res. 404, 355–360.
Mao, X., Barger, S.W., 1998. Neuroprotection by dehydroepiandrosterone-sulphate: role of an NFκB-like factor. Neuroreport 9, 759–763.
Mathis, C., Paul, S.M., Crawley, J.N., 1994. The neurosteroid pregnenolone sulphate blocks NMDA antagonist-induced deficits in a passive avoidance memory task. Psychopharmacology 116, 201–206.
Maurice, T., Hiramatsu, M., Kameyama, T., Hasegawa, T., Nabeshima, T., 1994. Behavioural evidence for a modulating role of σ ligands in memory processes. II. Reversion of carbon monoxide-induced amnesia. Brain Res. 647, 57–64.
Maurice, T., Phan, V.L., Noda, Y., Yamada, K., Privat, A., Nabeshima, T., 1999a. The attenuation of learning impairments induced after exposure to CO or trimethyltin in mice by sigma (σ) receptor involves both σ_1 and σ_2 sites. Br. J. Pharmacol. 127, 335–342.
Maurice, T., Phan, V.L., Urani, A., Kamei, H., Noda, Y., Nabeshima, T., 1999b. Neuroactive neurosteroids as endogenous effectors for the sigma1 (σ_1) receptor: pharmacological evidences and therapeutic opportunities. Jpn. J. Pharmacol. 81, 125–155.
Maurice, T., Privat, A., 1997. SA4503, a novel cognitive enhancer with σ_1 receptor agonist properties, facilitates NMDA receptor-dependent learning in mice. Eur. J. Pharmacol. 328, 9–18.
Maurice, T., Roman, F.J., Privat, A., 1996. Modulation by neurosteroids of the in vivo (+)-[^{3}H]SKF-10,047 binding to σ_1 receptors in the mouse forebrain. J. Neurosci. Res. 46, 734–743.
Maurice, T., Su, T.P., Privat, A., 1998. $Sigma_1$ (σ_1) receptor agonists and neurosteroids attenuate β_{25-35}-amyloid peptide-induced amnesia in mice through a common mechanism. Neuroscience 83, 413–428.
Monnet, F.P., Mahe, V., Robel, P., Baulieu, E.E., 1995. Neurosteroids, via sigma receptors, modulate the [^{3}H]norepinephrine release evoked by *N*-methyl-D-aspartate in the rat hippocampus. Proc. Natl. Acad. Sci. U. S. A. 92, 3774–3778.
Nabeshima, T., Katoh, A., Ishimaru, H., Yoneda, Y., Ogita, K., Murase, K., Ohtsuka, H., Inari, K., Fukuta, T., Kameyama, T., 1991. Carbon monoxide-induced delayed amnesia, delayed neuronal death and change in acetylcholine concentration in mice. J. Pharmacol. Exp. Ther. 256, 378–384.
Park-Chung, M., Wu, F.S., Farb, D.H., 1994. 3α-hydroxy-5β-pregnan-20-one sulphate: a negative modulator of the NMDA-induced current in cultured neurons. Mol. Pharmacol. 46, 146–150.
Park-Chung, M., Wu, F.S., Purdy, R.H., Malayev, A.A., Gibbs, T.T., Farb, D.H., 1997. Distinct sites for inverse modulation of *N*-methyl-D-aspartate receptors by sulfated steroids. Mol. Pharmacol. 52, 1113–1123.
Phan, V.-L., Su, T.-P., Privat, A., Maurice, T., 1999. Modulation of steroidal levels by adrenalectomy/castration and inhibition of neurosteroid synthesis enzymes affect σ_1 receptor-mediated behaviour in mice. Eur. J. Neurosci. 11, 2385–2396.
Rothman, S.M., Olney, J.W., 1986. Glutamate and the pathophysiology of hypoxic–ischemic brain damage. Ann. Neurol. 19, 105–111.
Rupprecht, R., Hauser, C.A., Trapp, T., Holsboer, F., 1996. Neurosteroids: molecular mechanisms of action and psychopharmacological significance. J. Steroid Biochem. Mol. Biol. 56, 163–168.
Schumacher, M., Guennoun, R., Robel, P., Baulieu, E.E., 1997. Neurosteroids in the hippocampus: neuronal plasticity and memory. Stress 2, 65–78.
Smith, S.S., 1991. Progesterone administration attenuates excitatory amino acid responses of cerebellar Purkinje cells. Neuroscience 42, 309–320.
Urani, A., Privat, A., Maurice, T., 1998. The modulation by neurosteroids of the scopolamine-induced learning impairment in mice involves an interaction with sigma1 (σ_1) receptors. Brain Res. 799, 64–77.
Weaver, C.E., Marek, P., Park-Chung, M., Tam, S.W., Farb, D.H., 1997. Neuroprotective activity of a new class of steroidal inhibitors of the *N*-methyl-D-aspartate receptor. Proc. Natl. Acad. Sci. U. S. A. 94, 10450–10454.
Weaver, C.E., Wu, F.-S., Gibbs, T.T., Farb, D.H., 1998. Pregnenolone sulphate exacerbates NMDA-induced death of hippocampal neurons. Brain Res. 803, 129–136.
Wu, F.S., Gibbs, T.T., Farb, D.H., 1991. Pregnenolone sulphate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor. Mol. Pharmacol. 40, 333–336.