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The attenuation of learning impairments induced after exposure to CO or trimethyltin in mice by sigma (σ) receptor ligands involves both σ_1 and σ_2 sites

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1 Sigma (σ) receptor ligands were previously reported to alleviate learning and memory impairments on several pharmacological and pathological rodent models of amnesia. Such effect was demonstrated as involving the σ_1 subtype of σ receptor.

2 In this study, we characterized the pharmacological effect mediated by σ ligands on two lesional models of amnesia in mice: (1) the hypoxia-related learning and memory impairment model induced by repeated exposure to carbon monoxide (CO) gas; and (2) the intoxication with trimethyltin (1 mg kg^{-1}).

3 The selective σ_1 ligand PRE-084 (1 mg kg^{-1}) or the non-selective σ_1/σ_2 compounds DTG (0.1 mg kg^{-1}), BD1008 (3 mg kg^{-1}), and haloperidol (0.1 mg kg^{-1}) reversed significantly the spontaneous alternation deficits observed 7 days after exposure to CO or 14 days after intoxication with trimethyltin.

4 The selective σ_1 receptor antagonist NE-100 (1 mg kg^{-1}) was ineffective by itself, but blocked completely the PRE-084 effects, partially the DTG effects, and did not affect the effects induced by BD1008 or haloperidol.

5 A similar pharmacological profile was observed in the step-down type passive avoidance test performed 8 days after exposure to CO.

6 These results show that, in contrast to the previously reported amnesia models, the impairments induced after exposure to CO or intoxication with trimethyltin could be alleviated not only by σ_1 receptor agonists but also by σ_2 agonists. The particular pattern of neurodegeneration observed in these lesional models may explain these differences.

Keywords: σ_1 receptor; σ_2 sites; σ pharmacology; CO-induced amnesia; trimethyltin; learning and memory; mouse

Abbreviations: BD1008, *N*-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamine; CO, carbon monoxide; DTG, 1,3-di(2-tolyl)guanidine; NE-100; *N*,*N*-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)ethylamine; NMDA, N-methyl-D-aspartate; PRE-084, 2-(4-morpholino)ethyl-1-phenylcyclohexane-1-carboxylate hydrochloride; SAM, senescence-accelerated mouse

Introduction

The ligand specificity and autoradiographic distribution of sigma (σ) sites in both the central nervous system and peripheral tissues suggested that they belong to a unique receptor family. Binding strategies, using *in vitro* in *in vivo* bioassays, provided evidence for the existence of at least two subtypes of σ sites, denoted σ_1 and σ_2 (Quirion *et al*., 1992). The σ_1 protein was recently purified and its cDNA was cloned in several species (Hanner *et al*., 1996; Kekuda *et al*., 1996; Seth *et al*., 1997; 1998; Pan *et al*., 1998). The amino-acid sequences are structurally unrelated to known mammalian proteins, but shared some homology with a fungal sterol C_8-C_7 isomerase (Hanner *et al*., 1996). Little is however known about the nature of the σ_2 site. The major role of σ_1 receptors is likely to exert a potent neuromodulation on different neurotransmitter systems, including potentiation of several N-methyl-D-aspartate (NMDA) receptor-mediated responses (Monnet *et al*., 1990; 1992; Roman *et al*., 1991) and modulation of cholinergic systems (Siniscalchi *et al*., 1987; Matsuno *et al*., 1992). The cellular mechanism remains to be determined, but recent evidences clearly suggest a direct effect of σ_1 ligands on intracellular Ca^{2+} fluxes (Su *et al*., 1998). Such physiologic effects led to major behavioural consequences. Among others, selective σ_1 receptor ligands attenuated several behavioural deficits induced in rodents after stress (Kamei *et al*., 1996a, b; 1997; Song *et al*., 1997), or in models of depression (Matsuno *et al*., 1996) and amnesia (for review, see Maurice & Lockhart, 1997). Indeed, σ_1 receptor agonists, such as DTG, (+)-SKF-10047 (+)-pentazocine, PRE-084, or SA4503 attenuated the learning and memory impairments induced by selective blockade of the NMDA receptor activation with dizocilpine (Maurice *et al*, 1994a, b, d; Ohno & Watanabe, 1995; Maurice & Privat, 1997; Zou *et al*., 1998), or by antagonism of the muscarinic acetylcholine receptor with scopolamine (Earley *et al*., 1991; Matsuno *et al*., 1993; 1994; 1997). Beside these results concerning pharmacological amnesia models, σ_1 receptor agonists were also efficient in reversing the deficits observed in models more directly linked to pathology. This was shown in mice exposed to CO gas, in the senescence-accelerated mouse (SAM) model of age-related deficits, or in mice administered centrally with $\beta_{25\text{-}35}$-amyloid peptide (Maurice *et*

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al., 1994b; 1996; 1998). Moreover, due to the recent cloning of the protein and the availability of molecular biology tools, the implication of the σ_1 receptor in learning the memory processes could be firmly established using an *in vivo* antisense strategy (Maurice *et al*., 1997).

Recently, Couture & Debonnel (1998) reported that, similarly to σ_1 receptor ligands, σ_2 agonists, such as Lu 28-1798, BD1008 or ibogaine, are also able to potentiate the neuronal response to NMDA, in the CA_3 region of the rat dorsal hippocampus. These effects were insensitive to the σ_1 receptor antagonists haloperidol or NE-100 and to progesterone. Since the anti-amnesic effects of σ_1 receptor agonists could be considered as relevant to this cellular physiological mechanism, such results questioned about the putative anti-amnesic effects of σ_2 agonists. As regards the different pharmacological models of amnesia, the major involvement of σ_1 receptor is now widely accepted, mainly because more or less selective σ_1 receptor antagonists, such as haloperidol, BMY-14802 or NE-100, block efficiently the anti-amnesic effects of the agonists (Early *et al*., 1991; Matsuno *et al*., 1993; 1994; 1997; Maurice *et al*., 1994a, b, d; Ohno & Watanabe, 1995; Maurice & Lockhart, 1997; Maurice & Privat, 1997; Zou *et al*, 1998). It appeared however of interest to re-examine the σ receptor pharmacology in lesional models of amnesia that involve the neurotoxicity of excitatory amino acids, such as the deficits in mice exposed to CO gas or intoxicated with trimethyltin. Repetitive exposures to CO gas in a hypoxia-related model, that induces in mice a long-lasting but delayed amnesia measured 1 week after exposure. The hippocampal cholinergic system appears markedly affected by the hypoxic toxicity (Nabeshima *et al*., 1991). Histological studies have shown that seven days after exposure to CO, there was a moderate neuronal loss in the CA_1 region of the hippocampal formation which was augmented by increasing the severity of the CO exposure (Ishimaru *et al*., 1991). On the other hand, p.o. as well as i.p. administrations of trimethyltin in rodents induce progressive neurodegeneration in limbic structures, particularly the pyramidal cells of CA_1 and CA_4 (Hagan *et al*., 1988; Segal, 1988), together with severe behavioural impairments, such as learning deficits in water-maze or passive avoidance acquisition (Walsh *et al*., 1982a, b; Woodruff & Baisden, 1994). DTG and (+)-SKF-10047 were previously reported to attenuate, in a BMY-14802-sensitive manner, the deficits of spontaneous alternation and passive avoidance in mice exposed to CO (Maurice *et al*., 1994c). In this study, we thus characterized the anti-amnesic effects of selective σ_1 agonists, σ_1 antagonists and non-selective σ_1/σ_2 ligands on the learning deficits in mice exposed to CO or intoxicated with trimethyltin.

Methods

Animals

Male mice (ddY strain, Nihon SLC, Shizuoka, Japan or Swiss strain, 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 (light on at 08 00 h). Experiments were carried out between 10 00 and 18 00 h, in a soundproof and air-regulated experimental room. The use of laboratory animals and experimental protocols followed the guidelines approved by the Animal Care and Use Committee at Nagoya University (Nagoya, Japan) and by INSERM (Paris, France).

Drugs and administration procedures

2-(4-Morpholino)ethyl-1-phenylcyclohexane-1-carboxylate hydrochloride (PRE-084) was provided by Dr T.-P. Su (Unit on Pathobiology, Intramural Research Division, NIDA, NIH, Baltimore, MD, U.S.A.); 1,3-di(2-tolyl)guanidine (DTG) and trimethyltin chloride were from RBI (Natick, MA, U.S.A.); N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)ethylamine (NE-100) was from Taisho Pharmaceutical (Tokyo, Japan); haloperidol was from Dainippon Pharmaceutical (Osaka, Japan); spiperone was from Sigma (St-Louis, MO, U.S.A.); *N*-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamine (BD1008) was a gift from Dr W.D. Bowen (Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD, U.S.A.). DTG was prepared as a microsuspension in Cremophor EL (Sigma) 10% in saline solution by ultrasonic vibration. Other compounds were dissolved in saline. 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*., 1994c, d; 1998; Maurice & Privat, 1997) or unpublished data for BD1008.

Exposure to CO

Exposure to CO was carried out as previously described (Ishimaru *et al*., 1991; 1992; Nabeshima *et al*., 1991; Maurice *et al*, 1994b). 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 25 ml min^{-1}, and mice were exposed until they began grasping, i.e., between 50 and 70 s. This was done three times, with 1 h between each exposure. The animals were kept on a hot plate (Natsume KN-205D, Japan or SILAB, Montpellier, France) immediately after the first exposure and up to 2 h after the third, 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; 1992). Animals were then examined for spontaneous alternation behaviour, 7 days after exposure to CO, and for passive avoidance behaviour, 8 days after exposure to CO.

Intoxication with trimethyltin

Trimethyltin chloride was dissolved in saline and administered i.p. In preliminary experiments, the LD_{50} dose was estimated in Swiss mice at 1.7 mg kg^{-1} i.p. (not shown), which appeared much lower than in rats: 12.6 mg kg^{-1} (Brown *et al*., 1979). Mice received a single injection of trimethyltin at 1 mg kg^{-1}, or saline for controls, and the spontaneous alternation behaviour was examined after 14 days.

Spontaneous alternation performances

Spatial working memory performances were assessed by recording spontaneous alternation behaviour in the Y-maze (Maurice *et al*., 1994a, b; 1996). 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 checked visually. 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. The drugs were administered 30 min before the session, i.e., 7 days after CO exposure or 14 days after trimethyltin intoxication.

Step-down type passive avoidance test

Long-term memory was examined using the step-down type of passive avoidance task (Ishimaru *et al*., 1991; 1992; Nabeshima *et al*., 1991; Maurice *et al*, 1994b; 1996). The apparatus consisted in a transparent acrylic cage (30 $\times$ 30 $\times$ 40 cm high) with a gridfloor, inserted in a soundproof outer box (35 $\times$ 35 $\times$ 90 cm high). 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 gridfloor. Intermittent electric shock (1 Hz, 500 ms, 45 V DC) were delivered to the gridfloor using a pulse stimulator (Nihon Kohden SEN-3301, Japan) connected to an isolator (Nihon Kohden SS-201J). The test consistent in 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 gridfloor, shocks were delivered for 15 s. Step-down latency and the numbers of vocalizations and flinching reactions were measured. Shock sensitivity was evaluated by summing these two numbers. Mice that showed latencies ranging from 3–30 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 gridfloor. 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 3 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*., 1996; 1998). The drugs were administered 30 min before the first training, and once, injections not being repeated before the second training, or the retention test.

Statistical analyses

Results are expressed as means $\pm$ s.e.mean, excepting step-down latency, which are expressed in terms of medians and interquartile ranges. None of the vehicle used in this study, i.e., saline solution, DMSO 5% in saline, or cremophor EL 10% in saline, administered 30 min before the Y-maze session or the first passive avoidance training, affected the behaviours showed either by control animals or by CO-exposed ones (data not shown). For clarity, results are thus presented vs the untreated non-CO- or CO-exposed animals. 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 analysis of variance (F values). SDL 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 analysis of variance (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 χ^2 test. The levels for statistical significance were $P<0.01$, $P<0.05$.

Results

Effect of σ receptor ligands against the spontaneous alternation deficits in mice exposed to CO

Mice exposed to CO showed a progressive deterioration of working memory, which could be measured on spontaneous behaviour, 7 days after CO exposure, using the Y-maze test.

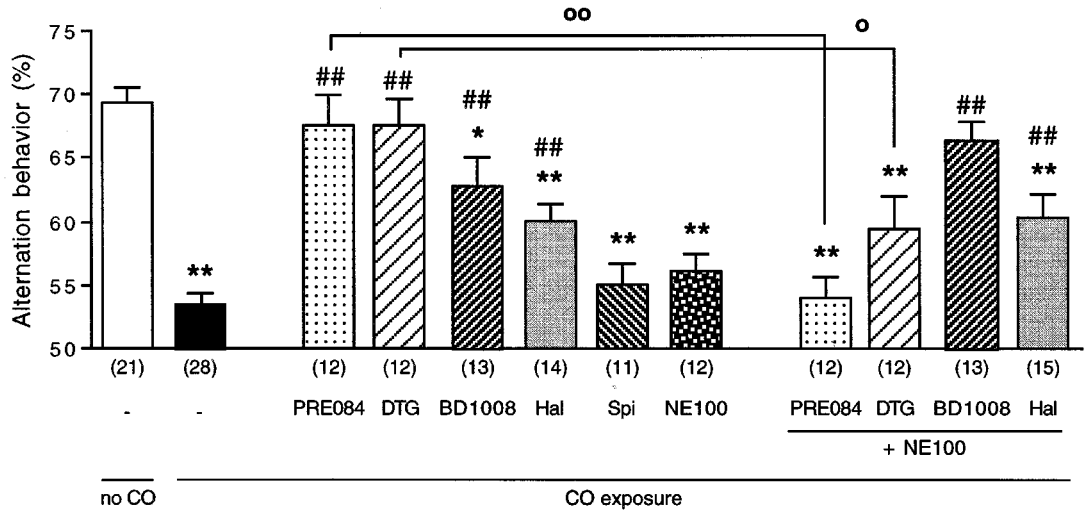

Figure 1 Effects of σ receptor ligands on the spontaneous alternation impairments in CO-exposed mice. Mice were exposed three consecutive times to CO (25 ml min^{-1}, 50–70 s) at 38°C, and alternation performances were examined 7 days after exposure. PRE-084 (1 mg kg^{-1}, s.c.), DTG (0.1 mg kg^{-1}, s.c.), BD1008 (3 mg kg^{-1}, s.c.), haloperidol (0.1 mg kg^{-1}, i.p.), spiperone (0.1 mg kg^{-1}, i.p.), and NE-100 (1 mg kg^{-1}, i.p.) were administered 30 min before the session. The number of animals per group is indicated below each column. Hal: haloperidol; Spi: spiperone; Sal: saline. $^{*}P<0.05$, $^{**}P<0.01$ vs the control group; $^{\#\#}P<0.01$ vs the untreated CO-exposed group; $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$; Dunnett's test.

Untreated mice not exposed to CO ($n=21$) exhibited an alternation percentage of $69.2\pm1.2\%$ (Figure 1) and performed 29 ± 1 arm entries during the 8 min session in the maze. Untreated mice exposed to CO ($n=28$) showed a significantly decreased percentage of alternation ($53.6\pm0.9\%$; $P<0.01$, Student's t-test; Figure 1), together with a mild increase in the number of arm entries (35 ± 1, $P<0.01$).

The σ receptor ligands significantly affected the spontaneous alternation deficits shown by mice exposed to CO ($F_{11,174}=13.04$, $P<0.01$, Figure 1). First, treatment with the selective σ_1 receptor agonist PRE-084 (Table 1) or the non-selective σ_1/σ_2 ligands DTG, BD1008, or haloperidol (Table 1) allowed significant attenuations of the alternation deficits (Figure 1). Spiperone, a drug presenting the same pharmacological profile as haloperidol except for σ receptor subtypes (Table 1), failed to affect the alternation deficits in mice exposed to CO. The selective σ_1 receptor antagonist NE-100 (Table 1) also failed to affect the alternation deficits observed after exposure to CO, in the $0.1-3$ mg kg^{-1} dose range (shown in Figure 1 for 1 mg kg^{-1}).

The co-administration of NE-100, at 1 mg kg^{-1}, together with the efficient ligands differentially affected their effects. NE-100 allowed a complete reversion of the PRE-084 effect ($P<0.01$ vs the PRE-084-treated CO-exposed group, Figure 1). The antagonist also significantly affected the DTG effect ($P<0.05$ vs the DTG-treated CO-exposed group, Figure 1). Finally, the effects of BD1008 and haloperidol were unaffected by NE-100. The DTG, BD1008, or haloperidol effects were not affected further by using a higher dose of NE-100 (3 mg kg^{-1}, data not shown).

Effect of σ receptor ligands against the spontaneous alternation deficits in mice intoxicated with trimethyltin

Mice intoxicated with trimethyltin showed progressive working memory, which could be quantified 14 days after the acute administration of trimethyltin. The spontaneous alternation behaviour was markedly affected in mice intoxicated with trimethyltin ($54.5\pm1.4\%$, $n=16$, Figure 2) as compared to saline treated animals ($69.8\pm1.5\%$, $n=14$, $P<0.01$, Student's t-test, Figure 2). The number of arm explored during the 8 min session was not affected by the treatment, since the intoxicated group performed 26 ± 2 arm entries as compared with 29 ± 2 for the control group ($P>0.05$).

The σ receptor ligands significantly affected the spontaneous alternation deficits showed by mice intoxicated with trimethyltin ($F_{11,183}=9.12$, $P<0.01$, Figure 2). The treatment with the selective σ_1 receptor agonist PRE-084 or the non-selective σ_1/σ_2 ligands DTG, BD1008, or haloperidol allowed significant attenuations of the alternation deficits (Figure 2). Spiperone and the σ_1 receptor antagonist NE-100 failed to

Table 1 Pharmacological profile of σ receptor ligands

	σ_1 sites		σ_2 sites		Selectivity
	K_i (nM)	Radiotracer	K_i (nM)	Radiotracer	(σ_2/σ_1 ratio)
PRE-084*	2.2	(+)-[^{3}H]-SKF-10047	13091	[^{3}H]-DTG	6319
DTG†	59.8	(+)-[^{3}H]-pentazocine	12.8	[^{3}H]-DTG	0.2
BD1008†	2.1	(+)-[^{3}H]-pentazocine	8.1	[^{3}H]-DTG	3.9
Haloperidol†	2.3	(+)-[^{3}H]-pentazocine	12.0	[^{3}H]-DTG	5.2
Spiperone‡$^{\#}$	1100	(+)-[^{3}H]-SKF-10047	670	[^{3}H]-DTG	0.6
NE-100$^+$	1.5	(+)-[^{3}H]-pentazocine	84.6	[^{3}H]-DTG	55

*McCann *et al*., 1994; †Hellewell *et al*., 1994; ‡Tam & Cook, 1984; $^{\#}$Weber *et al*., 1986; $^+$Chaki *et al*., 1994.

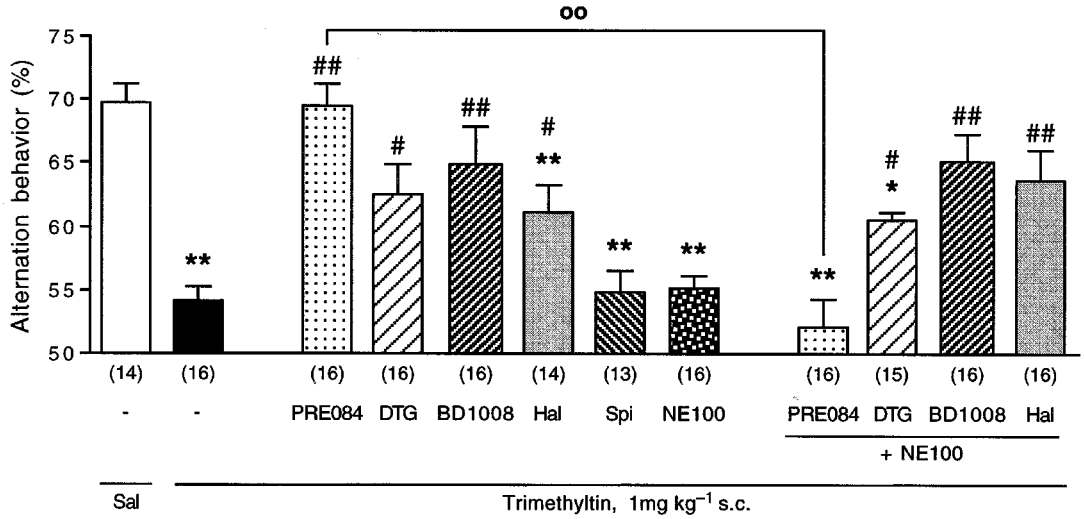

Figure 2 Effects of σ receptor ligands on the spontaneous alternation impairments in trimethyltin-intoxicated mice. Mice received an acute i.p. injection of trimethyltin (1 mg kg^{-1}), 14 days before the test. PRE-084 (1 mg kg^{-1}, s.c.), DTG (0.1 mg kg^{-1}, s.c.), BD1008 (3 mg kg^{-1}, s.c.), haloperidol (0.1 mg kg^{-1}, i.p.), spiperone (0.1 mg kg^{-1}, i.p.), and NE-100 (1 mg kg^{-1}, i.p.) were administered 30 min before the session. The number of animals per group is indicated below each column. Hal: haloperidol; Spi: spiperone; Sal: saline. *$P<0.05$, **$P<0.01$ vs the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs the untreated CO-exposed or trimethyltin-intoxicated group; $^{oo}P<0.01$; Dunnett's test.

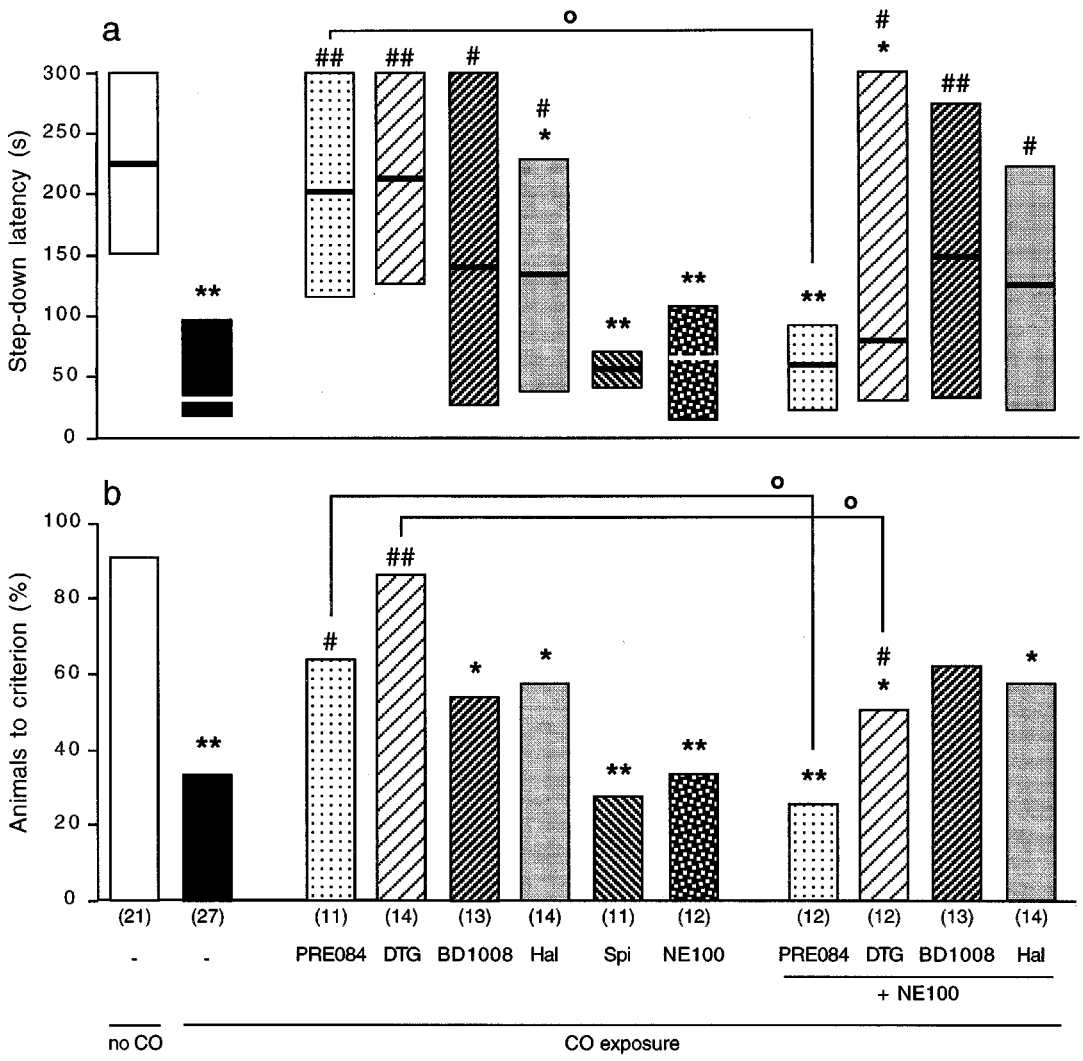

Figure 3 Effects of σ receptor ligands on the passive avoidance deficits in CO-exposed mice: (a) step-down latency and (b) percentage of animals to criterion. Mice were exposed three consecutive times to CO (25 ml min^{-1}, 50–70 s) at 38°C, and animals were trained for passive avoidance behaviour 8 days after exposure. Retention was examined 24 h after the training. PRE-084 (1 mg kg^{-1}, s.c.), DTG (0.1 mg kg^{-1}, s.c.), BD1008 (3 mg kg^{-1}, s.c.), haloperidol (0.1 mg kg^{-1}, i.p.), spiperone (0.1 mg kg^{-1}, i.p.), and NE-100 (1 mg kg^{-1}, i.p.) were administered 30 min before training. The number of animals per group is indicated below each column in (b). Hal: haloperidol; Spi: spiperone; $^{*}P<0.05$, $^{**}P<0.01$ vs the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs the untreated CO-exposed group; $^{\circ}P<0.05$; Dunn's test in (a) and χ^2 test in (b).

affect the alternation deficits induced by administration of trimethyltin (Figure 2).

The co-administration of NE-100 together with PRE-084 allowed a complete blockade of its effect ($P<0.01$, Figure 2). However, NE-100, at 1 mg kg^{-1} (Figure 2) or at 3 mg kg^{-1} (data not shown), failed to significantly affect the attenuations induced by DTG, BD1008, or haloperidol.

Effect of σ receptor ligands against the passive avoidance deficits in mice exposed to CO

Exposure to CO did not affect the mouse behaviour during the passive avoidance training sessions. Control animals not exposed to CO stepped down 4 s [interquartile range: 3–6 s] after the beginning of training and showed a shock sensitivity, measured by summing the number of vocalisations and flinching reactions of the animals during the electric shocks, of 19 ± 2. Similarly, animals exposed to CO showed a step-down latency of 4 s [3–5 s] ($P>0.05$, Mann-Whitney's test), and a shock sensitivity of 19 ± 1 ($P>0.05$, Student's t-test). However, during the retention test performed after 24 h, significant differences were observed between both groups. The control animals showed a step-down latency of 226 s [152–300 s], with an avoidance criterion of 91% ($n=21$), whereas the animals exposed to CO showed a latency of 35 s [18–96 s] ($n=27$, $P<0.01$), and an avoidance criterion of 34.6% ($P<0.01$, Figure 3).

During the passive avoidance training sessions, none of the treatments with the σ receptor ligands affected either the step-down latencies (in the 3–7 s range, $KW=13.04$, $P>0.05$) or the sensitivity to the shocks (in the 16–21 range, $F_{11,173}=1.01$, $P>0.05$), as compared to control or untreated animals exposed to CO. Differences were however observed during the retention test, both in terms of step-down latencies ($KW=44.94$, $P<0.01$, Figure 3a) or in the percentages of animals to criterion (Figure 3b). The treatment with PRE-084 or DTG allowed a significant reversion of the deficits observed after CO, as measured using both parameters. The treatment with BD1008 or haloperidol induced a significant attenuation of the decrease in latency in mice exposed to CO (Figure 3a).

The increase in the percentage of animals to criterion did not however appear significant (Figure 3b). Spiperone did not affect both parameters. NE-100, in the 0.1–3 mg kg^{-1} dose range, did not affect both parameters, as compared to mice exposed to CO (the 1 mg kg^{-1} dose effect being shown on Figure 3).

The co-administration of NE-100 with PRE-084 completely blocked its attenuating effect on both parameters ($P<0.05$ vs the PRE-084-treated CO-exposed group each, Figure 3a and b). Co-administration of NE-100 with DTG tended to attenuate the DTG effect on step-down latencies, but in a non-significant manner (Figure 3a). The percentage of animals to criterion was significantly decreased in the (NE−100+DTG)-treated group as compared to the group treated with DTG alone ($P<0.05$, Figure 3b). The increased latencies in CO exposed animals treated with BD1008 or haloperidol were not affected by the NE-100 co-administration.

Discussion

It has now been widely described that selective σ receptor agonists, selective for the σ_1 subtype, attenuate the learning and memory deficits observed in mice or rats in several pharmacological models. These anti-amnesic effects could be blocked by the σ_1 receptor antagonists BMY-14802, NE-100 or haloperidol, a non-selective σ_1/σ_2 ligand which act at σ_1 receptors as an antagonist (Matsuno *et al*., 1997; Maurice & Privat, 1997). We here extended the study of the σ pharmacology to two lesional models of amnesia, different from the previously described pharmacological models. First, the successive exposure of mice to CO gas induce, after 5–7 days, delayed amnesia in the Y-maze and passive avoidance tests and delayed neuronal death, that remains restricted to the CA_1 area of the hippocampal formation (Nabeshima 1991; Maurice *et al*., 1994c). The model could be regarded as an interesting amnesia model related to a direct hypoxic insult, and involving the neurotoxicity of excitatory amino acids (Ishimaru *et al*., 1992). Moreover, the σ ligands DTG and (+)-SKF-10047 were previously reported to reverse the CO-induced learning deficits (Maurice *et al*., 1994c). The involvement of σ_1 receptors was clearly evidenced by the observations that (−)-SKF-10047 was inactive and that BMY-14802 blocked the DTG effect. However, DTG, which is a non-selective σ_1/σ_2 agonist, allowed a complete reversion of the CO-induced deficits, although the drug, used in the same conditions, only partially attenuated the dizocilpine-induced deficits (Maurice *et al*., 1994b). Second, trimethyltin administration in rats or mice produces important damage in selective neuronal populations from the limbic structures in the brain (Brown *et al*., 1979; Chang & Dyer, 1983; Hagan *et al*., 1988). In particular, Chang & Dyer (1983) reported that administration of 6 mg kg^{-1} trimethyltin in rats induced necrotic changes in the CA_1 and CA_{3c} area of the hippocampus, the neurodegeneration becoming maximal 21 days after intoxication. The neurodegeneration process is likely to involve elevated glutamate and the hyperactivation of NMDA receptors (Naalsund & Fonnum, 1986). Consequently, intoxicated animals show marked learning and memory impairments, as observed for example by Walsh *et al*. (1982a, b) using the radial-arm maze and passive avoidance tests in rats. In this study, we clearly evidenced spontaneous alternation deficits in mice, 14 days after acute intoxication.

The pharmacology of the anti-amnesic effects induced by several σ ligands was thus characterized in these two lesional models. In Table 1, the relative K_i values of the different compounds are presented for the σ_1 sites, labelled using (+)-[^{3}H]SKF-10047 or (+)-[^{3}H]-pentazocine, and the σ_2 sites, labelled using [^{3}H]-DTG. The most selective drug in this study appears to be PRE-084. The drug is a selective σ_1 receptor agonist, that already showed marked anti-amnesic effects against the dizocilpine-induced deficits, the age-related disorders in SAM, or the deficits observed in mice administered centrally with $\beta_{25\text{-}35}$-amyloid peptide (Maurice *et al*., 1994d; 1996; 1998). In both the CO and trimethyltin amnesia models, PRE-084 allowed highly significant reversions of the short-term and long-term memory deficits, that appeared fully blocked by NE-100, confirming the involvement of σ_1 receptors.

In the CO model, the non-selective σ_1/σ_2 receptor agonist DTG showed an anti-amnesic effect partially sensitive to NE-100, whereas in the trimethyltin model, NE-100 failed to affect the effect of DTG. It can thus be concluded that the drug may exert its anti-amnesic effect in these models through the concomitant involvement of σ_1 and, mainly, σ_2 receptors. Haloperidol is a drug that presents a high affinity for either the σ_1 or σ_2 site (Hellewell *et al*., 1994; Table 1). The drug behaves as a potent σ_1 receptor antagonist in several physiological and behavioural tests, in particular regarding the anti-amnesic effects mediated by σ_1 ligands (Matsuno *et al*., 1997; Maurice & Privat, 1997; Maurice *et al*., 1998). On the other hand, it induces a marked dystonia after local injection in the rat red nucleus, behaving thus as a potent σ_2 agonist (Matsumoto *et al*., 1990). In the CO and trimethyltin models, the drug induced marked anti-amnesic effects on both tests. Since spiperone did not allow such anti-amnesic effect, it is likely that the effect of haloperidol involves an interaction with σ sites and more precisely σ_2 sites, since it appeared insensitive to the effect of NE-100.

As reported in Table 1, BD1008 is considered as a high affinity ligand for both the σ_1 and σ_2 sites, and as very selective towards binding to other receptors (Hellewell *et al*., 1994). However, the drug behaved as a weak antagonist in some physiological tests describing the σ_1 pharmacology, such as the NMDA-evoked [^{3}H]-noradrenaline release from rat hippocampal slices (Monnet *et al*., 1996) or the NMDA-evoked [^{3}H]-dopamine release from rat striatal slices (Gonzales-Alvear & Werling, 1994). BD1008 weakly antagonized the effects of (+)-pentazocine or DTG, but dose-dependently antagonised the effect of BD-737, suggesting that it acts on the same atypical σ receptor subtype as BD-737, distinct from both the σ_1 and σ_2 sites (Monnet *et al*., 1996). In addition, Couture & Debonnel (1998) reported that BD1008, such as Lu 28-179, is able to potentiate the NMDA-induced electric activity of the rat dorsal hippocampal neurones in a NE-100- and progesterone-insensitive manner. In their model, σ_1 receptor agonists, such as (+)-pentazocine, BD737, or igmesine, among others, induce a marked potentiation of the neuronal response to NMDA. The non-selective σ_1/σ_2 ligand DTG also potentiated the neuronal responses to NMDA, but at higher doses induced an epileptoid activity (Bergeron *et al*., 1995), that appeared due to the co-activation of both the σ_1 and σ_2 receptors (Couture & Debonnel, 1998). The observation that BD1008 and Lu 28-179 were able to potentiate the neuronal response to NMDA without generating an epileptoid activity suggested that both compounds behaved as σ_2 agonists devoid of σ_1 receptor agonist activity. Our results are in agreement with such observation, since the anti-amnesic effect induced by BD1008 appeared completely insensitive to the effect of NE-100, indicating that the drug behaved only as a σ_2 receptor agonist. The question remains to determine whether BD1008 also act as

a weak antagonist on the BD737-related σ site, as suggested by Monnet *et al*. (1996). The effect of the BD737 related drugs should be examined at the behavioural level. Furthermore, the results presented here, together with the previous reports by Monnet *et al*. (1996) and Couture & Debonnel (1998), strongly suggest that the classical classification of σ receptors as σ_1 and σ_2 subtypes is likely to become inadequate. Indeed, the K_i value reported in Table 1 for σ_1 sites may in fact correspond to different subtypes and the high affinity of BD1008 for (+)-^{3}H-pentazocine-labelled sites may rather reflect its affinity for the BD737-related site. This site, being less sensitive to haloperidol, may represent a subtype different from the σ_1 receptor (Monnet *et al*., 1996).

The results presented here suggest strongly that in particular amnesia models, not only σ_1 receptor ligands show marked anti-amnesic effects, but also σ_2 site agonists. The exact nature of this σ_2 site remains to be clarified. Matsumoto and co-workers (1990) showed that it is involved in motor and posture control. Our results reveal that it may also be involved in cognitive functions. Another point is that our results are in agreement with the recent report by Couture & Debonnel (1998), who used an *in vivo* electrophysiology technique to demonstrate that, similarly to σ_1 receptor agonists, the σ_2 receptor agonists are able to potentiate the neuronal response to NMDA in the rat hippocampus. This point strongly strengthens the idea that this physiological potentiation mediated by σ ligands may be involved in the mechanism of their anti-amnesic effect, as previously proposed (Maurice *et al*., 1994b; Maurice & Lockhart, 1997). It remains to determine why the σ_2 site ligands show such anti-amnesic effect in these particular models as compared to pharmacological amnesia models. The particular pattern of neurodegeneration, almost similar between CO exposure and trimethyltin intoxication, may be involved. Future experiments, using a specific σ_1 receptor polyclonal antibody prepared in the laboratory, will allow to quantify the susceptibility of σ sites to the insults in the different hippocampal areas (CA_1-CA_4, dentate gyrus), in order to determine the importance of the distribution of the different sites in this structure.

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