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European Journal of Pharmacology 486 (2004) 151–161
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Enhanced antidepressant efficacy of σ_1 receptor agonists in rats after chronic intracerebroventricular infusion of β-amyloid-(1–40) protein

Alexandre Urani[a,b,1], Pascal Romieu[a,2], François J. Roman[b], Kiyofumi Yamada[c,3], Yukihiro Noda[c], Hiroyuki Kamei[c,3], Hung Manh Tran[c,4], Taku Nagai[c], Toshitaka Nabeshima[c], Tangui Maurice[a,*,2]

[a] *INSERM U.336, Behavioural Neuropharmacology Group, Institut de Biologie, 4, bvd Henri IV, 34060 Montpellier, France*
[b] *PfizerGRD-Fresnes, 3/9, rue de la Loge, B.P. 100, 94265 Fresnes Cedex, France*
[c] *Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, Tsuruma-cho, Nagoya 466-8560, Japan*

Received 1 September 2003; received in revised form 4 December 2003; accepted 12 December 2003

Abstract

Treatment of depressive symptoms in patients suffering from neurodegenerative disorders remains a challenging issue, since few available antidepressants present an adequate efficacy during pathological aging. Previous reports suggested that selective σ_1 receptor agonists might constitute putative candidates. We here examined the pharmacological efficacy of igmesine and (+)-SKF-10,047 and the σ_1 receptor-related neuroactive steroid dehydroepiandrosterone sulfate, in rats infused intracerebroventricularly during 14 days with the β-amyloid-(1–40) protein and then submitted to the conditioned fear stress test. Igmesine and (+)-SKF-10,047 significantly reduced the stress-induced motor suppression at 30 and 6 mg/kg, respectively, in β-amyloid-(40–1)-treated control rats. Active doses were decreased, to 10 and 3 mg/kg, respectively, in β-amyloid-(1–40)-treated animals. The dehydroepiandrosterone sulfate effect was also facilitated, both in dose (10 vs. 30 mg/kg) and intensity, in β-amyloid-(1–40)-treated rats. Neurosteroid levels were measured in several brain structures after β-amyloid infusion, in basal and stress conditions. Progesterone levels, both under basal and stress-induced conditions, were decreased in the hippocampus and cortex of β-amyloid-(1–40)-treated rats. The levels in pregnenolone, dehydroepiandrosterone and their sulfate esters appeared less affected by the β-amyloid infusion. The σ_1 receptor agonist efficacy is known to be inversely correlated to brain progesterone levels, synthesized mainly by neurons that are mainly affected by the β-amyloid toxicity. The present study suggests that σ_1 receptor agonists, due to their enhanced efficacy in a nontransgenic animal model, may alleviate Alzheimer's disease-associated depressive symptoms.
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Keywords: σ_1 Receptor; Neuro(active)steroid; β-Amyloid-(1–40) protein; Alzheimer's disease; Depression; Conditioned fear stress; (Rat)

1. Introduction

Alzheimer's disease is the most common form of dementia among the elderly (Evans et al., 1989). Physiopathological features characteristic of Alzheimer's disease include abnormal extracellular accumulation of β-amyloid proteins into sensitive structures of the brain. Gradual deposition of β-amyloid protein in the form of neurotic plaques, apparition of neurofibrillary tangles as well as progressive cognitive deficits accompany the emergence of Alzheimer's disease (Selkoe, 1991). Among the most common and important complications of Alzheimer's disease is clinically relevant depression, which worsens patient disability and suffering. Prevalence of depression among Alzheimer's disease patients ranges from 0% to 86% depending on reports, but

* Corresponding author. CNRS FRE 2693, Université de Montpellier II, c.c. 090, place Eugène Bataillon, 34095 Montpellier Cedex 5, France. Tel.: +33-4-67-4-36-23; fax: +33-4-67-14-42-51.
E-mail address: maurice@univ-montp2.fr (T. Maurice).
[1] Present address: Laboratory of Biochemistry, Zentralinstitut für Seelische Gesundheit, J5 (Innenstadt), 68159 Mannheim, Germany.
[2] Present address: CNRS FRE 2693, Université de Montpellier II, c.c. 090, place Eugène Bataillon, 34095 Montpellier Cedex 5, France.
[3] Present address: Laboratory of Neuropsychopharmacology, Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan.
[4] Present address: Faculty of Pharmacy, University of Medicine and Pharmacy, 41-43 Dinh Tien Hang Str., Dist. 01, Ho Chi Minh City, Viet-Nam.

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doi:10.1016/j.ejphar.2003.12.018

is usually considered to average about 30–50% (Zubenko and Moossy, 1988; Wragg and Jeste, 1989; Zubenko, 2000; Purandare et al., 2001; Olin et al., 2002). Depression could also be observed in association with other types of degenerative dementia, such as Parkinson's disease, Huntington's chorea, Pick's disease or vascular dementia (Zubenko and Moossy, 1988). The cause of depression observed in Alzheimer's disease patients seems to be less genetic and more structural, related to functional declines, than classic depression seen in adults (Boland, 2000; Espiritu et al., 2001). In particular, the neurodegenerative pathologies provoke anatomic damages, reduction of cerebral blood flow or receptor dysfunctions in particular brain structures, including frontotemporal areas, hippocampus or the locus cœruleus (Zubenko and Moossy, 1988; Forstl et al., 1992; Sheline et al., 1996). Alzheimer's disease patients developing major depression thus show a particular neuropathological and neurochemical context, mainly characterized by a severe central cholinergic deficit, occurring in basal forebrain structures innervating the hippocampus and neocortex (Zubenko and Moossy, 1988; Forstl et al., 1992; Sheline et al., 1996). Present therapeutic strategies using classical antidepressant treatments have produced contradictory findings and did not satisfactorily lead to clear depression reduction (Boland, 2000; Lyketsos et al., 2003; Zubenko et al., 2003). Novel therapeutic approaches with preserved antidepressant efficacy are thus needed to treat depression in patients with neurodegenerative dementia.

We have demonstrated that chronic administration of β-amyloid protein into the cerebral ventricle, using a long-term mini-pump implantation, produced memory impairments without apparent neurodegeneration (Nitta et al., 1994; Yamada and Nabeshima, 2000; Tran et al., 2002). However, numerous neurochemical and neurophysiological alterations were observed after infusion of β-amyloid protein, such as impairment of long-term potentiation (Itoh et al., 1999); functional reduction of cholinergic and dopaminergic systems (Itoh et al., 1996); changes in the ciliary neurotrophic factors levels (Yamada et al., 1995); changes in the mRNA expression of brain-derived neurotrophic factor (BDNF) (Tang et al., 2000); induction of inducible nitric oxide (NO) synthase (iNOS) and overproduction of NO in the hippocampus (Tran et al., 2001); tyrosine nitration of synaptophysin (Tran et al., 2003); impairment of endogenous antioxidant system (Kim et al., 2003); and reduced activation of protein kinase C (PKC) (Olariu et al., 2002). These observations suggested in a convergent manner that β-amyloid toxicity resulted in functional deficits affecting neuronal responses and signalling pathways within the hippocampus, sustaining the marked memory impairments.

The σ_1 receptor agonists are potent antidepressant drugs acting through a unique mechanism that suggests their potential efficacy in Alzheimer's disease-related depression (Maurice, 2002). The σ_1 receptor is a 223-amino acid protein, cloned in several animal species (Hanner et al., 1996; Kekuda et al., 1996) that appeared devoid of analogy with any other known mammalian protein. Selective σ_1 receptor ligands exert a potent neuromodulation on intracellular Ca^{2+} mobilisations and excitatory neurotransmitter systems, including noradrenergic, glutamatergic and cholinergic responses (for review, see Maurice et al., 1999). Its endogenous effector remains unidentified, but the biological relevance of this receptor is supported by the correlation observed between the σ_1 binding affinity and functional and behavioural effects of drugs, and the interaction of several endogenous systems with this receptor, including peptides of the neuropeptide Y family, or neuro(active)steroids. In particular, pregnenolone or dehydroepiandrosterone behaved as σ_1 receptor agonists, while progesterone is a potent antagonist (Maurice et al., 1999). A similar crossed pharmacology between neuro(active)steroids and σ_1 receptor ligands has been observed in animal models of depression, forced swimming or conditioned fear stress, or in the σ_1 receptor involvement in cocaine-induced conditioned place preference (Noda et al., 2000; Urani et al., 2001; Romieu et al., 2003).

In a previous study, we have reported that the antidepressant-like efficacy of igmesine or PRE-084, two σ_1 receptor agonists, measured using the forced swim test, were potentiated in mice injected intracerebroventricularly (i.c.v.) with β_{25-35}-amyloid peptide (Urani et al., 2002). This enhanced efficacy was attributed to decreased progesterone levels in the hippocampus of β_{25-35} animals and suggested that σ_1 agonists, due to their enhanced efficacy, may allow to alleviate the depressive symptoms associated with Alzheimer's disease (Urani et al., 2002). In the present study, these observations were confirmed and extended using the Alzheimer's disease model of rats chronically infused with β-amyloid-(1–40) protein (Nitta et al., 1994, 1997; Yamada et al., 1995, 1999). We examined the antidepressant-like efficacy of σ_1 receptor agonists, and particularly igmesine and the σ_1 receptor-related neuroactive steroid dehydroepiandrosterone sulfate, on the conditioned fear stress response of rats, as previously reported (Nabeshima et al., 1985; Kamei et al., 1997). The effect of the β-amyloid infusion on brain neurosteroid levels was measured in basal and stressful conditions.

2. Materials and methods

2.1. Animals and treatment

Male Wistar rats (Charles River Japan, Yokohama, Japan or breeding centre of the Faculty of Pharmacy, Montpellier, France) weighing 200–230 g at the beginning of the experiments were used. They were housed two or three per cage under standard light–dark conditions (12-h light cycle starting at 08:00 h) at a constant temperature of 23 ± 1 °C. The animals had free access to food and water and they have been handled in accordance with the guidelines established

by the Institute for Laboratory Animal Research of Nagoya University and to the European Communities Council Directive of 24 November 1986 (86-609/EEC).

On the day of surgery, a cannula attached to a mini-osmotic pump was implanted in the rat right cerebral ventricle (A 20.3 mm, L 1.2 mm, V 4.5 mm) as previously described (Nitta et al., 1994) and β-amyloid-(1–40) (Feinchemikalien, Switzerland) was continuously infused at a dose of 0.3 nmol/12 μl/day for 14 days. Control animals received 0.3 nmol/12 μl/day of β-amyloid-(40–1), the reverse sequence of (1–40). Both peptides (1–40) and (40–1) were dissolved in 35% acetonitrile–0.1% trifluoroacetic acid (vehicle). We have previously confirmed that β-amyloid-(40–1) or vehicle itself has no effect on learning behaviour at this flow rate (Yamada et al., 1999). On day 14 after the start of β-amyloid infusion, rats were submitted to the conditioned fear stress procedure. After the first behavioural session, some animals were anaesthetized with pentobarbital 6%, transcardiacally perfused with 200 ml of saline solution and their brains were quickly removed from the skull. The cerebral cortex, hippocampus and cerebellum were immediately dissected out, and subsequently stored at −80 °C until assayed.

2.2. Drugs

(+)-*N*-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-ethyl-but-3-en-1-ylamine hydrochloride (igmesine, JO-1784) was synthesized at Institut de Recherche Jouveinal/Parke-Davis. Progesterone (4-pregnene-3,20-dione) was from Sigma/Aldrich (St. Louis, MO, USA). Dehydroepiandrosterone sulfate (5-androsten-3β-ol-17-one sulfate) and (+)-SKF-10,047 were from Research Biochemicals (Natick, MA, USA). *N*,*N*-Dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)ethylamine (NE-100) was provided by Taisho Pharmaceuticals (Tokyo, Japan). [1,2,6,7-^{3}H(*N*)]Progesterone (3589 GBq/mmol, 37 MBq/ml), [7-^{3}H(*N*)]pregnenolone ([^{3}H]pregnenolone, 777 GBq/mmol, 37 MBq/ml), [1,2,6,7-^{3}H(*N*)]dehydroepiandrosterone ([^{3}H]dehydroepiandrosterone, 2220 GBq/mmol, 37 MBq/ml) and [7-^{3}H(*N*)]dehydroepiandrosterone sulfate ([^{3}H]dehydroepiandrosterone sulfate, 592 GBq/mmol, 37 MBq/ml) were from New England Nuclear (Boston, MA, USA). The pregnenolone antibody was from AbCys (Paris, France); progesterone and dehydroepiandrosterone antibodies were from Biovalley (Marne-la-Vallée, France). Progesterone was suspended in sesame oil; other drugs were solubilised in distilled water or saline solution. Drugs were injected subcutaneously (s.c.) or intraperitoneally (i.p.), in a volume of 100 μl/20 g of body weight.

2.3. Conditioned fear stress procedure

The apparatus was a transparent acrylic rectangular cage (25 × 30 × 47 high cm) equipped with a metal wire floor. The cage was inserted in a sound-attenuated box and was illuminated with a 20-W bulb. Each rat was placed into the test cage and received intermittent electric shocks (0.1 Hz, 200 ms, 100 V DC) for 10 min through an isolated pulse stimulator (Nihon Koden, Tokyo, Japan). Each animal received electric footshocks in the range of 0.2–0.5 mA, because the current resistance of the animal varied between 200 and 500 kΩ (Kamei et al., 1997). The test session was performed 24 h after the first session. Animals were placed again into the test cage, but no footshock was delivered. The spontaneous motility of rats was measured using an infrared beams activity device (Scanet SV-10, Neuroscience, Osaka, Japan or Opto-varimex, Columbus Instruments, Columbus, OH, USA), in which the cage was inserted. The non-shocked control group was operated similarly, except for the absence of shock treatment. The σ_1 receptor ligands, or appropriate vehicle solutions, were administered 30 min before the test session.

2.4. Extraction and purification of neurosteroids

Brain samples were thawed, weighed and homogenized in ice-cold 10 mM phosphate buffer saline, pH 7.4. Recovery tracers ([^{3}H]progesterone, [^{3}H]pregnenolone, [^{3}H]dehydroepiandrosterone, [^{3}H]dehydroepiandrosterone sulfate, 50 Bq each) were added. Then, 10 ml of ethylacetate/isooctane, 1/1 vol/vol, was added and the tubes were vigorously stirred for 8 min. After centrifugation at 4000 × *g* for 5 min, the organic phase was removed and the extraction step was repeated twice. This organic phase was then defatted with a MeOH 90%/isooctane separation. The aqueous extracts containing unconjugated steroids were further purified by reverse-phase chromatography on C_{18} cartridges (Amersham, Les Ulis, France). The isooctane phases containing lipoidal derivatives were thrown away. Sulfate esters were hydrolysed. The aqueous phase from the first separation was

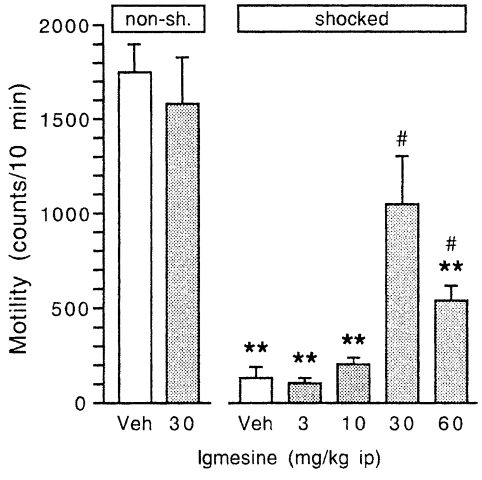

Fig. 1. Effect of the σ_1 receptor agonist igmesine in naive rats submitted to the conditioned fear stress. Igmesine was administered i.p. 30 min before the motility measurement. The number of animals per experimental group was $n = 4–6$. *$P < 0.05$, **$P < 0.01$ compared to the vehicle (Veh)-treated non-shocked (non-sh.) group; $^{\#}P < 0.05$ compared to the Veh-treated shocked group (Dunn's test).

brought to pH = 1.0 with a few drops of sulfuric acid and to a NaCl concentration of 20% by adding 2/1 vol/vol of a 30% NaCl solution. Extraction with ethylacetate was again performed as described above, and this extract, which contained steroid sulfates, was hydrolysed at 37 °C for 16 h. Ethylacetate extracts were washed once with 1 N NaOH (0.25 vol.) and twice with water (0.25 vol.). The extracts were finally dried.

The different steroids were separated using partition chromatography on Celite^{545} (Prolabo, Fontenay-sous-Bois, France) microcolumns, with propanediol, 1 g, as the stationary phase. Impregnated celite was settled in 5-ml disposable glass pipettes. Extracts were taken up in 1 ml of isooctane saturated with propanediol, and deposited onto the columns. Progesterone was eluted with 19-ml isooctane, pregnenolone with 15 ml of isooctane/benzene (7/3 vol/vol) and dehydroepiandrosterone with 20 ml of isooctane/benzene (1/1 vol/vol). The recovery of the different steroids added as tracers was routinely 60–80%.

After separation, each steroid was quantified by radioimmunoassay using specific antibodies presenting minimal cross-reactions. Measurements were performed in triplicate of four dilutions of each purified sample. Results are expressed as ng/g of tissue.

2.5. Statistical analysis

Results are expressed as mean ± S.E.M. Behavioural data were analyzed using the Dunn's multiple comparisons test after a non-parametric Kruskal–Wallis analysis of variance (ANOVA, KW values). Neurosteroid measurements were analyzed using a two-way ANOVA (F-values),

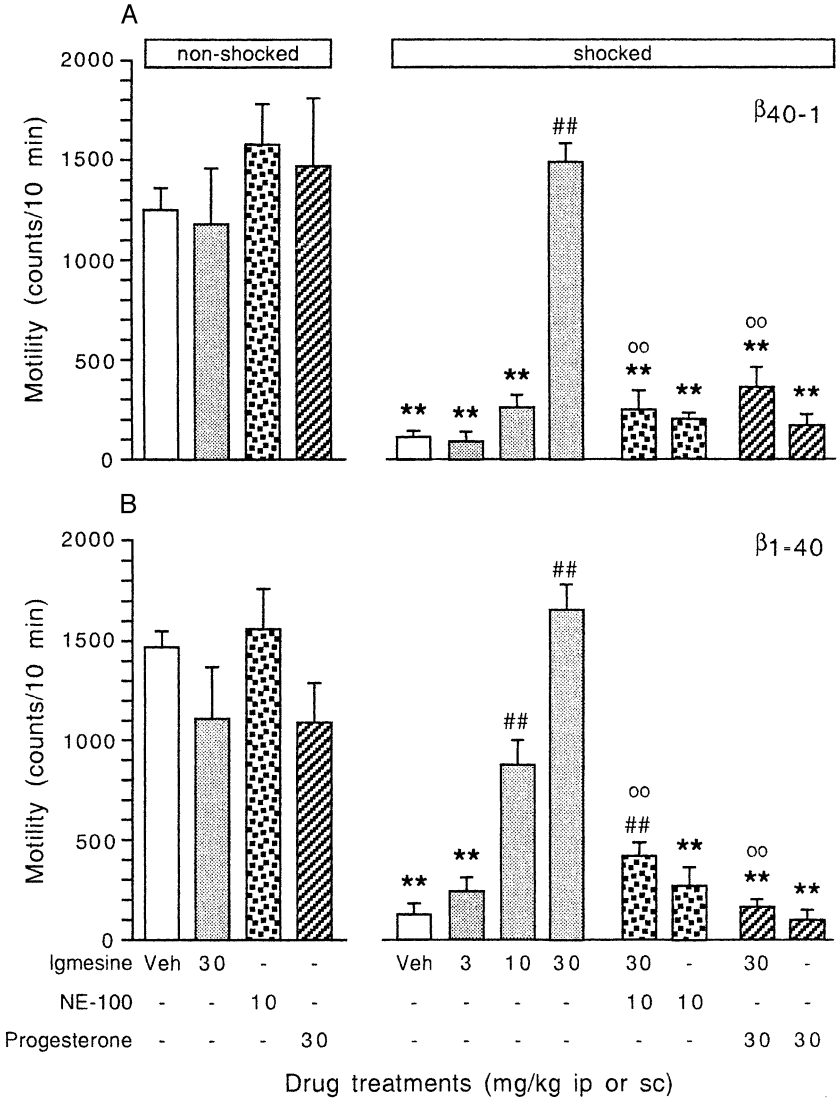

Fig. 2. Effect of igmesine on the conditioned fear stress response in rats infused during 14 days with: (A) β-amyloid-(40–1) or (B) β-amyloid-(1–40) protein. The σ_1 receptor antagonist NE-100 or the neuroactive steroid progesterone was injected i.p. or s.c., respectively, 15 min before igmesine, administered i.p. 30 min before the motility measurement. The number of animals per experimental group was $n = 4–6$. $^{**}P < 0.01$ compared to the vehicle (Veh)-treated non-shocked group; $^{\#\#}P < 0.01$ compared to the Veh-treated shocked group; $^{oo}P < 0.01$ compared to the igmesine (30)-treated shocked group (Dunn's test).

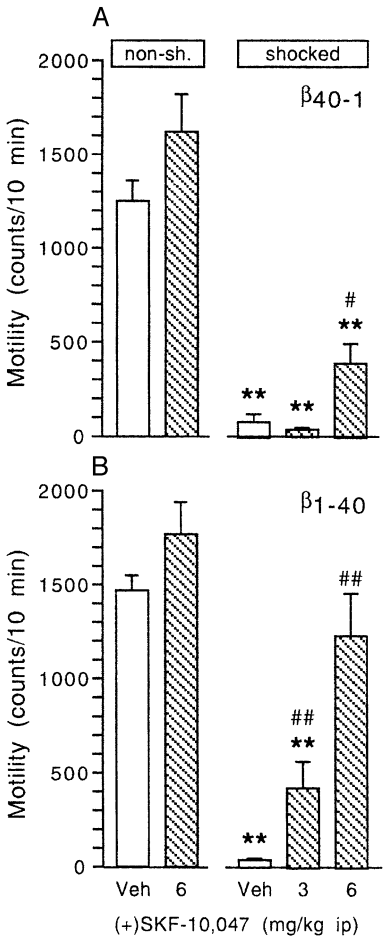

Fig. 3. Effect of the σ_1 receptor agonist (+)-SKF-10,047 on the conditioned fear stress response in rats infused during 14 days with: (A) β-amyloid-(40–1) or (B) β-amyloid-(1–40) protein. (+)-SKF-10,047 was administered i.p. 30 min before the motility measurement. The number of animals per experimental group was $n=5$. $^{**}P<0.01$ compared to the vehicle (Veh)-treated non-shocked (non-sh.) group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared to the Veh-treated shocked group (Dunn's test).

with the infusion treatment and exposure to shock as independent parameters, post-hoc comparisons being made using the Welch's test. The criteria for statistical significance was $P<0.05$.

3. Results

3.1. Effects of the σ_1 receptor agonists on conditioned fear stress in β-amyloid-infused rats

Treatment with the σ_1 receptor agonist igmesine, tested in the 3–60 mg/kg i.p. dose range, resulted in an attenuation of the highly significant decrease in motility observed in rats that experienced the unavoidable electric footshock (KW = 26.84, $P<0.001$; Fig. 1). At 30 and 60 mg/kg, igmesine induced a significant ($P<0.05$), but bell-shaped effect.

Rats infused chronically with either β-amyloid-(40–1) or β-amyloid-(1–40) protein exhibited, similarly as intact animals, a highly significant decrease of motility after shock (Fig. 2A and B). In control, β-amyloid-(40–1)-treated animals, the igmesine treatment attenuated the decrease of motility, in a similar dose–response effect as compared to non-infused animals (KW = 51.07, $P<0.0001$; Fig. 2A). The σ_1 receptor agonist induced a highly significant effect at 30 mg/kg. This effect was blocked by the selective σ_1 receptor antagonist NE-100 (10 mg/kg i.p.) or the neuroactive steroid progesterone (30 mg/kg s.c.) (Fig. 2A). In β-amyloid-(1–40)-treated animals, igmesine also attenuated the decrease of motility (KW = 54.64, $P<0.0001$; Fig. 2B). The effect was even potentiated, since the compound induced a highly significant effect at a lower dose, 10 mg/kg, as well as at 30 mg/kg (Fig. 2B). The maximal effect was blocked by NE-100 or progester-

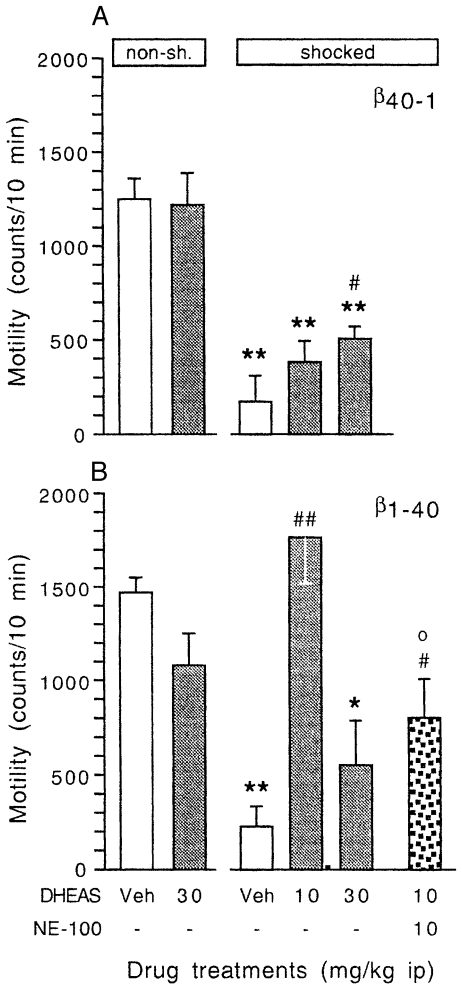

Fig. 4. Effect of the neuroactive steroid dehydroepiandrosterone sulfate on the conditioned fear stress response in rats infused during 14 days with: (A) β-amyloid-(40–1) or (B) β-amyloid-(1–40) protein. NE-100 was injected i.p. 15 min before dehydroepiandrosterone sulfate (DHEAS), administered s.c. 30 min before the motility measurement. The number of animals per experimental group was $n=5$–6. $^{*}P<0.05$, $^{**}P<0.01$ compared to the vehicle (Veh)-treated non-shocked group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared to the Veh-treated shocked group; $^{o}P<0.05$ compared to the dehydroepiandrosterone sulfate (30)-treated shocked group (Dunn's test).

one (Fig. 2B). None of the compounds, tested at their highest active dose, affected the motility in non-shocked groups (Fig. 2A and B).

The anti-stress effect of the prototypic σ_1 receptor agonist (+)-SKF-10,047 was also tested in rats infused with either β-amyloid-(40–1) or β-amyloid-(1–40) protein. In β-amyloid-(40–1)-treated animals, (+)-SKF-10,047 attenuated the decrease of motility ($KW = 20.55$, $P < 0.001$; Fig. 3A). The σ_1 receptor agonist induced a significant but limited attenuation at 6 mg/kg. In β-amyloid-(1–40)-treated animals, the efficacy of (+)-SKF-10,047 to increase the shocked rats motility was potentiated, since highly significant effects were measured at 3 and 6 mg/kg ($KW = 19.15$, $P < 0.001$). At the latter dose, a complete reversion was measured (Fig. 3B).

3.2. Effect of the neuroactive steroid dehydroepiandrosterone sulfate on conditioned fear stress in β-amyloid-infused rats

The anti-stress effect of the σ_1 receptor-related neuroactive steroid dehydroepiandrosterone sulfate was examined in rats infused with either β-amyloid-(40–1) or β-amyloid-(1–40) protein. In β-amyloid-(40–1)-treated animals, dehydroepiandrosterone sulfate attenuated the decrease of motility ($KW = 19.79$, $P < 0.001$; Fig. 4A). The steroid induced a significant but limited attenuation at 30 mg/kg. In β-amyloid-(1–40)-treated animals, the dehydroepiandrosterone sulfate efficacy to increase the shocked rats motility was potentiated, since a highly significant reversion was observed at the lower dose of 10 mg/kg ($KW = 17.70$, $P < 0.001$; Fig. 4B). This motility increase was significantly, but not fully, blocked by NE-100, confirming the involvement of the σ_1 receptor in this effect (Fig. 4B).

3.3. Neurosteroid levels in β-amyloid-infused rats

Progesterone levels were measured in several brain regions, the hippocampus, cortex and cerebellum, of rats infused with β-amyloid-(40–1) or (1–40) protein (Fig. 5). In the hippocampus, the two-way ANOVA resulted in a highly significant effect of the β-amyloid treatment [$F(1,12) = 67.63$, $P < 0.001$], but not of stress [$F(1,12) = 3.28$, $P = 0.10$]. However, the treatment × stress interaction was significant [$F(1,12) = 6.58$, $P < 0.05$]. Indeed, in control β-amyloid-(40–1)-treated rats, the stress significantly increased hippocampal progesterone level (Fig. 5A). In β-amyloid-(1–40)-treated animals, the non-shocked group showed a decreased basal progesterone level, that remained unchanged in the shocked group (Fig. 5A). In turn, shocked β-amyloid-(1–40)-treated animals presented almost one third the progesterone contents of shocked β-amyloid-(40–1)-treated ones. In the cortex, highly significant effects were measured for the β-amyloid treatment [$F(1,11) = 55.80$, $P < 0.001$] and stress [$F(1,11) = 10.13$, $P < 0.01$], but not for the treatment × stress interaction. β-Amyloid-(1–40)-treated animals exhibited significantly less progesterone levels (Fig. 5B), in basal as well as stress conditions. For both β-amyloid-(40–1)- and (1–40)-treated groups, the stress only moderately increased progesterone levels. Progesterone levels in the cerebellum appeared unchanged among experimental groups (Fig. 5C).

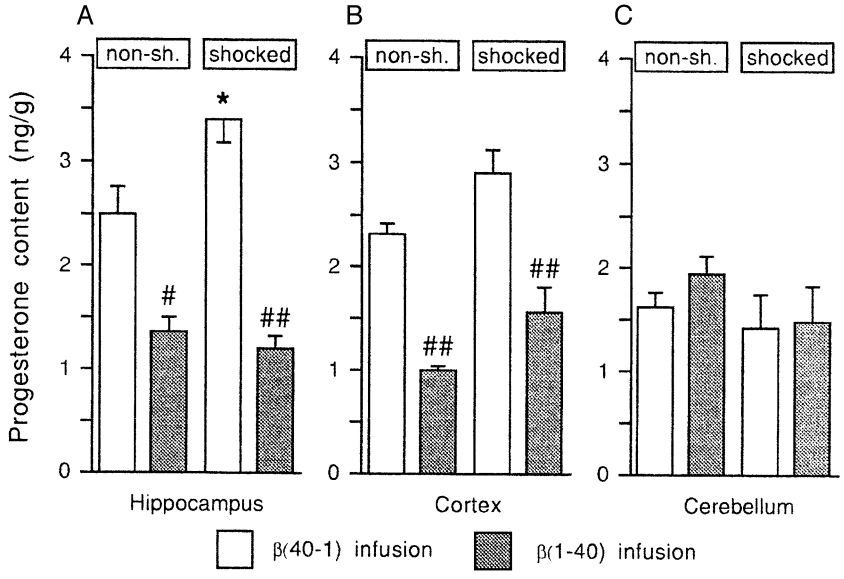

Fig. 5. Brain contents in progesterone in the hippocampus (A), cortex (B) or cerebellum (C) of rats infused during 14 days with β-amyloid-(40–1) or β-amyloid-(1–40) protein. Progesterone levels were measured in non-shocked or shocked animals, sacrificed 30 min after the test session in the conditioned fear procedure. The number of samples was $n = 3–4$ per condition. $^{*}P < 0.05$ compared to the respective non-shocked group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the respective β-amyloid-(40–1)-treated group (Welch's test).

Pregnenolone and dehydroepiandrosterone levels were measured in the different conditions in the hippocampus, cortex and cerebellum, while the levels in sulfate ester of each steroid was measured in the hippocampus (Figs. 6 and 7). In the hippocampus, significant effects for pregnenolone levels were measured for stress [$F(1,12)=11.57$, $P<0.01$] and the β-amyloid treatment × stress interaction [$F(1,12)=10.46$, $P<0.01$]. Indeed, in control β-amyloid-(40–1)-treated rats but not in β-amyloid-(1–40)-treated animals, stress significantly increased hippocampal pregnenolone level (Fig. 6A). In the cortex, a highly significant effect was measured only for stress [$F(1,11)=19.98$, $P<0.001$], indicating no change induced by the β-amyloid-(1–40)-treatment (Fig. 6B). Pregnenolone levels in the cerebellum appeared unchanged among experimental groups (Fig. 6C). In the hippocampus, pregnenolone sulfate levels varied significant after stress [$F(1,11)=18.19$, $P<0.01$], but no difference was measured between β-amyloid-(40–1)- and (1–40)-treated rats (Fig. 6D).

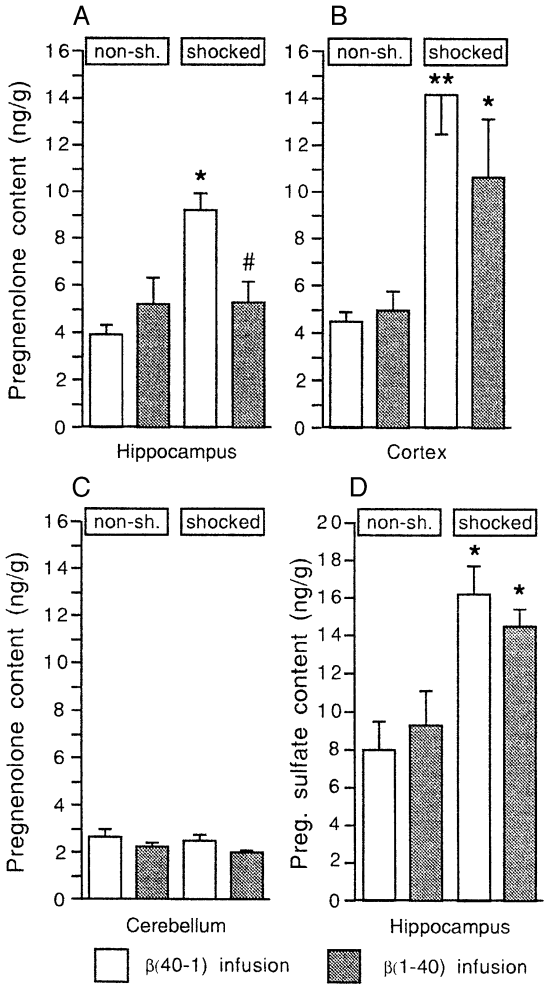

Fig. 6. Brain contents in pregnenolone in the hippocampus (A), cortex (B) or cerebellum (C) of rats infused with β-amyloid-(40–1) or β-amyloid-(1–40) protein. (D) Hippocampal content in pregnenolone sulfate in rats with β-amyloid-(40–1) or β-amyloid-(1–40) protein. Neurosteroid levels were measured in non-shocked or shocked animals, sacrificed 30 min after the test session in the conditioned fear procedure. The number of samples was $n=3–4$ per condition. $^{*}P<0.05$, $^{**}P<0.01$ compared to the respective non-shocked group; $^{\#}P<0.05$ compared to the respective β-amyloid-(40–1)-treated group (Welch's test).

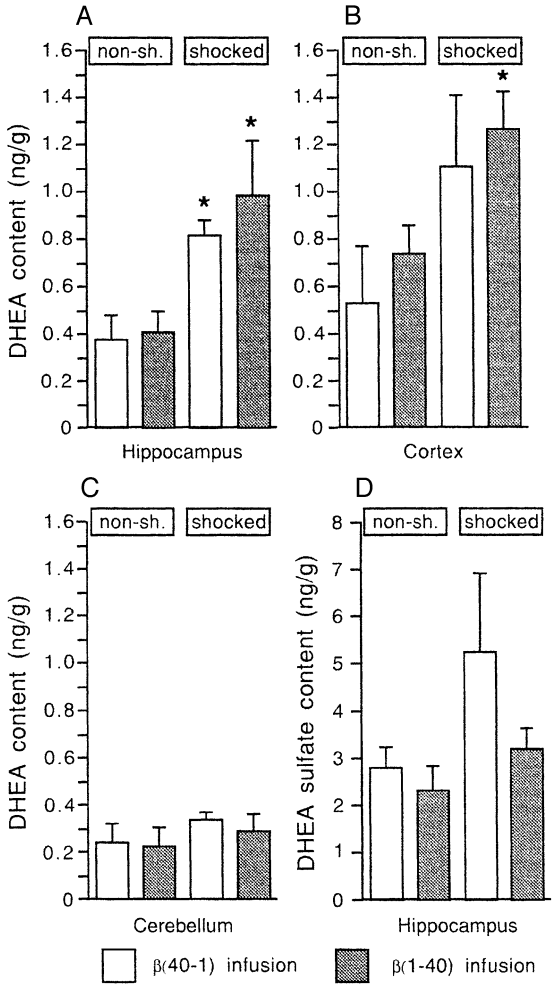

Fig. 7. Brain contents in dehydroepiandrosterone (DHEA) in the hippocampus (A), cortex (B) or cerebellum (C) of rats infused with β-amyloid-(40–1) or β-amyloid-(1–40) protein. (D) Hippocampal content in dehydroepiandrosterone sulfate (DHEAS) in rats with β-amyloid-(40–1) or β-amyloid-(1–40) protein. Neurosteroid levels were measured in non-shocked or shocked animals, sacrificed 30 min after the test session in the conditioned fear procedure. The number of samples was $n=3–4$ per condition. $^{*}P<0.05$ compared to the respective non-shocked group (Welch's test).

Dehydroepiandrosterone levels were much lower in all brain structures, and significant variations were only observed after stress in the hippocampus [$F(1,12)=13.48$, $P<0.01$; Fig. 7A] and cortex [$F(1,11)=6.57$, $P<0.05$; Fig. 7B]. No difference was measured between β-amyloid-(40–1)- and (1–40)-treated rats and in the cerebellum among all experimental groups (Fig. 7C). In the hippocampus, dehydroepiandrosterone sulfate levels failed to change significantly after stress [$F(1,11)=1.78$, $P=0.21$], or according to the β-amyloid-treatment [$F(1,11)=0.27$, $P=0.61$; Fig. 7D].

4. Discussion

Major depression affects between 30% and 50% of the patients who develop Alzheimer's disease and has serious consequences not only for the evolution of the patient, in terms of increased disabilities, but also for caregivers. Results of current antidepressant therapies have produced unsatisfactory findings without fully addressing the benefits of depression reduction (Boland, 2000; Espiritu et al., 2001; Lyketsos et al., 2003). Preclinical studies are thus requested to propose alternative strategies based on novel antidepressants that may present a preserved efficacy in the demented subject. We report here, using a non-transgenic rat model of Alzheimer's disease, induced by the chronic intracerebroventricular infusion of β-amyloid-(1–40) peptide, that the effect of the selective σ_1 receptor agonists, igmesine, (+)-SKF-10,047 or dehydroepiandrosterone sulfate, was enhanced in this model compared to control animals, in the conditioned fear stress test. As compared to β-amyloid-(40–1) peptide-infused control animals, the active dose of each compound was lower by two-to three-fold in β-amyloid-(1–40)-treated rats, with an increase of the intensity of the effect observed for (+)-SKF-10,047 or dehydroepidrosterone sulfate.

Selective σ_1 receptor agonists showed antidepressant efficacy in several animal models of behavioural despair, particularly the tail suspension test or the forced swim test (Matsuno et al., 1996; Ukai et al., 1998; Urani et al., 2001), suggesting that depressive symptoms may be alleviated by the drugs acting through this receptor. Indeed, a preliminary report outlined the potential clinical efficacy of igmesine in depression (Pande et al., 1998). This antidepressant efficacy may involve the wide-range neuromodulatory action, affecting both intracellular Ca^{2+} mobilisations and responses to neurotransmitters, including glutamatergic, cholinergic and monoaminergic systems, known to be involved in the physiopathological changes sustaining depressive states (Maurice et al., 1999). The conditioned fear stress paradigm has been originally reported by Fanselow (1980). Rats exhibit a marked suppression of motility when they are replaced in the same environment in which they previously experienced an aversive electric footshock. This motor suppression is regarded as a conditioned emotional response to the environment associated with the previous footshock. Indeed, when animals returned into the same environment in which they received the aversive shock, they exhibited a marked suppression of motility. However, when they were placed in a different environment, the difference in motility between shocked and non-shocked mice was not observed (Kameyama et al., 1985).

The conditioned fear stress-induced motor suppression could be attenuated by treatments with antidepressants acting as selective serotonin reuptake inhibitors, citalopram or fluvoxamine (Hashimoto et al., 1996; Inoue et al., 1996; Li et al., 2001), suggesting that the freezing behavior is mediated by serotonergic receptor inactivation (Inoue et al., 1996). However, the conditioned fear stress-induced motor suppression was poorly sensitive to anxiolytics, such as diazepam and chlordiazepoxide (Kameyama and Nagasaka, 1982; Nagasaka and Kameyama, 1983) but attenuated by the benzodiazepine antagonist flumazenil (Izumi et al., 1999). As a result, the conditioned fear stress model may be useful for investigating the pathogenesis of mood disorders, particularly those considered to be treatment resistant, and for developing novel therapeutic drugs. It has been shown that σ_1 receptors play an important role in conditioned fear stress response (for reviews, see Kamei et al., 1998; Maurice et al., 1999). Several σ_1 receptor agonists such as (+)-SKF-10,047 and dextromethorphan attenuated the conditioned fear stress-induced motor suppression in rodents, the effects being antagonized by the σ_1 receptor antagonist NE-100 (Kamei et al., 1996). In addition, dehydroepiandrosterone sulfate and pregnenolone sulfate attenuated the conditioned fear stress-induced motor suppression in mice, via their interaction with the σ_1 receptor (Noda et al., 2000). Progesterone behaved as a potent σ_1 receptor antagonist, since it antagonized the attenuating effects of (+)-SKF-10,047, dehydroepiandrosterone sulfate and pregnenolone sulfate, similarly to what was observed with NE-100 (Noda et al., 2000). In the present study, we confirmed that (+)-SKF-10,047 or dehydroepiandrosterone sulfate are active in the conditioned fear stress in control rats. In addition, the efficacy of igmesine was demonstrated.

The chronic infusion of β-amyloid-(1–40) at a dose of 300 pmol/day provoked numerous physiopathological changes and behavioural impairments reminiscent of Alzheimer's disease (Yamada and Nabeshima, 2000). Accumulation of β-amyloid-(1–40) in the hippocampus and cerebral cortex was evident immunohistochemically following a 14-day period of infusion (Nitta et al., 1997). An interesting observation in our study is that fear conditioning is not affected by β-amyloid peptide treatment-since the stress-induced motor suppression is the same in treated animals as in intact rats-unlike other kinds of more complex memory tests. Indeed, a significant impairment of spatial reference memory formation in a water maze and a deficit of passive avoidance performance was observed in β-amyloid-(1–40)-infused animals, which was accompanied by a mild, but significant, reduction of choline acetyltransferase activity in the hippocampus (Nitta et al., 1997). Impairment of long-term potentiation (Itoh et al., 1999) and reduced activation of protein kinase C (Olariu et al., 2002) were also observed. The chronic infusion of β-amyloid-(1–40), even at very low concentrations, directly inhibited various cholinergic neuronal functions independently of apparent neurotoxicity, suggesting a possible link between chronic infusion of β-amyloid-(1–40) burden and cholinergic dysfunction in Alzheimer's disease. Using an in vivo brain microdialysis technique, we observed that KCl-and nicotine-induced increase in acetylcholine and dopamine release in the hippocampus/cerebral cortex and the striatum, respectively, is markedly impaired by the chronic infusion of β-amyloid-(1–40), although the basal levels of these neurotransmitters

in the β-amyloid-(1–40)-infused rats did not differ from those in vehicle-infused control animals (Itoh et al., 1996). The reduction of nicotine-induced ACh release may be partly due to a decrease in affinity of nicotinic ACh receptors (Olariu et al., 2001, 2002). In addition, the chronic infusion of β-amyloid-(1–40) resulted in changes in the ciliary neurotrophic factor protein levels (Yamada et al., 1995) and in the mRNA expression of BDNF in the hippocampus (Tang et al., 2000). The latter playing a major role in both the etiology of major depression and Alzheimer's disease (Tsai, 2003). All these physiological disturbances may contribute to the onset of learning and memory deficits and support the idea that infusion of β-amyloid-(1–40) in rats provoked neurotoxic changes relevant to the physiopathology of Alzheimer's disease (Yamada and Nabeshima, 2000).

The importance of neurosteroids in mood disorders and depression has been demonstrated (for review, see Van Broekhoven and Verkes, 2003). Recent reports focused on the physiopathological significance of neurosteroids in Alzheimer's disease and related dementia (Wolkowitz et al., 1997, 2003; De Bruin et al., 2002; Brown et al., 2003; Weill-Engerer et al., 2002). On one hand, neurosteroids, and particularly dehydroepiandrosterone sulfate, are expected to elicit a marked neuroprotection in the brain. On the second hand, brain structural abnormalities related to Alzheimer's disease, both β-amyloid deposits and neurofibrillary tangles, which result from the aggregation of pathologic tau proteins, affect brain neurosteroid expression. Brown et al. (2000) reported that β-amyloid-(1–42) protein increased dehydroepiandrosterone levels in human glia-derived cell lines, after a 24-h application. The authors suggested that glial cells might be able to resist to the β-amyloid-induced toxicity because of their ability to produce dehydroepiandrosterone. Direct measurements of pregnenolone, pregnenolone sulfate, dehydroepiandrosterone, dehydroepiandrosterone sulfate, progesterone and allopregnanolone were performed in individual brain regions of Alzheimer's disease patients and aged non-demented controls, including hippocampus, amygdala, frontal cortex, striatum, hypothalamus and cerebellum (Weill-Engerer et al., 2002). A general trend towards decreased levels of all steroids was observed in brain regions of Alzheimer's disease patients compared to controls. Pregnenolone sulfate levels were significantly lower in the striatum and cerebellum; dehydroepiandrosterone sulfate levels were significantly reduced in the hypothalamus, striatum and cerebellum; and progesterone and allopregnanolone levels were markedly but non-significantly reduced in several brain structures, including the hypothalamus, striatum, frontal cortex, or amygdala. A significant negative correlation was found between the levels of cortical β-amyloid peptides and those of pregnenolone sulfate in the striatum and cerebellum and between the levels of phosphorylated tau proteins and dehydroepiandrosterone sulfate in the hypothalamus (Weill-Engerer et al., 2002). Since high levels of key proteins implicated in the formation of plaques and neurofibrillary tangles were correlated with decreased brain levels of pregnenolone sulfate and dehydroepiandrosterone sulfate, the authors supported the concept of a possible neuroprotective role of these neurosteroids in Alzheimer's disease.

In the present study, we chose to measure neurosteroid levels in the hippocampus and cortex, because of: (i) the demonstrated importance of these forebrain structures in depression (Reid and Stewart, 2001); (ii) the importance of the neurosteroid/σ_1 receptor interaction within these structures (Maurice et al., 1999); and (ii) their particular vulnerability to the β-amyloid-(1–40) infusion (Nitta et al., 1997). It must be however outlined that σ_1 receptor agonists ameliorated the conditioned fear stress response through the involvement of mesolimbic dopaminergic systems (Kamei et al., 1997), and thus basal ganglia structures may also be of interest.

We observed significant decreases in brain neurosteroid levels in β-amyloid-(1–40)-infused rats, either in basal conditions or after the fear stress. Pregnenolone and dehydroepiandrosterone sulfate levels failed to increase after stress in β-amyloid-(1–40)-infused rats. Most significantly, continuous β-amyloid-(1–40) infusion caused a marked decrease of progesterone levels in the hippocampus and cortex of rats, and these alterations were not affected by the conditioned fear stress. Interestingly, the β-amyloid-mediated neurotoxic process seems to differentially affect the activity of the different enzymes involved in the steroid biosyntheses. In particular, the 3β-hydroxysteroid dehydrogenase enzyme activity is likely to be mainly affected, consistently with the important decrease in progesterone measured in β-amyloid-(1–40) rats. Since progesterone, released in stressful situations, acts as an endogenous antagonist at the σ_1 receptor, an enhanced behavioural efficacy of the σ_1 receptor agonists was observed. This mechanism has recently been demonstrated through pharmacological manipulations of the progesterone levels (adrenalectomy/castration and administration of inhibitors of the enzymes involved in progesterone synthesis and metabolism) for both the memory and behavioural despair responses (Phan et al., 1999; Urani et al., 2002). However, although this proposed mechanism might serve as an interesting basis to design new, efficient antidepressants for indications such as the Alzheimer's disease-related depression, several points must be further examined. In particular, dehydroepiandrosterone sulfate efficacy was increased in β-amyloid-(1–40)-infused rats. This increase could be due to an interaction with σ_1 receptor since it was partially blocked by NE-100, but may also originate from parallel pathways to be elucidated. At the clinical level, Wolkowitz et al. (1997, 2003) reported that dehydroepiandrosterone, administered to the patients with treatment-resistant depression for 6 months, provoked a marked improvement in depression ratings (Wolkowitz et al., 1997), but more recently, in a randomized, double-bind, placebo-

controlled study, that it allowed only a transient effect on cognitive performances, narrowly missing significance (Wolkowitz et al., 2003). The lack of effect of dehydroepiandrosterone itself in Alzheimer's disease patients encourages the use of more selective synthetic compounds, such as σ_1 receptor agonists.

In summary, the present study showed an increased antidepressant efficacy of σ_1 receptor agonists in a non-transgenic model of Alzheimer's disease, induced by the chronic infusion of β-amyloid-(1–40) in rats. This effect was coherent with decreased brain level in neurosteroids, and particularly progesterone, as previously demonstrated in mice (Phan et al., 1999, 2002; Urani et al., 2001) and in humans (Wolkowitz et al., 2003). The present results confirmed previous observations in mice injected acutely into the lateral ventricle with aggregated β-amyloid-(25–35) peptide (Urani et al., 2002). Targeting the σ_1 receptor thus appears as a promising alternative for alleviating the depressive symptoms in Alzheimer's disease patients.

Acknowledgements

Supported by INSERM, Pfizer Global Research and Development and a Grant-in-Aid for scientific research (A) from the Ministry of Education, Science and Culture of Japan (No. 10044260).

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