Antioxidant Effects of 1,4-Dihydropyridine and Nitroso Aryl Derivatives on the Fe^{+3}/Ascorbate-Stimulated Lipid Peroxidation in Rat Brain Slices

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ABSTRACT. 1. Lipid peroxidation in rat brain slices was induced by Fe^{+3}/ascorbate.

2. Brain lipid peroxidation, as measured by malondialdehyde formation, was inhibited by all the tested nitro aryl 1,4-dihydropyridine derivatives over a wide range of concentrations. The time-course antioxidant effects of the most representative agents were assessed. On the basis of both time-course and IC_{50} experiments the tentative order of antioxidant activity on rat brain slices could be: nicardipine>nisoldipine>(*R,S/S,R*)-furnidipine>(*R,R/S,S*)-furnidipine>nitrendipine>nimodipine>nifedipine.

3. 1,4-Dihydropyridine derivatives that lack of a nitro group in the molecule (isradipine, amlodipine) also inhibited lipid peroxidation in rat brain slices but at higher concentrations than that of nitro-substituted derivatives.

4. All the tested nitroso aryl derivatives [2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester (NTP), nitrosotoluene, nitrosobenzene] were more potent inhibitors of lipid peroxidation than were the parent nitro compounds. In conclusion, on the basis of the IC_{50} values determined, the rank order of antioxidant potency for these derivatives can be established as: ortho-nitrosotoluene>NTP>nitrosobenzene.

KEY WORDS. Nitroaryl 1,4-dihydropyridines, nitroso derivatives, lipoperoxidation, antioxidant

INTRODUCTION

Lipid peroxidation has been invoked as a mechanism for neuropathological damage in both aging and neurodegenerative and other neuropsychiatric diseases (Floyd *et al.*, 1984; Halliwell *et al.*, 1984; Jenner, 1994; Olanow, 1993).

Oxidative damage to membrane lipids has been associated with brain ischemia (Flynn *et al.*, 1989; Rechrona *et al.*, 1982). The brain is a preferential target for the peroxidative process because it has a high content of polyunsaturated fatty acids (Halliwell *et al.*, 1984). Iron, hydrogen peroxide and ascorbate in combination or alone have been used in *in vitro* studies to stimulate lipid peroxidation in brain-tissue preparations (Andorn *et al.*, 1996; Gonçalves *et al.*, 1991). Fenton-type reactions, taking place in the reperfusion period (Rechrona *et al.*, 1982), have been implicated in the oxidative process. During this period, an increase in free radicals, leading to membrane lipid peroxidation, has been reported (Yoshida *et al.*, 1985).

Calcium antagonists are beneficial when given prior to experimentally induced ischemia, but not when given only in reperfusion (Ferrari *et al.*, 1993). Protective effects observed with nisoldipine do not require preischemic cardiodepression (Ferrari *et al.*, 1993), indicating that blockade of myocardial calcium channels is not require for this effect. Calcium antagonists also reduce oxidative injury in isolated membrane vesicles (Janero and Burghardt, 1989) and endothelial cells (Mak *et al.*, 1992). In addition, calcium channel blockers that inhibit the calcium influx are used in the pharmacological management of ischemia (Weiner, 1988).

Studies on cardiac (Janero and Burghardt, 1988) and liver (Engineer and Sridhar, 1989) membranes point to an antioxidant protective effect of these drugs that may contribute to their pharmacological activity. However, the biochemical basis of these membrane protective effects is not clear—namely, membranes from various brain areas have different susceptibilities to peroxidation (Zaleska and Floyd, 1985).

Nifedipine is unstable under daylight conditions (Squella *et al.*, 1989, 1990) and converts photochemically into 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylic acid dimethyl-ester (NTP). Other studies found that nifedipine had lower antioxidant potency than did NTP in phosphatidylcholine liposomes (Misik *et al.*, 1991; Ondrias *et al.*, 1989). The nitroso group of NTP reacts with unsaturated lipid, thus forming stable nitroxide radicals covalently attached to membrane lipids (Misik *et al.*, 1991; Ondrias *et al.*, 1989).

Taking into account the aforediscussed results, we have compared the antioxidant properties of NTP with other nitroso compounds, such as nitrosobenzene and ortho-nitrosotoluene. In conclusion, in this paper, we have assessed 1,4-dihydropyridine and some related nitroso aryl derivatives for their ability to prevent lipid peroxidation in rat brain slices in a nonenzymatic active oxygen-generating system.

MATERIALS AND METHODS

Chemicals

Nifedipine, nimodipine, nisoldipine and nitrendipine were from Chile Laboratories (Santiago, Chile); nicardipine was from Pharma Investi Laboratories (Santiago, Chile); (*R*,*R*/*S*,*S*)- and (*R*,*S*/*S*,*R*)-furnidipine enantiomers were from Alter Laboratories (Madrid, Spain). Nitrobenzene and nitrosobenzene were purchased from Merck; nitrosotoluene was synthesized according to a procedure previously reported by Varghese and Withmore (1983); and ascorbic acid and thiobarbituric acid were purchased from Sigma.

Nifedipine irradiation

An ethanol solution of pure nifedipine (10 mM) was irradiated with a 500 W UV lamp (360 nm) for 4 hr. Solution was evaporated to dryness *in vacuo*: and the residue was dissolved in chloroform and evaporated nearly to dryness. This product was assayed by HPLC (Waters chromatograph model 600, pulsed electrochemical detector model 464, photodiode array detector model 996) and GC-MS (Hewlett-Packard). Results from these analyses permit us to conclude that, under our irradiation conditions, nifedipine photodecomposed into one product, which was identified as NTP.

Tissue preparation

Rat brain slices were obtained from Wistar rats, by using a method previously described by Hajos *et al.* (1986). The slices were cut 0.4-mm thick and incubated at 37°C in a Krebs solution of the following composition (mM): NaCl (110), KCl (2), $CaCl_2$ (1), $NaHCO_3$ (25), $MgSO_4$ (1.19), KH_2PO_4 (1.18), glucose (10), sodium succinate (5) and TRIS-HCl (10) buffer, pH 7.4, continuously gassed with a mixture of 95% O_2/5% CO_2.

Tissue viability

1. Measurements of respiration: Tissue respiration was measured by incubating rat brain slices (2 mg protein/ml) in a 1.8-ml thermostatically controlled (37°C) and stirred chamber fitted with oxygen electrode (YSI oxygraph model 50, electrode Clark type). The chamber was filled with freshly gassed modified Krebs-Henseleit Buffer, pH 7.4. The rate of oxygen consumption by the rat brain slices was 109.3±12.5 *n*-at-gr O/min. In the presence of sodium azide (1 mM), the O_2 consumption was stopped.
2. Lactate dehydrogenase (LDH) release: Tissue injury was assessed by measuring the release of LDH from rat brain slices according to Bergmeyer and Bernt (1974). Measurements of LDH release in rat brain slices at zero time and after 30 min of incubation in the absence of Fe^{+3}/ascorbate were carried out.

The results from these experiments did not show significant differences in the release of LDH at these times.

Lipid peroxidation

Lipid peroxidation was assessed by measuring TBARS. The TBARS assay was performed by modifying a previously described method (Andorn *et al.*, 1988). Samples were prepared by placing 2.0±0.3 mg of protein by per milliliter of rat brain slices in a final volume of 2 ml with or without Fe^{+3}/ascorbate and other agents and incubating for 30 min at 37°C. The reaction was started by the addition of 12 μM Fe^{+3}/500 μM ascorbate. After 30 min of incubation at 37°C, the reaction was stopped by the addition of 1 ml trichloracetic acid at 20% TA. Then, 1 ml of thiobarbituric acid reagent (1%) was added to the mixture and heated in a boiling water bath for 15 min. The mixture was allowed to cool and then centrifuged at 1,700*g* for 20 min. Malondialdehyde values in the supernatant were calculated by using a molar extinction coefficient of 1.56×10^5/M cm at 532 nm. To minimize the possible contribution of iron thiobarbiturate complexes (Zhou and Sorenson, 1997) to the absorbance, reference cuvettes were prepared by adding Fe^{+3}/ascorbate at the same concentrations as the samples and incubating for 30 min at 37°C; then 1 ml of theobarbituric acid reagent (1%) was added to the mixture and heated in a boiling water bath for 15 min.

Time course of the antioxidant effect in rat brain slices

Nisoldipine was added at 5, 15, and 30 μM concentrations, and the antioxidant effect was assessed at different times (0, 5, 10, 20 and 30 min).

Some of the agents were assayed for comparative purposes at a fixed concentration of 20 μM, and their antioxidant effects were assessed at the same times as those of nisoldipine. Lipid peroxidation for both types of experiments was assayed in the same manner as heretofore described.

Determination of n-Octanol-water apparent partition coefficients (P)

Both *n*-octanol and water were presaturated with each other for at least 24 hr before the experiment. Volumes of 5 ml of each phase, with an initial 0.02 M in the octanol phase, were placed in 30-ml stoppered tubes and mixed for 24 hr at 37°C. Only the aqueous phase was analyzed for nitroso derivative content by UV–visual spectrophotometry at the following maxima: λ_{NTP}=274 nm, λ_{NB}=306 nm, $\lambda_{o\text{-}NT}$=314 nm, after separating each phase by centrifugation at 2000*g* for 15 min at 37°C. The concentrations in octanol were calculated by the differences between them and the total concentration. *P* values were measured in replicates of four.

Statistical analysis

Results are reported as mean±SEM values unless otherwise stated. The significance of differences between mean values was determined by two-tailed Student's *t*-test.

RESULTS

Lipid peroxidation in the presence of Fe^{+3}/ascorbate in rat brain slices was dependent on both incubation time (Fig. 1A) and protein concentration (Fig. 1B). Because values of steady-state TBARS were achieved in 30 min of incubation at 37°C, and 2.0 mg of protein/ml assay was on the linear part of the protein concentration versus TBARS curve for each sample, these conditions were selected for all of the study.

Stimulated lipid peroxidation (Fe^{+3}/ascorbate) was determined from rat brain slices in the presence of different agents. In Figure 2, typical concentration–effect curves for representative nitro aryl 1, 4-dihydropyridine derivatives are shown. As can be seen in Figure 2 and Table 1, all the tested compounds produced a concentration-dependent inhibitory effect on the lipid peroxidation initiated by the Fe^{+3}/ascorbate system. From Table 1, it is evident that nicardipine was the most potent antioxidant drug with an IC_{50} of 13.1 μM. On the other hand, the weakest drug was nifedipine, with an IC_{50} higher than 100 μM. It is worth noting that there is a significant difference between the enantiomers of furnidipine [1.57-fold (*R*,*S*/*S*,*R*)-

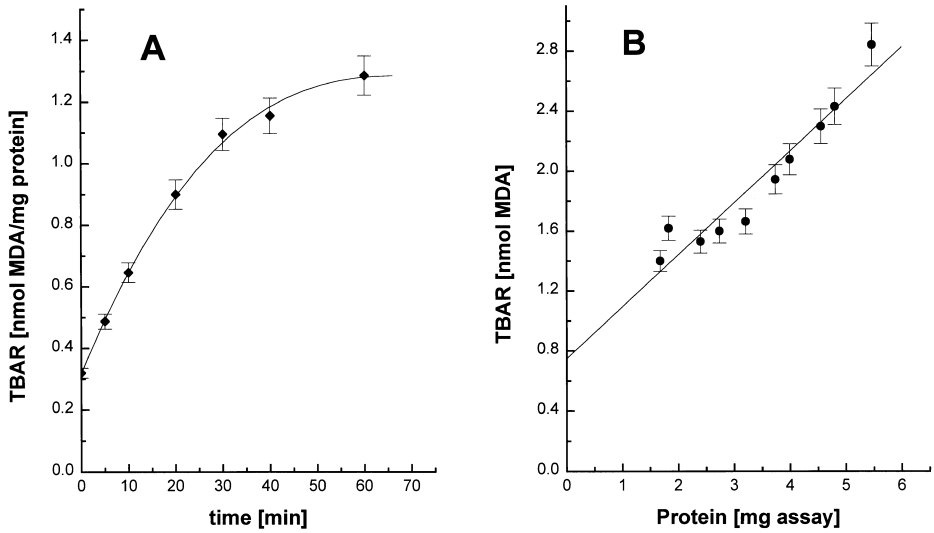

FIGURE 1. Dependence of Fe^{+3}/ascorbate-stimulated lipid peroxidation on both protein concentration and incubation time in rat brain slices. (A) The incubation time (at 37°C) was varied, using a protein concentration of 2 mg/ml in the TBARS assay (see Material and Methods). The data shown are mean±SEM values, n=4. (B) The protein concentration in the TBARS assay was varied, using an incubation time of 30 min at 37°C.

enantiomer versus (*R*,*R*/*S*,*S*)-enantiomer], indicating some degree of selectivity in the antioxidant effect of these compounds.

Figure 3A shows the time course of the antioxidant effect of nisoldipine at different concentrations. The effects were concentration dependent and, at 30 μM, this drug exhibited an efficient inhibitor character, retarding the onset of lipoperoxidation. Figure 3B shows the time course of the antioxidant effect of some 1,4-dihydropyridines at a 20 μM concentration. This concentration enables the observation of the antioxidant effect of all the derivatives studied. From these experiments, it can be concluded that nisoldipine and nicardipine were the most effective antioxidant agents, both producing a delay in the onset of the lipoperoxidation. The results from these experiments were consistent with those previously observed with 30 μM nisoldipine (Fig. 3A). In conclusion, these experiments confirmed the relative order of potency previously found from the concentration–effect curves.

The intrinsic activity was compared in Table 1. Nisoldipine had the maximum value, because it displayed 100% antioxidant effect. In contrast, nifedipine exhibited the lowest value for intrinsic activity (48% activity). Effects of nitro aryl derivatives on malondialdehyde (MDA) formation at a 25 μM concentration indicate that both nisoldipine and nicardipine exhibited the highest cytoprotective effects (approximately 70% inhibition), nimodipine being the weakest drug (25.3% inhibition). Moreover, we also tested other 1,4-dihydropyridines at this concentration, such as isradipine, amlodipine and felodipine. In this condition, only isradipine (12% inhibition) and amlodipine (25% inhibition) exerted antioxidant effects, felodipine being inactive. Other assayed calcium channel antagonists (i.e., verapamil and diltiazem) completely lacked inhibitory effects on lipid peroxidation.

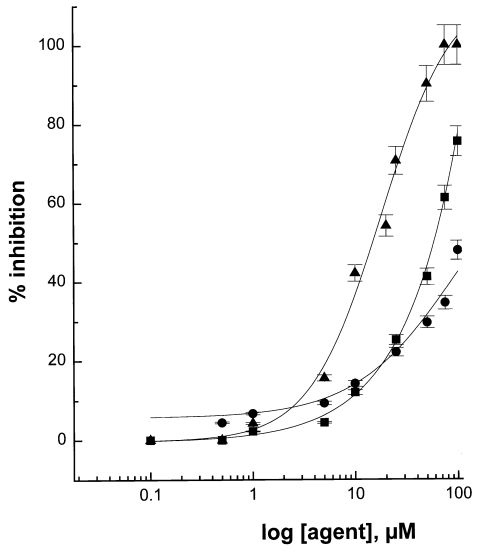

FIGURE 2. Concentration–effect curves for inhibition of Fe^{+3}/ascorbate-stimulated lipid peroxidation in rat brain slices by representative nitro aryl 1,4-dihydropyridines: (▲) nisoldipine, (■) nimodipine, (●) nifedipine.

The effect of Ca^{+2} channel blockers on the peroxidative process, measured by the production of MDA, was tested under different incubation conditions, so that any artifact from the TBA reaction could be ruled out. Three types of incubation experiments were performed: (1) a preincubation of 5, 10 and 15 min with the 1,4-dihydropyridine was followed by the addition of 0.5 mM ascorbic acid and 12 μM Fe^{+3} (2) the drug and the prooxidant mixture were added simultaneously to the incubation medium and (3) the prooxidant mixture was present from the start of the reaction and the drug was added in the last 5, 15 and 25 min. For this type of experiment, nisoldipine at a 50 μM concentration was selected. We found that addition of nisoldipine at different times (5, 10, 15 min) prior to generation of free radicals did not showed significant differences in the magnitude of the antioxidant effect exerted by this drug. In Figure 4, the incubation of nisoldipine at different times is shown. As can be seen, the addition of nisoldipine after free radical generation resulted in various degrees of significant protection. Thus, at 5 min of incubation, a 26.7% antioxidant effect is achieved. However, at 25 min of incubation, the effect increased to 72.5%.

As has been reported by us (Núñez-Vergara *et al.*, 1994; Squella *et al.*, 1989, 1990; Zanocco *et al.*, 1992) and other laboratories (Hayase *et al.*, 1994; Majeed *et al.*, 1987; Misik *et al.*, 1991) 1,4-dihydropyridines are unstable under daylight and UV light; thus we selected nifedipine to perform photodegradation studies. After 4 hr of irradiation [either artificial daylight or UV light (360 nm)], only one product identified as NTP was obtained. Additionally, for comparative purposes, we synthesized ortho-nitrosotoluene (see Materials and Methods). Comparisons of their antioxidant properties are shown in Figure 5, in which concentration–effect curves of two ni-

TABLE 1. Antioxidant effects of 1,4-dihydropyridine derivatives on the Fe^{+3}/ascorbate-stimulated lipid peroxidation in rat brain slices

1,4-DHP	IC_{50} (μM)	Intrinsic activity[a]	MDA (nmol/mg/ protein/30 min)[b]
o-Nitro-substituted derivatives			
Nisoldipine	14.8 ± 0.8	1.00	0.211 ± 0.007
R,S/S,R-furnidipine	26.2 ± 1.8	0.74	0.350 ± 0.009
R,R/S,S-furnidipine	41.2 ± 2.1	0.77	0.500 ± 0.011
Nifedipine	> 100	0.48	0.560 ± 0.010
m-Nitro-substituted derivatives			
Nicardipine	13.1 ± 0.9	0.75	0.221 ± 0.005
Nitrendipine	44.5 ± 1.5	0.77	0.560 ± 0.012
Nimodipine	59.0 ± 2.0	0.76	0.538 ± 0.010
Non-nitro-substituted derivatives			
Isradipine	> 100	0.33	0.650 ± 0.020
Amlodipine	> 100	0.40	0.500 ± 0.012
Felodipine	n.d.	—	0.720 ± 0.024

Abbreviations: 1,4-DHP, 1,4-dihydropyridine; MDA, malondialdehyde; n.d., not done.
[a] Calculated values considering their maxima effects.
[b] Values estimated at a 25 μM concentration of drug.
(Stimulated control = 0.720 ± 0.024 nmol MDA/mg protein/30 min).

troso aryl derivatives and their nitro parent compounds on Fe^{+3}/ascorbate-stimulated lipid peroxidation in rat brain slices are shown. From these curves, it is apparent that NTP derivatives displayed the maximum effect. In contrast, nitrosobenzene exerted only 56% inhibition. From these studies, the following relative antioxidant potencies can be established (Table 2): ortho-nitrosotoluene>NTP> nitrosobenzene. Studies with parent nitro compounds (i.e., nifedipine, *o*-nitrotoluene and nitrobenzene) showed that only nifedipine significantly inhibited lipoperoxidation (48%).

In conclusion, all nitro-substituted 1,4-dihydropyridine derivatives exhibited the most potent antioxidant activity on Fe^{+3}/ascorbate-stimulated lipid peroxidation in rat brain slices. Non-nitro-substituted 1, 4-dihydropyridines (i.e., isradipine, amlodipine and felodipine) were significantly less effective in their antioxidant effects (Table 1). On the other hand, the tested nitroso aryl derivatives, ortho-nitrosotoluene and NTP, showed more potent antioxidant effects than that of the parent nitro compounds.

DISCUSSION

In this study, we examined the antioxidant properties of a series of 1, 4-dihydropyridines and some nitroso aryl derivatives on Fe^{+3}/ascorbate-stimulated lipid peroxidation in rat brain slices. The addition of Fe^{+3}/ascorbate to rat brain slices increased lipid peroxidation by at least twofold over basal values at 30 min. In a recent paper (Andorn *et al.*, 1996), the addition of ascorbate (0.1 mM) was reported to increase lipid peroxidation by at least sixfold over basal values in membranes isolated from different regions of the human brain. The differences between these results may be due to the differences in the biological preparations (rat brain slices versus human

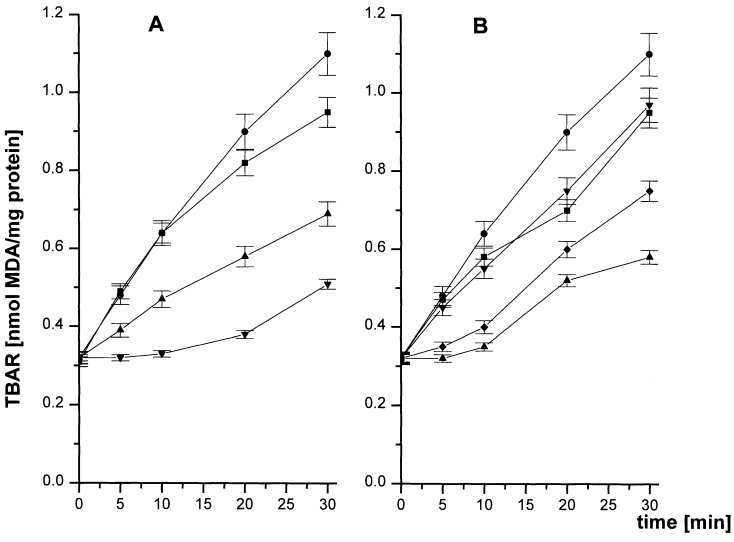

FIGURE 3. (A) Time course of formation of TBARS in the presence of different concentrations of nisoldipine: (●) control, (■) 5 μM, (▲) 15 μM, (▼) 30 μM. (B) Time course of formation of TBARS in the presence of different 1,4-dihydropyridine derivatives at a 20 μM concentration: (●) control, (▼) nifedipine, (■) nimodipine, (◆) nicardipine, (▲) nisoldipine.

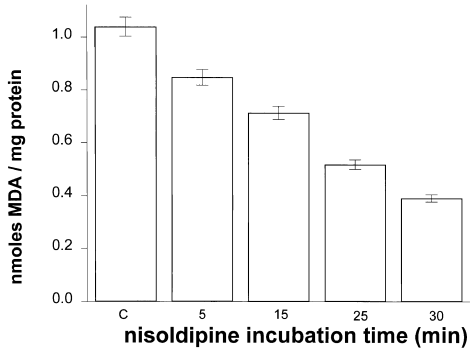

FIGURE 4. Dependence of MDA formation with incubation time of nisoldipine. All samples were incubated for 30 min with Fe^{+3}/ascorbate, nisoldipine being added to the system at different times.

brain membranes). We found that the presence either mannitol or thiourea, even at a 1 mM concentration, did not modify the levels of MDA produced by Fe^{+3}/ascorbate stimulation (data not shown). A similar results was described by Andorn *et al.* (1996) in membranes isolated from different regions of the human brain when the Fe^{+3}/ascorbate system was used for the induction of peroxidative damage. These authors demonstrated that hydroxyl radical or hydrogen peroxide were not implicated in the peroxidative damage, because mannitol or catalase failed to prevent lipid peroxidation. On the basis of our own results and these findings, it can be concluded that the mechanism by which Fe^{+3}/ascorbate promotes lipid peroxidation is mainly due to superoxide anion formation (Andorn *et al.*, 1996; Bendich *et al.*, 1986).

The results presented here demonstrate that members of the 1, 4-dihydropyridine class differ in their abilities to inhibit lipid peroxidation in rat brain slices. Amlodipine and isradipine exhibited weak antioxidant properties, felodipine being inactive (Table 1). Nitrendipine, nimodipine and nifedipine had an intermedial range of antiperoxidant activity. However, nisoldipine and nicardipine significantly reduced the lipid peroxidation initiated by the Fe^{+3}/ascorbate system in a concentration-dependent manner. In addition, the most potent antioxidant effects were obtained with nitro-substituted derivatives (i.e., nisoldipine and nicardipine) in contrast, the weakest derivatives (i.e., isradipine) corresponded to non-nitro-substituted derivatives. Therefore, apparently the inclusion of a nitro group in the molecule provides antiperoxidant properties, at least in our system. The aforedescribed results are in opposition to previously reported data (Janero and Burghardt, 1989). That study described the antiperoxidant activity of several dihydropyridines in myocardial-membrane phospholipids on lipid peroxidation stimulated by a system containing an Fe-ADP complex and an enzymatic reaction in which hypoxanthine/xantine oxidase generates superoxide anion formation. Those results show that nifedipine, nitrendipine and nimodipine were devoid of antioxidant activity. On the other hand, nisoldipine, nicardipine and felodipine significantly reduced the susceptibility of phospholipids to free radical attack in a concentration-dependent manner. It is worth noting that, in our study, felodipine was devoid of antiperoxidant activity. Nevertheless, nisoldipine and nicardipine also were the most potent derivatives, IC_{50} values being significantly lower in our study than in that of Janero and Burghardt (1989) (nicardipine 11.5-fold and nisoldipine 5.5-fold). On the basis of IC_{50} values, the following relative order of antioxidant potency can be established: nicardipine>nisoldipine>(*R*,*S*/*S*,*R*)-furnidipine>(*R*,*R*/*S*,*S*)-furnidipine>nitrendipine>nimodipine>nifedipine>non-nitro-substituted 1,4-dihydropyridine derivatives (isradipine, amlodipine, felodipine). Although the antiperoxidant IC_{50} values of the most potent derivatives were significantly greater than their IC_{50} values for the receptor-mediated depression of the slow action potential of heart-muscle cells (Triggle and Janis, 1987), these compounds can accumulate intracellularly to reach concentrations from several thousand- to several hundred thousandfold as high as the extracellular, aqueous concentrations (Chester *et al.*, 1987). To corroborate the relative order of antioxidant effectivity, time courses of lipoperoxidation experiments in the presence of the drugs were conducted. First, the time course of the antioxidant effect of one the most effective agents (nisoldipine) at different concentrations was assessed. Results from these experiments revealed that this drug, at a 30 μM concentration, delayed the onset of lipoperoxidation. At lower concentrations than its IC_{50}, this delay was absent. Probably, the latter result could be due to differences in drug partition between aqueous and lipid bilayer phases in the time scale of the experiments. Second, time courses of lipoperoxidation experiments in the presence of different drugs (nifedipine, nimodipine, nicardipine and nisoldipine), at a fixed 20 μM concentration, were conducted. Interestingly, the relative order of antioxidant potency at any time was not different from those obtained from concentration–effect curves; that is, nisoldipine and nicardipine were the most effective agents. On the other hand, we observed that both nisoldipine and nicardipine exhibited a retarding effect on the onset of lipoperoxidation that was similar (approximately 10 min). This time may be used to calculate the relative efficiencies of the drugs. Consistently, this result confirms that both nisoldipine and nicardipine were equipotent as antioxidants in this system. The other tested drugs did not show a delay in the onset of lipoperoxidation, probably owing to the fact that the concentrations used were lower than their IC_{50} values. However, differences in drug partition between aqueous and lipid bilayer phases in the time scale of the experiments cannot be discarded.

With the limited data available at present on the dihydropyridine partition coefficients, an absolute correlation cannot be made between antiperoxidant potency and lipophilicity. However, because

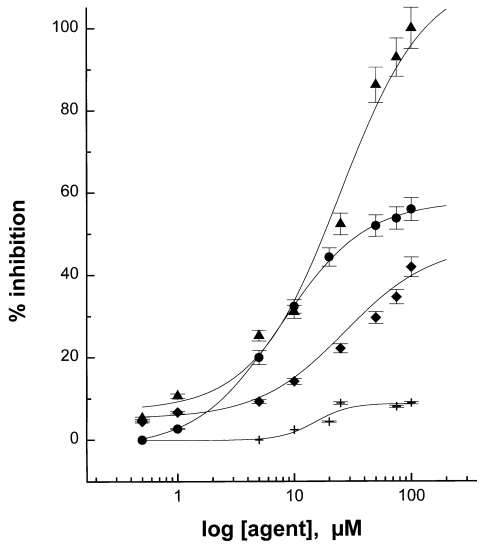

FIGURE 5. Concentration–effect curves for inhibition of Fe^{+3}/ascorbate-stimulated lipid peroxidation in rat brain slices by nitroso aryl derivatives and the parent compounds: (+) nitrobenzene, (▲) NTP, (●) nitrosobenzene, (◆) nifedipine.

TABLE 2. Antioxidant effects of nitroso aryl derivatives on Fe^{+3}/ascorbate-stimulated lipid peroxidation in rat brain slices

Nitroso derivative	IC_{50} (μM)	P^a
o-Nitrosotoluene	5.2 ± 0.8	80.3 ± 9.2
NTP	18.2 ± 2.1	50.5 ± 7.9
Nitrosobenzene	35.9 ± 1.9	75.0 ± 8.1

[a] Apparent partition coefficient octanol/water (50:50).

both nicardipine and nimodipine have similar membrane partition coefficients (Diez *et al.*, 1991) but very different antiperoxidant efficacies (Table 1), lipophillicity itself may be only one contributor to the dihydropyridine antiperoxidant action. Another factor to consider is the different intramembranous orientations of the various dihydropyridine calcium antagonists (Chester *et al.*, 1987). These factors may explain the significant differences between the antioxidant potencies in the different tested biological preparations (i.e., membranes, liposomes, microsomes, tissue). On the other hand, the antioxidant activity of 1,4-dihydropyridines also could be explained by their free radical scavenging properties. Our data show that the antioxidant effects of Ca^{2+} antagonists varied when the drugs were present either from the start of the Fe^{+3}/ascorbate-induced peroxidation or after initiation of the reaction (Fig. 4). These results could indicate a radical-trapping effect of the drugs (Gonçalves *et al.*, 1991).

Nifedipine is unstable under daylight and UV light conditions (Squella *et al.*, 1989, 1990). This property may affect its antioxidant activity. Therefore, in the present work, we studied the antioxidant activity of the photolytic product of nifedipine and other related nitroso derivatives, such as nitrosobenzene and ortho-nitrosotoluene. Under our experimental conditions (see Materials and Methods), irradiation of nifedipine with either artificial daylight or UV light (360 nm) yields NTP. This derivative had a more potent antioxidant effect than did nifedipine (Fig. 5; Tables 1 and 2). Additionally, the other tested nitroso derivatives (ortho-nitrosotoluene, nitrosobenzene) became potent antioxidants in our system. Attempts to correlate partition coefficients of nitroso derivatives with their antioxidant effects were not conclusive, because lipophillicity was in the same order of magnitude (Table 2). However, results with the parent nitro compounds differed. They were moderately effective (nifedipine) or completely inactive (nitrobenzene, ortho-nitrotoluene). Previous works (Misik *et al.*, 1991; Ondrias *et al.*, 1994) demonstrated that NTP had an antioxidant ability in a model of lipid peroxidation in low-density lipoproteins (LDLs) and phosphatidylcholine liposomes and was compared with parent nifedipine and with other nitroso spin traps such as 3,5-dibromo-4-nitrosobenzenesulfonic acid, nitrosobenzene and 2-methylnitrosopropane. Nifedipine (20–200 μM) did not inhibit lipid peroxidation either in LDL or in liposomes, whereas its photolytical product NTP was found to very effective at the same concentrations.

In conclusion, on the basis of the IC_{50} values determined in this study, the following rank order of antioxidant potency for the nitroso derivatives can be established: ortho-nitrosotoluene>NTP> nitrosobenzene. For an explanation of the aforediscussed results, there are two main mechanisms by which nitroso compounds may inhibit lipid peroxidation. First, because nitroxide radicals were found to inhibit lipid peroxidation, the nitroxide radicals formed in the reaction between nitroso compounds and the unsaturated lipids may act as membrane chain-breaking antioxidants. Second, nitroso compounds may inhibit lipid peroxidation by direct radical trapping with subsequent formation of stable nitroxide radicals.

CONCLUDING REMARKS

Results from this study demonstrated that nitro-substituted 1,4-dihydropyridines, such as nicardipine and nisoldipine, exhibited the most potent antioxidant activities on Fe^{+3}/ascorbate-stimulated lipid peroxidation in rat brain slices. In contrast, non-nitro-substituted 1,4-dihydropyridines, such as, isradipine and amlodipine, were significantly less effective in inhibiting lipid peroxidation. The order of antioxidant activity in rat brain slices expressed as 50% inhibition of peroxidation was: nicardipine>nisoldipine>(*R,S/R,S*)-furnidipine>(*R,R/S,S*)-furnidipine>nitrendipine>nimodipine>nifedipine.

On the other hand, NTP, a photolytic product from nifedipine, nitrosotoluene and nitrosobenzene, exhibited a significant antioxidant effect, being a more potent inhibitor of lipid peroxidation than the parent nitro compounds. On the basis of IC_{50} values determined, the following rank order of antioxidant potency for these derivatives was determined: ortho-nitrosotoluene>NTP>nitrosobenzene.

Finally, we demonstrated that nifedipine, under irradiation either with artificial daylight or UV light for 4 hr, only decomposes to NTP.

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