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Zocor Forte® (simvastatin) has a neuroprotective effect against LPS striatal dopaminergic terminals injury, whereas against MPP^+ does not

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ABSTRACT

Due to their potential role in preventing further deterioration of Parkinson's disease, anti-inflammatory strategies have attracted great interest. In this context, some studies point out the possible protective effect of anti-inflammatory compounds against the in vivo degeneration of dopaminergic neurons produced by lipopolysaccharide (LPS)-induced inflammatory processes and others. We have investigated the effect of the treatment of Zocor Forte® (simvastatin) in LPS and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurodegenerative models to identify neuroprotective drugs for Parkinson's disease. We have perfused different concentrations of LPS or 1 mM 1-methyl-4-phenylpyridinium ion (MPP^+) in the rat's striatum, 24 h after implanting a brain microdialysis probe, both with and without Zocor Forte® (simvastatin) treatment. Results show that LPS perfusion produced a decrease in the basal release of dopamine. Forty-eight hours after implanting the probe, we have perfused 1 mM MPP^+ to check the integrity of the dopaminergic terminals present around the cannula. Our model to study toxicity in the striatal dopaminergic terminals suggests that Zocor Forte® (simvastatin) could prevent the neurotoxic damage produced by LPS, but not that produced by MPP^+.

1. Introduction

Parkinson's disease is an age-related neurodegenerative disorder characterized by the progressive degeneration of nigrostriatal dopaminergic neurons. The etiology, as well as the precise mechanisms underlying the selective destruction of the nigrostriatal dopaminergic pathway, remains elusive. Current dopaminergic treatments improve the patients' motor symptoms (including resting tremor, slowness of movement, rigidity, and postural instability) and their quality of life during the early stages of Parkinson's disease, but they do not prevent the progression of the disease and they are associated with disabling side effects (Ogawa, 1994). There is an urgent need for more effective treatments that address the underlying neurodegenerative processes in Parkinson's disease. Although genetic factors account for some cases of Parkinson's disease (Gasser, 1998), the vast majority are considered idiopathic. Evidence from animal studies suggests the involvement of neuroinflammation in the pathogenesis of Parkinson's disease. In fact, inflammation has been implicated in the neurodegenerative process in animal models of the disease, created by numerous neurotoxic compounds, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine or rotenone (Liberatore et al., 1999; Cicchetti et al., 2002; Gao et al., 2002). Likewise, injections of lipopolysaccharide (LPS), a potent inflammation inductor, induced an acute inflammatory response with a strong macrophage/microglial reaction, causing, in *in vivo* and *in vitro* studies, the death of dopaminergic neurons (Herrera et al., 2000). For this reason, anti-inflammatory strategies have attracted much interest, due to their potential for preventing further deterioration of Parkinson's disease. In this context, some studies point out the possible protective effect of anti-inflammatory compounds against the *in vivo* degeneration of dopaminergic neurons produced by the inflammatory process induced by LPS (Castaño et al., 2002; Tomás-Camardiel et al., 2004).

Statins, inhibitors of the rate-limiting enzyme hydroxymethylglutaryl-coenzyme A reductase in cholesterol synthesis, are in wide clinical use to reduce serum cholesterol and triglyceride levels. Recent evidence shows that these drugs have other cholesterol-independent (or so-called pleiotropic; Kolovou et al., 2008) effects such as: reducing the risk of ischemic heart or coronary artery disease events and stroke (Berger et al., 2007; Miida et al., 2007) and they are also being recognized as having a potential application in diabetes mellitus, Alzheimer's disease and Parkinson's disease (DeKosky, 2005; Riad et al., 2007; Wolozin et al., 2007). Statins may also be beneficial in the treatment of inflammatory diseases. Several data suggest that statins may also have immunomodulatory effects relevant to the treatment of neuroinflammatory conditions (Kuipers and van den Elsen, 2007).

In this study, we investigated whether statins could exert a neuroprotective effect on the degenerative process following intrastriatal perfusion of LPS or 1-methyl-4-phenylpyridinium ion (MPP^+)

Abbreviations: GFAP, glial fibrillary acidic protein; LPS, lipopolysaccharide; MPP^+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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in rats. We used simvastatin, a highly lipophylic statin that crosses the blood–brain barrier readily (Caballero and Nahata, 2004). The conclusions of the present study are based on the assumption that MPP^+, as an active metabolite of MPTP, causes damage to dopaminergic neurons, due to its accumulation in the dopaminergic neurons, its inhibitory action on the respiratory chain, the generation of cytotoxic hydroxyl free radicals and, ultimately, neurotoxicity on the dopaminergic terminals followed by cellular death and the release of dopamine contained in vesicles into the extracellular space. It is plausible that the number of surviving dopaminergic terminals is related to the amount of dopamine obtained after MPP^+ perfusion, since there was a clear correlation index between the amount of dopamine measured after MPP^+ perfusion and the lack of tyrosine hydroxylase immunoreativity (Santiago et al., 2001). Working on this assumption, we have perfused different doses of LPS and MPP^+ 1 mM, with and without treatment of Zocor Forte® (simvastatin), 24 h after the implantation of a microdialysis probe (day 1). The possible toxic effect, with cellular death, produced by the LPS or MPP^+ perfusion, is studied by the extracellular output of dopamine produced with a MPP^+ perfusion 24 h later (day 2).

2. Materials and methods

2.1. Animals and drug treatment

Animals were male albino Wistar rats weighing 270–320 g at the time of probe implantation. The rats were kept, three or four per cage, at constant room temperature (22 ± 2 °C) and relative humidity (60%) with a 12-h light–dark cycle and unlimited access to food and water. Experiments were carried out in accordance with the Guidelines of the European Union Council (86/609/EU), following the Spanish regulations (BOE 67/8509-12, 1988) for the use of laboratory animals and approved by the Scientific Committee of the University of Sevilla.

The following drugs were dissolved in the perfusion fluid: 1-methyl-4-phenylpyridinium (MPP^+ iodide; Research Biochemical Inc., Natick, MA, U.S.A.) and lipopolysaccharide (LPS) from *Escherichia coli* Serotype 026:B6 (Sigma Chemical Co., St. Louis, MO, U.S.A.). Zocor Forte® (simvastatin, 5 mg/kg body weight) was dissolved in saline and injected intraperitoneally.

2.2. Surgery and brain dialysis

Animals were anesthetized with general chloral hydrate (400 mg/kg, i.p.) and local lidocaine (10% w/v in water) anesthesia and mounted in a stereotaxic apparatus (David Kopf Instruments) with the nose positioned 3.3 mm below the horizontal bar. Following a midline incision, the skull was exposed and 2 burr holes were drilled through which 2 probes were implanted in both corpus striata with coordinates from bregma point and dura (A/P +0.6, L/M ±2.5, V/D −6.0) (Paxinos and Watson, 1986). Following surgery, animals were housed individually in plastic cages (35×35×40 cm) and allowed to recover overnight, with free access to food and water.

Microdialysis in the corpus striatum was performed with an I-shaped cannula (Santiago and Westerink, 1990). The exposed tip of the dialysis membrane was 4 mm. The dialysis tube (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methalyl sulfonate copolymer (AN 69, Hospal, Barcelona, Spain). The dopamine in vitro recovery of the membrane was 20.3 ± 1.6% (N = 5).

The perfusion experiments were carried out 24 (day 1) and 48 h (day 2) after implantation of the probe (Santiago et al., 2001). Microdialysis and subsequent chemical analysis were performed using an automated on-line sample injection system (Westerink et al., 1987). The corpus striatum was perfused at a flow rate of 3.0 µl/min, using a microperfusion pump (model 22, Harvard Apparatus, South Natick, MA, U.S.A.), with a Ringer solution containing (in mM): NaCl, 140; KCl, 4.0; $CaCl_2$, 1.2; and $MgCl_2$, 1.0. With the help of an electronic timer, the injection valve was held in the load position for 15 min, during which the sample loop (40 µl) was filled with dialysate. The valve then switched automatically to the injection position for 15 s. This procedure was repeated every 15 min, which was the time needed to record a complete chromatogram. After establishing a steady baseline of levels in four consecutive samples (in fmol/min), drugs were administered and sampling was continued for 2.5 h thereafter. All drugs were dissolved in Ringer's solution. Chronic treatment with Zocor Forte® (simvastatin, 5 mg/kg body weight, i.p.) was carried out once a day, for five consecutive days, the first dose being delivered two days before the cannula implantation. Zocor Forte® (simvastatin) was injected every morning and, especially, 2 h before perfusion of drugs through the microdialysis cannula. On day 1, different doses of LPS were perfused for 1 h and 1 mM MPP^+ for 15 min without or with the treatment of Zocor Forte® (simvastatin). On day 2, 1 mM MPP^+ was perfused for 15 min.

At the end of the experiment rats were given an overdose of chloral hydrate, and the brain was fixed with 4% paraformaldehyde via intracardiac perfusion. Coronal sections (40 µm thick) were made, and dialysis probe placement localized according to the atlas of Paxinos and Watson (1986).

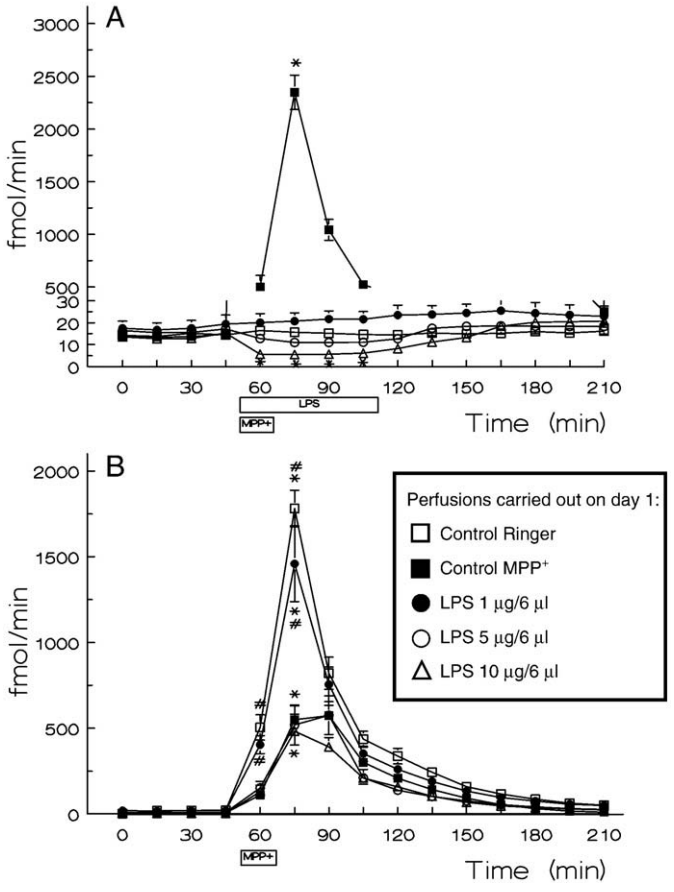

Fig. 1. A) Effect of perfusion of Ringer's solution; LPS (horizontal box) (1 µg/6 µl, 5 µg/6 µl and 10 µg/6 µl); or 1 mM MPP^+ (horizontal box) on the striatal extracellular output of dopamine on day 1. B) Effect of perfusion of 1 mM MPP^+ (horizontal box), 24 h later (day 2) after different treatments carried out on day 1, on striatal extracellular output of dopamine. Data are mean ± S.E.M. (vertical bars) values, expressed as fmol/min (N = 6–10 per group). Statistical significance (Kruskal–Wallis followed by Mann–Whitney U-test): *P<0.05, compared with its corresponding basal value. Only the statistical significance of the collection time with maximal effect of MPP^+ perfusion is shown. #P<0.05, comparing data with MPP^+ perfusion on day 2 at the same collection time (Student's t-test).

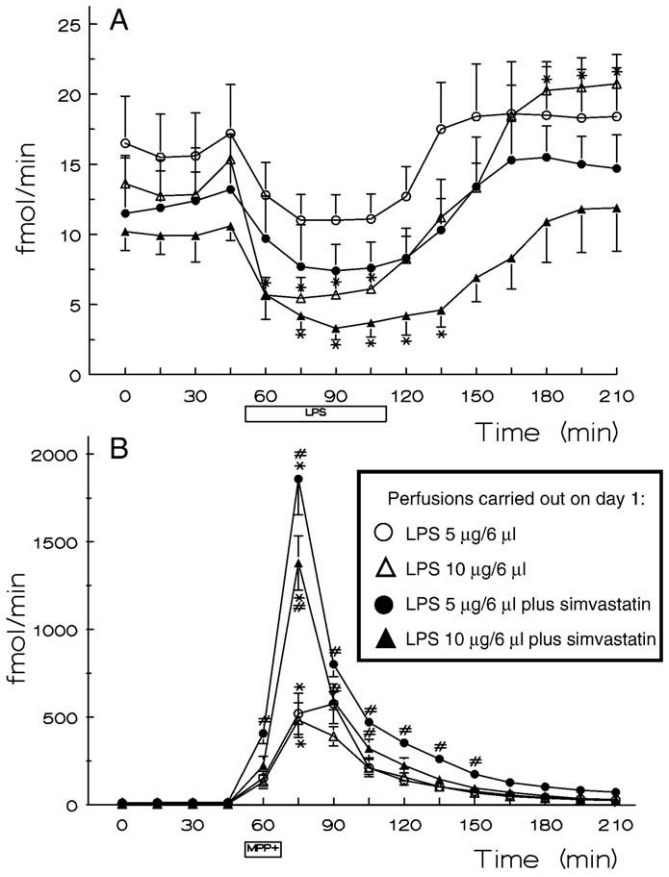

Fig. 2. A) Effect of perfusion (horizontal box) of 5 μg/6 μl and 10 μg/6 μl LPS in animals without or with treatment of Zocor Forte® (simvastatin) on the striatal dopamine release on day 1. B) Effect of perfusion (horizontal box) of 1 mM MPP^+, 24 h later (day 2) after different treatments carried out on day 1, on striatal extracellular output of dopamine. Data are mean ± S.E.M. (vertical bars) values, expressed as fmol/min ($N = 5$–8 per group). Statistical significance (Kruskal–Wallis followed by Mann–Whitney *U*-test): $^*P<0.05$, compared with the basal value. Only the statistical significance of the collection time with maximal effect of MPP^+ perfusion is shown. $\#P<0.05$, comparing data without Zocor Forte® (simvastatin) treatment at the same collection time (Student's *t*-test).

2.3. Chemical assays

Dopamine level in dialysates was analysed by HPLC with electrochemical detection. A Merck L-6200A intelligent pump was used in conjunction with a glassy carbon electrode set at 780 mV (ANTEC, The Netherlands). A Merck Lichrocart cartridge (125 mm × 4 mm) column filled with Lichrospher reverse-phase C_{18} 5 μM material was used. The mobile phase consisted of a mixture of 0.05 M of sodium acetate, 0.4 mM of 1-octanesulfonic acid, 0.3 mM of Na_2EDTA and 70 ml methanol/l, adjusted to pH 4.1 with acetic acid. All reactive agents and water were HPLC grade. The flow rate was 0.8 ml/min and the detection limit for dopamine was 5 fmol per injection.

2.4. Immunohistological evaluation: OX-6 and glial fibrillary acidic protein

Twenty-four hours after microdialysis experiments, rats were perfused through the heart under deep anaesthesia (chloral hydrate) with 150–200 ml of 4% paraformaldehyde in phosphate buffer, pH 7.4. The brains were removed, and then cryoprotected serially in sucrose in phosphate-buffered saline, pH 7.4; first in 10% sucrose for 24 h and then in 30% sucrose until sunk (2–5 days). The brains were then frozen in isopentane at −15 °C, and 30-μm sections were cut on a cryostat and mounted in gelatine-coated slides.

Primary antibodies used were: mouse-derived OX-6 (Serotec, Oxford, U.K.; 1:200) and anti-glial fibrillary acidic protein (anti-GFAP; Chemicon International Inc., USA; 1:300). OX-6 is directed against a monomorphic determinant of the rat major histocompatibility complex class II antigens, expressed by activated microglia but not for resting cells. In our system, an increase in OX-6 expression, as well as the loss of GFAP staining, is marker of cell damage caused by the neurotoxins. Conversely, neuroprotection will be achieved when a decrease of either neurotoxin-induced OX-6 expression or loss of GFAP immunostaining is found.

Incubations and washes for all the antibodies were in Tris buffer saline, pH 7.4, unless otherwise noted. All work was done at room temperature. Sections were washed and then treated with 0.3% hydrogen peroxide in methanol for 15 min, washed again, and incubated in a solution containing Tris buffer saline and 1% horse serum for 60 min in a humid chamber. Slices were drained and further incubated with the primary antibody in Tris buffer saline containing 1% horse serum and 0.25% Triton-X-100 for 24 h. Sections were then incubated for 2 h with biotinylated horse anti-mouse IgG (Vector, 1:200) followed by a second 1-h incubation with ExtrAvidin®-Peroxidase solution (Sigma, 1:100). The antibody was diluted in Tris buffer saline containing 0.25% Triton-X-100, and its addition was preceded by three 10-min rinses in TBS. The peroxidase was visualized with a standard diaminobenzidine/hydrogen peroxidase chromogen reaction for 5 min.

2.5. Quantification of tissue areas

Image analysis software (AnalySIS 3.1, Soft Imaging System Gmbh, Münster, Germany) coupled to a Polaroid DMC camera (Polaroid, Cambridge, MA, USA) attached to a Leika light microscope (Leika Mikroskopie, Wetzlar, Germany) was used for all the measures. Cells showing OX-6 immunoreactivity were counted by using five sections per animals systematically distributed through in the centre of the perfusion area in the striatum, far away from the tip of the cannula. In each section, a systematic sampling of the area occupied by the OX-6-positive cells was made from a random starting point with a grid adjusted to count five fields per section. An unbiased counting frame of known area (40×25 μm $= 1000$ μm^2) was superimposed on the tissue section image under a 100× oil immersion objective. The different types of OX-6-positive cells (displaying different shapes depending on their activation state) were counted as a whole and expressed as cells per mm^2. The measurements of areas lacking GFAP immunoreactivity were expressed as mm^2.

2.6. Expression of results and statistics

Difference between the average dialysate concentrations of the control and drug treatments was compared by Kruskal–Wallis analysis of variance by ranks, and, where appropriate (H value greater than the 95% confidence level), comparison of the means was carried out using Mann–Whitney *U*-test. The unpaired Student's *t*-test was used when comparing the values of the different treatments at the same collection time. For immunohistological evaluation, control and drug treatments were compared by ANOVA followed by the LSD post hoc test for multiple comparisons.

3. Results

3.1. Neurotoxic effect of LPS perfusion on striatal dopamine output on day 1

Twenty-four hours after implanting the probe (day 1), basal extracellular dopamine levels in the corpus striatum in the dialysates were 15.2 ± 1.3 fmol/min (mean ± S.E.M., number of animals: $N = 39$). There were no significant differences in basal extracellular

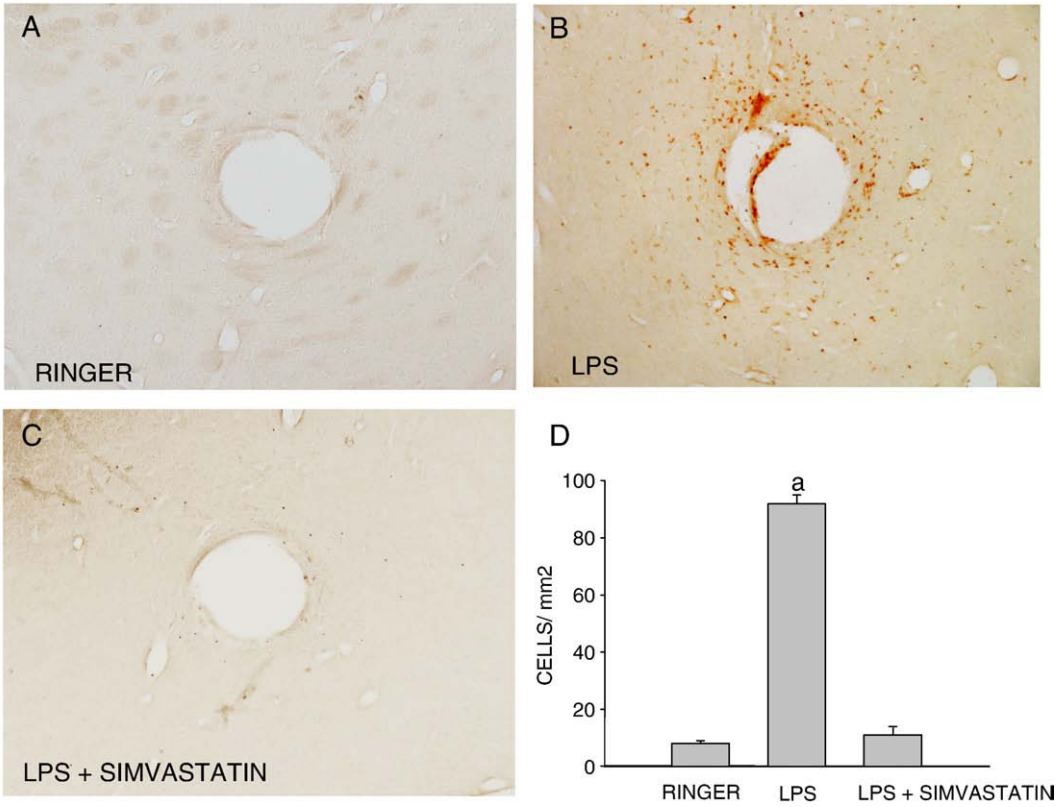

Fig. 3. Photomicrograph of horizontal sections (cryostat-cut sections) through striatum after OX-6 immunostaining on day 2. The striata were perfused on day 1 with: A) Ringer's solution. The microglial reaction is limited to the area around the cannula. B) 10 μg/6 μl LPS. An area showing a strong OX-6 immunoreactivity can be seen. C) 10 μg/6 μl LPS together with a Zocor Forte® (simvastatin) treatment (5 days, 5 mg/kg/day). The glial reaction is limited to the area around the cannula. D) Quantification of glial activation is expressed as OX-6 immunopositive cells per mm^2 for the treatments assayed. Numbers are mean ± S.D. of the five independent experiments and are expressed as cells per mm^2. Statistical significance: One-way ANOVA followed by LSD post hoc test for multiple comparisons. **a**, compared with animals perfused with Ringer's solution or with LPS previously treated with Zocor Forte® (simvastatin), $P<0.01$.

dopamine levels between treatment groups. The perfusion of Ringer's solution ($N=10$) or 1 μg/6 μl LPS ($N=6$) over a 1 hour period did not produce any significant effect on dopamine release. As has been previously described by many investigators, the perfusion of 1 mM MPP^+ ($N=9$) for 15 min produced a large increase in the extracellular output of dopamine (Fig. 1A). The 1-hour perfusion of higher doses of LPS (5 μg/6 μl, $N=8$; and 10 μg/6 μl, $N=6$) produced a decrease in dopamine release, it only being statistically significant in the case of the highest dose of LPS (Fig. 2A).

Forty-eight hours after surgery (day 2), dopamine basal release was similar, even higher, than that observed on day 1 in the case of perfusion Ringer's solution (19.8 ± 1.2 fmol/min, $N=10$) and 1 μg/6 μl LPS (18.6 ± 0.8 fmol/min, $N=6$). However, dopamine basal release was lower in the case of perfusion on day 1 of 1 mM MPP^+ (5.9 ± 0.6 fmol/min, $N=9$) and with high doses of LPS (5 μg/6 μl: 8.4 ± 0.8 fmol/min, $N=8$; and 10 μg/6 μl: 4.0 ± 0.6 fmol/min $N=6$). Perfusion of 1 mM MPP^+ for 15 min on day 2 produced a significant increase in the extracellular output of dopamine, it being similar to the case of perfusion on day 1 of Ringer's solution only (Fig. 1B) and 1 μg/6 μl LPS (Fig. 1B). Perfusion on day 1 of 1 mM MPP^+ and 5 and 10 μg/6 μl LPS produced a maximal extracellular output of dopamine around 500 fmol/min on day 2 (Fig. 1B).

3.2. Neuroprotective effect of Zocor Forte® (simvastatin) treatment on LPS perfusion in the striatal dopaminergic terminals

On day 1, basal release of dopamine during Zocor Forte® (simvastatin) treatment was 11.2 ± 1.1 fmol/min ($N=10$). The perfusion of 5 and 10 μg/6 μl LPS produced a transitory decrease in the dopamine release, both with and without treatment of Zocor Forte® (simvastatin) (Fig. 2A), and it only being statistically significant in the case of the highest dose of LPS.

The effect of 1 mM MPP^+ perfusion for 15 min on extracellular dopamine output on day 2 is depicted in Fig. 2B. Treatment with Zocor Forte® (simvastatin) completely prevented the neurotoxic effect of LPS produced on day 1, since the maximal extracellular output of dopamine was very similar to that obtained with the Ringer's solution perfusion (see Fig. 1B).

3.3. Neuroprotective effect of Zocor Forte® (simvastatin) on LPS perfusion on the microglial and astroglial population

Fig. 3 shows the number of OX6 expressing cells on day 2. The LPS perfusion produced an activation of microglial population around 90 expressing cells/mm^2. The treatment with Zocor Forte® (simvastatin) greatly reduced (by a factor of eight) the number of expressing cells, which was similar to the Ringer's solution perfusion (Fig. 3).

The perfusion of LPS produced a loss of GFAP immunostaining on day 2 as compared with Ringer's perfusion, which was reduced with Zocor Forte® (simvastatin) treatment (Fig. 4).

3.4. Lack of Zocor Forte® (simvastatin) treatment's neuroprotective effect on MPP^+ perfusion in the striatal dopaminergic terminals

On day 1, basal dopamine release during Zocor Forte® (simvastatin) treatment was 15.4 ± 1.4 fmol/min ($N=9$). The perfusion of 1 mM MPP^+ for 15 min, with the previous Zocor Forte® (simvastatin) treatment, produced the highest increase in the extracellular output of dopamine (Fig. 5A).

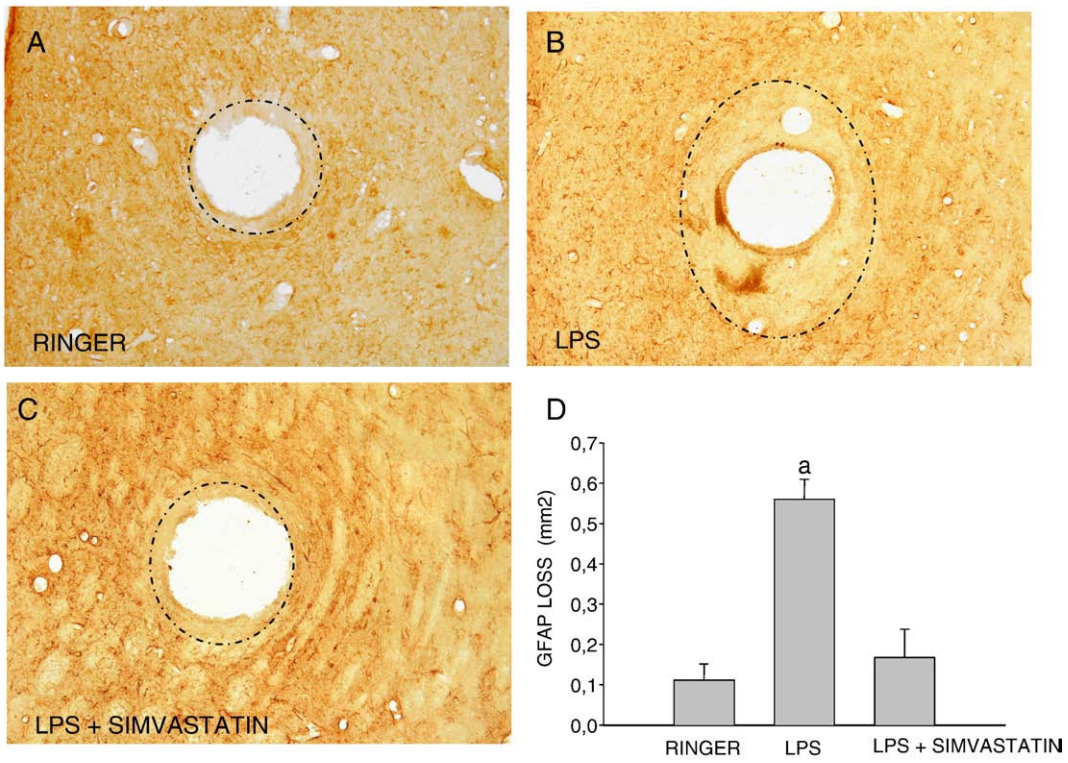

Fig. 4. Photomicrograph of horizontal sections (cryostat-cut sections) through striatum after anti-GFAP monoclonal antibody staining on day 2. The striata were perfused on day 1 with: A) Ringer's solution. No damage can be observed. B) 10 μg/6 μl LPS. The area lacking GFAP immunoreativity is clearly visible. C) 10 μg/6 μl LPS together with a Zocor Forte® (simvastatin) treatment (5 days, 5 mg/kg/day). No damage can be observed. D) Quantification of the areas lacking GFAP immunoreactivity for the treatments assayed. Numbers are mean ± S.D. of five independent experiments and are expressed as GFAP loss in mm^2. Statistical significance: One-way ANOVA followed by the LSD post hoc test for multiple comparisons. **a**, compared with animals perfused with Ringer's solution or with LPS previously treated with Zocor Forte® (simvastatin), $P<0.01$.

The effect of 1 mM MPP^+ perfusion for 15 min on extracellular dopamine output on day 2 is depicted in Fig. 5B. Treatment with Zocor Forte® (simvastatin) did not prevent the neurotoxic effect of MPP^+, even though a perfusion of 1 mM MPP^+ with Zocor Forte® (simvastatin) treatment on day 1 produced, on day 2, a smaller, statistically significant, increase in the maximal extracellular output of dopamine as compared to output without Zocor Forte® (simvastatin) treatment (Fig. 5B).

4. Discussion

The present study shows that an acute perfusion on day 1 of the lowest dose of LPS (1 μg/6 μl) for 1 h through a microdialysis probe implanted in the striatum does not produce any damage to the dopaminergic terminals. This is due to the fact that the maximum peak of extracellular dopamine output obtained after 1 mM MPP^+ perfusion on day 2 was very similar to that obtained for control animals perfused with Ringer's solution only on day 1. This conclusion is based on the hypothesis that the amount of extracellular dopamine output after 1 mM MPP^+ perfusion is an index of dopaminergic terminal disruption, as has been previously demonstrated (Santiago et al., 2001). However, the perfusion of higher doses of LPS (5 and 10 μg/6 μl) for 1 h on day 1 produced a clear and significant reduction in the amount of extracellular dopamine output obtained after perfusion of 1 mM MPP^+ on day 2. This could be indicative of damage to the dopaminergic terminals due to LPS intrastriatal perfusion. *In vivo* and *in vitro* studies have shown that microglia activators such as LPS (Herrera et al., 2000; Iravani et al., 2002; Hunter et al., 2007) or thrombin (Carreño-Müller et al., 2003) can produce the death of dopaminergic neurons and, consequently, reproduce the symptoms of Parkinson's disease.

It is accepted that inflammation has an important role in the pathogenesis of brain diseases (Wersinger and Sidhu, 2006; Wyss-Coray, 2006). Inflammation is as an attractive pharmacological target; it progresses over several days after injury and the intervention with anti-inflammatory agents may avoid intolerable side-effects (Barone and Feuerstein, 1999). Statins may have immunomodulatory effects relevant to the treatment of neuroinflammatory conditions (Kwak et al., 2000).

The present study showed that Zocor Forte® (simvastatin) attenuates LPS-induced dopamine degeneration in the striatum of the rat. Current evidence indicates that statins are neuroprotective both *in vitro* (Bösel et al., 2005) and *in vivo* (Endres et al., 2004). Therefore, Zocor Forte® (simvastatin) could exert its protection against the neurotoxic effect of LPS through its anti-inflammatory effect. These results agree with the anti-inflammatory effect described for statins (Stüve et al., 2003; Hernández-Romero et al., 2008). Moreover, there is a wide consensus on the idea that the anti-inflammatory actions of simvastatin (statins) are independent of cellular levels of cholesterol, postulated to arise from a reduction of isoprenoid intermediates in the cholesterol biosynthetic pathway (Masamura et al., 2003; Liao and Laufs, 2005). Hernández-Romero et al. (2008) treated animals with mevalonic acid by intraventricular injection and only found a slight, non significant, inhibition of the protective effect of simvastatin, supporting the idea that the anti-inflammatory actions described for simvastatin do not depend on the reduction of the cellular levels of cholesterol.

We found that Zocor Forte® (simvastatin) reduces the number of microglia/macrophages expressing major histocompatibility complex class II antigens as revealed by immunostaining with OX-6 (Fig. 4A). Recent studies have shown that statins suppress several kinds of microglial functions, including cell shape determination, cytoskeletal organization, phagocytosis and production of proinflammatory

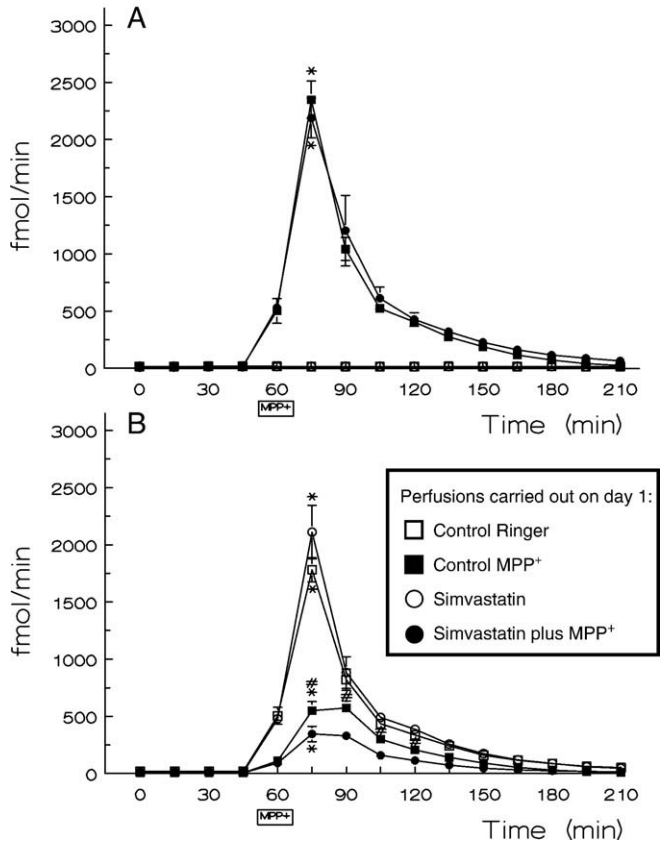

Fig. 5. A) Effect of perfusion of Ringer's solution alone or with 1 mM MPP^+ (horizontal box) in animals without or with treatment of Zocor Forte® (simvastatin) on the striatal extracellular output of dopamine on day 1. B) Effect of perfusion (horizontal box) of 1 mM MPP^+, 24 h later (day 2) after different treatments carried out on day 1, on striatal extracellular output of dopamine. Data are mean ± S.E.M. (vertical bars) values, expressed as fmol/min ($N = 4–10$ per group). Statistical significance (Kruskal–Wallis followed by Mann–Whitney *U*-test): $*P<0.05$, compared with the basal value. Only the statistical significance of the collection time with maximal effect of MPP^+ perfusion is shown. $\#P<0.05$, comparing data with Zocor Forte® (simvastatin) treatment at the same collection time (Student's *t*-test).

cytokines (Townsend et al., 2004; Cordle and Landreth, 2005; Kuipers et al., 2006). Zocor Forte® (simvastatin) also prevented the loss of astroglial GFAP produced after the striatal perfusion of LPS (Fig. 4B). We have also reported similar results with intranigral injections of LPS and simvastatin treatment (Hernández-Romero et al., 2008). In this previous paper, statins exerted a greater protective effect than dexamethasone, which was unable to prevent LPS-induced astrocytes loss (Castaño et al., 2002). We described similar effects for minocycline in an *in vivo* study on the degeneration of dopaminergic neurons induced by the injection of LPS in substantia nigra (Tomás-Camardiel et al., 2004). Interestingly, simvastatin had a greater effect than minocycline.

The fact that Zocor Forte® (simvastatin) treatment did not protect against MPP^+ toxicity could be indicative that this neuroprotective effect described for LPS is primary due to its anti-inflammatory effect. In a previous paper, we have shown the effect of perfusion of MPP^+ on OX-42 and GFAP immunostaining (Matarredona et al., 2001). Our results coincide with the previous study of Kreisler et al. (2007) who found that simvastatin and atorvastatin have a negative effect against MPTP toxicity, but they disagree with other authors (Selley, 2005). Differences in the methodology could account for these discrepancies.

Qian et al. (2007) reported that sinomenine, a natural dextrorotatory morphinan analog, has protective effects in both the LPS and the MPP^+ model, in spite of both compounds having a different mode of action to produce a neurotoxic damage. The LPS model leads to the direct activation of microglia which results in the production of superoxide that mediates neurotoxicity through activation of inflammatory processes (Herrera et al., 2000). However, MPP^+ could work both directly to kill a subset of dopaminergic neurons, through the inhibition of respiratory chain (Ramsay et al., 1991) and also indirectly to activate microglia through reactive microgliosis. Our results with Zocor Forte® (simvastatin) clearly support the hypothesis that MPP^+ mainly works directly, acting through the inhibition of the mitochondrial respiratory chain in order to kill dopaminergic neurons.

In conclusion, the present study demonstrates that Zocor Forte® (simvastatin) protects rat striatum dopaminergic terminals against LPS toxicity, whereas it has no effect on the MPP^+-induced neurotoxicity. The neuroprotective effects of statins such as experimental autoimmune encephalomyelitis, stroke and Alzheimer's disease have been previously described (Bösel et al., 2005; DeKosky, 2005; Berger et al., 2007; Miida et al., 2007), but this is the first report of such an effect on LPS dopamine toxicity in rat striatum by in vivo microdialysis.

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