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Behavioural and neurochemical effects of phosphatidylserine in MPTP lesion of the substantia nigra of rats

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Abstract

The present study investigated the effects of intranigral MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) infusion on rats treated with phosphatidylserine and evaluated in two memory tasks and on striatal dopamine levels. The results indicated that MPTP produced a significant decrease in the avoidance number in comparison to sham-operated and non-operated rats submitted to a two-way avoidance task. MPTP-lesioned rats exhibited an increase in the latencies to find the platform in cued version of the water maze in comparison to sham-operated and non-operated animals. The tested toxin reduced striatal dopamine levels in comparison to sham-operated and non-operated groups. A final surprising result was that phosphatidylserine was unable to reverse the cognitive deficits produced by MPTP or the reduction of striatal dopamine levels. In conclusion, the data suggest that MPTP is a good model to study the early impairment associated with Parkinson's disease and phosphatidylserine did not improve the memory impairment induced by MPTP.

Keywords: Phosphatidylserine; MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); Memory; (Rat); Parkinson's disease; Dopamine

1. Introduction

Parkinson's disease is a progressive neurodegenerative pathology with a prevalence of 1–2% in people over the age of 50 (Shastry, 2001). The cardinal clinical manifestations of Parkinson's disease include bradykinesia, rest tremor, rigidity, gait abnormalities and postural instability. These symptoms may be accompanied by autonomic dysfunction as well as psycho-organic disturbances such as depression, slowness of affect (Birkmayer and Riederer, 1985; Valldeoriola et al., 1997), and occasionally prominent dementia in the early stages of the illness (Birkmayer and Riederer, 1985; Valldeoriola et al., 1997; Calne, 2001).

Research into the pathogenesis of Parkinson's disease has been rapidly advanced by the development of animal models which also permit the investigation of new treatments. In particular, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a toxin that causes parkinsonism in man and is the most widely used and the best investigated model of Parkinson's disease (Gerlach and Riederer, 1996). The mechanism for the neurotoxicity of MPTP involves its conversion by monoamine oxidase B to the metabolite 1-methyl-4-phenylpyridinium (MPP^+) in glial cells (Chiba et al., 1984). MPP^+ is selectively taken up by striatal dopaminergic terminals via the neuronal dopamine transporter (DAT) (Gainetdinov et al., 1997); within dopaminergic neurons, it inhibits complex I of the mitochondrial respiratory chain and induces energy depletion (Ramsay et al., 1986).

Numerous neurochemistry studies have shown that MPTP administration predominately damages the nigrostriatal pathway, causing cell loss in the substantia nigra (Heikkila et al., 1984; Sundström et al., 1987; German et al., 1996; Gevaerd et al., 2001b) and dopamine depletion in the neostriatum (Sonsalla and Heikkila, 1986; Sundström et al., 1987; Da Cunha et al., 2001) mimicking in animals the

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neurochemical alterations observed in Parkinson's disease. On the other hand, data from behavioural studies show some discrepancies caused by differences in strain, MPTP administration schedule and regimen of administration. However, in most of these studies, a decrease of locomotion and/or rearing was observed following MPTP administration (Sedelis et al., 2001). In addition to these deficits in motor function, many authors have shown that this toxin produces cognitive impairment. In particular, Miyoshi et al. (2002) have demonstrated that the intranigral administration of MPTP impaired learning of a spatial working memory task and also learning in a cued version of the water maze. Similarly, Da Cunha et al. (2001) and Gevaerd et al., (2001a,b) showed that this toxin impaired the performance of rats submitted to the two-way active avoidance task.

Phosphatidylserine is an acidic phospholid that is a natural component of the brain cephalic fraction and represents the major phospholipid of brain synaptic membranes (Breckenridge et al., 1972). Phosphatidylserine appears to be located on the outer membrane surface (Smith and Loh, 1976). The effect of phosphatidylserine on learning and memory is well known and some authors have demonstrated that the effects of phosphatidylserine in behavioural or neurochemical studies are related to cognitive function (Zanotti et al., 1986; Raiteri et al., 1989; Claro et al., 1999). Our group has demonstrated that phosphatidylserine was able to reverse reserpine-induced amnesia in rats evaluated in a passive avoidance task, a model of Parkinson's disease related memory impairment (Alves et al., 2000).

The aim of the present study was evaluate the effects of phosphatidylserine on MPTP-lesioned rats observed in a two-way active avoidance task and a cued version of the water maze. Moreover, the neurochemical effects of these pharmacological manipulations were also investigated by the detection of striatal dopamine levels.

2. Materials and methods

2.1. Animals

Male Wistar rats from our colony weighing 280–320 g at the beginning of the experiments were used. The animals were maintained in a temperature-controlled room (22 ± 2 °C) on a 12 h light–dark cycle (lights on 7:00 a.m.) with free access to food and water. All the behavioural experiments were conducted between 2:00 and 6:00 p.m. The animals used in this study were maintained and handled in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals Resources, National Research Council, USA.

2.2. Surgery

The animals were divided into the following groups: non-operated animals, sham-operated animals and MPTP-lesioned group. The MPTP-lesioned rats received atropine sulphate (0.4 mg/kg, intraperitoneal [i.p.]) and penicillin G-procaine (20.000 U in 0.1 ml, intramuscular) and were anesthetized with sodium thiopental (40 mg/kg). MPTP–HCl (Sigma, St. Louis, MO, USA; 1 μmol in 2.1 μl of saline, 0.35 μl/min) was bilaterally administered through a 30-gauge needle according to the following coordinates: anteroposterior (AP): − 5.0 mm from bregma; mediolateral (ML): ± 2.1 mm from midline; dorsoventral (DV): − 7.7 mm from skull, adapted from Paxinos and Watson (1986). Sham operations followed the same general procedure except that the needle was lowered to the same substantia nigra pars compacta coordinates (see above) and removed without injection. After surgery, the animals were left in a temperature-controlled chamber until they recovered from anaesthesia, when they were returned to their home cages and allowed to recover for 21 days before the beginning of the behavioural task and biochemical assays.

2.3. Treatment

Phosphatidylserine (kindly provided by Trb Pharma, Brazil) was purified from bovine brain. According to Fidia's Technical file on BROS®, the main fatty acids present in the preparation are C16:0 (2.7%), C18:0 (39.5%), C18:1 (35.3%), C20:1 (6.1%), C22:1 (6.4%), C24:1 (3.3%) and C22:6 (6.7%), and their purity is 92%. The material was dissolved in distilled water and sonicated. The phosphatidylserine suspension was injected i.p. at a dose of 50 mg/kg. Saline (0.9% NaCl), 1 ml/kg, was used as the control solution for phosphatidylserine.

After surgery, the animals were treated with phosphatidylserine or saline. Thus, the rats were divided at random into six groups: (1) non-operated/saline; (2) non-operated/phosphatidylserine; (3) sham-operated/saline, (4) sham-operated/phosphatidylserine, (5) MPTP-lesioned/saline and (6) MPTP-lesioned/phosphatidylserine.

2.4. Two-way active avoidance task

Fourteen days after surgery, the rats were treated with saline or phosphatidylserine for 7 days and submitted to a two-way active avoidance task, as described previously (Da Cunha et al., 2001). The active avoidance test apparatus was an automated shuttle box (GEMINI Avoidance System, San Diego Instruments, San Diego, CA) with a dark front glass and a floor made of parallel 5 mm calibre stainless steel bars spaced 15 mm apart. The box (23 × 50 × 23 cm) is divided into two compartments of the same size by a wall with a guillotine door that remains opened during the test. In the training session, after 3 min of habituation, 30 sound cues (conditioned stimulus, 1.5 kHz, 60 dB, maximum duration 10 s) were paired with a subsequent 0.4 mA footshock unconditioned stimulus, maximum duration (5 s) until the animal crossed to the other compartment. The animal could avoid the shock by

crossing to the other side during the presentation of the conditioned stimulus (active avoidance). The time between each conditioned stimulus presentation varied randomly, ranging from 10 to 50 s. The number of active avoidances, the latency to cross to the other side of the box after the beginning of each conditioned stimulus, and the number of inter-trial crossings between the two box compartments were recorded automatically by the apparatus. The test session, conducted 24 h later, was identical to the training one, except for a 2-min habituation time. Acquisition was estimated by the increased number of avoidances in the training session. Memory consolidation was estimated by an increase in the number of avoidances in the test session compared to the training session. The number of inter-trial crossings in the training session gave an estimate of the ambulatory behaviour of the animals (Ribeiro et al., 1999). The reaction time to the footshock unconditioned stimulus was considered as the latency to escape it in the five first trials (training session). The reaction time to the conditioned stimulus (sound cue) was considered as the mean latency to perform active avoidances in the test session. The apparatus was washed with a water–alcohol (5%) solution before behavioural testing to eliminate possible bias due to odours left by previous rats.

2.5. Water maze task

After surgery the rats were treated with saline or phosphatidylserine for 21 days before being submitted to the water maze task. This task was conducted in a water maze that consisted of a round tank 170 cm in diameter, 50 cm in height and a water depth of 32 cm. The water temperature was maintained at 23 ± 2 °C. The four starting positions were located at the intersections of imaginary quadrants. Several distal visual cues were placed on the walls of the water maze room. In the experiments, the tank was video-taped and the latency to reach the escape platform was recorded. An image analyser (CEFET, Curitiba, Brazil) was used to measure the swimming speed of the rats in the pool.

The animals were submitted to a cued version of the water maze that consisted of 4 training days, four consecutive trials per day, during which the rats were left in the pool facing the wall and allowed to swim freely to a transparent acrylic platform (11×14 cm) submersed 2 cm below to the water surface. This platform had a cue consisting of a 7 cm diameter white ball attached to the top of the platform and protruding above the water. The platform was placed on the center of one of the four quadrants in the tank. The position of the platform was always changed in each trial of the day. All trials were terminated when the rat found the platform or during a maximum period of 60 s, after which the animal was gently guided to platform. After the animal escaped to the platform it was allowed to remain on it for 20 s and was then removed from the tank for 30 s before being placed in the next random initial position. The protocol of this memory task followed previously described procedures (Miyoshi et al., 2002).

2.6. Determination of striatal dopamine levels

Twenty four hours after the water maze task, the animals were sacrificed by decapitation. The striatum was rapidly dissected on dry ice, weighed and stored at − 70 °C until the determination of dopamine levels. The endogenous levels of dopamine were assayed by reversed-phase high performance liquid chromatography with electrochemical detection, as described by Hallman and Jonsson (1984). Briefly, a C18 reverse phase column (Shim-pack, CLC-ODS 150×4.6 mm; Shimadzu), an amperometric detector (Amtec, Decade) and a liquid chromatography workstation Class-Vp 5032 (Shimadzu) were used. Frozen tissue was homogenized in 300 μL of 0.1 M perchloric acid and centrifuged ($12{,}000 \times g$, 10 min), and 20 μl of the supernatant was injected into the chromatograph. The mobile phase flow rate was 0.9 ml/min and its composition was: 7.85 g citric acid, 235 ml twice-distilled water, 35 mg octyl sodium sulphate, 10 ml acetonitrile, and 5 ml tetrahydrofluran. The pH was adjusted to 3.0 with NaOH (10 M). Detector sensitivity was 2 nA, and the oxidation potential was fixed at 0.85 V using a glassy carbon working electrode vs. an Ag/AgCl reference electrode. The peak areas of the external standards were used to quantify the sample peaks. The values obtained were expressed as ng/g tissue wet weight.

2.7. Statistical analysis

Active avoidance and water maze task data were analysed by two-way analysis of variance (ANOVA) with treatment as one factor and the session day as the second factor (repeated measure). For the session in the active avoidance, escape latencies for the individual day of training in the water maze, neurochemical data, inter-trial crossings, footshock and sound cue were analysed by one-way ANOVA. The training and test session in the active avoidance were analyzed by one-way ANOVA with repeated measurements. Differences between groups were further analysed by the post hoc Duncan test and were considered to be statistically significant when $P < 0.05$. The values were reported as mean ± S.E.M.

3. Results

Fig. 1 shows the effect of phosphatidylserine on MPTP-treated rats in the active avoidance task. Two-way ANOVA showed that there was no interaction between the treatments and times [$F(5,46) = 1.62$; $P = 0.17$] evaluated in the present study. However, there was a significant difference between treatment groups

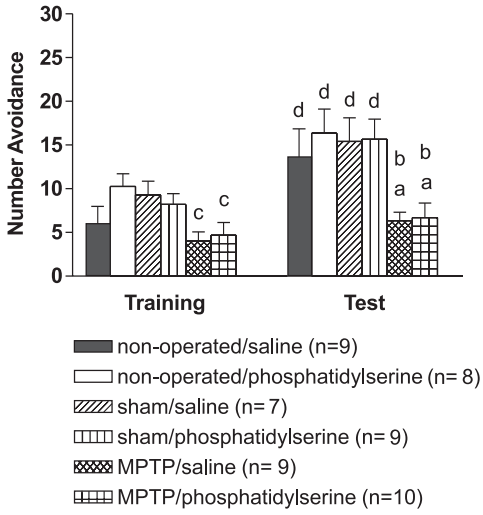

Fig. 1. Effects of administration of phosphatidylserine (50 mg/kg) in rats treated with intranigral MPTP on avoidance number in a two-way active avoidance task. The values represent the mean ± S.E.M. [a]$P<0.05$ compared to the non-operated/saline and sham/saline groups; [b]$P<0.05$ compared to the non-operated/phosphatidylserine and sham/phosphatidylserine groups; [c]$P<0.05$ compared to the sham/saline and non-operated/phosphatidylserine groups. [d]$P<0.05$ compared to the same group in the training session. One-way ANOVA followed by the Duncan test.

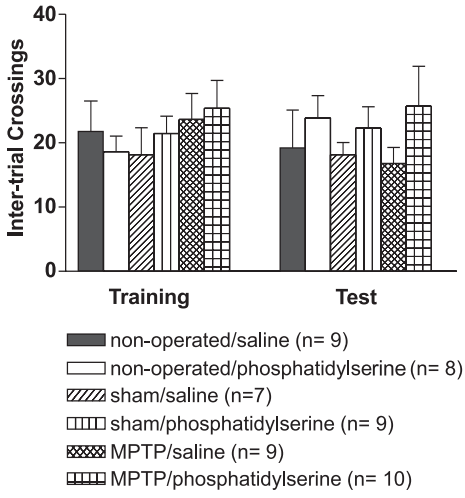

Fig. 2. Effects of administration of phosphatidylserine (50 mg/kg) in rats treated with intranigral MPTP on inter-trial crossings in the two-way active avoidance task. The values represent the mean ± S.E.M. No significant differences were observed between groups.

[$F(5,46)=4.49$; $P<0.01$] and between the training and test sessions [$F(1,46)=40.32$; $P<0.01$]. One-way ANOVA indicated that MPTP produced a significant decrease in avoidance number in the training session in comparison to sham-operated/saline and non-operated/phosphatidylserine rats [$F(5,46)=2.88$; $P<0.05$]. In the test session performed 24 h after training, again the results showed that MPTP produced a significant decrease in this parameter when the rats were compared to the sham-operated (saline and phosphatidylserine) and non-operated (saline and phosphatidylserine) groups [$F(5,46)=4.06$; $P<0.01$]. One-way ANOVA with repeated measurements showed that in test session the sham-operated and non-operated groups exhibited a significant increase in avoidance number in comparison to the performance in the training session [$F(1,46)=40.32$; $P<0.001$]. Phosphatidylserine did not affect the performance of the MPTP-lesioned, sham-operated and non-operated animals in the training or test sessions.

The data about the inter-trial crossings are presented in Fig. 2. The results show that the MPTP-lesioned rats did not differ from the sham-operated or non-operated animals in the training and test sessions [$F(5,46)=0.5$; $P=0.76$ and $F(5,46)=0.62$; $P=0.67$, respectively]. Phosphatidylserine-treated groups also did not differ from the respective saline-treated rats. No significant differences were observed between groups in the sensitivity of the animals to the footshock [$F(5,46)=1.04$; $P=0.4$] and in the reaction time to the conditioned stimulus [$F(5,46)=2.08$; $P=0.08$].

The effects of MPTP lesion and phosphatidylserine treatment on striatal dopamine levels are illustrated in Fig. 3. One-way ANOVA showed that MPTP-lesioned rats presented a significant reduction in dopamine levels in comparison to sham-operated and non-operated animals [$F(5,50)=4.60$; $P<0.01$]. In addition, no significant change was observed in the striatal dopamine levels of the groups that received saline or phosphatidylserine.

Table 1 illustrates the effects of intranigral MPTP administration in rats and the treatment with phosphatidylserine in the cued version of the water maze. Two-way ANOVA showed that there was an interaction between

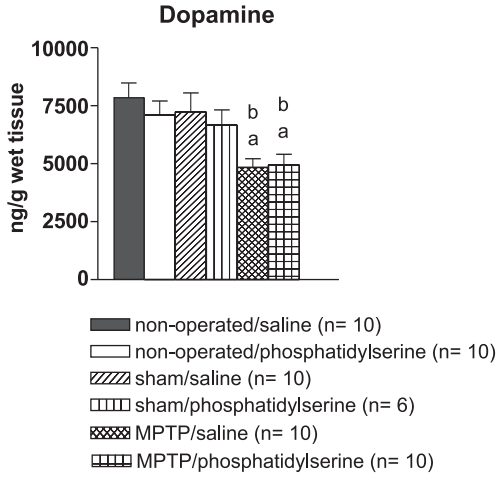

Fig. 3. Effects of administration of phosphatidylserine (50 mg/kg) in rats treated with intranigral MPTP on striatal dopamine levels. The values represent the mean ± S.E.M. [a]$P<0.05$ compared to the non-operated/saline and sham/saline groups; [b]$P<0.05$ compared to the non-operated/phosphatidylserine group and sham/phosphatidylserine group. One-way ANOVA followed by the Duncan test.

Table 1
Effects of administration of phosphatidylserine (50 mg/kg) in rats treated with MPTP intra-nigral in the latency time (s) on cued version of the water maze task

Training	1st day	2nd day	3rd day	4th day
Non-operated/saline (12)	31 ± 2.33	8 ± 0.90	7 ± 0.74	7 ± 0.99
Non-operated/phosphatidylserine (12)	29 ± 3.54	10 ± 1.47	8 ± 1.19	7 ± 1.00
Sham/saline (12)	33 ± 2.39	8 ± 0.64	7 ± 0.45	7 ± 0.90
Sham/phosphatidylserine (12)	31 ± 2.48	8 ± 1.13	6 ± 0.65	6 ± 0.79
MPTP/saline (14)	41 ± 2.84[a,b]	13 ± 1.90[a,c]	10 ± 1.19[c]	7 ± 0.59
MPTP/phosphatidylserine (13)	42 ± 2.33[a,b]	18 ± 2.69[a,b]	10 ± 1.09[c,d]	6 ± 0.57

The values represent the mean ± S.E.M. (one-way ANOVA followed by Duncan test).
[a] $P<0.05$ compared to the non-operated/saline and sham/saline groups.
[b] $P<0.05$ compared to the non-operated/phosphatidylserine and sham/phosphatidylserine groups.
[c] $P<0.05$ compared to the sham/phosphatidylserine group.
[d] $P<0.05$ compared to the non-operated/saline group.

treatments and time [$F(15,207)=2.84$; $P<0.001$]. Moreover, there was a significant difference in the groups of treatments [$F(5,69)=7.47$; $P<0.001$] and in the days of training [$F(3,207)=394.02$; $P<0.001$]. One-way ANOVA showed that the MPTP-lesioned animals exhibited a significant increase in the latencies to find the platform in comparison to the sham-operated (saline and phosphatidylserine) and non-operated (saline and phosphatidylserine) groups on the first and second day of training [$F(5,69)=4.39$; $P<0.01$ and $F(5,69)=5.73$; $P<0.001$, respectively]. On the third day of training, MPTP-lesioned rats exhibited a significant increase in this parameter when compared to the sham-operated/phosphatidylserine group, and the MPTP/phosphatidylserine group exhibited a significant increase in escape latencies compared to the non-operated/saline group [$F(5,69)=3.08$; $P<0.05$]. On the other hand, there were no differences in the latencies to find the platform during the fourth day of training in this maze between groups [$F(5,69)=0.60$; $P=0.69$]. Phosphatidylserine treatment did not change the performance of MPTP-lesioned rats in finding a platform during the 4 days of training compared to sham-operated and non-operated animals.

Table 2 illustrates the effects of intranigral MPTP administration in rats and the treatment with phosphatidylserine in the swim speed on the cued version of the water maze. Two-way ANOVA showed that there was no difference in the swim speed between groups and days of training [$F(5,69)=0.44$; $P=0.81$].

4. Discussion

Our data indicate that MPTP-lesioned rats can be considered a good animal model to study the early memory impairment associated with Parkinson's disease. The bilateral intranigral injection of this neurotoxin produced a reduction of performance of the animals in the active avoidance task which was observed in terms of a decrease of the avoidance score, but not of the inter-trial crossings. Moreover, this lesion also produced impairment in the cued test of the animals evaluated in the water maze. A significant reduction of striatal dopamine levels was verified in MPTP-lesioned rats. These data corroborate previous studies from our group (Da Cunha et al., 2001; Gevaerd et al., 2001a,b; Miyoshi et al., 2002). Contrary to our prediction, phosphatidylserine, a phospholipid commonly used in the treatment of age-associated memory impairment (Drago et al., 1981; Zanotti et al., 1989; Crook et al., 1991), did not reverse either the cognitive deficit caused by MPTP or the striatal dopamine depletion produced by this neurotoxin.

The course of appearance of the cognitive deficits in Parkinson's disease is a controversial issue but there is increasing awareness that cognitive dysfunction constitutes an important non-motor symptom that is a source of disability. Many authors have demonstrated that it can occur in the early stages of the disease when the motor symptoms yet not so evident (Schneider and Pope-Coleman, 1995; Calne, 2001). On the other hand, some studies

Table 2
Effects of administration of phosphatidylserine (50 mg/kg) in rats treated with MPTP intra-nigral in the swim speed (cm/s) on cued version of the water maze task

Training	1st day	2nd day	3rd day	4th day
Non-operated/saline (12)	30 ± 1.03	26 ± 1.39	22 ± 1.29	23 ± 1.42
Non-operated/phosphatidylserine (12)	28 ± 1.11	26 ± 1.48	24 ± 1.27	23 ± 0.83
Sham/saline (12)	28 ± 0.98	27 ± 1.08	25 ± 1.13	25 ± 1.40
Sham/phosphatidylserine (12)	27 ± 1.02	28 ± 1.79	25 ± 1.32	26 ± 1.78
MPTP/saline (14)	27 ± 0.96	26 ± 1.19	25 ± 1.15	23 ± 1.38
MPTP/phosphatidylserine (13)	27 ± 0.88	27 ± 1.14	25 ± 1.07	23 ± 0.99

The values represent the mean ± S.E.M (two-way ANOVA).

have shown that it is frequent in the later stages of the disease, and it has been suggested that cognitive impairment is due to the natural course of Parkinson's disease (Marttila and Rinne, 1976; Lieberman et al., 1979; Pirozzolo et al., 1982). In fact, a consensus for this question is shown by the observations of Rabinstein and Shulman (2001). These authors proposed that cognitive dysfunction in Parkinson's disease spans a continuum that ranges from circumscribed cognitive impairment that may be observed relatively early in the disease process to global dementia in the late stages of the disease.

The present results show a memory impairment of MPTP-lesioned rats in the two-way active avoidance system observed as a reduction both in the avoidance number and in the training and test session as shown in Fig. 1. However, the inter-trial crossings (Fig. 2) were not affected by the administration of this toxin to rats, indicating that in this schedule of administration MPTP did not reduce motor function. Indeed, according to Schneider and Pope-Coleman (1995), specific cognitive dysfunction pre-dates motor dysfunction in a chronic, slowly progressing Parkinson model in monkeys and supports the contention that cognitive deficits in Parkinson's disease may precede the motor signs of the disorder and may not be caused by them. In the majority of studies, a decrease of locomotion and/or rearing following treatment with MPTP was found (Sedelis et al., 2001). Furthermore, as suggested by Tanila et al. (1998), the effects of MPTP on motor behaviour have been found to be less reproducible and very much dependent on the level of stress of the animals.

Our data indicate that cognitive function is impaired in MPTP-lesioned rats, as also reported by other investigators. Many studies have shown that MPTP-lesioned rats submitted to the two-way active avoidance task may be used as a model of memory impairment caused by lesion of the nigrostriatal pathway (Da Cunha et al., 2001; Gevaerd et al., 2001a,b). In a task which required active avoidance of an aversive stimulus cued by a conditioned stimulus, MPTP produced impaired retention (Georgiev and Kambourova, 1991). MPTP-treated mice exhibited impaired alternation performance in a complex T-maze task which indicated a reduction in spatial working memory (Tanila et al., 1998). Thus, the present data suggest that the cognitive impairment induced by MPTP observed in the two-way active avoidance task is probably related to deficits in the memory acquisition and retention process. Certainly, these results are not related to an MPTP effect on the reduced sensitivity of footshock and sound perception, since this toxin did not change the response of the rats to unconditioned or conditioned stimulus.

Unexpectedly, phosphatidylserine (50 mg/kg) was not able to reverse the memory impairment observed in the current study. In a previous investigation, an acute dose pre or post-training of this phospholipid attenuated the amnesia in reserpine-treated rats submitted to an inhibitory avoidance task (Alves et al., 2000).

The phosphatidylserine effect on memory consolidation was not evaluated in the present study, because this drug was administered to rats always after the surgery and before the behavioural and neurochemical studies. In fact, there is a small possibility that phosphatidylserine may induce an enhancement of arousal or attention in these animals. However the present data suggest that this effect has not occurred. Phosphatidylserine did not change the performance of the animals in the training session. The enhancement of performance was observed only in the test session for the sham or control groups which received phosphatidylserine and this result was probably due to an effect in the acquisition or memory consolidation. Moreover, if phosphatidylserine produced an enhancement of arousal or attention, the groups treated with this drug would show an increase in the response of inter-trial crossings or swim speed, which did not occurred. Thus, the time to react to the shock and the perception of conditioned stimulus were not affected by phosphatidylserine treatment. Thus, in the cued test, an absence of phosphatidylserine effect was observed in the first day of training and only the MPTP lesioned rats exhibited an increased in the time spent to reach the platform.

In addition, the sham/phosphatidylserine group did not differ from control group in the performance on these two memory tasks indicating that phosphatidylserine did not improve the performance of non lesioned rats. It is possible that they are reaching ceiling performance. This is an interestingly question that needs further studies. Anyway, the main objective of this investigation was evaluated if phosphatidylserine could reverse the memory impairment associated to MPTP lesion of the substantia nigra of rats.

The effect of phosphatidylserine on learning and memory is well known. Chronic treatment with phosphatidylserine (50 mg/kg/day for 7–12 weeks) improved both spatial behaviour and passive avoidance retention of aged impaired rats (Zanotti et al., 1989). Moreover, Drago et al. (1981) have demonstrated that i.p. (5, 10 and 20 mg/kg) or i.c.v. (5, 10 and 20 μg/2 μl) injections of phosphatidylserine facilitated the acquisition of active avoidance behaviour in the shuttle box and pole jumping test situations, with a consequent improvement in the retention of active and passive avoidance responses. In this line, the postnatal administration of phosphatidylserine (50 mg/kg to mothers for 30 days) to C57BL/6 mice resulted in improvement of memory processes in adulthood, as assessed in the passive avoidance task (Fagioli et al., 1989). Likewise, Zanotti et al. (1986) and Claro et al. (1999) demonstrated that phosphatidylserine antagonizes scopolamine-induced amnesia in adult rats tested in a passive avoidance condition and plus-maze discriminative avoidance task, respectively. Indeed, Drago et al. (1981) showed that phosphatidylserine did not change the responsiveness to an electrical footshock by aged rats. Furthermore, some authors showed that phosphatidylserine treatment did not affect locomotion in aged rats (Drago et al., 1981) and C57BL/6

mice (Ammassari-Teule et al., 1990) submitted to the open field test.

The cued version of the water maze was employed to evaluate the effect of the lesion induced by MPTP on striatum-dependent memory (Packard and McGaugh, 1992; Milner et al., 1998; Miyoshi et al., 2002). The results showed that the MPTP-lesioned animals exhibited a significant increase in the latencies to find the platform in comparison to the sham-operated and non-operated groups on the first and second day of training (Table 1). Furthermore, the administration of MPTP into the substantia nigra pars compacta of rats produced a significant increase in this parameter when compared to the sham-operated/phosphatidylserine group and the MPTP-lesioned group treated with phosphatidylserine exhibited a significant increase in escape latency in comparison to the non-operated/saline group on the third day of training. These data indicate that this toxin impaired the learning of the rats in this task. However, there were no differences in the latencies to find the platform on the fourth day of training in this maze between groups, a fact suggesting that after overtraining the animals are able to learn this task. Moreover, there is no difference of the swim speed between the groups of rats during the 4 days of training (Table 2). This important information might corroborate that the deficit observed in MPTP lesioned rats was truly cognitive and non motor.

The present data corroborate those reported by Miyoshi et al. (2002) who demonstrated that MPTP impaired learning in a cued version and also in a spatial working memory version of the water maze. Moreover, phosphatidylserine (50 mg/kg) treatment did not change the performance of the MPTP-lesioned in finding a platform during the 4 days of training rats when compared to sham-operated and non-operated animals. The present data corroborate those reported by Blokland et al. (1999) who demonstrated that phosphatidylserine (15 mg/kg) did not affect the psychomotor or spatial discrimination performance of rats in the water maze task. On the other hand, chronic phosphatidylserine (50 mg/kg, v.o.) improved spatial memory in aged rats in the water maze test when the animals were evaluated at 7 or 12 weeks after the phosphatidylserine in comparison to the same rats before treatment (Zanotti et al., 1989). One hypothesis to explain the difference in the effect of phosphatidylserine on these tasks is related to differences in the schedule of treatment, the age and strain of the animals, and the kinds of memory tested. The cued version of the water maze was performed to evaluate the habit learning (Packard and McGaugh, 1992) which is dependent on the integrity of the striatum while the spatial reference memory version is related to the hippocampus (Morris et al., 1982).

Cognitive deficits are observed in Parkinson's disease patients even at the beginning of the disease, and during its late course 10–30% of the patients will develop dementia (Brown and Marsden, 1984; Owen et al., 1995). It was suggested that these deficits are a consequence of dysfunction of the caudate nucleus (Taylor et al., 1986; Gotham et al., 1988). On this basis, we evaluated the effects of intra-nigral MPTP infusion on striatal dopamine levels. The data showed that this toxin produced a significant reduction in this catecholamine in comparison to sham-operated and non-operated rats (Fig. 3). The nigrostriatal dopamine depletion in the present study was 38% and was consistent with earlier studies (Da Cunha et al., 2001; Gevaerd et al., 2001b; Miyoshi et al., 2002). The reduction of 38% in striatal dopamine levels of MPTP lesioned rats verified in the present study can explain why motor disabilities were not observed in the present study.

An important factor for the appearance of motor parkinsonian signs is the extent of striatal dopamine depletion, since a loss of 80% is commonly assumed to be necessary to observe symptoms across species (Heikkila and Sonsalla, 1992). On the other hand, Vingerhoets et al. (1994) pointed out that clinical features begin to emerge when there is a 40–60% reduction of nigral neurons and striatal dopamine. Differences in rat strain, experimental conditions, and MPTP dose might account for the discrepancy. In fact, a chronic low dose of MPTP in monkeys resulted in a decrease of approximately 90% in striatal dopamine and in its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Schneider, 1990). Moreover, in C57BL/6 mice that received four injections of MPTP (10 mg/kg) intraperitoneally, the striatal dopamine concentrations were markedly decreased in 82% 3 days after the toxin injection as compared with the saline-treated group (Araki et al., 2001). Lindner et al. (1999) demonstrated that young (2 months old) and middle-aged rats (12 months old) that received bilateral striatal infusions of 6-OHDA exhibited a dopamine depletion of 53% and 77%, respectively. One possible explanation for the discrete reduction observed in the present study may have been related to the interval between MPTP administration and dopamine detection which was 21 days. Perhaps, this result had some influence by causing a compensatory increase in dopamine turnover in the surviving dopaminergic neurons. In this sense, of particular interest are the studies of Tanji et al. (1999) who investigated the chronological changes in dopamine receptors and uptake sites in the striatum and substantia nigra of mouse brain. They demonstrated that dopamine uptake sites had slightly recovered by 21 days, a finding possibly indicates that dopaminergic neurons recover or regenerate in the chronic phase.

The other explanation for the mild dopamine depletion verified is associated with the incomplete lesion produced by MPTP. In this way, many authors have indicated that, despite the high depletion of dopamine, the lesion of dopaminergic neurons is partial. Gevaerd et al. (2001b) demonstrated that bilateral intranigral MPTP produced a 40% reduction in striatal dopamine compared to control and a 57% decrease of number of tyrosine hydroxylase immunoreactive neurons in the substantia nigra. Lindner et al. (1999) observed that bilateral striatal infusions of 6-OHDA

reduced striatal dopamine levels by 53% in young rats (2 months old) and by 77% in middle-aged rats (12 months old) and a 65% and 67% destruction of dopaminergic cells in the substantia nigra, respectively.

Phosphatidylserine was not able to restore striatal dopamine levels in MPTP lesioned rats. In addition, no significant change was observed in the levels of striatal dopamine of the groups that receive saline or phosphatidylserine. This result was surprising for us because many authors have shown that this phospholipid increases catecholamine turnover. In fact, intravenous injection of phosphatidylserine increased the in vivo turnover rate of norepinephrine in the hypothalamus and of dopamine in the striatum (Toffano et al., 1978). Mazzari and Battistela (1980) verified that the release of dopamine due to removal of external K^+ is enhanced by the preincubation of striatal synaptosomes of rats with phosphatidylserine and suggested that the effect of phosphatidylserine may be due to an increased rate of Ca^{2+} influx which then results in enhanced dopamine release. Moreover, chronic treatment with phosphatidylserine (15 mg/kg for 30 days) increased the K^+-evoked release of dopamine in the striatum and in the cerebral cortex of old animals. This effect seems to be selective since norepinephrine and serotonin release was not affected (Raiteri et al., 1989).

On the other hand, in an experimental procedure similar to the current study, Gevaerd et al. (2001b) showed that benserazide/L-DOPA restored the striatal dopamine levels in MPTP lesioned rats. The present data suggest that phosphatidylserine did not probably change the dopamine turnover in rats. In addition, Maggione et al. (1990) showed that phosphatidylserine was able to reverse depressive symptoms in elderly women, but this effect was not correlated to catecholamine levels.

According to Lindner et al. (1999), the predictive validity of pre-clinical models is dependent on the extent to which the model simulates the clinical condition. Thus, Parkinson's disease is characterized by an array of classic symptoms which emerge in patients with incomplete nigrostriatal cell loss and partial striatal dopamine depletion. These facts and the present data suggest that the current model, MPTP-lesioned rats, can be considered a good animal model to evaluate the memory impairment associated with early stages of the Parkinson's disease.

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