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Pre- and post-treatment with pirlindole and dehydropirlindole protects cultured brain cells against nitric oxide-induced death

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Abstract

We have previously shown that pirlindole and dehydropirlindole, two monoamine oxidase type-A inhibitors, protect cultured brain cells against iron-induced toxicity through a mechanism unrelated to monoamine oxidase type-A inhibition. The current study was performed to test whether the protective effect of pirlindole and dehydropirlindole could be extended to a nitric oxide (NO)-induced insult. A comparison with other monoamine oxidase inhibitors (brofaromine, moclobemide and deprenyl) and with trolox was made. In a first series of experiments, rat hippocampal or cortical cultured cells were exposed to a drug for 3 h, then 5 μM sodium nitroprusside, a NO donor, was added and the incubation was continued for 16 h. Cell survival assessment showed that pirlindole, dehydropirlindole and trolox significantly protected cultures against NO-induced toxicity in a concentration-dependent manner with respective EC_{50}'s of 7, 3 and 17 μM. Similarly, pirlindole, dehydropirlindole or trolox, at a concentration of 50 μM, significantly decreased both intracellular peroxide production and lipoperoxidation. Other drugs were ineffective. In a post-hoc treatment protocol (3- or 6-h pre-incubation in the presence of sodium nitroprusside, then addition of one of the above mentioned compounds), only pirlindole and dehydropirlindole significantly improved cell survival in a concentration-dependent manner with respective EC_{50}'s of 9 and 4 μM. The maximal protection in terms of cell survival was 90% and 78% after 3 and 6 h, respectively. They also reduced the production of both lipoperoxides and endoperoxides. Our results show that pirlindole and dehydropirlindole protect neurons against NO-induced toxicity at pharmacologically relevant concentrations. Moreover, their protective effect is still apparent when they are applied after the start of the insult. Therefore, our preclinical study suggests a new strategy that may be efficient to reduce NO-induced damage in the central nervous system.

Keywords: Nitric oxide (NO); Antidepressant; Antioxidant; Nerve cell culture; Neuroprotection; Monoamine oxidase A inhibitor

1. Introduction

It is well established that nitric oxide (NO) is implicated in various physiological functions such as blood pressure regulation (Furchgott and Zawadzki, 1980; Moncada et al., 1988), immune response (Snyder and Bredt, 1992) and synaptic plasticity (Hölscher, 1997).

On the other hand, it has also been shown that excessive production of NO is involved in the progression of brain injury during or following hypoxia/ischemia (Ashwal et al., 1998; Buisson et al., 1993; Forman et al., 1998; Holtz et al., 2001; Iadecola, 1997; Leker et al., 2001; Rodrigo et al., 2001) and perhaps in other central nervous system diseases (Youdim et al., 1993, 1994).

NO is synthesized by three distinct forms of NO synthase [NOS, EC. 1.14.13.39., neuronal NO synthase (nNOS), endothelial NO synthase (eNOS) and inducible NO synthase (iNOS)], using L-arginine as substrate (Garthwaite, 1991). NO can cause cell death by inducing lipid peroxidation or by activating the nuclear poly-(ADP)-ribosy synthase enzyme (also named poly-(ADP)-ribosy polymerase) (Dawson et al., 1994; Pieper et al., 1999; Szabo and Dawson, 1998).

Moreover, this gas is a very reactive and unstable free radical (Stamler et al., 1992). During the reperfusion phase, it reacts with the superoxide anion ($O_2^{-\circ}$) to form peroxynitrite ($ONOO^-$), a powerful oxidant (VanDyke, 1997). Peroxynitrite decomposition could lead to the production of the very reactive hydroxyl radical (OH°) and the NO_2° radical (Mouithys-Mickalad et al., 1998).

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During hypoxia/ischemia, glutamate released from neurons (Choi, 1990) activates glutamate receptors that, in turn, increase the intracellular Ca^{2+} concentration and NOS activity (Ashwal et al., 1998; Holtz et al., 2001; Forman et al., 1998; Iadecola, 1997; Leker et al., 2001; Rodrigo et al., 2001). In addition, it has been shown that, in some cell types, oxidative glutamate toxicity requires monoamine metabolism as a source of free radicals (Maher and Davis, 1996). Taken together, these cellular modifications contribute to neuronal death and cerebral injury (Altavilla et al., 1999; Kohno et al., 1997).

We have recently shown that two reversible inhibitors of monoamine oxidase (EC 1.4.3.4.) type-A, pirlindole (pirazidole; 1,10-trimethylene-8-methyl-1,2,3,4-tetrahydropyrazino [1,2,-*a*] indole hydrochloride) and its dehydroderivative, dehydropirlindole are able to protect cultured brain cells against iron-induced oxidative stress. This protection was unrelated to monoamine oxidase type-A inhibition because two other monoamine oxidase type-A inhibitors, moclobemide and brofaromine were not protective despite their effective inhibition of the enzyme (Boland et al., 2002b). Similar results have been published concerning the protective effect of other monoamine oxidase type-A inhibitors in a model of oxidative glutamate toxicity (Maher and Davis, 1996).

In view of these data, we tested the hypothesis that pirlindole and dehydropirlindole also afford protection against NO-induced toxicity. Their effect was compared to that of various other drugs: brofaromine and moclobemide, two other monoamine oxidase type-A inhibitors as well as deprenyl (selegiline), an inhibitor of monoamine oxidase type-B known to be protective in certain experimental models or neurodegenerative diseases (Sano et al., 1997; The Parkinson Study Group, 1993). We also compared their effect with that of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is a water-soluble derivative of vitamin E, known to protect neuronal cells against iron-induced oxidative stress (Boland et al., 2000). Evaluation of cell protection was done by measuring cell death, intracellular peroxide production and lipid peroxidation. Compounds that were found to be effective in a pre-treatment protocol were also tested in a post-treatment protocol in which they were applied 3 or 6 h after the start of the exposure to NO.

2. Materials and methods

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, Publication No. (NIH) 85-23, revised 1985.

2.1. Hippocampal and cortical cell primary culture

Primary cell cultures were performed following a method previously described (Boland et al., 2000). Briefly, brain cortices or hippocampi were removed from embryonic 17- to 18-day Wistar rats. After isolation of the areas of interest, cells were dissociated enzymatically [0.25% trypsin in phosphate buffered salt solution (PBS)] and then mechanically, using a narrowed Pasteur pipette. The cells were seeded (cortex: 80,000 cells/cm^2, hippocampus: 50,000 cells/cm^2) into poly-L-ornithine-coated plates containing culture medium (modified Eagle's medium supplemented with 16 mM KCl, 26 mM sodium bicarbonate, 55 mM glucose, 1 mM L-glutamine, 1 mM pyruvate and 10% fetal calf serum).

After a 4–6 h incubation, the medium was replaced with fresh medium. Cultures were maintained in a humidified atmosphere (5% CO_2/95% air) at 37 °C without any renewing of the medium. All experiments were performed with cells that had been kept in culture for 7–10 days without any replacement of the culture medium.

2.2. Drugs and NO exposure

To induce NO production, we used sodium nitroprusside [SNP, $Na_2Fe(CN)_5NO{\cdot}2H_2O$] which is known to be a NO donor (Cuajungco and Lees, 1998; Farinelli et al., 1996; Garthwaite and Garthwaite, 1988; Haby et al., 1994; Medvedev et al., 1992; Ohki et al., 1995; Tabuchi et al., 1996; Wei and Quast, 1998; Boland et al., 2002a).

Two types of protocols were used.

In the first protocol, exposure to the drugs and sodium nitroprusside was performed as follows: 7- to 10-day-old cell cultures were washed three times with Locke's solution (154 mM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5 mM HEPES and 10 mM glucose, pH 7.2, supplemented with 10 mg/l gentamycin) (Blanc et al., 1997; Mattson et al., 1995). The cells were then immediately exposed to the drugs for a 3-h pre-incubation in Locke's solution. Finally, after pre-incubation, 5 μM sodium nitroprusside (from a 400-μM stock solution prepared just before use in double-distilled sterile water), was added and the incubation was prolonged for either 8 (intracellular peroxide measurement) or 16 h (cell death determination and lipid peroxidation assessment).

The second protocol was as follows. After the triple wash with Locke's solution, we exposed either hippocampal (cell death and intracellular peroxide production measurements) or cortical (lipoperoxidation measurements) cultured cells first to 5 μM sodium nitroprusside alone for 3 or 6 h in the same solution. Then, the tested drugs were added to the cells and the incubation was continued for either 5 h [intracellular peroxide measurements (3-h experiments)] or 16 h [cell death determination (3- and 6-h experiments) and lipid peroxidation assessment (3-h experiments)].

2.3. Assessment of cell death

Cell death was assessed by the usual lactate dehydrogenase (E.C.1.1.1.27, LDH) activity assay that measures the

quantity of LDH released by dying cells (Boland et al., 2000).

Briefly, the extracellular medium was removed at the end of the experiment. Total releasable LDH was obtained by submitting the cells to a freeze–thaw cycle. A phosphate buffer containing β-nicotinamide adenine dinucleotide (0.2 mg/ml) and sodium pyruvate (0.1 mg/ml) was added to the incubating medium sample. The absorbance decrease was then immediately determined for 6 min at 340 nm (Cecil Spectrophotometer). Values were expressed as percentages of the total releasable LDH.

2.4. Measurement of intracellular peroxides

The level of intracellular peroxides was quantified by fluorescence with 2,7-dichloro-fluorescein diacetate (DCF, Molecular Probes) (Boland et al., 2000).

Cells were loaded for 50 min with 100 μM DCF. At the end of the incubation, the cells were washed once and the relative levels of fluorescence were quantified using a fluorescence plate reader (485 nm excitation and 538 nm emission, Spectra Max Gemini XLS, Molecular Devices) (Blanc et al., 1997). Values were expressed as percentages of the fluorescence in control cultures.

2.5. Measurement of lipid peroxidation

The fluorescence of thiobarbituric acid reactive substances was used as a measure of membrane lipoperoxidation. This test estimates the level of malonyl dialdehyde precursors (Blanc et al., 1997).

The level of lipoperoxidation was estimated following a method described previously (Boland et al., 2000). Briefly, after 16 h of incubation, hippocampal or cortical cells (50 mm dishes) were washed with ice-cold PBS supplemented with 0.5 mM dithiothreitol. Cells were scraped and the samples were sonicated; aliquots were removed for protein determination. Trichloroacetic acid (5%) and the thiobarbituric acid reactive substances reagent were added. The solution was incubated at 95 °C for 30 min. After cooling, isobutanol was added, the samples were vigorously mixed then centrifuged at 1800 rpm for 10 min. The fluorescence of the upper organic phase was quantified using a plate reader (544 nm excitation and 590 nm emission, Spectra Max Gemini XLS, Molecular Devices). Values of thiobarbituric acid reactive substances fluorescence were expressed as the percentage of the fluorescence level in control cultures.

2.6. Drugs

Pirlindole, brofaromine and moclobemide were gifts from Therabel research, Brussels, Belgium; Novartis, Brussels, Belgium and Roche, Brussels, Belgium, respectively. Dehydropirlindole was a generous gift from Prof. Delarge, Laboratory of Medicinal Chemistry, University of Liège. Trolox and deprenyl were purchased from Sigma.

2.7. Data analysis

All data are presented as means and S.E.M. values.

Curve fitting was carried out using SigmaPlot© 2.0 software and the standard equation:

$$E = E_{max}/[1 + (EC_{50}/x)^h],$$

where x is the concentration of drug and h is the Hill coefficient.

For experiments involving multiple treatment conditions, statistical comparisons were based on the analysis of variance (ANOVA) followed by Dunn's post-hoc test for pairwise comparisons. For these comparisons, the sodium nitroprusside group served as the reference. Student's t-test was used for data analysis in experiments involving comparisons between two types of treatments.

3. Results

3.1. Protective effect of pirlindole, dehydropirlindole and trolox

Hippocampal cells exposed to the NO-induced oxidative stress were significantly protected in a concentration-dependent manner by pirlindole and dehydropirlindole as shown in Fig. 1A. A significant decrease in cell mortality was observed from the 5-μM concentration for both pirlindole and dehydropirlindole. The maximal protective effect was reached at 50 and 20 μM for pirlindole (88% protection) and dehydropirlindole (100% protection), respectively. Calculated EC_{50}'s were 7.1 ± 1.2 μM for pirlindole and 3.3 ± 0.9 μM for dehydropirlindole; respective Hill coefficients were 1.14 ± 0.07 and 1.22 ± 0.08. We observed a decrease in cell protection with 100-μM pirlindole. This was due to its toxicity at high concentrations, as shown previously (Boland et al., 2002b). We performed similar experiments with brofaromine and moclobemide, two monoamine oxidase type-A inhibitors and deprenyl, a monoamine oxidase type-B inhibitor that is known to be neuroprotective in some models. Fig. 1B shows that none of these drugs was able to protect the cells exposed to sodium nitroprusside.

In order to compare the potency of pirlindole and dehydropirlindole to that of a typical free radical scavenger acting as a stoichiometric antioxidant, we assessed the effect of trolox under the same experimental conditions. As shown in Fig. 1A, trolox protected cells in a concentration-dependent manner starting at 10 μM, with a maximal effect at 100 μM (100% protection). Its EC_{50} was 17.4 ± 2.7 μM. This value is significantly higher than the values for pirlindole ($P < 0.05$) and dehydropirlindole ($P < 0.05$). The Hill coefficient for trolox was 1.40 ± 0.05.

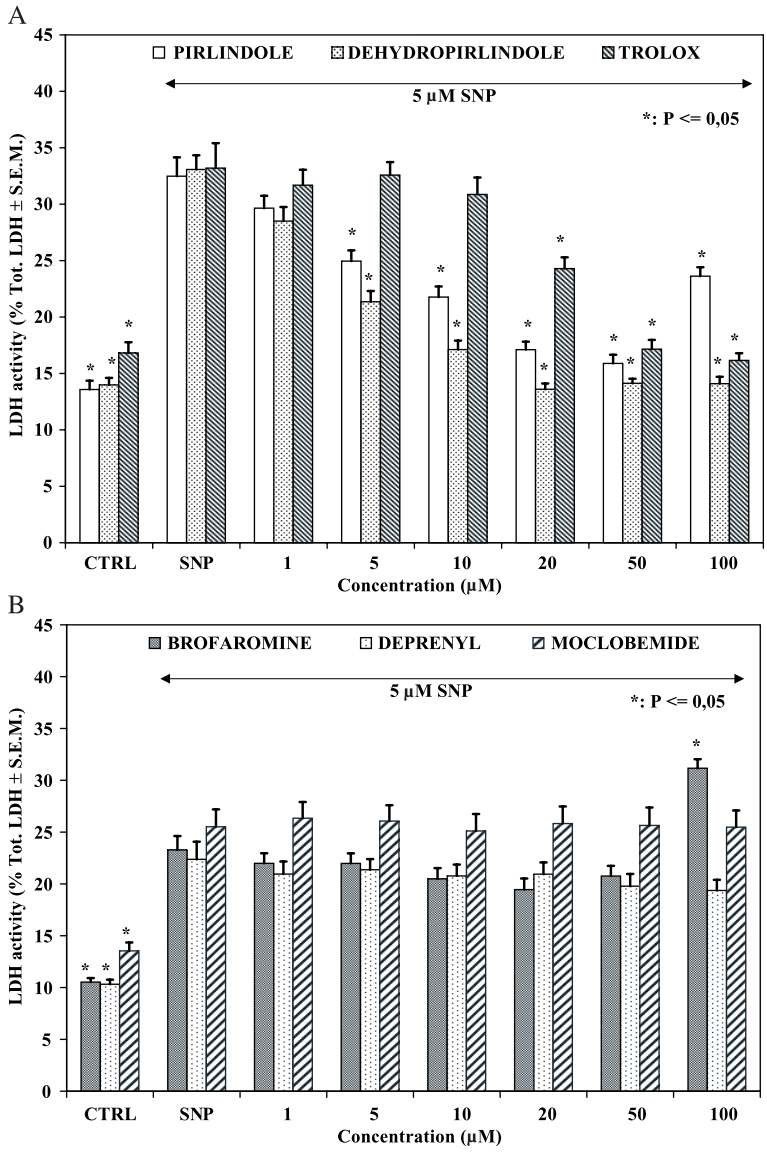

Fig. 1. (A) Protective effect of pirlindole, dehydropirlindole and trolox. Cell death assessment [lactate dehydrogenase (LDH)] of cultured hippocampal cells exposed to 5-µM sodium nitroprusside (SNP) in the absence or in the presence of various concentrations of each compound for 16 h. Number of wells: 15–29. (B) Lack of protective effect of moclobemide, brofaromine and deprenyl. Same protocol as in (A). Number of wells: 15–24.

3.2. Production of intracellular peroxides (DCF assay)

In order to assess whether monoamine oxidase inhibitors or trolox could regulate the intracellular production of peroxides, we performed DCF assays. After 3 h of preincubation in the presence of pirlindole, dehydropirlindole, trolox, moclobemide, brofaromine or deprenyl at a concentration of 50 µM, hippocampal cells were exposed to 5 µM sodium nitroprusside for an 8-h incubation; DCF measurements were then performed. Results are summarized in Fig. 2A. The addition of sodium nitroprusside to the incubating medium induced a marked increase (2.3 times, $P<0.05$) in the production of intracellular peroxides. When drugs were used in combination with sodium nitroprusside, only pirlindole, dehydropirlindole and trolox induced a significant effect on the endoperoxide production. Indeed, trolox inhibited the production of intracellular peroxides by 89%. However, the inhibition was significantly less marked for pirlindole and dehydropirlindole (49% and 54%, respectively) than for trolox ($P<0.05$). Moclobemide, brofaromine and deprenyl did not significantly modify the intracellular peroxide production (Fig. 2A).

3.3. Modulation of lipoperoxidation

We determined the effect of the various drugs, tested at 50 µM, on lipoperoxidation when hippocampal or cortical cells were incubated in the presence of sodium nitroprusside for 16 h.

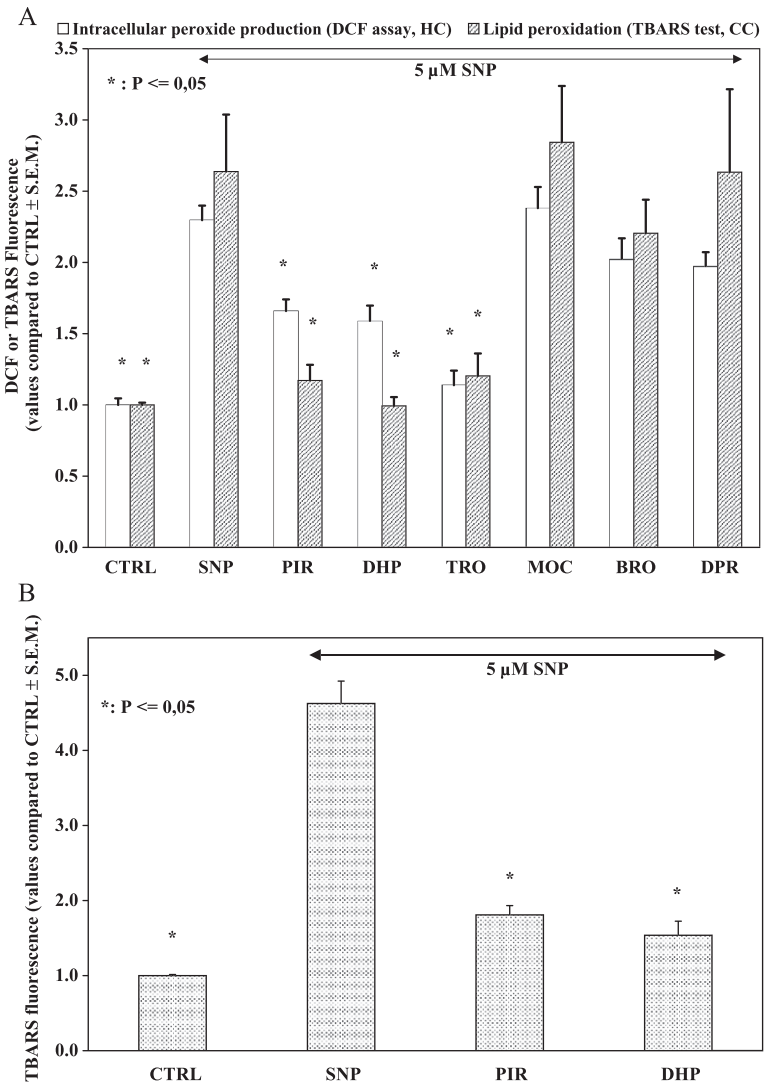

Fig. 2. (A) Measurement of intracellular peroxide production [DCF assay, hippocampal cells (HC)] and lipoperoxidation [thiobarbituric acid reactive substances (TBARS) test, cortical cells (CC)] on cells exposed to 5 µM sodium nitroprusside (SNP) in the presence or in the absence of monoamine oxidase inhibitors (50 µM) or trolox (50 µM) for either 8 h (DCF) or 16 h (TBARS). Number of wells (DCF) = 26–54, number of experiments (TBARS) = 4–7. Abbreviations: PIR: pirlindole, DHP: dehydropirlindole, TRO: trolox, MOC moclobemide, BRO brofaromine, DPR deprenyl. (B) Measurement of lipoperoxidation in hippocampal cells (TBARS test) exposed to 5 µM sodium nitroprusside (SNP) in the presence or in the absence of monoamine oxidase inhibitors (50 µM) during 16 h. Number of experiments = 5. Abbreviations: as in (A).

As shown in Fig. 2A, incubation of cortical cultures in the presence of NO induced a significant increase in lipoperoxidation (2.64 fold increase, $P < 0.05$). Pirlindole, dehydropirlindole and trolox completely blocked the NO-induced lipoperoxidation ($P < 0.05$) while the other drugs were not effective.

In order to determine whether the above reagents were also efficient in hippocampal cells, we performed the same experiments with these cells, using pirlindole and dehydropirlindole (Fig. 2B). Under these conditions, sodium nitroprusside induced a significant increase of the lipoperoxidation (4.63 fold, $P < 0.05$) which was 1.75 times higher than the one observed with cortical cells. Nevertheless, as in cortical cells, lipoperoxidation was markedly reduced by pirlindole and dehydropirlindole ($P < 0.05$). Because of the similarity of the effects of both drugs in the two structures, further experiments concerning assessment of lipoperoxidation were performed using cortical cells in order to reduce the number of animals utilized in the experiments.

3.4. Post-hoc treatment with pirlindole or dehydropirlindole improves cell survival

Because brain infarction can usually be treated only several hours after its onset, it was important to examine whether pirlindole and dehydropirlindole still have a protective effect when they are used after the induction of the oxidative stress. Therefore, we exposed cultured nerve cells

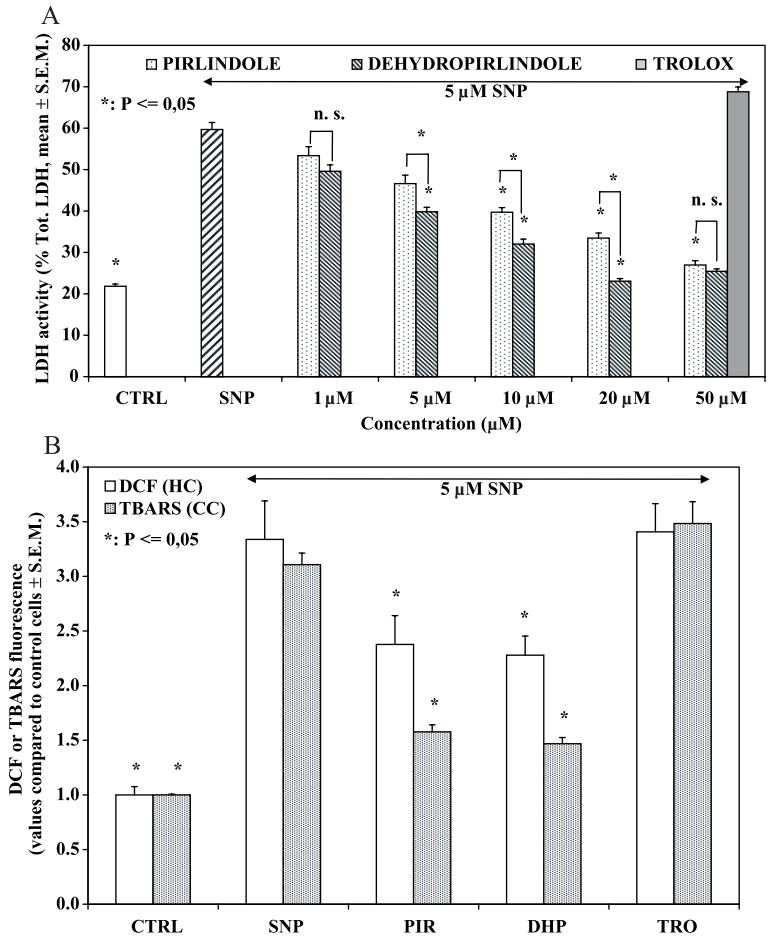

Fig. 3. (A) Pirlindole and dehydropirlindole, but not trolox, are protective when applied after the induction of the oxidative stress. Cell death estimation [lactate dehydrogenase (LDH), hippocampal cells]: cells were exposed to 5 µM sodium nitroprusside (SNP) for 3 h followed by the addition of the monoamine oxidase inhibitors (1–50 µM) or trolox (50 µM) during 16 h. Number of wells: 13–44. (B) Pirlindole and dehydropirlindole, but not trolox, decrease intracellular peroxide production [DCF assay, hippocampal cells (HC)] and lipoperoxidation [thiobarbituric acid reactive substances (TBARS) test, cortical cells (CC)] in a 3-h post-hoc treatment model: cells were exposed to 5 µM sodium nitroprusside (SNP) for 3 h, followed by the addition of the monoamine oxidase inhibitors (50 µM) or trolox (50 µM) for either 5 (DCF) or 16 h (TBARS). Number of wells: DCF: 18–23; number of experiments: TBARS: 15–16. Abbreviations: as in Fig. 2A.

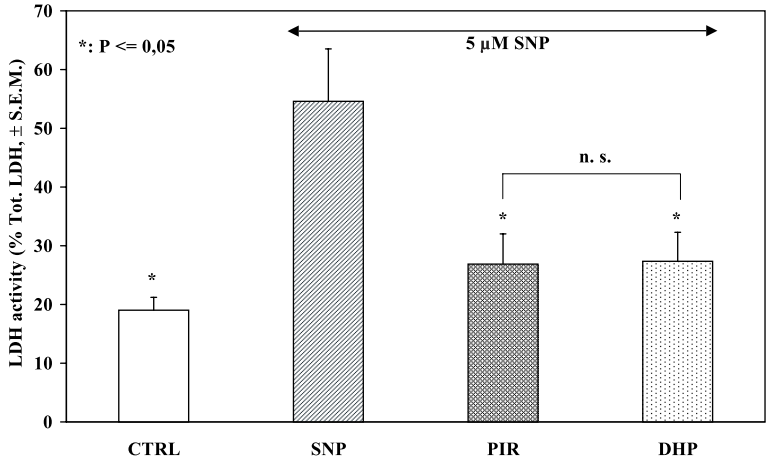

Fig. 4. Pirlindole and dehydropirlindole are protective when applied 6 h after the induction of the oxidative stress. Cell death estimation [lactate dehydrogenase (LDH)]: cells were exposed to 5 µM sodium nitroprusside (SNP) for 6 h followed by the addition of the drugs (50 µM) for 16 h. Number of wells: 18–19.

(hippocampal cells: LDH and DCF experiments-cortical cells: measurement of the fluorescence of thiobarbituric acid reactive substances) to sodium nitroprusside (5 μM) for 3 h before adding pirlindole, dehydropirlindole or trolox.

As shown in Fig. 3A, only pirlindole and its dehydroderivative were able to significantly protect ($P<0.05$) hippocampal cells from the NO-induced oxidative stress under these conditions. The LDH experiments showed that the protection was concentration-dependent and almost complete: 88% and 91% protection; the EC_{50}'s were 8.7 and 3.8 μM for pirlindole and dehydropirlindole, respectively. Dehydropirlindole was significantly more potent than pirlindole (Fig. 3A).

Similarly, only pirlindole and dehydropirlindole induced a significant decrease in the production of both peroxides (45%, $P<0.05$, DCF measurements) and lipoperoxidation (75%, $P<0.05$, measurements of thiobarbituric acid reactive substances) (Fig. 3B).

Trolox was not able to protect cells from the NO-induced aggression in any of the post-treatment experiments.

Because end stages or cell disintegration can begin from 6 h to several days after the ischemic insult (Lipton, 1999), we performed experiments in which pirlindole or dehydropirlindole was added to the hippocampal cells 6 h after sodium nitroprusside. After a 16-h incubation, cell death was estimated by measuring LDH activity. The results of these experiments are summarized in Fig. 4. Even with such a long delay, both pirlindole and dehydropirlindole (50 μM) still increased cell survival by 78% and 77%, respectively ($P<0.05$).

4. Discussion

Our experiments showed that, among the drugs tested, only pirlindole, dehydropirlindole and trolox were able to protect cultured hippocampal cells against a NO-induced cell death when these compounds were used before induction of the oxidative stress. However, when they were added 3 or 6 h after the induction of the oxidation, only pirlindole and dehydropirlindole were protective. Moreover, it appeared that their protective effect was related to their ability to decrease the production of both intracellular and lipid peroxides.

These results are similar to those obtained in a previous study with iron as the toxic substance (Boland et al., 2002b). We had shown in that study that the protection against iron was unrelated to monoamine oxidase type-A inhibition because, as in the present study, moclobemide and brofaromine were not protective despite their effective inhibition of the enzyme (Boland et al., 2002b). Taken together these results support the fact that the cell survival improvement provided by pirlindole and its dehydroderivative against an oxidative stress is not directly related to the inhibition of the enzyme. Hence, it can be hypothesized that these substances act as either stoichiometric antioxidants (such as vitamin E) or chelators (see below).

On the other hand, this protective effect of pirlindole and dehydropirlindole is pharmacologically relevant because the EC_{50}'s values for the protective effect were close to the EC_{50}'s for monoamine oxidase type-A inhibition obtained in the same model (2 μM) (Boland et al., 2002b).

Intracellular peroxide production (DCF assay) data showed a significant decrease in NO-induced endoperoxide production by pirlindole, dehydropirlindole and trolox. Trolox was the most powerful for restricting endoperoxide production (89% decrease) while the two monoamine oxidase type-A inhibitors decreased this production by 50%. Our previous data concerning protection against iron-induced cell death by pirlindole, dehydropirlindole and trolox were similar: we observed a decrease of the intracellular peroxide production by 42%, 46% and 72%, respectively.

The presence of intracellular peroxides can induce lipid peroxidation. Therefore, we determined the effect of the different substances on lipoperoxidation (thiobarbituric acid reactive substances test). We found that only pirlindole, dehydropirlindole and trolox significantly limited NO-induced lipid peroxide production. The ability of these three compounds to decrease lipoperoxidation was similar and allowed an almost complete blockade of lipid peroxidation. These data are consistent with those of our previous study showing that the same compounds reduce significantly iron-induced lipid peroxidation. However, in our previous study, the inhibition of lipoperoxidation was not complete (a mean of 30% for pirlindole and dehydropirlindole), perhaps because iron-induced oxidative stress is more severe than that induced by sodium nitroprusside.

We compared the protective effect of pirlindole and dehydropirlindole to that of the analog of vitamin E, trolox. We showed that the EC_{50} of trolox was 2.5–5.5 times higher than that of pirlindole or dehydropirlindole, respectively. It appears, therefore, that trolox was less efficient than these compounds to protect cells exposed to NO oxidative stress. Interestingly, the EC_{50} obtained for trolox in the present model was close to that obtained when cells were incubated in the presence of 2-μM iron (19 μM) (Boland et al., 2002b).

The monoamine oxidase type-B inhibitor, deprenyl, was also tested. We found that deprenyl was unable to afford any protection to the cells. However, Maruyama et al. showed a protective effect of deprenyl (20 μM) on dopaminergic neuroblastoma cells SH-SY5Y exposed to 3-Morpholinosydnonimine (SIN-1) or (±)-(*E*)-4-ethyl-2-[(*Z*)-hydroxyimino]-5-nitro-3-hexen-1-yl-nicotinamide (NOR-4), a peroxynitrite donor and a NO donor, respectively (Maruyama et al., 1998, Maruyama and Naoi, 1999). This difference between results might be linked to the experimental models used. More recently, it has been shown that rasagiline, a selective inhibitor of monoamine oxidase type-B exerts protection against various types of neurotoxic

stress by mechanisms unrelated to monoamine oxidase type-B inhibition, namely by preventing the fall in mitochondrial membrane potential (the first step of cell death) or by increasing the activity of antioxidant enzymes (Youdim et al., 2001a,b; Youdim and Weinstock, 2001).

Perhaps the most significant finding of our study was the ability of pirlindole and dehydropirlindole to protect cultured brain cells (improvement of cell survival, decrease in lipoperoxide and endoperoxide production) against NO-induced oxidative stress when they are used in a post-hoc treatment (3 and 6 h post-treatment), while trolox is ineffective under these experimental conditions. However, they are not the only compounds to be protective on a post-hoc use. Indeed, various groups have shown, using various experimental models, that some antagonists of NMDA receptors (Dizocilpine: MK-801, AHN649: a 3-amino analog of dextromethorphan), basic Fibroblast Growth Factor (bFGF), vitamin B3 (nicotinamide), taurine, resveratrol and tirilazad mesylate are protective against diverse aggressions such as hypoxia-ischemia, hydrogen peroxide, NO or peroxinitrite (Hagberg et al., 1994; Fici et al., 1996; Pringle et al., 1997; Lock et al., 1997; Ay et al., 1999; Mark et al., 1999; Tortella et al., 1999; Bastianetto et al., 2000; Sakakibara et al., 2000; Kearns and Dawson, 2000; Yuan et al., 2001). However, to our knowledge, this is the first demonstration that post-treatment with pirlindole and dehydropirlindole protects cultured brain cells against NO-induced death.

It seems clear that the protective effect of pirlindole and dehydropirlindole is, at least in part, linked to the fact that they reduce or block both intracellular peroxide production and lipid peroxide formation. However, because pirlindole and dehydropirlindole are protective post-hoc, whereas trolox is not, we suggest that the mechanisms involved in the protection by these substances are different. This is particularly true for the post-hoc treatment experiments.

On the one hand, trolox probably acts as a stoichiometric scavenger, each molecule of trolox being able to react with one molecule of free radical. This may explain the fact that its protective effect is obtained at rather high concentrations and only when a 3-h pre-treatment period is used.

On the other hand, pirlindole and dehydropirlindole may have the following effects: Firstly, they may act as chelators of free radicals, each molecule being able to interact with many molecules of free radicals, thereby blocking their massive production. Secondly, they may repair the damage produced downstream of the free radical production. Thirdly, they may trigger the induction of protective biochemical pathways. The later two mechanisms are reminiscent of what has been suggested for rasagiline (Youdim and Weinstock, 2001). Clearly, additional studies need to be performed to test these hypotheses.

It has been shown recently that the expression of NOS is rapidly increased in the early stage of permanent ischemia (Leker et al., 2001). When ischemia experiments are performed, the number of neuronal NOS-immunoreactive neurons rises markedly during the first minutes of the experiment and this persists for hours (Holtz et al., 2001). In addition, some models have been used to show the importance of modulating either the expression of NOS or the production of NO during or after ischemia. Indeed, delayed cell death can be reduced by the use of NOS inhibitors (N_{ω}-methyl-L-arginine: L-NMMA, N_{ω}-nitro-L-arginine methyl ester: L-NAME) administered either intraventricularly or intraperitoneally to rodents (Kohno et al., 1995, 1997; Forman et al., 1998; Nanri et al., 1998; Rodrigo et al., 2001). Such an approach cannot be used in humans because of its invasiveness. Because several monoamine oxidase inhibitors are currently used in therapeutics, our study suggests a new strategy that may be effective to reduce NO-induced damage of ischemia–reperfusion in the central nervous system.

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