



0091-3057(94)00208-8

PRE-084, a σ Selective PCP Derivative, Attenuates MK-801-Induced Impairment of Learning in Mice

TANGUI MAURICE,*¹ TSUNG-PING SU,† DANIEL W. PARISH,‡
TOSHITAKA NABESHIMA§ AND ALAIN PRIVAT*

*INSERM U. 336, Ecole Nationale Supérieure de Chimie, 8, rue de l'Ecole Normale,
34053 Montpellier Cedex 1, France

†National Institute on Drug Abuse—Intramural Research Program, P.O. Box 5180, Baltimore, MD 21224

‡BioCAD Corporation, 545 Oakmead Parkway, Sunnyvale, CA 94086

§Department of Neuropsychopharmacology and Hospital Pharmacy,
Nagoya University School of Medicine, Showa ku, Nagoya 466, Japan

Received 22 March 1994

MAURICE, T., T.-P. SU, D. W. PARISH, T. NABESHIMA AND A. PRIVAT. *PRE-084, a σ selective PCP derivative, attenuates MK-801-induced impairment of learning in mice.* PHARMACOL BIOCHEM BEHAV 49(4) 859-869, 1994. — We investigated the effect of the σ selective PCP derivative PRE-084 on the impairment of learning induced in mice by the noncompetitive NMDA antagonist MK-801. Learning capacities were evaluated using the spontaneous alternation in a Y-maze test for spatial working memory, the step-down passive avoidance and the elevated plus-maze test for long-term memory. At doses about 1 mg/kg IP, PRE-084 significantly attenuated MK-801 (0.2 mg/kg IP) induced impairment of learning. The dose-response curve was bell-shaped which is typical for cognition enhancers. The effect of PRE-084 was antagonized by BMY-14802 (10 mg/kg IP) and suppressed by a chronic treatment with haloperidol (4 mg/kg/day SC for 7 days). Furthermore, PRE-084 did not affect scopolamine (1 mg/kg SC) induced amnesia but significantly attenuated mecamylamine (10 mg/kg IP) induced amnesia. These results show that σ sites mediate a modulation of the NMDA receptor complex-dependent learning processes and may similarly affect the cholinergic nicotinic memory processes. Moreover, the PCP derivative PRE-084, acting selectively at σ sites, reverses the amnesia induced by a drug acting at the PCP site.

σ sites	PRE-084	N-methyl-D-aspartate receptor	MK-801-induced amnesia	Chronic treatment
Passive avoidance test		Y-maze test	Mecamylamine	Amnesia models

A RECENT proposal for classification has divided σ sites into two different classes (25): the σ_1 site, at which ligands like (+)-pentazocine, (+)-N-allyl-normetazocine ((+)-SKF 10,047), and dextrometorphan bind with a high affinity and stereoselectivity, and the σ_2 site, which has lower affinity and is not stereoselective for these ligands. Both 1,3-di-(2-tolyl)guanidine (DTG) and haloperidol are nonselective drugs and bind with a similar high affinity to the two classes of sites (25,34,42). Further, it has been shown that haloperidol, rimcazole, α -(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol (BMY-14802) and N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-ethylamine hydrochloride (NE-100) appear to act as antagonists in several behavioral

tests (23,35,36). On the other hand, it is now clear that these sites are different entities from opiate receptors, for which they were initially classified, and from the high affinity phenylpiperidine (PCP) binding site (26). The PCP site is located inside the ion channel associated with the N-methyl-D-aspartate (NMDA) type of glutamate receptor. Drugs, such as PCP, its more selective derivative TCP, or MK-801, bind with high affinity to this site and lead to a noncompetitive antagonism of the NMDA type of glutamatergic neurotransmission (1,18).

Although the pharmacological roles of σ sites are not yet well understood, evidences indicate that they may have several biological functions (32,41). Among them, a facilitatory role

¹ To whom request for reprints should be addressed.

of σ ligands on some NMDA responses has been reported. In vitro, nanomolar concentrations of (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine((+)-3-PPP) and (+)-*N*-cyclopropylmethyl-*N*-methyl-1,4-diphenyl-1-ethyl-but-3-en-1-ylamine hydrochloride (JO-1784) potentiated the NMDA-induced release of [³H]norepinephrine from rat hippocampal slices (19, 27). In vivo, low doses of DTG, JO-1784, or (+)-pentazocine selectively enhanced the NMDA-induced activation of pyramidal CA3 dorsal hippocampal neurons in the rat (20,21). In search of a behavioral relevance of these results, we recently reported that the MK-801-induced amnesia could be significantly attenuated by σ ligands, such as DTG, (+)-SKF 10,047, and (+)-pentazocine, at doses below 1 mg/kg in mice (16,17). All these effects could be reversed by σ antagonists and presented the affinity order and drug stereoselectivity of the σ_1 subtype of σ sites. Furthermore, a similar modulation may affect the cholinergic nicotinic-dependent memory processes, since DTG allowed a marked attenuation of the amnesia induced by the nicotinic antagonist mecamylamine, but not by the muscarinic antagonist scopolamine (16).

Since the PCP derivative 2-(4-morpholinoethyl 1-phenylcyclohexane-1-carboxylate hydrochloride (PRE-084) was recently described as a new, highly selective σ ligand, with an IC₅₀ of 44 nM (33), we investigated its effect on the NMDA receptor complex-dependent learning processes in mice. The purpose of this study was to determine if PRE-084 could be effective in attenuating the MK-801-induced impairment of learning, on short-term and long-term memory tests. The implication of σ sites was examined in antagonism studies with the purported σ antagonist BMY-14802 (35,36) and using a subchronic treatment with haloperidol, which is known to induce a downregulation of the σ sites (8). Furthermore, the selectivity of the putative PRE-084 effect was also examined on the cholinergic-dependent memory processes.

METHODS

Animals

For the in vitro binding experiments, male Wistar rats (Iffa-Credo, L'Arbresle, France), weighing 250–300 g, were used. For the behavior experiments, male Swiss mice (Iffa-Credo, L'Arbresle, France), aged 5–6 weeks and weighing 33–40 g, were used. They were housed in plastic cages, with free access to standard laboratory food and water, and kept in a regulated environment (23 ± 1°C), under a 12-hr light/dark cycle (light on at 0700). Mice were used for the experiments after they were adapted to laboratory conditions for at least 3 days and were naive to each test used in the present study. Experiments were carried out between 1000 and 1800 in a semi-soundproof laboratory, in which mice were habituated at least 30 min before each experiment.

In Vitro Binding Assays

[³H]TCP binding was performed as described by Vignon et al. (40) on rat brain membrane homogenates in a 5 mM Tris-HCl buffer (pH 7.6). Affinity of PRE-084 for the NMDA receptor-linked PCP sites was examined at equilibrium in competition experiments with 5 nM [³H]TCP, in the presence of 100 μM unlabelled TCP for the determination of nonspecific binding. [³H]BTCP binding was performed as described by Vignon et al. (39) on rat striatal membranes in a 50 mM Na₂HPO₄ buffer (pH 7.4). Affinity of PRE-084 for the dopamine transporter-associated BTCP sites was examined at equilibrium in competition experiments with 0.5 nM [³H]BTCP, in

the presence of 10 μM unlabelled BTCP for the nonspecific binding.

Spontaneous Alternation Performance

Spatial working memory performance was assessed by recording spontaneous alternation behavior in a Y-maze, as previously described (7,29). 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. Mice tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the mice know which arm they have already been visited. The series of arm entries, including possible returns into the same arm, was recorded 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) × 100. For example, if the arms were called A, B, and C and if the mouse performed ABCACBACCAB; the total number of arm entries would be 11 and the percent alternation 66.7.

Step-Down Type Passive Avoidance Test

The step-down type of passive avoidance task was used to examine the long-term memory based on negative reinforcement, as previously described (3,16). The apparatus consisted of a transparent acrylic cage (30 × 30 × 40 cm high) with a grid floor, inserted in a semi-soundproof outer box (35 × 35 × 90 cm high). The cage was illuminated with a 15 W lamp during the experimental period. A wooden platform (4 × 4 × 4 cm) was fixed in the center of the grid floor. Electric shocks (1 Hz, 500 msec, 40 V DC) were delivered to the grid floor with an isolated pulse stimulator (Model 2100, A-M Systems, Inc., Everett, WA). When the mouse was placed in the test cage, the current resistance varied from 100 to 250 kOhm. Therefore, each mouse received electric shocks varying from 0.15 to 0.38 mA. Training was carried out in two similar sessions. Each mouse was placed on the platform in the center of the cage. When the mouse stepped down and placed all its paws on the grid floor, shocks were delivered for 15 s. Step-down latency (SDL), and the number of flinching reactions and vocalizations were measured. Animals showing SDL in the criterion range (3–30 s) during the first training session were used for the second session and the retention test. More than 95% of the animals met this criterion. The second session was carried out 90 min after the first. Animals staying 60 s on the platform were considered as remembering the task and did not receive electric shocks anymore. The retention test was carried out 24 h after training, in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 s.

Elevated Plus-Maze Test

The elevated plus-maze was used to evaluate spatial long-term memory, following the procedure described by Itoh et al. (5,6). The apparatus consisted of two open arms (23.5 × 8 cm), painted white, and two enclosed arms (23.5 × 8 × 20 cm high), painted black. The arms extended from a central platform (8 × 8 cm), and the maze was elevated to a height of 50 cm above the floor. A fine white line was drawn in the

middle of the floor of the enclosed arms. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. The time taken by the mouse to move into one of the enclosed arms, its two front paws crossing the white line, was recorded as the transfer latency for the first day. If the animal did not enter an enclosed arm within 90 s, it was gently pushed on the back into one enclosed arm and the transfer latency was assigned as 90 s. The mouse was allowed to move freely for at least 10 s so that it could remember the presence of the enclosed arms, and it was then gently taken out and returned to its home cage. Retention was examined 24 h later. Each mouse was again placed into the maze and the transfer latency was recorded, up to 90 s.

Drugs and Treatments

[³H]TCP (55 Ci/mmol) and [³H]BTCP (48 Ci/mmol) were prepared by Amersham (Les Ulis, France). TCP and BTCP were gifts from Dr J.-M. Kamenka (Montpellier, France). (+)-MK-801 maleate was from R.B.I. (Natick, MA); haloperidol (Haldol®) was from Janssen Labs (France); scopolamine and mecamlamine were from Sigma (Sigma Chemical Corp., St Quentin Fallavier, France); BMY-14802 was a gift from Dr B. Lockhart (Montpellier, France). PRE-084 was synthesized as described elsewhere (D. W. Parish et al., in preparation). For behavior experiments, drugs were dissolved in saline, except BMY-14802, which was dissolved in a minimal volume of 0.1N HCl and then in saline, pH being adjusted to 4 with 1N NaOH. Drugs or the respective vehicle were injected in a volume of 100 μ l/20 g of body weight. For chronic treatments, mice received SC injections of haloperidol (2 mg/kg) or saline solution twice daily, at 1100 and 1700, for 7 days. They were then examined for mnemonic capacities, spontaneous alternation performances, and passive avoidance 24 h after the last injection.

Statistical Analysis

Results are expressed as the means \pm SEM, except for the step-down latencies, which are expressed in terms of medians and interquartile ranges. The data did not show a normal distribution, since upper cut-off times were set (nonparametric data). They were analyzed by the Kruskal-Wallis analysis of variances by ranks. If there were significant H values, post hoc comparisons were made by using the nonparametric Dunn's multiple comparisons test. The criterion for statistical significance was $p < 0.05$.

RESULTS

Binding Data

In preliminary binding experiments, we checked the selectivity of PRE-084 for the σ sites by assessing its binding to the two other main PCP targets, namely the dopamine uptake system and the high affinity PCP site. At the concentration of 10 μ M, PRE-084 inhibited only $30.4 \pm 5.7\%$ ($n = 3$) of the specific binding of [³H]BTCP to rat striatal membrane preparations, and therefore exhibited an $IC_{50} > 10 \mu$ M (Fig. 1A). On the other hand, at 100 μ M, PRE-084 did not induce any significant inhibition of the binding of [³H]TCP from rat cerebrium membrane preparations (Fig. 1B).

Effect of PRE-084 on the MK-801-Induced Impairment of Working Memory

The administration of PRE-084, in the 0.1–3 mg/kg dose range, did not affect the spontaneous alternation behavior in

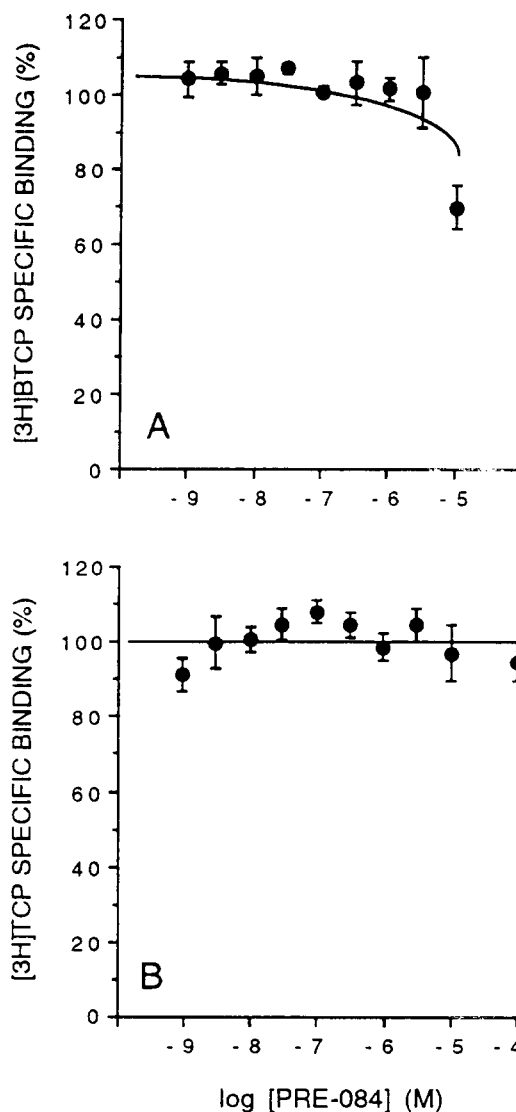


FIG. 1. Effect of PRE-084 on the in vitro binding of (A) [³H]BTCP to rat striatal membrane preparations and of (B) [³H]TCP to rat cerebrium membrane preparations. Increasing concentrations of PRE-084 were incubated in presence of 0.5 nM [³H]BTCP or 5 nM [³H]TCP. Results are the mean \pm SEM of three independent determinations, each performed in triplicate.

the Y-maze when compared to saline controls. Mice exhibited an alternation percentage about 70% (Fig. 2A), and performed from 25 to 30 arm entries within the 8-min session (Fig. 2B). Preadministration of MK-801 (0.2 mg/kg IP) to mice induced a marked decrease in spontaneous alternation performance (Fig. 2A), with a concomitant increase in the total number of arm entries (Fig. 2B), which reflected the hyperlocomotor activity induced by the drug. The preadministration of increasing doses of PRE-084 attenuated the MK-801 (0.2 mg/kg) induced decrease in alternation, as shown in Fig. 2A. The dose-response curve was bell-shaped, and attenuation was significant at the doses of 0.3 and 1 mg/kg. The MK-801-induced increase in the number of arm entries was not affected by the PRE-084-pretreatment (Fig. 2B).

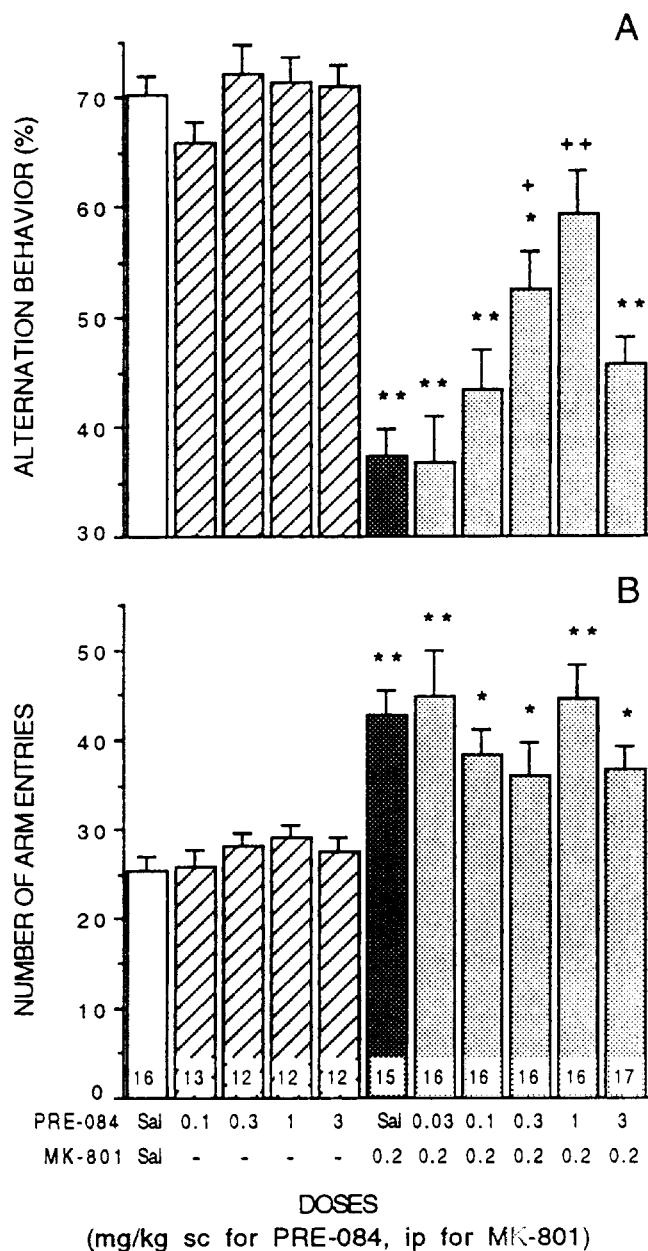


FIG. 2. Dose-response effect of PRE-084 on alternation behavior in MK-801-treated mice: (A) alternation performances, (B) total number of arm entries. PRE-084 (0.03–3 mg/kg SC) was administered 30 min before the test, or 10 min before MK-801 (0.2 mg/kg IP), which was administered 20 min before the test. The number of mice used is shown in the columns in (B). * $p < 0.05$; ** $p < 0.01$ vs. (Sal + Sal)-treated group; † $p < 0.05$; †† $p < 0.01$ vs. (Sal + MK-801)-treated group (Dunn's test).

Effect of PRE-084 on the MK-801-Induced Impairment of Learning

In the step-down type passive avoidance task, the treatments with PRE-084 (0.3–3 mg/kg), MK-801 (0.2 mg/kg), or the combination of PRE-084 plus MK-801, did not change the sensitivity to the electric shocks in terms of number of vocalizations or of flinching reactions during each training

session, compared to the control group (Table 1). The SDL during the first training was not affected by the PRE-084 treatment, and slightly but nonsignificantly lowered for all the MK-801-treated groups (Table 1), reflecting the hyperlocomotor activity of the drug. During the second training session, the control and the PRE-084-treated groups exhibited a significant increase in SDL, that was not observed with the MK-801-treated groups (Table 1). It must be noted that the SDL exhibited group treated with PRE-084 (1 mg/kg) and MK-801 did not significantly differ from the SDL of the control group.

During the retention test, control mice exhibited SDL of 225 s [170–300] ($n = 17$), that was not affected by the treatments with PRE-084 alone (0.1–3 mg/kg) alone (Fig. 3). SDL was drastically decreased to 17 s [14–33] ($n = 17$) with the MK-801 (0.2 mg/kg) treatment, and the preadministration of PRE-084 (0.1–1 mg/kg) prior to MK-801 led to a significant increase in SDL (Fig. 3). This effect also showed a bell-shaped curve.

In the elevated plus-maze task, control mice exhibited a transfer latency into the enclosed arms of 59 ± 5 s ($n = 30$) on the first day, decreasing to 24 ± 2 s on the second day, indicating that the mice remembered the presence of the enclosed arms. The treatments with PRE-084 (0.3–3 mg/kg) alone did not affect the transfer latencies on both days (Fig. 4A and B). The MK-801 (0.2 mg/kg) treatment did not modify the transfer latency on the first day (58 ± 6 s, $n = 27$; Fig. 4A). However, it resulted in a marked amnesia, since the transfer latency on the second day was not decreased ($p < 0.01$ vs. latency of control mice on the second day; Fig. 4B). It remained in the same order as the latency of naive animals: 52 ± 5 s. On the first day, the PRE-084-pretreatment did not modify the transfer latencies in either MK-801-treated or control mice. However, a marked decrease in the mean transfer latencies on the second day was observed for the groups pretreated with 0.3, 1, and 3 mg/kg of PRE-084 (Fig. 4B), although the Kruskal–Wallis analysis did not show any significance of the PRE-084-pretreatment ($H = 7.23$, $p = 0.12$).

Antagonism of the Effect of PRE-084 by BMY-14802

When BMY-14802 was administered IP at doses between 1 and 10 mg/kg, 30 min before each test, it did not affect the behavior in the Y-maze or the passive avoidance performances as compared to control groups (16). In the Y-maze test, BMY-14802 (10 mg/kg IP) did not change the MK-801 (0.2 mg/kg)-induced decrease in alternation (Fig. 5A), and a nonsignificant attenuation of the MK-801-induced hyperlocomotion was observed (Fig. 5B). When it was administered simultaneously with PRE-084 (1 mg/kg SC), it significantly prevented the attenuating effect of PRE-084 on MK-801-induced decrease in alternation performances (Fig. 5A). Alternation percentages were similar for the (BMY-14802 + PRE-084 + MK-801)-treated group and for the (Veh + Sal + MK-801)-treated group, showing a complete antagonism by BMY-14802 (Fig. 5A). The same nonsignificant attenuation of hyperlocomotion from MK-801-treated group was observed (Fig. 5B). A similar result was obtained in the passive avoidance test. BMY-14802 (10 mg/kg IP) alone did not affect the MK-801 (0.2 mg/kg)-induced decrease in SDL, but, when it was administered simultaneously with PRE-084 (1 mg/kg SC), it significantly prevented the increase in SDL induced by PRE-084 (Fig. 5C).

Effect of a Chronic Treatment with Haloperidol

Mice were chronically treated with haloperidol (4 mg/kg/day SC) or saline solution for 7 days. They were examined 24

TABLE 1
EFFECT OF THE SUCCESSIVE ADMINISTRATIONS OF PRE-084 (0.1-3 mg/kg s.c.) AND
MK-801 (0.2 mg/kg i.p.) DURING PASSIVE AVOIDANCE TRAINING SESSIONS

Treatments	n	SDL Median [I.R.] (sec)	Vocalizations Mean ± SEM	Flinching Reactions Mean ± SEM
<i>First training session:</i>				
Saline + Saline	17	8[4.0-15.0]	6 ± 1.5	11 ± 0.8
PRE-084 (0.3)	12	7[4.0-11.0]	5 ± 1.2	10 ± 1.1
PRE-084 (1)	11	4[3.0-9.8]	3 ± 1.7	9 ± 0.9
PRE-084 (3)	12	6[3.3-10.0]	2 ± 1.11	1 ± 1.0
Saline + MK-801	17	3[3.0-6.5]	5 ± 1.4	10 ± 1.0
PRE-084 (0.1) + MK-801	18	3[3.0-4.3]	4 ± 1.2	10 ± 0.9
PRE-084 (0.3) + MK-801	18	3[3.0-5.0]	4 ± 1.1	10 ± 0.7
PRE-084 (1) + MK-801	17	5[3.0-8.3]	6 ± 1.6	9 ± 1.0
PRE-084 (3) + MK-801	18	3[3.0-6.5]	5 ± 1.3	11 ± 0.8
ANOVA		$H = 9.97$ $p > 0.05$	$H = 2.49$ $p > 0.05$	$H = 2.59$ $p > 0.05$
<i>Second training session:</i>				
Saline + Saline		57[11.0-60.0]§	4 ± 1.9	8 ± 1.4
PRE-084 (0.3)		47[10.0-58.3]‡	2 ± 1.2	10 ± 1.0
PRE-084 (1)		48[15.0-60.0]§	3 ± 0.9	9 ± 1.1
PRE-084 (3)		52[17.3-60.0]§	4 ± 1.5	8 ± 0.4
Saline + MK-801		5[3.0-5.5]†	8 ± 1.7	10 ± 1.3
PRE-084 (0.1) + MK-801		5[4.0-8.0]†	2 ± 0.7	5 ± 0.7
PRE-084 (0.3) + MK-801		6[4.0-26.0]*	5 ± 1.5	9 ± 1.4
PRE-084 (1) + MK-801		9[4.8-14.3]	4 ± 1.6	9 ± 1.
PRE-084 (3) + MK-801		3[3.0-5.5]†	6 ± 1.5	10 ± 0.9
ANOVA		$H = 38.75$ $p < 0.01$	$H = 10.05$ $p > 0.05$	$H = 10.27$ $p > 0.05$

* $p < 0.05$; † $p < 0.01$ vs. (Sal + Sal)-treated group; ‡ $p < 0.05$; § $p < 0.01$ vs. the same treatment group in the first training session (Bonferroni multiple comparison test).

h after the last administration of haloperidol, for alternation behavior in the Y-maze test (Fig. 6A and B) or for passive avoidance performances (Fig. 6C). The alternation performances of control mice and the decrease in alternation induced by MK-801 (0.2 mg/kg IP), as observed in the chronically saline-treated groups, was unchanged by the chronic haloperidol treatment (Fig. 6A). However, the attenuating effect of PRE-084 (1 mg/kg SC) on the MK-801-induced impairment of alternation, which was observed in the chronically saline-treated groups, was completely abolished by the chronic haloperidol treatment (Fig. 6A). It must be noted that the chronic haloperidol treatment also led to a significant decrease in the hyperlocomotion induced by MK-801, as observed for both the (Sal + MK-801)- and (PRE-084 + MK-801)-treated groups (Fig. 6B). A similar result was obtained in the passive avoidance test. The increasing effect of PRE-084 (1 mg/kg SC) on the SDL exhibited by MK-801-treated mice, which was observed in the chronically saline-treated groups, was completely abolished by the chronic haloperidol treatment (Fig. 6C).

Selectivity of the Effect of PRE-084 in Cholinergic Amnesia Models

A series of experiments addressed the selectivity of the anti-amnesic effect of PRE-084 (1 mg/kg SC) on pharmacological models of amnesia involving the cholinergic systems. Both scopolamine (1 mg/kg SC) and mecamylamine (10 mg/kg IP) significantly decreased alternation performances (Fig. 7A). The PRE-084 (1 mg/kg SC) pretreatment did not affect the

scopolamine-induced decrease in alternation, but completely prevented the mecamylamine-induced decrease (Fig. 7A). Mild decreases in the total number of arm entries were observed with the scopolamine treatment, although not significant for the (scopolamine + saline)-treated group, and with the mecamylamine treatment (Fig. 7B). Similarly, both drugs induced decreases in SDL as compared to saline-treated groups (Fig. 7C). The scopolamine-induced amnesia was not affected by the PRE-084-pretreatment, whereas the mecamylamine-induced decrease in SDL was significantly attenuated (Fig. 7C).

DISCUSSION

This study showed that the high affinity σ ligand PRE-084, at the low mg/kg dose range, significantly attenuated the impairment of learning induced in mice by systemic administration of MK-801. MK-801 exerts its amnesic action by binding to the high affinity PCP site, which is localized within the ion channel associated with the NMDA type of glutamate receptors. The drug induces a noncompetitive antagonism of the learning processes, that depend on the activation of the NMDA receptor in the hippocampus (22,38). PRE-084, a morpholine PCP derivative with a carboxyl ethylene insertion between the cyclohexyl and the morpholine rings (33), appears to be a selective σ compound. Besides its weak affinities for receptor sites such as dopamine D_2 , muscarinic cholinergic, 5-HT₂, α and β adrenoceptors, as previously described (33), we reported that the drug did not bind to the PCP sites, la-

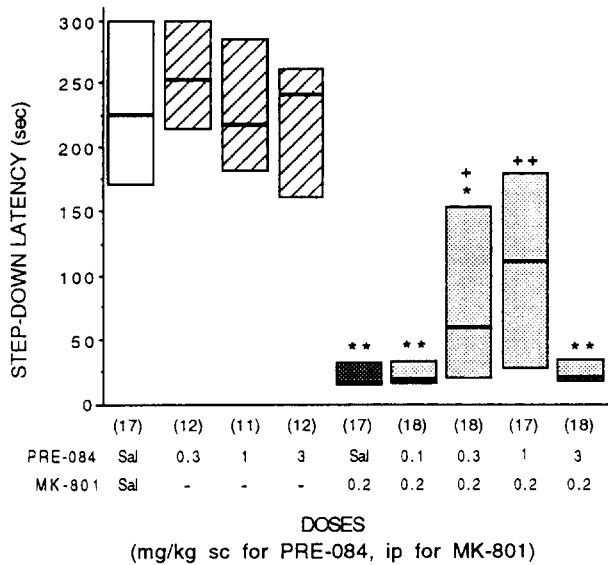


FIG. 3. Dose-response effect of PRE-084 on the MK-801-induced amnesia in the step-down passive avoidance test. PRE-084 (0.1–3 mg/kg SC) was administered 30 min before the first training, or 15 min before MK-801 (0.2 mg/kg IP), which was administered 30 min before the first training. A second training was repeated 90 min after, and retention was examined 24 h after the first training. Results show the median and interquartile range of number of mice indicated below each bar. * $p < 0.05$; ** $p < 0.01$ vs. (Sal + Sal)-treated group; † $p < 0.05$; †† $p < 0.01$ vs. (Sal + MK-801)-treated group (Dunn's test).

belled with [3 H]TCP, nor to the BTCP sites, which are linked to the dopamine uptake complex (39). These results clearly show that PRE-084 can be considered as a PCP derivative selective for σ sites, to which it binds with a high affinity [$IC_{50} = 44$ nM (34)] and devoid of affinity for the other main molecular targets of PCP.

At doses about 1 mg/kg, PRE-084 did not affect the mnemonic capacities by itself, but attenuated the MK-801-induced impairment of learning. This effect was first shown on the spontaneous alternation behavior in the Y-maze, a test predictive of spatial working memory (7,29). We also used two different long-term memory tests, the step-down passive avoidance (3) and the elevated plus-maze test (5,6). In all tests, PRE-084, at the doses of 0.3 and 1 mg/kg SC, attenuated the MK-801-induced impairment of learning in a significant manner, except in the elevated plus-maze task. Moreover, the effect was only partial, leading to 55%–67% antagonism according to the test, and bell-shaped, since the dose of 3 mg/kg was always without effect.

This attenuation is likely to involve sigma sites, since: (a) PRE-084 is a highly selective σ drug; (b) BMY-14802, the purported σ antagonist (35,36), behaved as an antagonist, having no effect by itself on the MK-801-induced impairment of learning, and antagonizing the effect of PRE-084; (c) the similarity of the effect of PRE-084 and the previously described effects of DTG, (+)-pentazocine and (+)-SKF-10,047 on MK-801-induced impairment of learning in mice (16,17); (d) the chronic treatment with haloperidol induces a downregulation of sigma sites (8,11), and suppressed the effect of PRE-084. Finally, we previously reported a stereoselectivity with both the pentazocine and SKF-10,047 isomers, the (+)-isomers being effective whereas the (-)-isomers were ineffec-

tive (16). Consequently, this σ effect may involve the σ_1 type of σ sites, according to the classification proposed by Quirion et al. (25).

Very little is known about the general behavioral effects of PRE-084. In our tests, neither mnemonic, nor locomotor effects were observed when PRE-084 was administered alone to mice, in the 0.1–3 mg/kg range. Furthermore, PRE-084 did not affect the general behavior in an open-field paradigm (un-

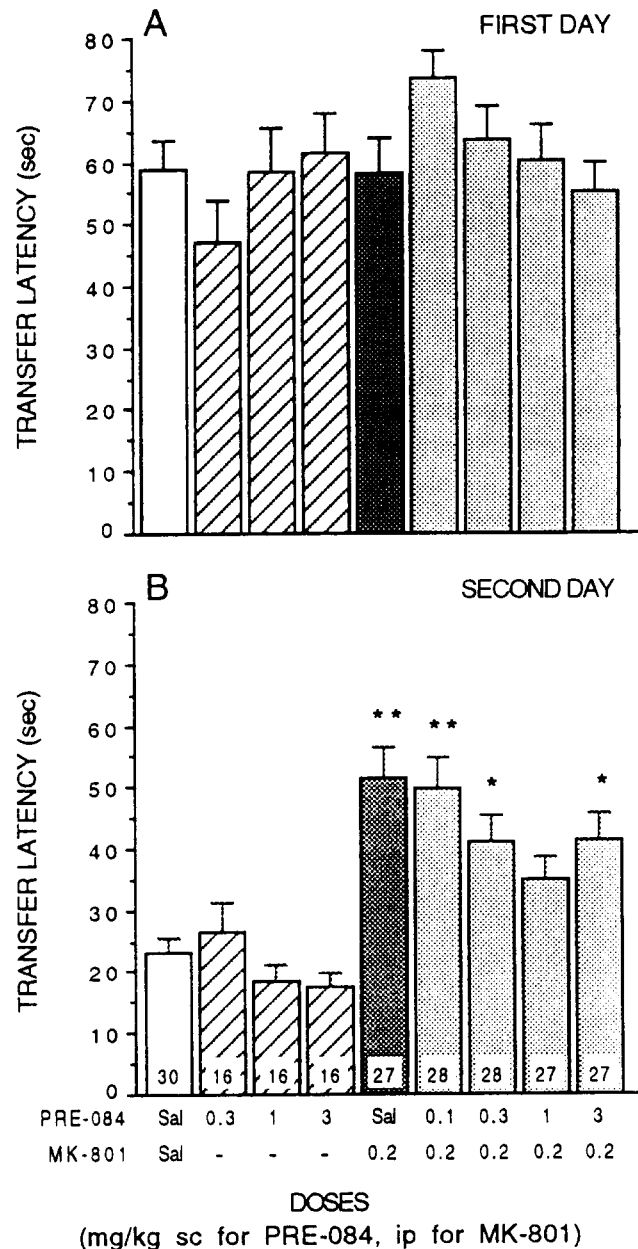


FIG. 4. Dose-response effect of PRE-084 on the MK-801-induced amnesia in the elevated plus-maze test. Transfer latencies to the enclosed arms are shown for (A) the first day and (B) the second day. PRE-084 (0.1–3 mg/kg SC) was administered 30 min before the test on the first day, or 15 min before MK-801 (0.2 mg/kg IP), which was administered 30 min before the test. The number of mice used is shown in the columns. * $p < 0.05$; ** $p < 0.01$ vs. (Sal + Sal)-treated group (Dunn's test).

published preliminary result). We used different behavioral tests, to assess the mnemonic capacities through different paradigms. First, the determination of spontaneous alternation performance in the Y-maze allowed the concomitant observation of locomotion, in term of the total number of arms entered during the 8-min session. It must be noted that this parameter cannot be consider as strictly dependent on locomotion, since several other behaviors are implicated in the ability of the animal to visit the maze, such as exploratory activity or

attention. However, PRE-084 did not affect the number of arm entries by itself, and did not modify the MK-801-induced hyperactivity. Second, the step-down passive avoidance task is based on a negative reinforcement: intermittent electric shocks. The measurement of the number of vocalizations and flinching reactions during the two training sessions allowed to quantify any possible pro- or analgesic action of the drug. As reported, the PRE-084-treatments did not affect the sensitivity of the animals to the shocks. Finally, we also used the elevated

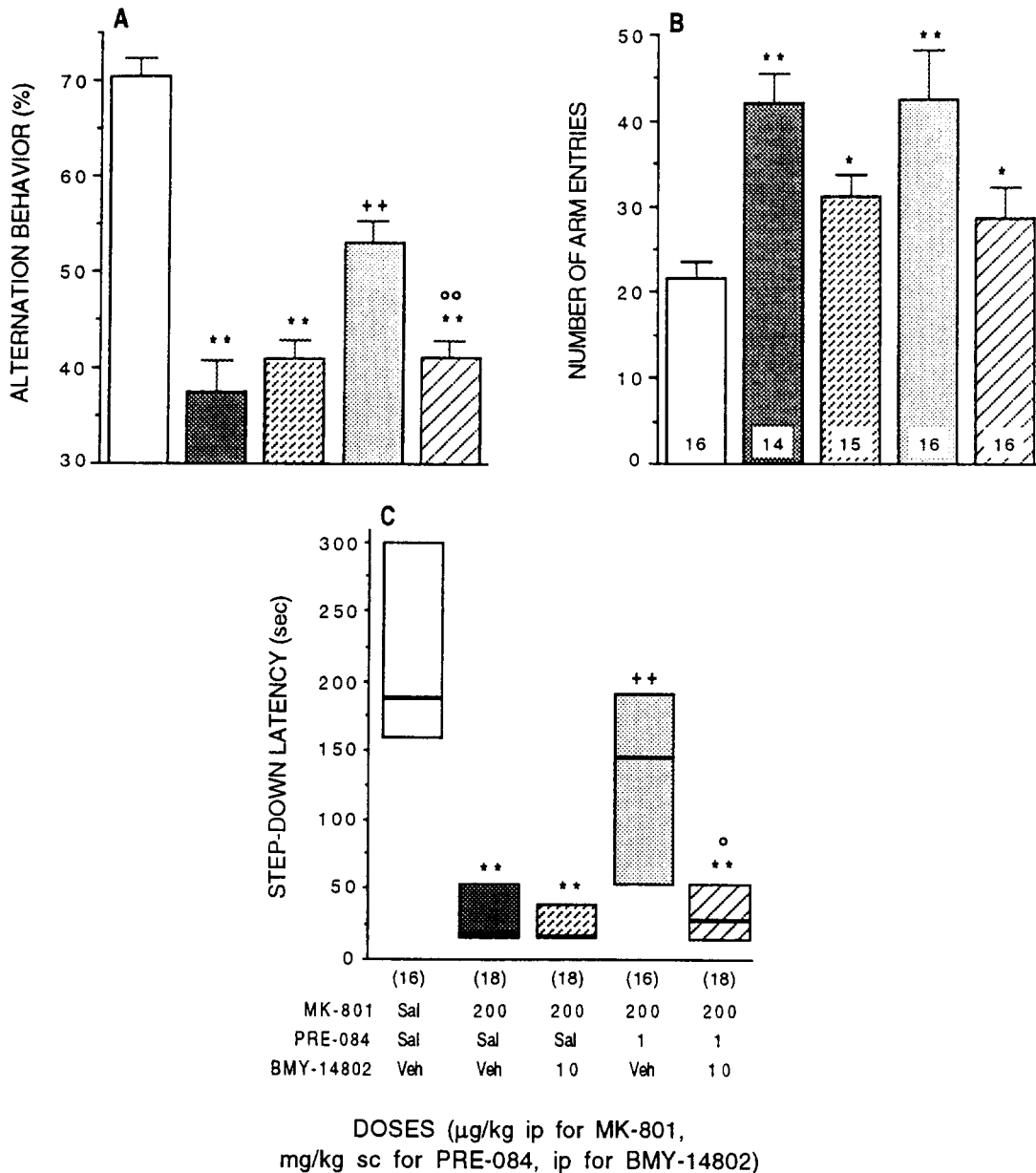


FIG. 5. Antagonism by BMY-14802 of the PRE-084-effect on MK-801-induced amnesia: (A) alternation performances; (B) number of arm entries in the Y-maze test; and (C) SDL in the passive avoidance test. For the Y-maze test, BMY-14802 (10 mg/kg IP) was administered simultaneously with PRE-084 (1 mg/kg SC), which was administered 10 min before MK-801 (0.2 mg/kg IP), 20 min before the test. For the passive avoidance test, BMY-14802 and PRE-084 were administered 15 min before MK-801, 30 min before the first training. * $p < 0.05$; ** $p < 0.01$ vs. (Veh + Sal + Sal)-treated group; †† $p < 0.01$ vs. (Veh + Sal + MK-801)-treated group; ° $p < 0.05$; °° $p < 0.01$ vs. (Veh + PRE-084 + MK-801)-treated group (Dunn's test).

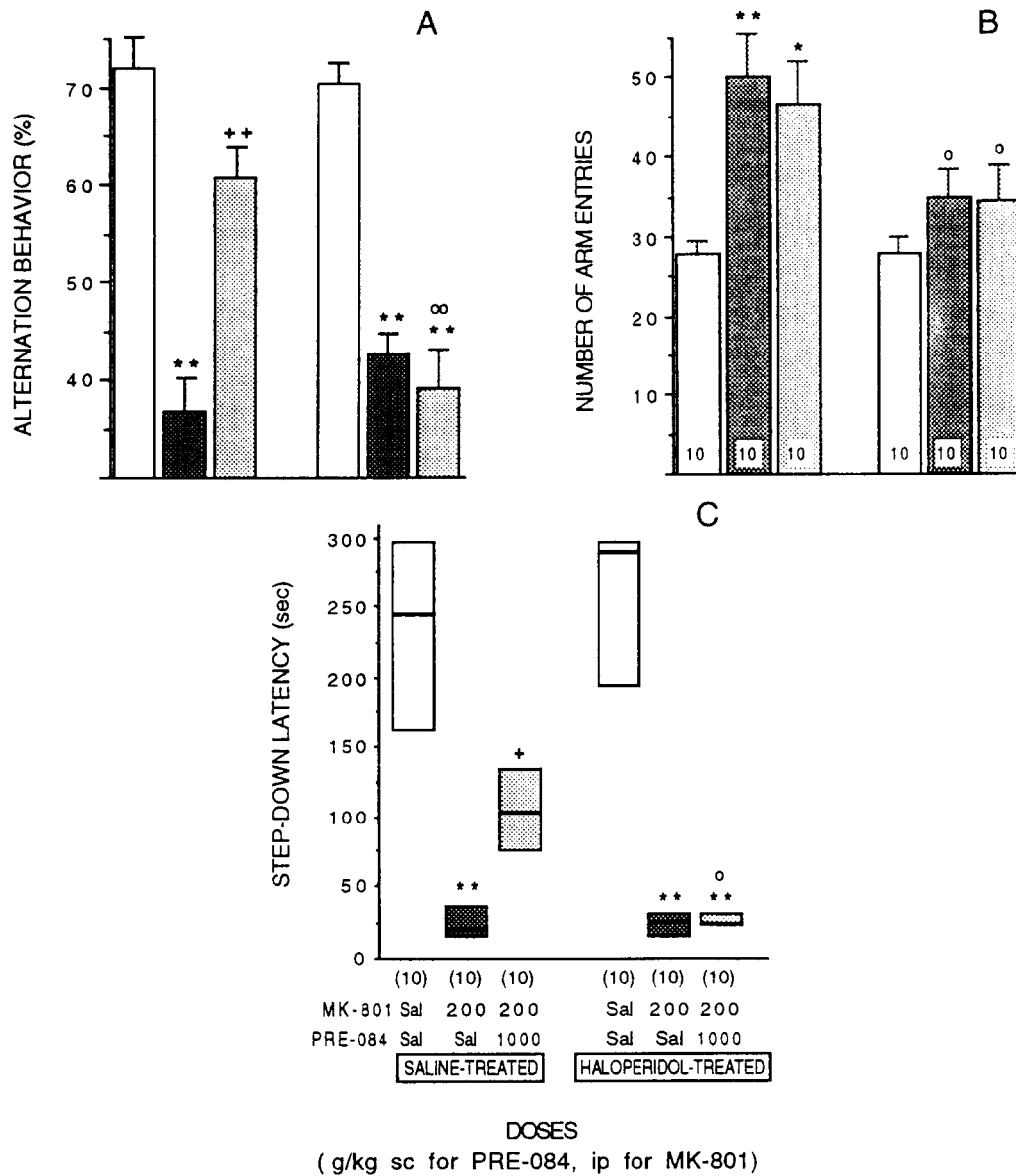


FIG. 6. Effect of PRE-084 on the MK-801-induced amnesia in mice chronically treated with haloperidol: (A) alternation performances; (B) number of arm entries in the Y-maze test; and (C) SDL in the passive avoidance test. Haloperidol (2 mg/kg SC) or saline solution (100 μ l/20 g body weight) was administered twice a day for 7 days. Twenty-four hours after the last administration of haloperidol, PRE-084 (1 mg/kg SC) was administered 10 min before MK-801 (0.2 mg/kg IP), given 20 min before the Y-maze test session or 15 min before MK-801, given 30 min before the first passive avoidance training. * $p < 0.05$; ** $p < 0.01$ vs. resp. (Sal + Sal)-treated group; † $p < 0.05$; †† $p < 0.01$ vs. resp. (Sal + MK-801)-treated group; ^o $p < 0.05$; ^{oo} $p < 0.01$ vs. the same treatment group chronically treated with saline (Dunn's test).

plus-maze test, with a protocol adapted for measuring the mnemonic capacities (5,6). This test is based on the exploration of the maze, and on the aversive behavior of mice for the clear and open arms. However, the protocol does not allow to quantify exactly the anxious state of the animal. In summary, we can conclude that all these tests indicated in a convergent manner, that PRE-084 led to an attenuation of the MK-081-induced amnesia, without affecting mnemonic capacities by itself, and without altering other behaviors, such as locomo-

tion, exploration, or pain sensation. For comparison, at the same low mg/kg range used in the present experiments, a selective σ ligand such as DTG did not elicit direct behavioral effects. Previous studies in the rat reported that DTG induced PCP-like stereotyped behavior, convulsions and ataxia, at doses above 16 mg/kg (42); it reduced the number of arm entries in the radial-arm maze and the number of line crossings in an open-field, at doses between 4 and 8 mg/kg (28); and it prevented the scopolamine-induced amnesia, at 4 mg/kg (2).

However, no direct behavioral effects were evidenced for this drug, in the $\mu\text{g}/\text{kg}$ range that we used to initially describe the attenuating effect on MK-801-induced amnesia (16,17).

A particular aspect of this study addressed the influence of a chronic treatment with haloperidol on the attenuating effect of σ ligands on the MK-801-induced amnesia. Itzhak and Alerhand (8) reported first that a chronic treatment with haloperidol, similar to the protocol used in this study, induced a complete elimination of σ sites, an upregulation of D_2 dopaminergic receptors, and no change in PCP sites, after even 14

or 21 days of treatment. A downregulation of σ sites after chronic haloperidol treatment was also described by Karbon and Naper (11) in the guinea pig brain. However, Manalack and Beart (13) reported a contradictory result with DTG, since chronic treatments with DTG or with the purported σ antagonist rimcazole induced an upregulation of σ sites in rat. Thus, the agonist or antagonist mode of action of DTG on σ sites remains uncertain. However, studies using haloperidol led to convergent results, indicating that the drug behaves as an agonist and induces downregulation of σ sites. In accordance with

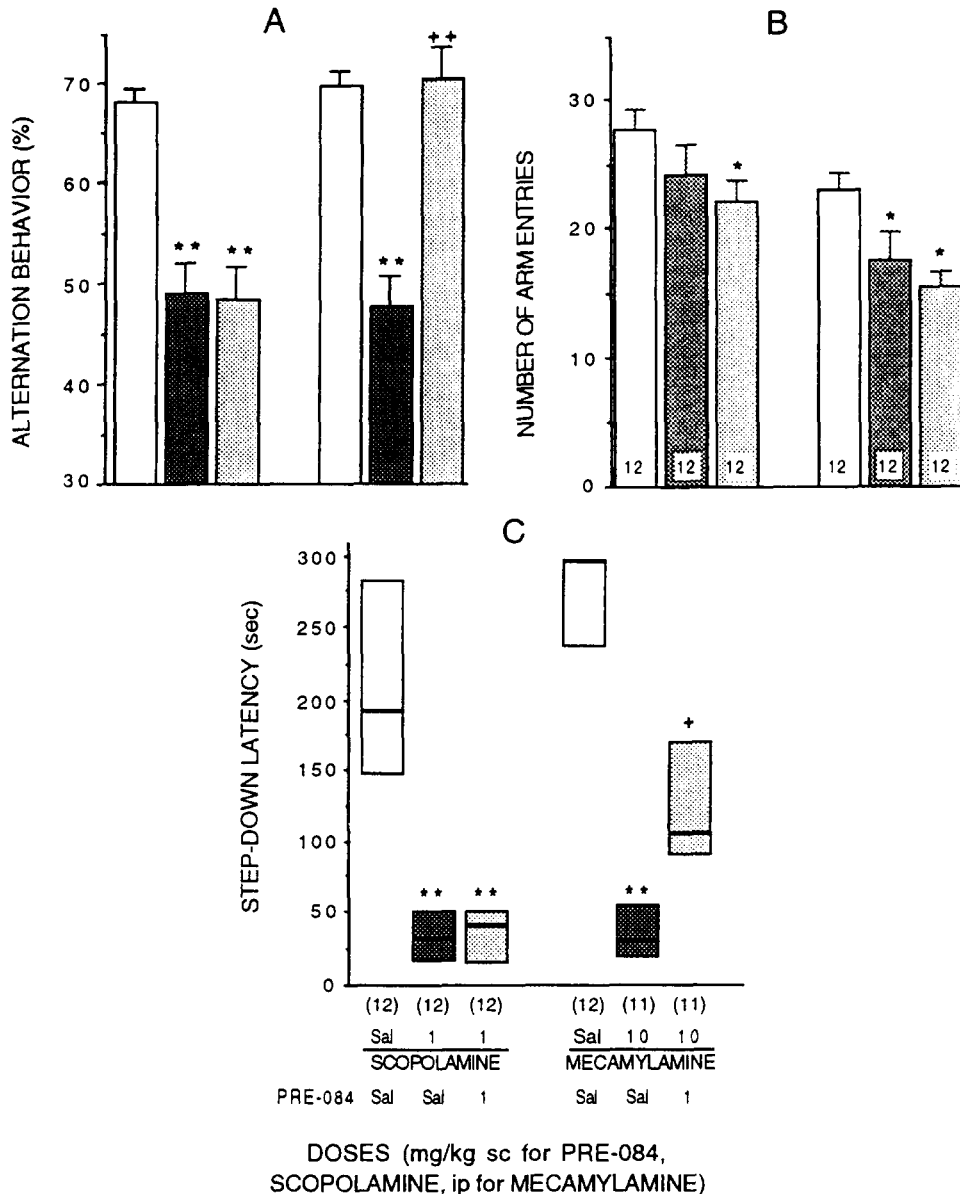


FIG. 7. Effect of PRE-084 on the amnesia induced by scopolamine or mecamlamine: (A) alternation performances; (B) number of arm entries in the Y-maze test; and (C) SDL in the passive avoidance test. PRE-084 (1 mg/kg SC) was administered 10 min before scopolamine (1 mg/kg SC) or mecamlamine (10 mg/kg IP), which was given 20 min before the Y-maze test session or 15 min before the amnesic drugs, given 30 min before the first passive avoidance training. * $p < 0.05$; ** $p < 0.01$ vs. resp. (Sal + Sal)-treated group; † $p < 0.05$; †† $p < 0.01$ vs. (Sal + mecamlamine)-treated group (Dunn's test).

these biochemical data, we observed a complete disappearance of the attenuating effect of PRE-084 on the MK-801-induced impairment of learning. Interestingly in haloperidol-treated mice, the MK-801-induced hyperlocomotor activity during the Y-maze session was significantly decreased. This may reflect the decreased activity in the striatal dopaminergic systems, involved in locomotion through the nigrostriatal pathways (4). On the other hand, Ulas et al. (37) clearly reported that a chronic haloperidol treatment (1 mg/kg/day for 21 days) did not change NMDA receptors in the hippocampus. This is in accordance with our observation that the chronic haloperidol treatment did not affect the extent of the impairment of learning induced by MK-801. Since a downregulation of σ sites also did not affect the extend of MK-801-induced impairment of learning, it may be concluded that the NMDA-dependent mnemonic processes may be more likely under a phasic regulation by σ sites rather than under a tonic one. The lack of effect of σ antagonists on the MK-801 impairment of learning is in accordance with this observation.

Moreover, the attenuating effect of PRE-084 on the MK-801-induced impairment of learning through σ sites supports the findings of some previous *in vitro* and *in vivo* studies. First, it was reported that the NMDA-evoked [3 H]norepinephrine release from rat hippocampal slices could be stimulated by the σ ligands JO-1784 and (+)-3-PPP at nanomolar concentrations (19,27). The same authors reported that the NMDA-induced firing activity of CA3 pyramidal hippocampal neurons could be increased by low doses of σ ligands (20,21). The exact nature of these regulations of the NMDA type of glutamatergic neurotransmission by σ ligands in the hippocampus remains to be determined, but our results indicate that it may mediate the effect on the NMDA-dependent mnemonic processes.

An important issue remains to determine whether σ ligands modulate both the nicotinic cholinergic systems and the NMDA systems in a similar manner. We reported here that, at the same dosage that affected the MK-801-induced impairment of learning, PRE-084 did not affect the amnesia induced by scopolamine, but was able to attenuate the amnesia induced by mecamlamine. It was previously reported that σ ligands could attenuate the scopolamine-induced amnesia in the rat, but at higher doses than we used in this study (2). The effect of σ ligands on the muscarinic systems may therefore be

unrelated to the attenuation we reported in this study, and may involve complex and indirect interactions, that remain to be examined (2). On the other hand, the modulation of the nicotinic systems could directly or indirectly involve the regulation of the NMDA receptor activation. Several studies reported that the NMDA system regulates acetylcholine release from septal, cortical, striatal or nucleus accumbens slices (9,12,31). In particular, MK-801 induced a dose-dependent inhibition of acetylcholine release. On the other hand, a direct interaction could be evoked. For example, (+)-SKF-10,047 was shown to increase acetylcholine release from guinea-pig cortical slices (30) and from rat frontal cortex *in vivo* (14,15). JO-1784 potentiated the KCl-evoked [3 H]acetylcholine release from rat hippocampal slices *in vitro* and high affinity [3 H]choline uptake from mouse hippocampal synaptosomes *in vivo* (10). These effects involved σ sites since they were stereoselective and reversed by haloperidol. Finally, Paul et al. (24) reported that σ ligands were able to inhibit the nicotinic receptor-mediated catecholamine release in adrenal chromaffin cells *in vitro*, through an interaction that may closely involve the receptor-associated ionophore. Even if these results appear to account for opposite biological effects, it seems that σ ligands may directly modulate the nicotinic cholinergic receptor in a similar manner to that operating in the NMDA receptor complex. The exact regulatory mechanisms between σ sites and these two different receptor complexes enhance the possible interest in the action of σ ligands on cognitive functions.

In conclusion, this study showed that the PCP derivative PRE-084 efficiently attenuated MK-801-induced impairment of learning in mice, by selectively acting at σ sites. This effect illustrates the functional phasic facilitation by σ ligands on the NMDA receptor activation previously described in electrophysiology and superfusion studies in the hippocampus.

ACKNOWLEDGEMENTS

Thanks are due to Jean Bayle for his dexterity in elaborating the apparatus used for behavioral testing; to Drs. Brian Lockhart and Jacques Vignon for their critical reading of the manuscript; to Pr. Tsutomu Kameyama and to Dr. Masayuki Hiramatsu for helpful discussions before initiating these experiments; and to Dr. Jean-Marc Kamenka for his generous gift of TCP and BTCP. This study was supported by the Institut de Recherche sur la Moëlle Epinière and the Association Française contre les Myopathies.

REFERENCES

1. Anis, A.; Berry, S. C.; Burton, N. R.; Lodge, D. The dissociative anesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by *N*-methyl-D-aspartate. *J. Pharmacol. Exp. Ther.* 79:565-575; 1983.
2. Earley, B.; Burke, M.; Leonard, B. E.; Gouret, C. J.; Junien, J. L. Evidence for an anti-amnesic effect of JO 1784 in the rat: A potent and selective ligand for the sigma receptor. *Brain Res.* 546: 282-286; 1991.
3. Hiramatsu, M.; Koide, T.; Ishihara, S.; Shiotani, T.; Kameyama, T.; Nabeshima, T. Involvement of the cholinergic system in the effect of nefiracetam (DM-9384) on carbon monoxide (CO)-induced acute and delayed amnesia. *Eur. J. Pharmacol.* 216:279-285; 1992.
4. Ishikawa, J.; Meltzer, H. Y. Apomorphine does not reverse reduced basal dopamine release in rat striatum and nucleus accumbens after chronic haloperidol treatment. *Brain Res.* 507:138-142; 1990.
5. Itoh, J.; Nabeshima, T.; Kameyama, T. Utility of an elevated plus-maze for dissociation of amnesic and behavioral effects of drugs in mice. *Eur. J. Pharmacol.* 194:71-76; 1991.
6. Itoh, J.; Nabeshima, T.; Kameyama, T. Utility of an elevated plus-maze for the evaluation of memory in mice: effect of neuroleptics, scopolamine and electroconvulsive shock. *Psychopharmacology* 101:27-33; 1990.
7. Itoh, J.; Ukai, M.; Kameyama, T. Dynorphin A-(1-13) potently prevents memory dysfunctions induced by transient cerebral ischemia in mice. *Eur. J. Pharmacol.* 234:9-13; 1993.
8. Itzhak, Y.; Alerhand, S. Differential regulation of σ and PCP receptors after chronic administration of haloperidol and phencyclidine in mice. *FASEB J.* 3:1868-1872; 1989.
9. Jones, S. M.; Snell, L. D.; Johnson, K. M. Inhibition by phencyclidine of excitatory amino acid-stimulated release of neurotransmitter in the nucleus accumbens. *Neuropharmacology* 26:173-179; 1987.
10. Junien, J.-L.; Roman, F. J.; Brunelle, G.; Pascaud, X. JO-1784, a novel σ ligand, potentiates [3 H]acetylcholine release from rat hippocampal slices. *Eur. J. Pharmacol.* 200:343-345; 1991.
11. Karbon, W.; Naper, K. Differential regulation of [3 H](+)-3-PPP and [3 H]DTG labeled sigma binding sites in guinea pig brain membranes by subchronic haloperidol administration. *Soc. Neurosci.* 16:1137; 1990 (Abstr.).
12. Lodge, D.; Johnston, G. A. R. Effect of ketamine on amino

- acid-evoked release from rat cerebral cortex in vitro. *Neurosci. Lett.* 56:371-375; 1985.
13. Manallack, D. T.; Beart, P. M. Up-regulation of rat cortical σ receptors after subchronic administration of rimcazole and 1,3-di-(2-tolyl)guanidine. *Neuropharmacology* 27:1201-1203; 1988.
 14. Matsuno, K.; Mastunaga, K.; Mita, S. Increase in extracellular acetylcholine level by sigma ligands in rat frontal cortex. *J. Pharmacol. Exp. Ther.* 265:851-859; 1993.
 15. Matsuno, K.; Mastunaga, K.; Senda, T.; Mita, S. Increase in extracellular acetylcholine level in rat frontal cortex induced by (+)N-allylnormetazocine as measured by brain microdialysis. *Brain Res.* 575:315-319; 1992.
 16. Maurice, T.; Hiramatsu, M.; Itoh, J.; Kameyama, T.; Hasegawa, T.; Nabeshima, T. Behavioral evidence for a modulating role of sigma ligands in memory processes: I. Attenuation of dizocilpine (MK-801)-induced amnesia. *Brain Res.* 647:44-56; 1994.
 17. Maurice, T.; Hiramatsu, M.; Itoh, J.; Kameyama, T.; Hasegawa, T.; Nabeshima, T. Low dose of 1,3-di-(2-tolyl)guanidine (DTG) attenuates MK-801-induced spatial working memory impairment in mice. *Psychopharmacology* 114:520-522; 1994.
 18. McLennan, H.; Lodge, D. The antagonism of amino acid-induced excitation of spinal neurones in the cat. *Brain Res.* 169:83-90; 1979.
 19. Monnet, F. P.; Blier, P.; Debonnel, G.; De Montigny, C. The modulation by sigma ligands of NMDA-evoked [3 H]noradrenaline release involve Gi/o proteins. *Soc. Neurosci.* 17:1340; 1991 (Abstr.).
 20. Monnet, F. P.; Debonnel, G.; De Montigny, C. In vivo electrophysiological evidence for a selective modulation of N-methyl-D-aspartate-induced neuronal activation in rat CA3 dorsal hippocampus by sigma ligands. *J. Pharmacol. Exp. Ther.* 261:123-130; 1992.
 21. Monnet, F. P.; Debonnel, G.; Junien, J.-L.; De Montigny, C. N-methyl-D-aspartate-induced neuronal activation is selectively modulated by sigma receptors. *Eur. J. Pharmacol.* 179:441-445; 1990.
 22. Morris, R. G. M.; Anderson, E.; Lynch, G. M.; Baudry, M. Selective impairment of learning and blockage of long-term potentiation by a N-methyl-D-aspartate receptor antagonist, AP5. *Nature* 319:774-776; 1986.
 23. Okuyama, S.; Imagawa, I.; Ogawa, S.; Araki, H.; Tanaka, M.; Muramatsu, M.; Otomo, S. A potential antipsychotic nature of NE-100, a novel sigma receptor antagonist: preclinical neuropharmacological profiles. *Soc. Neurosci. Abstr.* 19:598; 1993.
 24. Paul, I. A.; Basile, A. S.; Rojas, E.; Youdim, M. B. H.; De Costa, B.; Skolnick, P.; Pollard, H. B.; Kuijpers, G. A. J. Sigma receptors modulate nicotinic receptor function in adrenal chromaffin cells. *FASEB J.* 7:1171-1178; 1993.
 25. Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J.-L.; Musacchio, J. M.; Rothman, R. B.; Su, T.-P.; Tam, S. W.; Taylor, D. P. A proposal for the classification of sigma binding sites. *Trends Pharmacol. Sci.* 13:85-86; 1992.
 26. Quirion, R.; Chicheportiche, R.; Contreras, P. C.; Johnson, K. M.; Lodge, D.; Tam, S. W.; Woods, J. H.; Zukin, S. R. Classification and nomenclature of phencyclidine and sigma receptor sites. *Trends Pharmacol. Sci.* 10:444-446; 1987.
 27. Roman, F. J.; Pascaud, X.; Duffy O.; Junien, J.-L. Modulation by neuropeptide Y and peptide YY of NMDA effects in hippocampal slices: Role of sigma receptors. In: Kameyama, T.; Nabeshima, T.; Domino, E. F., eds. NMDA related agents: Biochemistry, pharmacology and behavior. Ann Arbor, MI: NPP Books; 1991:211-218.
 28. Rückert, N. G. H.; Schmidt, W. J. The σ ligand 1,3-di-(2-tolyl)guanidine in animal models of schizophrenia. *Eur. J. Pharmacol.* 233:261-267; 1993.
 29. Sarter, M.; Bodewitz, G.; Stephens, D. Attenuation of the scopolamine-induced impairment of spontaneous alternation behavior by antagonist but not inverse agonist and agonist β -carbolines. *Psychopharmacology* 94:491-495; 1988.
 30. Siniscalchi, A.; Cristofori, P.; Veratti, E. Influence of N-allylnormetazocine on acetylcholine release from brain slices: Involvement of muscarinic receptors. *Arch. Pharmacol.* 336:425-429; 1987.
 31. Snell, L. D.; Johnson, K. M. Characterization of the inhibition of excitatory amino acid-induced neurotransmitter release in the rat striatum by phencyclidine-like drugs. *J. Pharmacol. Exp. Ther.* 238:938-946; 1986.
 32. Su, T.-P. σ receptors: Putative links between nervous, endocrine and immune systems. *Eur. J. Biochem.* 200:633-636; 1991.
 33. Su, T.-P.; Wu, X. Z.; Cone, E. J.; Shukla, K.; Gund, T. M.; Dodge, A.L.; Parish, D. W. Sigma compounds derived from phencyclidine: identification of PRE-084, a new, selective sigma ligand. *J. Pharmacol. Exp. Ther.* 259:543-548; 1991.
 34. Tam, S. W.; Cook, L. Sigma opiates and certain antipsychotic drugs mutually inhibit (+)-[3 H]SKF 10,047 and [3 H]haloperidol binding in guinea pig brain membranes. *Proc. Natl. Acad. Sci. USA* 81:5618-5621; 1984.
 35. Taylor, D. P.; Dekleva, J. Potential antipsychotic BMY 14802 selectively binds to sigma sites. *Drug Dev. Res.* 11:65-70; 1987.
 36. Taylor, D. P.; Eison, M. S.; Moon, S. L.; Yocca, F. D. BMY-14802: A potential antipsychotic with selective affinity for s-binding sites. *Adv. Neuropsychiat. Psychopharmacol.* 1:307-315; 1991.
 37. Ulas, J.; Nguyen, L.; Cotman, C.W. Chronic haloperidol treatment enhances binding to NMDA receptors in rat cortex. *Neuroreport* 4:1049-1051; 1993.
 38. Venable, N.; Kelly, P. H. Effects of NMDA receptor antagonists on passive avoidance learning and retrieval in rats and mice. *Psychopharmacology* 100:215-221; 1990.
 39. Vignon, J.; Pinet, V.; Cerruti, C.; Kamenka, J.-M.; Chicheportiche, R. [3 H]N-[1-(2-Benzo(b)thiophenyl)cyclohexyl]piperidine ([3 H]BTCP): A new phencyclidine analog selective for the dopamine uptake complex. *Eur. J. Pharmacol.* 148:427-436; 1988.
 40. Vignon, J.; Privat, A.; Chaudieu, I.; Thierry, A.; Kamenka, J.-M.; Chicheportiche, R. [3 H]Thienyl-phencyclidine ([3 H]TCP) binds to two different sites in rat brain. Localization by autoradiographic and biochemical techniques. *Brain Res.* 378:133-141; 1986.
 41. Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; De Costa, B.; Rice, K. C. Sigma receptors: Biology and function. *Pharmacol. Rev.* 42:355-402; 1990.
 42. Weber, E.; Sonders, M.; Quarum, M.; McLean, S.; Pou, S.; Keana, J. F. W. 1,3-Di-(2-[3 H]tolyl)guanidine: a selective ligand that label sigma-type receptors for psychotomimetic opiates and antipsychotic drugs. *Proc. Natl. Acad. Sci. USA* 83:8784-8788; 1986.