Effect of L-arginine/nitric oxide pathway on MPP⁺-induced cell injury in the striatum of rats

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1 Protection against 1-methyl-4-phenylpyridinium ion (MPP⁺) neurotoxicity by two nitric oxide-related compounds, N^ω-nitro-L-arginine (L-NOARG) and L-arginine, was studied in the corpus striatum by means of two MPP⁺ perfusions separated by 24 h. Dopamine extracellular output after the second MPP⁺ (1, 5 and 10 mM) perfusion was considered as an index of the dopaminergic neurone damage produced by the first MPP⁺ (1, 5 and 10 mM) perfusion.

2 L-NOARG, systemically administered (10 mg kg⁻¹, i.p., every 12 h for 4 days), failed to prevent the neurotoxic action of MPP⁺ (1 and 10 mM). On the contrary, the neurotoxic effect of MPP⁺ was increased by L-NOARG administration.

3 L-Arginine (10 mM) perfused 2 h before MPP⁺ perfusion did not protect against the neurotoxic action of high MPP⁺ concentrations (5 and 10 mM). At the highest MPP⁺ concentration used (10 mM), the increase in the extracellular output of dopamine after the second MPP⁺ perfusion was slower in L-arginine-treated rats than in control and L-NOARG-treated rats.

4 When MPP⁺ (1 mM) was perfused 2 h after L-arginine (10 mM) perfusion, there was a clear protection against MPP⁺ neurotoxicity. In the second MPP⁺ (1 mM) perfusion, the increase in the extracellular output of dopamine in L-arginine-treated rats was twice as high as for the control rats.

5 The results indicate that NO may exert a protective mechanism in the presence of a low ambient redox.

Keywords: Dopamine; microdialysis; striatum; 1-methyl-4-phenylpyridinium ion (MPP⁺); L-arginine; N^ω-nitro-L-arginine

Introduction

The neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces an extensive and quite selective destruction of dopaminergic neurones in the substantia nigra pars compacta and dramatic decrease of dopamine content in the caudate and putamen nuclei (Burns *et al.*, 1983; Langston *et al.*, 1984). The toxic action of MPTP is ultimately due to its principal metabolite, 1-methyl-4-phenylpyridinium ion (MPP⁺) (Langston *et al.*, 1984; Markey *et al.*, 1984). MPP⁺ is accumulated intraneuronally by a high-affinity dopamine uptake mechanism (Javitch *et al.*, 1985; Schinelli *et al.*, 1988). The toxic mechanism of MPP⁺ may involve its accumulation within mitochondria (Ramsay *et al.*, 1986) and inhibition of mitochondrial respiration supported by NADH-linked substrates (Nicklas *et al.*, 1985). Intrastriatal MPP⁺ perfusion causes a massive release of glutamate and aspartate (Westerink *et al.*, 1987). Therefore, the involvement of excitatory amino acids in the mechanism of MPTP/MPP⁺ neurotoxicity has been postulated. The effects of MK-801, a non-competitive antagonist of the N-methyl-D-aspartate (NMDA)-type receptor, on MPTP and MPP⁺ toxicity are still controversial. It was reported that MK-801 was able to prevent the dopamine neuronal degeneration induced by intranigral (Turk *et al.*, 1991) and striatal (Santiago *et al.*, 1992) perfusion of MPP⁺ in rats, and the MPTP-induced Parkinsonism in primates (Zuñiga *et al.*, 1992). In contrast, it was also noted that MPTP-induced neurotoxicity was not prevented by MK-801 (Sonsalla *et al.*, 1992). The activation of NMDA receptors stimulates, via calcium-calmodulin-dependent mechanisms, the synthesis of nitric oxide (NO), which, in turn, increases guanosine 3':5'-cyclic monophosphate (cyclic GMP) formation (East & Garthwaite, 1991; Garthwaite, 1991). For this reason, NO is actually considered as a putative mediator of neurotoxicity. Studies in a stroke model in mice have shown that 1 mg of N^ω-nitro-L-arginine (L-NOARG) per kilogram of body weight provides up to 75% protection, whereas MK-801 provides only 50–60% protection (Nowicki *et al.*,

1991). In order to verify a possible involvement of NO in NMDA-mediated neurotoxicity, we have investigated, using the microdialysis technique, a potential protective effect against MPP⁺ perfusion of either a 4 day systemic treatment with L-NOARG or perfusion with 10 mM L-arginine.

Methods

Animals and drug treatment

Male albino Wistar rats were used for the experiments. The rats were housed in plastic cages (35 × 35 × 40 cm) and allowed free access to food and water.

The following drugs were used: MPP⁺ iodide (Research Biochemical Inc., Natick, MA, U.S.A.) and L-arginine and N^ω-nitro-L-arginine (Sigma Chemical Co., St. Louis, MO, U.S.A.). MPP⁺ iodide (1, 5 and 10 mM) and L-arginine (10 mM) were dissolved in the perfusion fluid. N^ω-nitro-L-arginine was dissolved in saline and injected intraperitoneally (10 mg kg⁻¹, every 12 h) for 4 days before MPP⁺ perfusion. L-Arginine perfusion started 2 h before and lasted 3 h after each MPP⁺ perfusion.

Surgery and brain dialysis

Microdialysis in the corpus striatum was performed with an I-shaped cannula (Santiago & Westerink, 1990). The exposed tip of the dialysis membrane was 4 mm. The dialysis tube (i.d.: 0.22 mm; o.d.: 0.31 mm) was prepared from polyacrylonitrile/sodium methallyl sulphonate copolymer (AN 69, Hospital, Bologna, Italy). The *in vitro* recovery of the membrane (*n* = 5) was: 20.3 ± 1.6%, for dopamine; 32.4 ± 2.7%, for 3,4-dihydroxyphenylacetic acid (DOPAC); and 30.5 ± 2.8%, for homovanillic acid (HVA). The probe was implanted into the corpus striatum [A/P 0.6, L/M 2.8, V/D 6.0, from bregma point and dura] during general chloral hydrate (400 mg kg⁻¹, i.p.) and local lignocaine (10% w/v in water) anaesthesia.

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The perfusion experiments were carried out 24–48 h after implantation of the probe. Brain dialysis was performed with a fully automated on-line system as has been described previously (Westerink *et al.*, 1987). In brief, two polyethylene tubes (i.d. = 0.28 mm) were connected to the outlets of the dialysis tube. One tube (45 cm in length) was connected to the perfusion pump, and the other (45 cm in length) to the injection valve of the high performance liquid chromatography (h.p.l.c.) apparatus. From the time drugs (MPP⁺ or L-arginine) were included in the Ringer solution delivered by the perfusion pump to the time when the first sample with drugs was injected onto the column, there was a time lag of about 30 min; the data presented are corrected to account for this time lag. 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 μ l) was filled with dialysate. The valve then switched automatically to the injection position for 15 s. This procedure was repeated every 15 min – the time needed to record a complete chromatogram. The corpus striatum was perfused with Ringer solution at a flow rate of 3.0 μ l min⁻¹ (perfusor VI, B. Braun, Melsungen, Germany). The composition of the Ringer solution was (in mM): NaCl 140, KCl 4.0, CaCl₂ 1.2 and MgCl₂ 1.0.

At the end of the experiment, the rat was given an overdose of chloral hydrate, and the brain was fixed with 4% paraformaldehyde via intracardiac perfusion. Coronal sections (40 μ m thick) were cut, and placement of the dialysis

probe localized according to the atlas of Paxinos & Watson (1986).

Chemical assays

Dopamine, DOPAC and HVA were quantitated by h.p.l.c. with electrochemical detection. A Kontron 420 pump was used in conjunction with a glassy carbon electrode set at -780 mV (ANTEC, The Netherlands). A Merck Lichrocart cartridge (125 mm $\times$ 4 mm) column filled with Lichrospher reverse-phase C₁₈ 5 μ m material was used. The mobile phase consisted of a mixture of 0.05 M sodium acetate, 0.4 mM 1-octanesulphonic acid, 0.3 mM Na₂EDTA and 70 ml methanol l⁻¹, adjusted to pH 4.1 with acetic acid. The flow rate was 0.8 ml min⁻¹ and the detection limit for dopamine, DOPAC and HVA was 5, 5 and 20 fmol per injection, respectively.

Expression of results and statistics

The average of the last four stable samples before drug-treatment was used as the basal value and was defined as 100%. All values given are expressed as percentages of basal values. Difference between the average dialysate concentrations of the basal values and drugs treatment was compared by Kruskal-Wallis analysis of variance by ranks, and, where appropriate (H value greater than the 95% confidence level),

Figure 1 Effect of 1-methyl-4-phenylpyridinium ion (MPP⁺) perfusion (black bar), at concentrations of 10 mM (a) and 1 mM (b), on the extracellular output of dopamine in control (□), N^ω-nitro-L-arginine-L (Δ) and L-arginine- (○) treated rats. Data are mean $\pm$ s.e.mean, expressed as fmol min⁻¹ ($n = 4-6$). * $P < 0.05$; ** $P < 0.01$, compared with the basal values (Kruskal-Wallis followed by the Mann-Whitney's test).

Figure 2 Effect of 1-methyl-4-phenylpyridinium ion (MPP⁺) (10 mM) perfusion (black bar), 24 h after a first MPP⁺ (10 mM) perfusion, on the extracellular output of dopamine (a) and DOPAC (b) in control (□), N^ω-nitro-L-arginine (L-NOARG) (Δ) and L-arginine- (○) treated rats. Data are mean $\pm$ s.e.mean, expressed as fmol min⁻¹ ($n = 4-6$). * $P < 0.05$; ** $P < 0.01$, compared with the basal values (Kruskal-Wallis followed by the Mann-Whitney's test). * $P < 0.05$; ** $P < 0.01$, comparing control data with L-NOARG or L-arginine data at the same collection time (Student's t test).

means were compared by the Mann-Whitney U-test. Student's *t* test was used to compare control and L-NOARG or L-arginine data at the same collection time.

Results

Basal release of dopamine was unaffected by either the systemic administration of L-NOARG (10 mg kg⁻¹, i.p., every 12 h) for 4 days or the perfusion of L-arginine (10 mM) for 2 h prior to MPP⁺ perfusion. The dopamine basal release values were as follows (in fmol min⁻¹): 19.0 ± 2.8 (*n* = 16), for control rats; 16.8 ± 1.5 (*n* = 9), for L-NOARG-treated rats; and 24.1 ± 2.5 (*n* = 14), for L-arginine-treated rats.

The first perfusion of MPP⁺, for 15 min, produced a similar increase in the extracellular output of dopamine for all concentrations of MPP⁺ used (1, 5 and 10 mM) both in control animals and in those treated with L-NOARG or L-arginine. Figure 1 shows the effect of MPP⁺ (1 and 10 mM) perfusion on the extracellular output of dopamine in control, L-NOARG- and L-arginine-treated rats. However, after MPP⁺ perfusion, there were similar decreases in the extracellular outputs of DOPAC and HVA, the extent of which was dependent upon the concentration of MPP⁺ used, regardless of the treatment of the rats (data not shown).

After the perfusion of MPP⁺ (1, 5 or 10 mM), L-NOARG-treated rats received two further intraperitoneal injections of

L-NOARG (10 mg kg⁻¹, every 12 h) and L-arginine-treated rats were perfused with L-arginine (10 mM) for 3 h.

One day later, every experimental group received the same treatment as on the previous day. In the control animals, the second perfusion with MPP⁺ (1 mM) resulted in a maximum output of dopamine of approximately 16% of that after the first MPP⁺ (1 mM) perfusion. However, this output was 10 times higher than that induced by the second perfusion with MPP⁺ (10 mM), which was in turn only 2.5% of the level induced by the first perfusion with MPP⁺ (10 mM). Similar trends were found for DOPAC. The basal levels of DOPAC output after the second MPP⁺ perfusion with respect to the first one, were about 50% and 15% for doses of MPP⁺ 1 and 10 mM, respectively.

Figure 2 shows that the second 15 min perfusion with MPP⁺ (10 mM), performed 24 h after perfusion with the same, induced a significant increase in the extracellular output of dopamine (Figure 2a). Dopamine content in control animals reached the highest levels, and although they were not statistically significant when compared with the maximum output of dopamine in L-NOARG-treated rats, they were statistically significant with respect to the levels in L-arginine-treated rats. Basal levels of DOPAC were higher in control rats (corresponding to the highest release of dopamine) compared with L-NOARG- and L-arginine-treated rats (Figure 2b), but the difference was not significant. After perfusion with MPP⁺ (10 mM), DOPAC output underwent a

Figure 3 Effect of 1-methyl-4-phenylpyridinium ion (MPP⁺, 1 mM) perfusion (black bar), 24 h after a first MPP⁺ (1 mM) perfusion, on the extracellular output of dopamine (a) and DOPAC (b) in control (□), N^ω-nitro-L-arginine (L-NOARG) (Δ) and L-arginine- (○) treated rats. Data are mean ± s.e.mean, expressed as fmol min⁻¹ (*n* = 5–6). **P* < 0.05; ***P* < 0.01, compared with the basal values (Kruskal-Wallis followed by the Mann-Whitney's test). #*P* < 0.05; ***P* < 0.01, comparing control data with L-NOARG or L-arginine data at the same collection time (Student's *t* test).

Figure 4 Effect of 1-methyl-4-phenylpyridinium ion (MPP⁺, 5 mM) perfusion (black bar), 24 h after a first MPP⁺ (5 mM) perfusion, on the extracellular output of dopamine (a) and DOPAC (b) in control (□) and L-arginine (○) treated rats. Data are mean ± s.e.mean, expressed as fmol min⁻¹ (*n* = 4–6). **P* < 0.05; ***P* < 0.01, compared with the basal values (Kruskal-Wallis followed by the Mann-Whitney's test).

decrease. This decrease was statistically significant in control animals (Figure 2b). The effect of MPP⁺ perfusion (1, 5 and 10 mM) on HVA output was similar to the effect on extracellular output of DOPAC (data not shown).

Perfusion with MPP⁺ (1 mM) for 15 min, performed 24 h after the first perfusion with MPP⁺ (1 mM) showed marked differences in the maximum extracellular output of dopamine between the different experimental groups studied (Figure 3a). When compared at the same collection time, control and L-NOARG-treated rats had similar profiles of dopamine, although dopamine output was higher and statistically significant in the former with respect to the latter group. The increase in the extracellular output of dopamine due to MPP⁺ perfusion was higher and faster in rats treated with L-arginine. The maximum output of dopamine took place 15 min after MPP⁺ perfusion in L-arginine-treated rats and lasted 15 min more in control and L-NOARG-treated rats (Figure 3a). As observed for the maximum output of dopamine after MPP⁺ perfusion, the highest basal value of DOPAC after the second MPP⁺ perfusion was found in rats treated with L-arginine, followed by that of control rats. The lowest basal value of DOPAC was found in rats treated with L-NOARG (Figure 3b). The effect of perfusion with MPP⁺ (1 mM) on the DOPAC output was similar for the three experimental groups (Figure 3b). Treatment with L-arginine produced a different effect on dopamine extracellular output depending on the concentration of MPP⁺ used (1 and 10 mM). For this reason, 24 h after a first perfusion with MPP⁺ (5 mM) for 15 min, control and L-arginine-treated rats underwent a second perfusion with the same. Under these conditions, there were no statistical differences in the maximum extracellular output of dopamine between either group (Figure 4a). Basal DOPAC output was higher in control rats, but not statistically significant compared with L-arginine-treated rats (Figure 4b).

Discussion

It is well documented that MPP⁺, as an active metabolite of MPTP, causes damage to dopaminergic neurones. It is plausible that this effect is related to the amount of dopamine released after MPP⁺ perfusion. Working on this assumption, we have perfused MPP⁺ twice with an interval of 24 h between them. On the first day, MPP⁺ perfusion produced a maximum output of dopamine which remained fairly constant for the three doses applied (1, 5 and 10 mM). The toxic effect, with cellular death, produced by the first MPP⁺ perfusion is indicated by the output of dopamine and its metabolites produced with a second MPP⁺ perfusion 24 h later. This second MPP⁺ perfusion showed that the maximum output of dopamine differed, depending on the dose applied. The maximum output of dopamine with 1 mM MPP⁺ perfusion in control rats was 10 times higher than that produced with 10 mM MPP⁺, which was about 2.5% of that of the first day. Similar behaviour was found for DOPAC and HVA output. These results indicate that the toxic effect of MPP⁺ depends on its concentration.

It has been reported that the toxic effect of MPP⁺ is due to its accumulation in the dopaminergic neurones (Ramsay *et al.*, 1986), its inhibitory action on the respiratory chain (Nicklas *et al.*, 1985) and the generation of cytotoxic hydroxyl free radicals (Chiueh *et al.*, 1992). However, a massive release of glutamate and aspartate could be involved in the toxic effect of MPP⁺ (Carboni *et al.*, 1990). Moreover, MK-801, a non-competitive antagonist of the glutamate NMDA-type receptor, is able to prevent the dopamine neuronal degeneration induced by MPTP/MPP⁺ neurotoxicity (Turski *et al.*, 1991; Santiago *et al.*, 1992). Activation of NMDA receptors stimulates the synthesis of nitric oxide (NO), which is actually considered as a putative mediator of neurotoxicity (East & Garthwaite, 1991; Garthwaite, 1991). Therefore, probably through NO increase, there is a MPP⁺ secondary

toxicity due to glutamate. Taking into account the protective effect of MK-801 (Turski *et al.*, 1991; Santiago *et al.*, 1992), we expect that the inhibition of the enzyme, nitric oxide synthase (NOS), should give similar results to those described in other circumstances. Ischaemia produces, like MPP⁺, neuronal death with a previous high output of dopamine and excitatory amino acids (Baker *et al.*, 1991). Likewise, it has been reported that MK-801 protects against neuronal death produced by ischaemia (Rod *et al.*, 1989; Demerlé-Pallardi *et al.*, 1991). Nowicki *et al.* (1991) have reported a higher protective effect for L-NOARG than for MK-801 in a stroke model in mice.

We inhibited the NOS by systemic administration of L-NOARG for 4 days (Dwyer *et al.*, 1991). After this treatment, animals were perfused twice with MPP⁺ (1 or 10 mM). Under these conditions, we found an increase in the toxicity with both MPP⁺ concentrations assayed. The second MPP⁺ perfusion produced greater damage in animals treated with L-NOARG than in control animals, the output of dopamine in the former group being about 75% of the latter group in both cases (1 or 10 mM). Likewise, DOPAC basal levels were lower in animals treated with L-NOARG than in control animals.

These data seem to demonstrate that the toxic action provoked by MPP⁺ through activation of NMDA receptors is not produced by an increase of NO synthesis. Similar data have been given for other neurodegenerative processes. Lener-Natoli *et al.* (1992) showed that the NOS inhibitors failed to protect hippocampal neurones against NMDA toxicity in diethylether anaesthetized rats. Moncada *et al.* (1992) failed to demonstrate any protective effect of N^ω-nitro-L-arginine methyl ester (L-NAME) in the ischaemic model. In addition, in a comparative study of the effect of NOS inhibitors, MK-801, and methyl blue (guanylate cyclase inhibitor), Demerlé-Pallardi *et al.* (1991) found that, while all these compounds decreased the cyclic GMP levels, only MK-801 was able to reduce neuronal death.

Our results with L-NOARG treatment seem to indicate that this inhibitor of NOS fails to protect against the neuronal death produced by MPP⁺ and, in some cases, increases the damage. For this reason, we have tried to study, in similar conditions, the effect of L-arginine, a substrate of NOS. We have found that the effect of L-arginine depends on the MPP⁺ concentration used.

Perfusion of MPP⁺ (10 mM) seems to produce higher damage in animals treated with L-arginine than in control animals. The maximum output of dopamine was lower (half) in the former compared with the latter animals. This damage was also higher than that produced in L-NOARG-treated rats. Twenty-four hours after the first MPP⁺ perfusion, DOPAC output was lower in L-arginine-treated rats compared to control and L-NOARG-treated rats. However, opposite effects seemed to be observed, when a lower MPP⁺ concentration (1 mM) was used. After the second MPP⁺ (1 mM) perfusion, the maximum output of dopamine in L-arginine-treated rats was twice that of control animals. It is worthwhile noting that the immediate rise of dopamine seen after MPP⁺ perfusion in L-arginine-treated rats is probably caused by the death of the neuronal elements surrounding the cannula. It is, therefore, of interest that, after MPP⁺ (1 mM) perfusion the maximum output of dopamine in L-arginine-treated rats had a similar time-profile on the first and second days, but, with 5 and 10 mM of MPP⁺, there was a delay in the maximum output of dopamine on the second day compared with the first day. These results seem to indicate that the higher delay in dopamine maximum output on the second day demonstrates higher neuronal death surrounding the cannula on the first day. Therefore, there is a protection with L-arginine against MPP⁺ (1 mM) neurotoxicity. Likewise, DOPAC basal levels, 24 h after the first MPP⁺ (1 mM) perfusion, were significantly higher than in control animals.

All of these findings are consistent with the neuroprotec-

tive and neurotoxic role of NO. The redox versatility of NO allows for its interconversion from neuroprotective to neurotoxic species by change in the ambient redox milieu. It has been recently reported (Lipton *et al.*, 1993) that NO[•] mediated neurotoxicity is engendered by reaction with superoxide anion (O₂^{•-}) and not by NO[•] alone. In contrast, the neuroprotective effects of NO result from downregulation of NMDA-receptor activity by reaction with thiol group(s) of the receptor's modulatory site. This reaction is not mediated by NO[•] itself, but occurs under conditions supporting S-nitrosylation of NMDA receptor thiol.

Free radical formation enhanced by MPP⁺ in the striatum appears to be dose-dependent (1–10 mM) (Chiueh *et al.*, 1992). Therefore, there is an ambient redox that is higher with MPP⁺ 10 mM than with MPP⁺ 1 mM. Taken together with our results, it is inferred that, after arginine and MPP⁺ 10 mM perfusion, the NO produced could be chemically reduced to NO[•] and react with superoxide anion and become neurotoxic. In contrast, with arginine and MPP⁺ 1 mM the

NMDA receptor's redox modulatory site can be downregulated by the NO[•] produced and thus prevent excessive influx of Ca²⁺ and its associated neurotoxic effects (Lei *et al.*, 1992; Stamler *et al.*, 1992).

In conclusion, these results seem to indicate that the NO could be an important protector mechanism when there is not a high ambient redox, since with a low concentration of MPP⁺ (1 mM) the neurotoxic action mediated by glutamate release, through NMDA receptors, could be regulated by NO. In fact, nitroprusside (which spontaneously generates NO) and L-arginine inhibit NMDA receptors (Manzoni *et al.*, 1992; Manzoni & Bockaert, 1993) and protect cerebellar cells from oxygen radical-induced damage (Garthwaite & Garthwaite, 1988).

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References

- BAKER, A.J., ZORNOW, M.H., SCHELLER, M.S., YAKSH, T.L., SKILLING, S.R., SMULLIN, D.H., LARSON, A.A. & KUCZENSKI, R. (1991). Changes in extracellular concentrations of glutamate, aspartate, glycine, dopamine, serotonin, and dopamine metabolites after transient global ischemia in the rabbit brain. *J. Neurochem.*, **57**, 1370–1379.
- BURNS, R.S., CHIUEH, C.C., MARKEY, S.P., EBERT, M.H., JACOBOWITZ, D.M. & KOPIN, I.J. (1983). A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 4546–4550.
- CARBONI, S., MELIS, F., PANI, L., HADJICONSTANTINO, M. & ROSSETTI, Z.L. (1990). The non-competitive NMDA-receptor antagonist MK-801 prevents the massive release of glutamate and aspartate from rat striatum induced by 1-methyl-4-phenylpyridinium (MPP⁺). *Neurosci. Lett.*, **117**, 129–133.
- CHIUEH, C.C., KRISHNA, G., TULSI, P., OBATA, T., LANG, K., HUANG, S.-J. & MURPHY, D.L. (1992). Intracranial microdialysis of salicylic acid to detect hydroxyl radical generation through dopamine autooxidation in the caudate nucleus: effect of MPP⁺. *Free Radical Biol. Med.*, **13**, 581–583.
- DEMERLÉ-PALLARDY, C., LONCHAMPT, M.O., CHABRIER, P.E. & BRAQUET, P. (1991). Absence of implication of L-arginine/nitric oxide pathway on neuronal cell injury induced by L-glutamate or hypoxia. *Biochem. Biophys. Res. Commun.*, **181**, 456–464.
- DWYER, M.A., BREDT, S. & SNYDER, S.H. (1991). Nitric oxide synthase: irreversible inhibition by L-NG-nitroarginine in brain in vitro and in vivo. *Biochem. Biophys. Res. Commun.*, **176**, 1136–1141.
- EAST, S.J. & GARTHWAITE, J. (1991). NMDA receptor activation in rat hippocampus induces cyclic GMP formation through the L-arginine nitric oxide pathway. *Neurosci. Lett.*, **123**, 17–19.
- GARTHWAITE, J. (1991). Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci.*, **14**, 60–67.
- GARTHWAITE, G. & GARTHWAITE, J. (1988). Cyclic AMP and cell death in rat cerebellar slices. *Neuroscience*, **26**, 321–326.
- JAVITCH, J.A., D'AMATO, R.J., STRITTMATTER, S.M. & SNYDER, S.H. (1985). Parkinson-inducing neurotoxin 1-methyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 2173–2177.
- LANGSTON, J.W., FORNO, L.S., REBERT, C.S. & IRWIN, I. (1984). Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the squirrel monkey. *Brain Res.*, **292**, 390–394.
- LEI, S.Z., PAN, Z.-H., AGGARWAL, S.K., CHEN, H.-S.V., HARTMAN, J., SUCHER, N.J. & LIPTON, S.A. (1992). Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. *Neuron*, **8**, 1087–1099.
- LENER-NATOLI, M., RONDOUIN, G., DE BOCK, F. & BOCKAERT, J. (1992). Chronic NO synthase inhibition fails to protect hippocampal neurones against NMDA toxicity. *NeuroReport*, **3**, 1109–1112.
- LIPTON, S.A., CHOI, Y.-B., PAN, Z.-H., LEI, S.Z., CHEN, H.-S.V., SUCHER, N.J., LOSCALZO, J., SINGEL, D.J. & STAMLER, J.S. (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature*, **364**, 626–632.
- MANZONI, O. & BOCKAERT, J. (1993). Nitric oxide synthase activity endogenously modulates NMDA receptors. *J. Neurochem.*, **61**, 368–370.
- MANZONI, O., PREZEAU, L., MARIN, P., DESHAGER, S., BOCKAERT, J. & FAGNI, L. (1992). Nitric oxide-induced blockade of NMDA receptors. *Neuron*, **8**, 653–662.
- MARKEY, S.P., JOHANNESSEN, J.N., CHIUEH, C.C., BURNS, R.S. & HERKENHAM, M.A. (1984). Intraneuronal generation of a pyridinium metabolite may cause drug-induced parkinsonism. *Nature*, **311**, 464–467.
- MONCADA, C., LEKIEFFRE, D., ARVIN, B. & MELDRUM, B. (1992). Effect of NO synthase inhibition on NMDA-induced and ischaemia-induced hippocampal lesions. *NeuroReport*, **3**, 530–532.
- NICKLAS, W.J., VYAS, I. & HEIKKILA, R.E. (1985). Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenylpyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Life Sci.*, **36**, 2503–2508.
- NOWICKI, J.P., DUVAL, D., POIGNET, H. & SCATTON, B. (1991). Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. *Eur. J. Pharmacol.*, **204**, 339–341.
- PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates* (second edition). San Diego: Academic Press.
- RAMSAY, R.R., DADGAR, J., TREVOR, A. & SINGER, T.P. (1986). Energy driven uptake of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by brain mitochondria mediates the neurotoxicity of MPTP. *Life Sci.*, **39**, 581–588.
- ROD, M.R. & AUER, R.N. (1989). Pre- and postischemic administration of dizocilpine (MK-801) reduces cerebral necrosis in the rat. *Can. J. Neurol. Sci.*, **16**, 340–344.
- SANTIAGO, M., VENERO, J.L., MACHADO, A. & CANO, J. (1992). In vivo protection of striatum from MPP⁺ neurotoxicity by N-methyl-D-aspartate antagonists. *Brain Res.*, **586**, 203–207.
- SANTIAGO, M. & WESTERINK, B.H.C. (1990). Characterization of the in vivo release of dopamine as recorded by different types of intracerebral microdialysis probes. *Naunyn-Schmied. Arch. Pharmacol.*, **342**, 407–414.
- SCHINELLI, S., ZUDDAS, A., KOPIN, I.J., BARKER, J.L. & DI PORCIO, H. (1988). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine metabolism and 1-methyl-4-phenylpyridinium uptake in dissociated cell cultures from the embryonic mesencephalon. *J. Neurochem.*, **50**, 1900–1907.
- SONSALLA, P.K., ZEEVALK, G.D., MANZONI, L., GIOVANNI, A. & NICKLAS, W.J. (1992). MK-801 fails to protect against the dopaminergic neuropathology produced by systemic 1-methyl-1-phenyl-1,2,3,6-tetrahydropyridine in mice or intranigral 1-methyl-4-phenylpyridinium in rats. *J. Neurochem.*, **58**, 1979–1982.

- STAMLER, J.S., SINGEL, D.J. & LOSCALZO, J. (1992). Biochemistry of nitric oxide and its redox-activated forms. *Science*, **258**, 1898–1902.
- TURSKI, L., BRESSLER, K., RETTIG, K.J., LÖSCHMANN, P.A. & WACHTEL, H. (1991). Protection of substantia nigra from MPP⁺ neurotoxicity by N-methyl-D-aspartate antagonists. *Nature*, **349**, 414–418.
- WESTERINK, B.H.C., DAMSMA, G., ROLLEMA, H., DE VRIES, J.B. & HORN, A.S. (1987). Scope and limitations of in vivo brain dialysis: a comparison of its applications to various neurotransmitter systems. *Life Sci.*, **41**, 1763–1776.
- ZUDDAS, A., OBERTO, G., VAGLINI, F., FASCETTI, F., FORNAI, F. & CORSINI, G.U. (1992). MK-801 prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in primates. *J. Neurochem.*, **59**, 733–739.

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