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# Antioxidant Properties of the Triaminopyridine, Flupirtine

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**ABSTRACT.** Flupirtine is a triaminopyridine-derived centrally acting analgesic, which interacts with mechanisms of noradrenergic pain modulation. Recently, it has been found to display neuroprotective effects in various models of excitotoxic cell damage, global and focal ischemia. Although this profile suggests that flupirtine acts as an antagonist of the *N*-methyl-*D*-aspartate (NMDA) and glutamate-triggered $Ca^{2+}$ channel, there is no direct interaction with the receptor. In this paper, we examined whether flupirtine can act as an antioxidant and prevent free radical-mediated structural damage. Flupirtine at 5–30 μM inhibited ascorbate/$Fe^{2+}$ (1–10 μM)-stimulated formation of thiobarbituric reactive substances, an indicator of lipid peroxidation, in rat brain mitochondria. Interestingly, we found an increasing effectiveness of the drug at higher iron concentrations. Additionally, higher concentrations of flupirtine also provided protection against protein oxidation, as demonstrated by a decrease in protein carbonyls formed after treatment of rat brain homogenates with ascorbate/$Fe^{2+}$. In PC12 cell culture, flupirtine at 10–100 μM was able to attenuate $H_2O_2$-stimulated cell death and improve the survival by 33%. BIOCHEM PHARMACOL **56**;10:1323–1329, 1998. © 1998 Elsevier Science Inc.

**KEY WORDS.** flupirtine; free radicals; antioxidants; mitochondria; protein oxidation; PC12 cell culture

The triaminopyridine derivative flupirtine is a centrally acting analgesic [1] which has been in use since 1986 in pain therapy. Additionally, it shows muscle-relaxant and minor sedative effects [2]. The antinociceptive properties of flupirtine have been attributed to interactions with noradrenergic and γ-aminobutyric acid-mediated mechanisms [3], whereas the muscle relaxation seems to be mediated via mechanisms closely associated to NMDA‖-responsive subtype of the glutamate receptor [4]. In rats, flupirtine and the competitive NMDA receptor antagonist 2-amino-7-phosphonovalerate show a similar profile of depressant effects on the monosynaptic Hoffmann reflex and the polysynaptic flexor reflexes and could be clearly distinguished from 6,7-dinitroquinoxaline-2,3-dione, an antagonist of NMDA-insensitive glutamate receptors that only affects the Hoffmann reflex [5]. The same authors also demonstrated that the depressant effect of flupirtine is significantly reduced by coadministration of NMDA, but not by α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid, an antagonist of non-NMDA glutamate receptors.

Several recent studies indicate that flupirtine also exhibits neuroprotective activity in models linked to NMDA receptor-mediated excitotoxic brain damage. Perovic *et al.* [6] demonstrated that flupirtine protects cultured cortical primary neurons against NMDA and the human immunodeficiency virus protein gp120. Hippocampal neurons treated with flupirtine are less sensitive to glutamate toxicity and do not show the typical increase in intracellular $Ca^{2+}$ levels [7]. Furthermore, flupirtine prevents the decrease in neural activity induced by retinal ischemia [8] and protects against global [9] and focal cerebral ischemia [7]. This impressive profile of protective effects may suggest some similarity between flupirtine and noncompetitive NMDA antagonists such as MK-801, PCP, or aminoadamantanes [10]. Flupirtine, however, does not bind directly to the NMDA receptor nor to any other part of the channel, and is devoid of the typical psychotomimetic side-effects [11] that prevent a therapeutic use of MK-801 and PCP. Instead, flupirtine seems to act on some mechanism downstream of the receptor. Recent studies suggest an involvement in antioxidant and antiapoptotic pathways: In hNT neurons, flupirtine attenuates the typical glutathione depletion after toxic insults with glutamate or β-amyloid protein. In toxin-stimulated cells, the glutathione and Bcl-2 levels were improved by factors of 2.5 and 6, respectively, [12] if they had been pretreated with flupirtine. It is

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‖ *Abbreviations*: LPO, lipid peroxidation; MDA malonic dialdehyde; MPTP, methylphenyltetrahydropyridine; NMDA, *N*-methyl-*D*-aspartate; PCP, phencyclidine; and TBA, thiobarbituric acid.

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not known, however, whether flupirtine can act as a free radical scavenger, or prevent the formation of free radicals through interaction with cellular iron. An impressive line of evidence underlines the importance of free radical-mediated damage in the process of neurodegeneration [13–16]. Factors leading to an excessive formation of free oxygen radicals have been observed in Parkinson's disease: a buildup of free redox-reactive iron [17, 18], defects in mitochondrial respiration [19, 20] a decrease in glutathione [21], and shifts in radical deactivating enzymes such as superoxide dismutase or glutathione peroxidase [22]. In the nigrostriatal neurons affected in Parkinson's disease, dopamine metabolism by monoamine oxidases [23, 24], dopamine autoxidation [25], and the neuromelanin-iron interaction [26] contribute to free radical formation.

In the development of neuroprotective strategies, free radical as well as excitotoxic mechanisms have to be taken into account. In this study, we investigated whether flupirtine has antioxidative properties which can be exploited to protect tissue against free radical-mediated damage. Apart from minor sedation, flupirtine does not display any side-effects, and may thus be a promising candidate for an application in neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease as well as against trauma and stroke-induced ischemic brain damage.

## MATERIALS AND METHODS

### *Fine Chemicals*

Flupirtine was kindly donated by ASTA Medica. All other fine chemicals were of the highest quality available from Sigma Chemical Co. 2,4-Dinitrophenylhydrazine (DNPH) was purchased from Fluka as a preparation with 30% water for safe transport. Stock solutions of oxidizable compounds (flupirtine, $FeSO_4$, ascorbic acid, apomorphine) were prepared in water or ethanol degassed by sonication immediately prior to use (The concentration of ethanol did not exceed 0.1%). Control experiments were carried out with addition of the respective solvents. Cell culture media and sera were obtained from Biologic Industries. A pheochromocytoma (PC) 12 cell line was a kind donation from D. Ofer.

### *Isolation of Mitochondria*

Male Sprague–Dawley rats (300–450 g) were killed by decapitation. The brains were immediately extracted and cooled in ice-cold isotonic 10 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose, 2 mM EDTA (sodium salt) and 2% BSA free of fatty acids (isolation buffer). A crude mitochondrial fraction was prepared from the brain by differential centrifugation [27] and stored at −18° in 10 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose. The protein concentration in the suspension was 50–55 mg of protein/mL, as measured by the biuret reaction.

### *Determination of Thiobarbituric Acid-reactive Substances*

All experiments were carried out in triplicate. 7.5 μL of mitochondrial preparation (equivalent to 0.40 mg of protein) were suspended in 750 μL of 25 mM Tris-HCl (pH 7.4) containing 50 μM ascorbic acid [28]. Samples of the tested drugs were added to the suspension and the reaction was started by the addition of $FeSO_4$ (from a 1.0 mM stock solution). The sample was allowed to stand at ambient temperature for 2 hr and incubation was stopped by the addition of 750 μL of 20% (w/v) trichloroacetic acid. The samples were centrifuged in a benchtop centrifuge; 500 μL of the supernatant were mixed with 500 μL of 0.5% (w/v) TBA and heated to 95° for 30 min. The absorption of TBA derivatives was measured photometrically at $\lambda = 532$ nm. A possible interference of the examined drugs with the thiobarbituric acid test was ruled out by control experiments (addition of drugs after incubation).

### *Measurement of Protein Oxidation*

Mitochondrial suspension equivalent to 1 mg of protein was incubated in 1 mL of 100 mM Tris-HCl (pH 7.4) containing 15 mM ascorbic acid, 250 μM $FeSO_4$, and protease inhibitors (0.5 mg/mL of leupeptin, 0.7 mg/mL of pepstatin, 0.5 mg/mL of apoprotonin) for 1 hr. Two control samples were left without iron. For the assay of radical scavenging activity, a 10 mM stock solution of flupirtine and apomorphine in ethanol was prepared and 10 μL added to the reaction mixture (final concentration 100 μM) prior to incubation. One control sample was treated with 20 μL of 1 M sodium borohydride in 100 mM NaOH to completely reduce all carbonyl groups. Assay for protein carbonyls was performed as described [29]. Briefly, four 200 μL of aliquots of the reaction mixture were withdrawn. Excess DNA was precipitated by the addition of 1% streptomycin sulfate. Three aliquots were mixed with 400 μL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl, while the remaining aliquot was treated with 2 M HCl. The samples were allowed to stand for 1 hr at ambient temperature, mixed with 500 μL of 20% (w/v) trichloroacetic acid, and centrifuged for 5 min. The precipitate was subsequently washed with 500 μL of 10% (w/v) trichloroacetic acid and twice with 500 μL of ethyl acetate/ethanol (1:1), until the washing solution of the 2,4-dinitrophenylhydrazine-treated samples was colorless. Finally, the samples were dissolved in 1.0 mL of 6 M guanidine hydrochloride (pH 2.3) containing 20 mM $KH_2PO_4$. The absorption of the HCl-treated sample was measured photometrically at $\lambda = 280$ nm to determine the protein concentration. The concentration of protein carbonyl 2,4-dinitrophenylhydrazine derivatives was monitored at $\lambda = 366$ nm, using the HCl-treated sample as a reference. A molar absorption $\epsilon = 22000\ M^{-1}\ cm^{-1}$ was used to calculate concentration of derivatized carbonyl sites.

### *PC12 Cell Culture*

PC12 cells were grown in 250-mL culture flasks in Dulbecco's modified Eagle's medium with 1 mg/mL of glucose, supplemented with glutamine (250 mg/L), antibiotics (streptomycin, penicillin), and serum [5% fetal calf serum (FCS), 10% horse serum] at 37° with 5% $CO_2$. On confluence, the culture medium was removed, and the cells were detached by trypsinization (0.25% trypsine) and counted in a hemocytometer. The viability was assessed by trypan blue exclusion. After careful resuspension in Dulbecco's modified Eagle's medium (with glutamine and antibiotics, but without sera), $10^4$ cells/well were placed in 94-well plates, pre-coated with collagen (10 $\mu g/cm^2$). Cells were allowed to attach overnight before every experiment.

Flupirtine was dissolved in PBS and added to the cells 15 min prior to the addition of $H_2O_2$. After incubation at 37° for 2 hr, the medium was exchanged with Dulbecco's modified Eagle's medium containing antibiotics and glutamine but no serum. The cell survival was determined after 12 hr by addition of 0.50 mg/mL of methylthiazolidinodiphenyltetrazolium bromide (MTT) after careful washout of the added drug. The efficacy of the washing was checked in a well with nonviable cells which had been killed with an excess of $H_2O_2$ (10 mM). Photometrical determination of the colored formazan product was carried out in a Perkin–Elmer Dual Wavelength Eliza–Reader at $\lambda$ = 570/650 nm.

### *Statistical and Mathematical Analysis*

All experiments were carried out at least in triplicate, while eight replicates were performed in cell culture experiments. The obtained data were compared by two-way ANOVA, followed by Mann–Whitney *U*-tests when necessary.

$EC_{50}$ values were obtained by regression analysis of the concentration/effect data (Fig. 1) with the help of INPLOT® (GraphPad) scientific graphic program.

## RESULTS

### *Effects of Flupirtine on the Formation of TBA Reactive Substances*

The treatment of mitochondria with ascorbic acid and $FeSO_4$ leads to free radical formation (mainly hydroxyl radicals) by Fenton reactions, with oxidation of the polyunsaturated fatty acids in biologic membranes. These reactions yield a variety of lipid breakdown products, such as MDA, as well as compounds that release MDA under acidic conditions (e.g. lipid hydroperoxides). These can be detected with the TBA assay. The amount of TBA-reactive compounds formed in the system depends on the concentration of both ascorbic acid and iron, the conditions described above having been optimized to reach a maximum effect. Such a system can be used to measure the ability of antioxidants to protect biologic lipids from free radical damage. In the experiments presented here, formation of TBA-reactive compounds in the mitochondria was induced in the presence of 50 $\mu$M ascorbic acid. With concentrations of $FeSO_4$ between 0.5 and 10 $\mu$M, we observed a steady increase in TBA-reactive products, with the reactions being complete within 120 min. With less than 0.5 $\mu$M Fe, no formation of TBA products was detectable, while at concentrations exceeding 10 $\mu$M no further increase could be observed (data not shown).

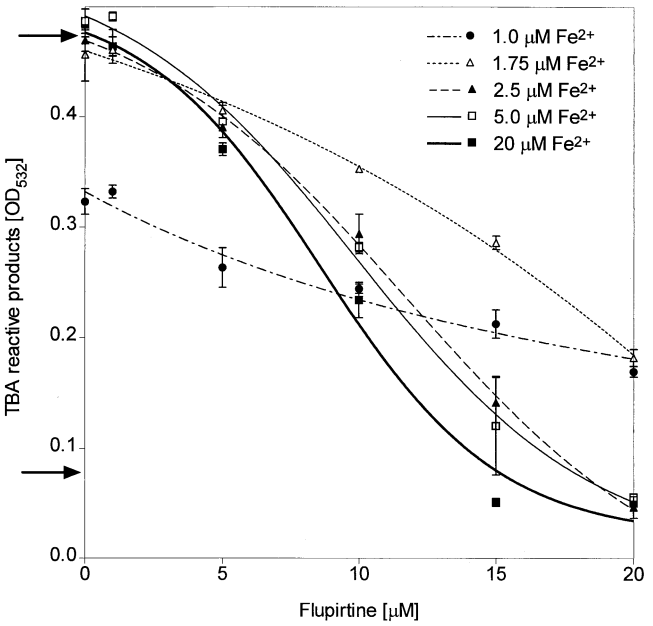


**FIG. 1. Inhibitory effect of flupirtine on free radical reactions in rat brain mitochondria. Rat brain mitochondria were incubated with ascorbic acid (50 $\mu$mol/L) and flupirtine/$FeSO_4$ as indicated. After 2 hr, samples were treated with TBA as described in the Materials and Methods section, and the absorption at $\lambda$ = 532 nm was measured (mean ± SEM., N = 5). $OD_{532}$ = 0.5 is equivalent to 2.01 nmol MDA/$mg_{protein}$. The lower arrow indicates the background activity of mitochondria in buffer (0.078 ± 0.002), the upper arrow represents mitochondria, ascorbate and 10 $\mu$M $FeSO_4$, with 10 $\mu$M flupirtine added before the TBA reaction (0.472 ± 0.012). Comparison of the curves by two-way ANOVA showed that $P < 0.005$ in all cases. Regression analysis was carried out on the basis of least square fits with the help of the INPLOT graphics program. $EC_{50}$ values were calculated on the basis of these regressions.**

The addition of flupirtine significantly inhibited radical reactions at drug concentrations as low as 5 $\mu$M (see Fig. 1). This effect was concentration-dependent (Fig. 1, Table 1): In the presence of 5 $\mu$M $FeSO_4$, 20 $\mu$M of flupirtine reduced radical damage by 90%, i.e. to levels lower than the background activity without ascobate and iron. Flupirtine was more potent as an antioxidant than the dihydropyridine $Ca^{2+}$ channel antagonist nimodipine, and was approximately as active as dopamine or the iron chelator desferrioxamine (see Table 2). Among the compounds tested, only apomorphine was more active [30]. No antioxidant effect was observed for the noncompetitive NMDA antagonists phencyclidine and MK-801 (data not shown).

Figure 1 clearly shows that the efficacy of flupirtine was iron-dependent: at 1 $\mu$M $FeSO_4$, it showed only weak effects ($EC_{50}$ = 24.3 $\mu$M) while in the presence of 5 $\mu$M

**TABLE 1. Inhibition of ascorbate/iron-induced formation of thiobarbituric reactive substances by flupirtine at different $Fe^{2+}$ concentrations**

| $FeSO_4$(μM)* | 0.5 | 1 | 2.5 | 5 | 10 |
|---|---|---|---|---|---|
| $IC_{50}$ [μM]† | 24.3 ± 2.21 | 13.9 ± 1.16 | 11.7 ± 1.00 | 8.55 ± 1.2 | 8.17 ± 1.12 |
| Max. inhib.‡ [%] | 60 ± 2 | 76 ± 2 | 89 ± 2 | 92 ± 2 | 88 ± 1 |
| Hill coefficient§ | 1.1 ± 0.1 | 1.5 ± 0.3 | 2.9 ± 0.4 | 4.7 ± 0.8 | 4.5 ± 0.7 |

* Concentration of ascorbate was 50 μM in all cases.
† Obtained from regression data (mean ± SE, $n = 6$), for concentration of flupirtine see Fig. 1.
‡ Maximum inhibition as determined from a triplicate experiment (mean ± SEM).
§ Apparent values (mean ± SE, $n = 5$), as obtained from the slope of the cooperativity plot (Hill plot), log [drug] versus log $[I/(I_{max}-1)]$.

$Fe^{2+}$, it was three times more active ($EC_{50} = 8.55$). Thus far, it is not clear whether this effect is caused directly by the iron or by iron-induced free radicals.

### *Attenuation of Protein Carbonyl Formation by Flupirtine*

Measuring protein carbonyl formation is a more specific but less sensitive method to assay free radical damage in a biologic system. Under conditions of oxidative stress, proline, arginine, lysine and threonine residues are converted into aldehydes and ketones, which can be labeled with specific reagents such as 2,4-dinitrophenylhydrazine (DNPH).

Having found that protein oxidation required more stringent conditions than LPO, we consequently had to increase the concentrations of ascorbate and iron to obtain a measurable oxidation of the mitochondrial proteins. With 250 μM ferrous iron and 15 mM ascorbic acid, a carbonyl content of 4.73 $nmol/mg_{protein}$ was obtained, a more than five-fold increase as compared with the controls (Table 3). The content of protein carbonyls could be reduced with flupirtine and with apomorphine [30]. It was impossible, however, to reach the control values.

### *Flupirtine-induced Improvement of the Survival of PC12 Cells after Treatment with $H_2O_2$*

PC12 cells undergo rapid cell death after insults with a wide variety of toxic agents (e.g. glutamate [31], complex I inhibitors [32], l-DOPA [33], and peroxynitrite [34]). They also respond to $H_2O_2$, which kills the cells with $EC_{50} = 0.5 \pm 0.1$ mM within 24 hr. The actual lethal damage takes less than 2 hr. Treatment with flupirtine during that time improved the survival from 58% (as obtained with 0.4 mM $H_2O_2$) to 78%. With 0.6 mM $H_2O_2$ (decreasing survival to 42%), no significant protection could be obtained with flupirtine (see Fig. 2). Flupirtine was not toxic to the cells in the tested concentration range (0.5–1000 μM).

## DISCUSSION

In the recent literature, the triaminopyridine derivative flupirtine has been identified as a potential antiapoptotic [35, 36] and neuroprotective drug acting on intracellular $Ca^{2+}$ [7], glutathione [37], and Bcl-2 levels [12]. Additionally, we were able to show that flupirtine can also act as a potent antioxidant in rat brain mitochondria and in cell culture with the potential to protect lipids and proteins from free radical damage. With the available data, iron chelation by flupirtine does not appear to be a major contributor to the observed effects. In this case, maximum efficacy would be expected for low iron concentrations. Flupirtine shows exactly the opposite effect (see Fig. 1, Table 1). The interpretation that flupirtine is a true scavenger directly interacting with free radicals is more consistent with our data. Indeed, flupirtine can undergo multistep oxidation via quinone intermediates, leading to a polymeric end product (Pergande G, unpublished data). In this respect, it appears to be similar to catecholamines such as dopamine, apomorphine or homovanillic acid, which also have antioxidant properties [38, 39]. Chelation does not always abolish the proxidant effects of iron salts;

**TABLE 2. Inhibition of ascorbate/iron-induced LPO by various antioxidants***

| | Apomorphine | | Dopamine | Desferrioxamine | Nimodipine |
|---|---|---|---|---|---|
| $FeSO_4$(μM)† | 2.5 | 5.0 | 2.5 | 2.5 | 2.5 |
| $IC_{50}$ [μM]† | 0.28 ± 0.02 | 0.61 ± 0.02 | 6.59 ± 0.20 | 2.78 ± 0.04 | 134 ± 8.0 |
| Max. inhib.§ [%] | 92 ± 1 | 93 ± 2 | 93 ± 1 | 75 ± 1 | 63 ± 1 |
| Hill coefficient‖ | 1.7 ± 0.1 | 4 ± 0.3 | 1.0 ± 0.1 | 0.9 ± 0.15 | ND |

* Drugs were dissolved in buffer or ethanol (apomorphine). Control experiments ($OD_{532}$); 0.082 ± 0.004, mitochondrial background; 0.456 ± 0.009, 2.5 μM $FeSO_4$; 0.489 ± 0.010, 5.0 μM $FeSO_4$; 0.449 ± 0.012, 2.5 μM $FeSO_4$ + 0.1% ethanol. All the examined drugs showed no effect if added directly before the TBA reaction.
† Concentration of ascorbate was 50 μM in all cases.
‡ Obtained from regression data (mean ± SE, $n = 6$).
§ Maximum inhibition as determined from a triplicate experiment (mean ± SEM).
‖ Apparent values (mean ± SE, $n = 5$), as obtained from the slope of the cooperativity plot (Hill plot), log [drug] versus log $[I/(I_{max}-1)]$.

**TABLE 3. Inhibition of protein carbonyl formation by apomorphine and flupirtine**

| | Protein carbonyls [$nmol_{carbonyl}/mg_{protein}$] |
|---|---|
| Control | 1.48 ± 0.10 |
| Ascorbate/iron | 3.05 ± 0.15 |
| + apomorphine (100 μM) | 1.80 ± 0.18 |
| + flupirtine (100 μM) | 2.45 ± 0.10 |

$n = 5$ in all experiments.

sometimes chelators activate iron (e.g. citrate or ADP). In PC12 cell culture, the iron chelator Desferal does not protect against $H_2O_2$-induced cell death, but the apomorphine shows a strong antioxidant effect [40]. It is still a matter of debate whether PC12 cell death induced by $H_2O_2$ is apototic or necrotic. Recent data show that apoptosis after trophic withdrawal leads to five times more DNA fragmentation than is provoked by $H_2O_2$ treatment [41].

The polyunsaturated fatty acids in biologic membranes are extremely sensitive to free radical mediated-peroxidation. The TBA assay primarily reflects LPO, especially the formation of MDA and its precursors. Unlike LPO, protein oxidation requires metal binding and proteins without a metal binding site (such as BSA) are considerably less sensitive. Modification of amino acid residues can be observed primarily in the close neighborhood of the bound metal [42]. Consequently, these processes are relatively

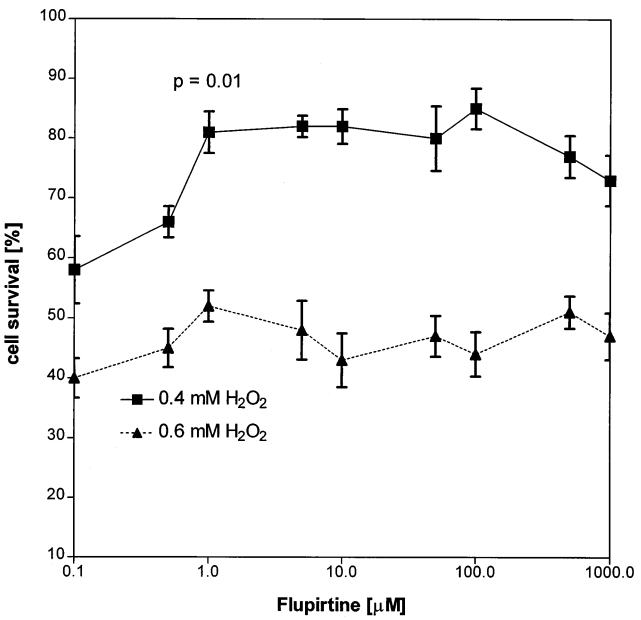


**FIG. 2. Flupirtine partially protects PC12 cells from $H_2O_2$-toxicity. Cells were planted with a density of 10000 $cm^{-1}$ in 96-well microtiter plates and allowed to adhere for 24 hr. After pretreatment with flupirtine for 2 hr, $H_2O_2$ was added and cell viability determined with the methylthiazolidinodiphenyltetrazolium bromide assay 12 hr later. Data are presented as % cell survival with respect to controls, mean ± SEM. (N = 8). All drug was removed by careful washing prior to the methylthiazolidinodiphenyltetrazolium bromide assay.**

insensitive toward antioxidants. Therefore, a partial reduction of protein oxidation as afforded by apomorphine or flupirtine (Table 3) can still be regarded as relevant.

Reactive oxygen species (e.g. $O_2^{\cdot-}$, $HO^{\cdot}$ or $H_2O_2$) which damage membranes, proteins and DNA have been implicated in Parkinson's disease and other neurodegenerative diseases [43]. They are formed as a side product of cellular respiration [44] and by redox enzymes such as xanthine oxidases [45] or monoamine oxidases [24]. Free iron, which is increased in the *substantia nigra* of Parkinson's disease patients [17], can activate less $O_2^{\cdot-}$ and $H_2O_2$ and convert them into highly toxic $HO^{\cdot}$ radicals [23]. These can cause severe structural damage to cells and tissue, especially if antioxidant defense mechanisms are impaired or the rate of radical formation increased. Lipids, DNA and proteins likewise can suffer modification and degradation, with cell death as the final result.

Several other mechanisms also play a role in neurodegeneration (for a recent review see Götz *et al.* [46]). In addition to free radicals, glutamate and aspartate neurotoxicity (excitotoxicity) are of importance, as well as the action of exo- and endogenous neurotoxins (e.g. MPTP, 6-hydroxydopamine, and tetrahydroisoquinoline). There is increasing biochemical evidence that these pathways are closely related. It has been demonstrated *in vivo* that NMDA-receptor antagonists like memantine or CPP, which are able to control cell damage caused by excitatory amino acids, also decrease the toxic effect of the respiratory toxin MPTP [47, 48]. Neurotrophins and growth factors also protect neurons against excitatory amino acids but do not affect the expression of the NMDA receptor or binding of glutamate. Instead, they cause a rise in catalase activity [49] and glutathione biosynthesis [50], thus providing improved defense against oxidative stress.

Excitotoxic insults and oxidative stress likewise cause a rise in intracellular free $Ca^{2+}$, the former by stimulation of influx, the latter by damaging mitochondria and decreasing their storage capacity for $Ca^{2+}$. The $Ca^{2+}$ channel inhibitor nimodipine prevents free radical processes in isolated mitochondria [28], although it is less active than flupirtine (see Table 2). The close interdependence of free radical formation and the potentially toxic deregulation of cellular $Ca^{2+}$ and the implication in neurodegeneration is well documented [51, 52]. Flupirtine, as an agent with three properties relevant for neuroprotection, can interrupt the fatal cascade at several points: it may prevent the rise in intracellular $Ca^{2+}$ after excitotoxic insults [7], provide antiapoptotic protection through induction of Bcl-2 and glutathione [12], and, as shown by the data presented here, prevent free radical-induced oxidative damage to lipids and proteins.

Unlike other antioxidants such as α-tocopherol or desferrioxamine, flupirtine easily passes the blood-brain barrier. Our data show that flupirtine is more active as a radical scavenger under conditions of oxidative stress (see Table 1 and Fig. 1), although we do not understand the chemical background of this effect. This effect can be a major

therapeutic advantage, as flupirtine will work with maximal efficiency only at sites of radical formation, thus limiting the probability of side-effects. Flupirtine can also be beneficial for the treatment of ischemia and reperfusion after stroke, a condition which has been associated with increased free radical formation and excitotoxic tissue damage [8, 53]. Additionally, flupirtine was able to ameliorate the motor symptoms after reserpine-induced dopamine depletion in rats [54], a model with relevance for the treatment of Parkinson's disease. Further work to examine the neuroprotective effects of flupirtine in rodent models of MPTP and methamphetamine toxicity is in progress.

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