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Phosphatidylserine reverses reserpine-induced amnesia

Célia S.D. Alves [a], Roberto Andreatini [a], Cláudio da Cunha [a], Sérgio Tufik [b], Maria A.B.F. Vital [a,*]

[a] *Laboratorio de Fisiologia e Farmacologia do SNC-Centro Politecnico, Department of Pharmacology, Federal University of Paraná, Jardim das Americas-Curitiba-PR, Caixa Postal 19.031, CEP 81.531-990, Brazil*

[b] *Department of Psychobiology, Federal University of São Paulo, Sao Paulo, Brazil*

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Abstract

The effects of phosphatidylserine (PS) were studied in rats treated with reserpine (1 mg/kg) immediately after training in the passive avoidance task. In experiment I, phosphatidylserine (25 mg/kg) was administered 30 min before or immediately after training. Acute pre- or post-treatment with phosphatidylserine was effective in reversing the amnestic effect of reserpine in test trials performed 24 h and 1 week after training. Experiment II was performed to determine if the long-term pretreatment with phosphatidylserine (25 mg/kg) for 7 days is able to protect the rats against the amnestic effects of reserpine in this task. The data show that phosphatidylserine reverses the impairment induced by reserpine in trials performed 24 h and 1 week after training. These results indicate that the memory deficits associated with catecholamine depletion caused by reserpine can be attenuated by acute pre- or post-training or by long-term pretreatment with this phospholipid.

Keywords: Phosphatidylserine; Reserpine; Memory; Passive avoidance

1. Introduction

Phosphatidylserine (PS) 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). Phosphatidylserine can modify glucose metabolism (Bruni et al., 1976), catecholamine (Toffano et al., 1976) and acetylcholine release (Mantovani et al., 1976), NMDA receptor density and function in animals with age-related deficits (Cohen and Müller, 1992) and can increase the muscarinic acetylcholine receptor density (Gelbmann and Müller, 1991). These effects have been correlated to the behavioral changes observed following chronic and acute administration of this compound. Particularly with respect to memory, acute administration of phosphatidylserine antagonizes scopolamine-induced amnesia in adult rats tested in a passive avoidance situation (Zanotti et al., 1986). Moreover, phosphatidylserine administration facilitated the acquisition of active avoidance behavior in aged rats submitted to the shuttle-box and pole jumping test (Drago et al., 1981). Phosphatidylserine administration during postnatal development also improves memory in adult mice tested in a passive avoidance task (Fagioli et al., 1989). Besides, phosphatidylserine attenuates scopolamine-induced amnesia in a discriminative avoidance task in mice (Claro et al., 1999).

Catecholamine participation in the events of memory processes is well known, showing that the dopaminergic system plays an important role in these processes (Packard and White, 1991; Goldman-Rakic, 1998).Thus, dopaminergic manipulations have a modulatory effect on the memory process, perhaps during the consolidation phase (McGaugh, 1989). On the one hand, this evidence is based on studies of facilitation of memory retrieval under the action of a number of dopaminergic stimulating agents (Packard and White, 1991; Bevilaqua et al., 1997). On the other hand, the catecholamine depletion induced by reserpine causes deficits in the performance of shock-avoidance tasks. Walsh and Palfai (1978) showed that reserpine, when administered after the training trial, is able to produce retrograde amnesia in animals submitted to the avoidance task.

* Corresponding author. Tel.: +55-41-366-31-44 ext. 222; fax: +55-41-266-2042.

E-mail address: vital@bio.ufpr.br (M.A.B.F. Vital).

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Reserpine interferes with the storage of monoamines in intracellular granules, with monoamine depletion in nerve terminals (Carlsson, 1975) and induction of transient hypolocomotion and muscular rigidity, thus providing a pharmacological model of Parkinsonism (Colpaert, 1987). The cognitive decline occurring in Parkinson's disease is recognized as a frequent and important feature of the illness. The present experiments were carried out to evaluate the effects of PS in rats treated with reserpine immediately after the training trial in the passive avoidance task.

2. Materials and methods

2.1. Animals

Male Wistar rats from our colony aged 3 months at the beginning of the study were randomly housed in groups of five in polypropylene cages at 20–23°C, on a 12-h light–dark cycle (lights on 7:00 a.m.) with free access to food and water. The animals used in this study were maintained and handled according to the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

2.2. Drugs

Phosphatidylserine S (BROS®) purified from bovine brain was kindly provided by TRB Pharma (Brazil) and was dissolved in distilled water and sonicated. The PS suspension was injected intraperitoneally (i.p.) at a dose of 25 mg/kg. 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%. Saline (0.9% NaCl) was used as the control solution for PS. Reserpine, 1 mg/kg (Sigma), was dissolved in 50 μl of glacial acetic acid and distilled water and administered i.p. The control solution for reserpine (vehicle) consisted of the same amount of acetic acid and water as used in the reserpine solution. All solutions were injected in a volume of 1 ml/kg.

2.3. Procedures

2.3.1. Experiment I

The following drug administration schedules were carried out. (1) Pre-training administration: Phosphatidylserine or saline was administered 30 min before training in the passive avoidance task. Immediately after training the rats received reserpine or vehicle and were returned to their home cages. (2) Post-training administration: The animals were submitted to training and received phosphatidylserine or saline injection immediately after, all animals were treated with reserpine or vehicle 30 min later. In both cases, the test trial was performed 24 h and 1 week after training.

2.3.2. Experiment II

The rats were treated with phosphatidylserine or saline for 7 days, and on the 8th day were submitted to the passive avoidance task (training). Immediately after shock exposure, an injection of reserpine or vehicle was administered. The test trials were performed 24 h and 1 week after training.

2.4. Apparatus

Memory and learning were assessed in the Gemini Avoidance apparatus (San Diego Instruments, San Diego, CA). The principle of this task is that animals exposed to aversive stimulation (mild electric shock) in a particular place will avoid going there on a second occasion (passive avoidance). The apparatus employed was a two-way shuttle-box with a guillotine door placed between the two modular testing chambers. One chamber was illuminated by a 40 W light while the other remained dark. In the training trial, the animals were individually placed in the illuminated chamber facing the closed guillotine door. After the animal turned around 180°, the door was opened and the latency to enter the dark chamber was computed. When the rat entered the darkened chamber, the door was noiselessly lowered and a 0.6 mA foot shock was applied through the grid floor for 2 s. After the exposure to shock, the rat was immediately removed from the apparatus and then returned to its home cage. Retention trials (without the foot shock) were performed 24 h and 1 week after the training trial. Finally, the latency to enter the dark compartment in the test sessions (24 h and 1 week after training) was considered as a retention score and a ceiling of 600 s was imposed.

2.5. Statistical analysis

The inhibitory avoidance data were analyzed by one-way Kruskal–Wallis analysis of variance (ANOVA) followed by the Mann–Whitney *U*-test. To evaluate the inter-trials variation, Friedman ANOVA followed by the Wilcoxon test was used.

3. Results

3.1. Acute PS treatment

3.1.1. Pre-training administration

There was a significant difference between groups in the 24 h ($H(3,58) = 9.29$; $P < 0.025$) and 1 week ($H(3,58) = 8.44$; $P < 0.037$) trials (Fig. 1). Reserpine (saline + reserpine) had an amnestic effect represented by

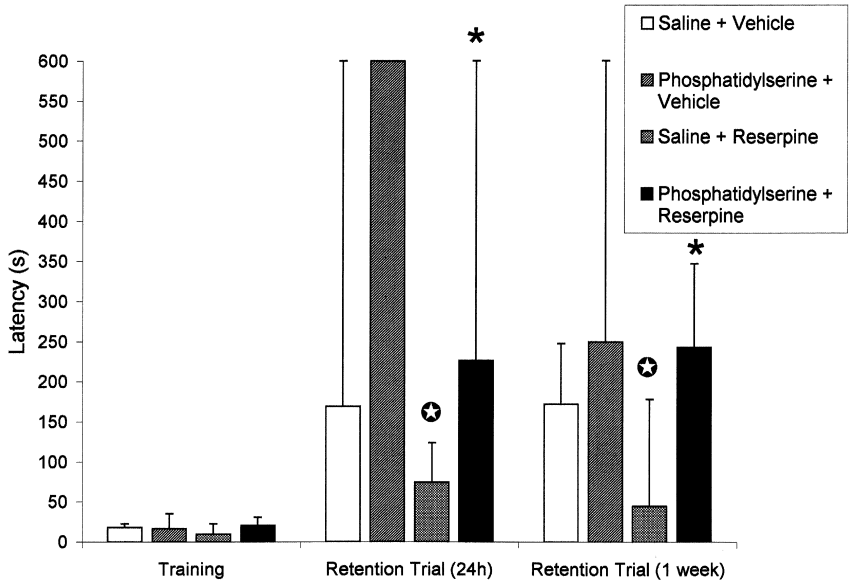

Fig. 1. Effects of acute PS (25 mg/kg) pre-training administration on reserpine-induced impairment in the passive avoidance task for rats. Data represent the median (±interquartile range) latency (s) to enter the dark compartment in the test session. ✪ $P < 0.05$ compared to the saline + vehicle group; ★$P < 0.01$ compared to the saline + reserpine group.

a significant impairment in the avoidance response (decreased latency) in comparison to control animals (saline + vehicle) in the 24 h ($U = 60.0$, $P = 0.049$) and 1 ($U = 46.50$, $P < 0.05$) week trials. On the other hand, phosphatidylserine administered 30 min before training attenuated the reserpine-induced amnestic effects, so that the median values of latencies of the PS + reserpine group did not differ from the control group (saline + vehicle) both in the 24 h ($U = 92.0$, $P = 0.40$) and 1 week ($U = 67.5$, $P = 0.06$) trials. In addition, the latencies of the rats treated with phosphatidylserine + reserpine were significantly higher than those of the rats of the saline + reserpine group in the 24 h trial ($U = 55.0$, $P < 0.01$). There was a significant difference in the latencies of the saline + vehicle and saline + phosphatidylserine groups at 1 week ($U = 46.5$, $P = 0.03$) but not at 24 h ($U = 76.5$, $P = 0.48$). There was a significant difference between phosphatidylserine + vehicle and saline + reserpine ($U = 43.5$, $P = 0.012$) in test trials performed at 24 h but not at 1 week. Finally, there was a significant difference for all groups between the training and the trials at 24 h and 1 week. However, only in the saline + phosphatidylserine group was a signif-

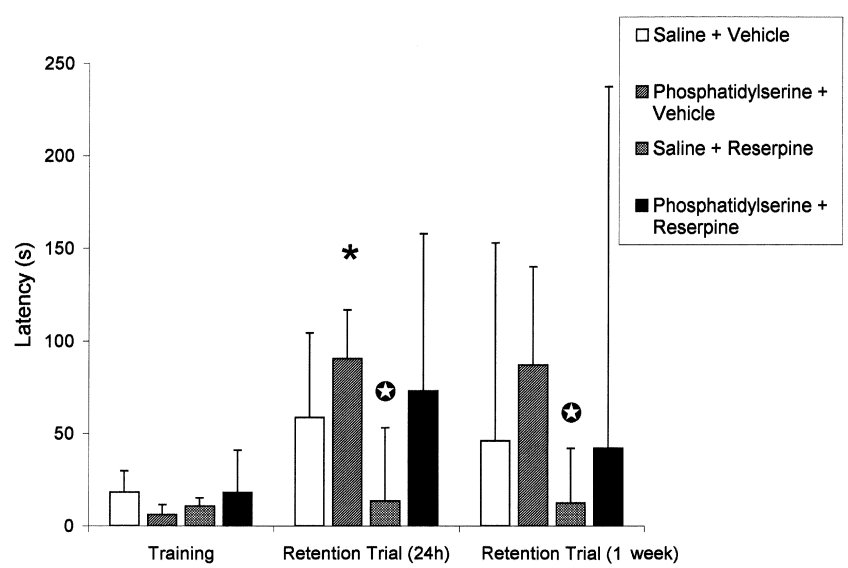

Fig. 2. Effects of acute PS (25 mg/kg) post-training administration on reserpine-induced impairment in the passive avoidance task for rats. Data represent the median (±interquartile range) latency (s) to enter the dark compartment in the test session. ✪ $P < 0.05$ compared to the saline + vehicle group; ★$P < 0.01$ compared to the saline + reserpine group.

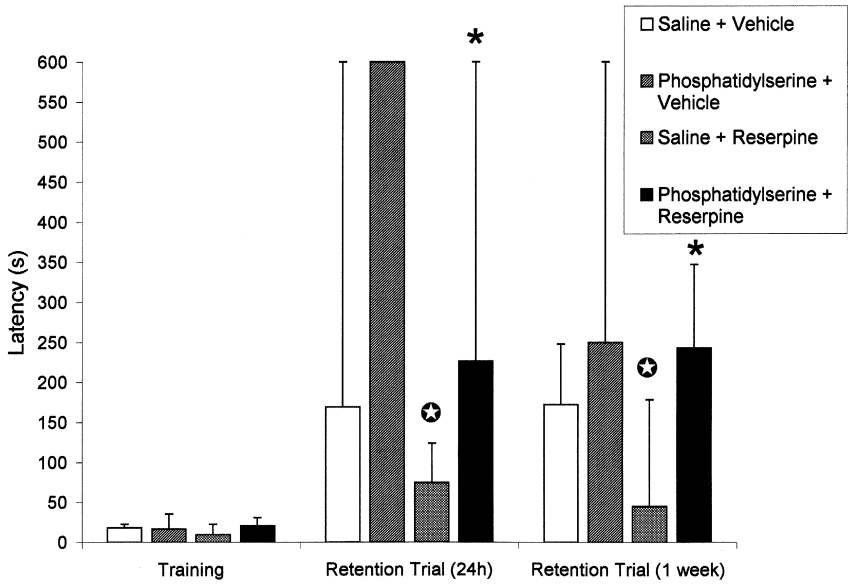

Fig. 3. Effects of long-term pretreatment with PS (25 mg/kg for 7 days) on reserpine-induced impairment in the passive avoidance task for rats. Data represent the median (±interquartile range) latency (s) to enter the dark compartment in the test session. ✪ $P < 0.001$ compared to the saline + vehicle; ★ $P < 0.01$ compared to the saline + reserpine group.

icant difference observed between the 24 h and 1 week trials ($z = 3.059$, $P = 0.002$).

3.1.2. Post-training administration

There was no difference in the latencies of animals which received phosphatidylserine immediately after training ($H = 6.86$, $P = 0.07$) in the training session (Fig. 2). Conversely, there was a significant decrease in the latency of the rats treated with reserpine (saline + reserpine) when compared to control (saline + vehicle) in the 24 h ($U = 36.0$, $P < 0.001$) and 1 week ($U = 47.0$, $P < 0.01$) trials. On the other hand, phosphatidylserine-treated rats (phosphatidylserine + reserpine) showed latencies that did not differ from those of the control group (saline + vehicle) in the 24 h ($U = 110.0$, $P = 0.91$) and 1 week ($U = 102.0$, $P = 0.67$) trials. Additionally phosphatidylserine-treated rats differed significantly from the reserpine group (saline + reserpine) in the 24 h ($U = 47.0$, $P = 0.006$) but not in the 1 week trial ($U = 57.5$, $P = 0.02$). Finally, there was no difference in the latencies of the saline + vehicle and saline + phosphatidylserine groups (24 h: $U = 108.5$, $P = 0.86$; 1 week: $U = 103.0$, $P = 0.69$).

3.2. Long-term PS pre-treatment

Fig. 3 shows the effects of phosphatidylserine (25 mg/kg, i.p.) administered for 7 days on reserpine-induced impairment in the passive avoidance task. Long-term treatment with phosphatidylserine or saline did not induce any difference in avoidance latency in the training session among groups ($H = 0.806$, $P = 0.84$). On the other hand, the reserpine group (saline + reserpine) showed impairment in the 24 h ($U = 24.0$, $P < 0.001$) and 1 week ($U = 34.0$, $P < 0.003$) trials. The animals that received prolonged phosphatidylserine treatment (phosphatidylserine + reserpine) did not differ from the control group (saline + vehicle) in the 24 h ($U = 92.0$, $P = 0.78$) and 1 week ($U = 68.5$, $P = 0.27$) trials.

Moreover, the latencies of rats treated with phosphatidylserine + reserpine were significantly higher than those of the rats of the reserpine group (saline + reserpine) in both trials sessions (24 h: $U = 15.5$, $P < 0.001$; 1 week: $U = 28.5$, $P < 0.001$). Finally, there was a significant difference for all groups between the training session and the 24 h and 1 week trials. On the other hand, only the saline + phosphatidylserine group showed a significant difference between the 24 h and 1 week trials ($z = 2.36$, $P < 0.01$).

4. Discussion

The present results show that acute administration of phosphatidylserine (pre- or post-training) was able to antagonize for up to 1 week the amnestic effects of reserpine after the training session. As can be observed in Figs. 1 and 2, treatment with reserpine induced an impairment in the passive avoidance task. Phosphatidylserine (reserpine + phosphatidylserine) administered pre- and post-training produced a significant increase in the latencies compared to saline + reserpine rats. However, phosphatidylserine per se did not produce a significant difference in this parameter, so that no difference was found when the saline + phosphatidylserine group was compared to the saline + vehicle group. Thus, it was observed in the pre-training study in phosphatidylserine + vehicle treated rats that the

retention scores of this group at 1 week were significantly lower than at 24 h. One possible explanation for this point is that the animals that received phosphatidylserine learned that, in retest situations, they would not receive the shock. Although that data from the passive avoidance test are usually dispersed (see for example, Angelucci et al., 1999), in the present study, the values for the saline + vehicle controls during the pre-training and post-training did not differ statistically ($U = 132.0$; the two-tailed $P = 0.2517$, Mann–Whitney test). In this study post-training phosphatidylserine administration was restricted to no more than 2 min after training and reserpine was always administered 30 min after training. Previous studies have shown that drugs administered after a training session of a memory task can enhance storage by improving the memory consolidation process. In this case, the later the drug is administered after the end of the training session, the lower is its effect. In the present study, phosphatidylserine reversed the memory impairing effect of immediate post-training reserpine administration even when administered 30 min after training. This result suggests that the effect of phosphatidylserine could be even greater if the drug was administered few minutes after training. More recent studies have shown that some drugs affecting, for example, intracellular hippocampal cAMP levels, present peaks of effects on memory storage, the first appearing immediately after training and the second appearing hours after training (Bevilaqua et al., 1997). This suggests that phosphatidylserine can reverse the amnestic effects of reserpine by acting on later events occurring in the memory consolidation processes.

These results suggests that even in the acute treatment, phosphatidylserine reverses the effects of reserpine in the memory consolidation process. Mazzari et al. (1982) demonstrated that ^{14}C-phosphatidylserine is able to cross the brain blood barrier, and despite its limited penetration, 0.25% of total radioactivity was recovered in the brain between 5 and 30 min when ^{14}C-phosphatidylserine was injected intravenously, most of it (90–95%) as authentic phosphatidylserine. The cited study indicates that phosphatidylserine can be present in the brain within this time range, which was similar to that used in the present study.

The results of long-term phosphatidylserine administration were similar to those of acute treatment (Fig. 3). Again this phospholid was able to antagonize the amnestic effects of reserpine on the passive avoidance task in both trials. This effect was evident for up to 1 week and the animals recovered memory spontaneously because the differences observed between vehicle- and reserpine-treated rats were not evident at 2 weeks after training (data not shown).

Catecholaminergic neurons play an important role in learning and memory, as shown by the finding that dopamine depletion induced by 6-hydroxydopamine causes impairment of performance in maze learning and conditional discrimination (Heffner and Seiden, 1983; Archer et al., 1988). In this respect, many authors have demonstrated that reserpine induced retrograde amnesia in a variety of paradigms (Weiskrantz and Wilson, 1956; Dismukes and Rake, 1972; Rake, 1973; Allen et al., 1974). Despite the behavioral effects produced by reserpine administration, such as ptosis, diarrhea and hypokinesia, the test trial performed 24 h after administration of the drug showed that the rats are capable to cross from the illuminated compartment of the avoidance apparatus to the dark side. Similar findings were reported by Kurtz and Palfai (1977), who used a discriminative escape situation and found that reserpine impaired retention when given immediately but not 90 min following the training session. Moreover, they found that reserpine did not produce state-dependent learning in the passive avoidance task. This is an important issue since reserpine may cause norepinephrine release that is known to cause state dependency (Castellano et al., 1993). In the present study, as done in the study by Kurtz and Palfai (1977), reserpine was always administered after training and thus could not cause state-dependence. Thus, this drug treatment resulted in retrograde amnesia for both passive and active avoidance training (Rake, 1973; Kurtz and Palfai, 1977). Therefore, as suggested by Walsh and Palfai (1978), reserpine injection might alter neurobiological mechanisms critically involved in the formation of long-term memory. In this respect, the results of the present study suggest that reserpine produced amnesia 24 h after the training session and this effect was observed up to 1 week later. In addition, our data showed that this amnesia was transient in both experiments and the animals recovered spontaneously. According to Sahgal (1993) some authors have extended the basic procedure by re-testing the same animals not only at 24 h, but for several subsequent days thereafter. No additional shock trials are given, and the aim appears to be to obtain a forgetting curve of reentry latencies plotted against days. On the first retention test day, the subject seems to have learned that shock is no longer present, and this suggests that retention data may be contaminated during the subsequent days. As a whole, the above facts seem to suggest that catecholamines, mainly dopamine, have an important role in cognitive function.

The effect of phosphatidylserine on learning and memory is well known and some authors have demonstrated that the effects of phosphatidylserine in behavioral or neurochemical studies are related to cognitive function. Chronic treatment with phosphatidylserine (50 mg/kg a day for 7–12 weeks) improved both spatial behavior 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 20mg/2μl) injections of phosphatidylserine facilitated the acquisition of active avoidance behavior in the shuttle-box and pole jumping test situations, with a consequent improvement in the retention of active and passive avoidance responses. In addition, 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) demonstrated that phosphatidylserine antagonizes scopolamine-induced amnesia in adult rats tested in a passive avoidance condition. These effects have been related to the behavioral changes observed following acute and chronic administration of this compound. It seems relevant to point out that no substantial difference was observed between phosphatidylserine treated and control rats in the responsiveness to electrical footshock (Drago et al., 1983).

The mechanism(s) of action of phosphatidylserine in reversing reserpine-induced amnesia in the passive avoidance task is unknown, although several hypotheses can be proposed. The one possible mechanism of action may involve the effects of phosphatidylserine on the increase of some neurotransmitter systems related to cognitive function. Intravenous injection of phosphatidylserine increases the in vivo turnover rate of norepinephrine in the hypothalamus and of dopamine in the striatum (Toffano et al., 1978). Thus, phosphatidylserine (15 mg/kg for 30 days) was also able to restore the age impairment in dopamine turnover in both striatum and limbic area. In this way, 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 could 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 serotonine release was not affected (Raiteri et al., 1989). Considering that reserpine interferes with dopamine, norepinephrine and serotonine storage, and these neurotransmitters are related to cognitive function, the above facts suggest that probably dopamine is the main catecholamine restored by phosphatidylserine. In addition to effects on the dopaminergic systems, phosphatidylserine also affected the cholinergic systems (Mantovani et al., 1976). Thus, it is not clear how phosphatidylserine can ameliorate the amnesia induced by reserpine but it may be possible that this effect is partially mediated by the enhancement of acetylcholine levels in the cerebral cortex of rats and/or by glucose accumulation, as proposed by Maggioni et al. (1990). Moreover, Gelbmann and Müller (1991) have demonstrated that a 21-day treatment with phosphatidylserine partially restored the density of muscarinic acetylcholine receptors in several regions of the aged (18 months) mouse brain. This effect was dose-dependent with a 15–28% increase of receptor density for i.p. phosphatidylserine doses between 10 and 40 mg/kg. However, although alterations in cholinergic and dopaminergic systems are probably related to the improving effects of phosphatidylserine on memory, the participation of other neurotransmitters cannot be ruled out. For instance, NMDA receptor-mediated glutamatergic neurotransmission is also important for learning, memory and cognitive function as reported by many authors (Izquierdo and Medina, 1997). As demonstrated by Cohen and Müller (1992), chronic phosphatidylserine ameliorates age-associated deficits of the NMDA receptor in the forebrain of aged mice. Phosphatidylserine produced a positive allosteric modulation of NMDA receptor function and a partial increase in receptor density. Thus, considering that these neurotransmitter systems play an important role in different steps of memory processes, it is reasonable to attribute the reversal of the amnestic effects of reserpine by phosphatidylserine to a restoration of the levels of one or more of the above neurotransmitters.

It is interesting to note that reserpine administration to rats and mice is used as a animal model of parkinsonism (Colpaert, 1987; Gerlach and Riederer, 1996). Besides the locomotor symptoms of Parkinson's disease, memory impairment is frequently found in these patients (Valldeoriola et al., 1997). The amnestic effect of reserpine could be a behavioral approach to the study of this cognitive symptom of Parkinson's disease. In this sense, the present results suggest that phosphatidylserine could be a potential drug treatment for Parkinson's disease memory deficits. This proposal is in accordance with the cholinergic, dopaminergic and glutamatergic effects of phosphatidylserine, since these neurotransmitters play an important role in the pathophysiology of Parkinson's disease (Gerlach and Riederer, 1996).

In conclusion, the results obtained in the present experiments provide suggestive evidence that phosphatidylserine can attenuate cognitive disfunction in a simple rat model of amnesia. It is therefore plausible to suggest that this amnesia is related, in a consistent manner, to certain degenerative diseases of the central nervous system that occur in humans. The mechanism underlying the effects of phosphatidylserine on memory remains to be investigated. Further experiments, now in progress, might help to understand these facts.

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References

Allen, C., Allen, B.S., Rake, A.V., 1974. Pharmacological distinctions between "active" and "passive" avoidance memory formation as shown by manipulation of biogenic amine active compounds. Psychopharmacologia 34, 1–10.

Angelucci, M.E.M., Vital, M.A.B.F., Cesario, C., Zadusky, C.R., Rosalen, P.L., Cunha, D., Cunha, C., 1999. The effect of caffeine in animal models of learning and memory. Eur. J. Pharmacol. 373, 135–140.

Archer, T., Danysz, W., Fredriksson, A., Jonsson, G., Luthman, J., Sundström, E., Teiling, A., 1988. Neonatal 6-hydroxydopamine-induced dopamine depletions: motor activity and performance in maze learning. Pharmacol. Biochem. Behav. 31, 357–364.

Bevilaqua, L., Ardenghi, P., Schröder, N., Bromberg, E., Schmitz, P.K., Schaeffer, E., Quevedo, J., Bianchin, M., Walz, R., Medina, J.H., Izquierdo, I., 1997. Drugs acting upon cyclic adnosine monophosphate/protein kinase A signalling pathway modulate memory consolidation when given late after training into rat hippocampus but not amygdala. Behav. Pharmacol. 8, 331–338.

Breckenridge, W.C., Gombos, G., Morgan, I.G., 1972. The lipid composition of adult rat brain syynaptosomal plasma membranes. Biochem. Biophys. Acta 266, 695–707.

Bruni, A., Toffano, G., Leom, A., Boarato, E., 1976. Pharmacological effects of phosphatidylserine liposomes. Nature 260, 331–333.

Carlsson, A., 1975. Monoamine-depleting drugs. Pharmac. Ther 1, 393–400.

Castellano, C., Introinicollison, I.B., McGaugh, J.L., 1993. Interaction of beta-endorphin and gabaergic drugs in the regulation of memory storage. Behav. Neur. Biol. 60, 123–128.

Claro, F.T., Silva, R.H., Frussa-Filho, R., 1999. Bovine brain phosphatidylserine attenuates scopolamine-induced amnesia. Physiol. Behav. 67, 551–554.

Cohen, S.A., Müller, W.E., 1992. Age-related alterations of NMDA-receptor properties in the mouse forebrain: partial restoration by chronic phosphatidylserine treatment. Brain Res. 584, 174–180.

Colpaert, F.C., 1987. Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rat. Neuropharmacol. 26, 1431–1440.

Dismukes, R.K., Rake, A.V., 1972. Involvement of biogenic amines in memory formation. Psychopharmacologia 23, 17–25.

Drago, F., Canonico, P.L., Scapagnini, U., 1981. Behavioral effects of phosphatidylserine in aged rats. Neurobiol. Aging 2, 209–213.

Drago, F., Toffano, G., Danielli, L.C.R., Continella, G., Scapagnini, U., 1983. Phosphatidylserine facilitates learning and memory process in aged rats. In: Samuel, D. (Ed.), Aging of the Brain. pp. 308–316.

Fagioli, S., Castellano, C., Oliverio, A., Pavone, F., Populin, R., Toffano, G., 1989. Phosphatidylserine administration during postnatal development improves memory in adult mice. Neurosci. Lett. 101, 229–233.

Gelbmann, C.M., Müller, E., 1991. Chronic treatment with phosphatidylserine restores muscarinic cholinergic receptor deficits in the aged mouse brain. Neurobiol. Aging 13, 45–90.

Gerlach, M., Riederer, P., 1996. Animal models of Parkinson's disease: an empirical comparison with the phenomenology of the disease in man. J. Neural Transm. 103, 987–1041.

Goldman-Rakic, P.S., 1998. The cortical dopamine system: role in memory and cognition. Adv. Pharmacol. 42, 707–711.

Heffner, T.G., Seiden, L.S., 1983. Impaired acquisition of an operant response in young rats depleted of brain dopamine in neonatal life. Psychopharmacology 79, 115–119.

Izquierdo, I., Medina, J.H., 1997. Memory formation: the sequence of biochemical events in the hippocampus and its connection to acitivity in other brain structures. Neurobiol. Learn. Mem. 68, 285–316.

Kurtz, P., Palfai, T., 1977. Effects of reserpine on retention of escape reversal in mice: absence of state-dependent learning. J Comp. Physiol. Psychol. 11, 393–406.

Maggioni, M., Picotti, G.B., Bondiolotti, G.P., Panerai, A., Cenacchi, T., Nobile, P., Brambilla, F., 1990. Effects of phosphatidylserine therapy in geriatric patients with depressive disorders. Acta Psychiatr. Scand. 81, 265–270.

Mantovani, P., Pepeu, G., Amaducci, L., 1976. Investigation into the relationship between phospholipids and brain acetylcholine. Adv. Exp. Med. Biol. 72, 285–292.

Mazzari, S., Battistela, A., 1980. Phosphatidylserine effect on dopamine release from striatum synaptosomes. In: Di Benedetto, C. (Ed.), A Multidisciplinary Approach to Brain Development. Elsevier/North-Holland Medical Press, Amsterdam, pp. 145–154.

Mazzari, S., Zanotti, A., Orlando, P., Raciti, R., Toffanno, G., 1982. Pharmacokinetics and pharmacodynamics of phosphatidylserine liposomes in mice. In: Antolini, A. (Ed.), Transport in Biomembranes: "Model System and Reconstruction". Raven Press, New York, pp. 257–263.

McGaugh, J.L., 1989. Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. Annu. Ver. Neurosci. 12, 255–287.

Packard, M.G., White, N.M., 1991. Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. Behav. Neurosci. 105, 295–306.

Raiteri, M., Marchi, M., Maura, G., Ferlito, M., Fontana, G., Robino, G., 1989. Selective recovery of neurotransmitter release in aged rat brain after chronic phosphatidylserine. In: Freysz, L., Hawthorne, J.N., Toffano, G. (Eds.), Neurochemical Aspects of Phospholipid Metabolism. Liviana Press, Padova, Italy, pp. 219–224.

Rake, A.V., 1973. Involvement of biogenic amines in memory formation: the central nervous system indole amine involvement. Psychopharmacologia 29, 91–100.

Sahgal, A., 1993. Passive avoidance procedures. In: Sahgal, A. (Ed.), Behavioural Neuroscience: A Practical Approach vol. I Oxford Univ. Press, Oxford, pp. 49–56.

Smith, A., Loh, H.H., 1976. The topographical distribution of phosphatidylethanolamine and phosphatidylserine in synaptosomal plasma membrane. Proc. West. Pharmacol. Soc. 19, 147–151.

Toffano, G., Leon, A., Benvegnu, D., Boarato, E., Azzone, G.F., 1976. Effects of brain cortex phospholipids on catecholamine content of mouse brain. Pharmac. Res. Commun. 8, 581–590.

Toffano, G., Leon, A., Mazzari, S., Savoini, G., Teolato, S., Orlando, P., 1978. Modification of noradrenergic hypothalamic system in rats injected with phosphatidylserine liposomes. Life Sci. 23, 1093–1102.

Valldeoriola, F., Nobbe, F.A., Tolosa, E., 1997. Treatment of behavioural disturbances in Parkinson's disease. J. Neural Transm. 51, 175–204.

Walsh, T.J., Palfai, T., 1978. Time-dependent effects of reserpine on retention of passive avoidance. Pharmacol. Biochem. Behav. 8 (2), 103–105.

Weiskrantz, L., Wilson, W.A., 1956. Effects of reserpine on learning and performance. Science 123, 1110–1118.

Zanotti, A., Aporti, F., Rubini, R., Toffano, G., 1986. Reversal of scopolamine-induced amnesia by phosphatidylserine in rats. Psychopharmacology 90, 274–275.

Zanotti, A., Valzelli, L., Toffano, G., 1989. Chronic phosphatidylserine treatment improves spatial memory and passive avoidance in aged rats. Psychopharmacology 99, 316–321.