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Apomorphine is a highly potent free radical scavenger in rat brain mitochondrial fraction

Michael Gassen, Yelena Glinka, Bilha Pinchasi, Moussa B.H. Youdim *

Department of Pharmacology, Bruce Rappaport Family Research Institute, Faculty of Medicine, Technion, Haifa, Israel

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Abstract

Ergoline-derived dopamine receptor agonists, like pergolide or bromocryptine, have recently attracted attention as potential neuroprotective drugs. The classical mixed type dopamine D_1 and D_2 receptor agonist apomorphine, although used clinically in the therapy of Parkinson's disease, has never been examined for any properties related to neuroprotection. In this paper, we examine the effects of 0.1–100 μM apomorphine on ascorbate/iron-stimulated free radical processes in rat brain mitchondrial fraction. Lipid peroxidation as assayed by the thiobarbituric acid reaction can be completely inhibited by submicromolar concentrations of apomorphine (0.3 μM with 2.5 μM Fe^{2+} and 0.6 μM with 5.0 μM Fe^{2+}), which proved to be more than twice as effective as desferrioxamine and twenty times as compared with dopamine. The inhibition of lipid peroxidation in mitochondria correlates with an increased rate of apomorphine oxidation. The formation of protein carbonyls, which is generally less sensitive to antioxidants, could be significantly reduced by apomorphine. In the model system we employed, apomorphine was more active than dopamine, desferrioxamine, or pergolide in preventing the formation of thiobarbituric reactive substances. The time course of the reaction suggests that apomorphine acts as a radical scavenger and that its iron chelating properties may not be of major importance. Since oxidative stress has been implicated in Parkinson's disease, the role of apomorphine as a neuroprotective is worthy of examination.

Keywords: Apomorphine; Free radical; Lipid peroxidation; Protein carbonyl; Iron; Ascorbate; Thiobarbituric acid

1. Introduction

The introduction of L-DOPA (3,4-dihydroxyphenylacetic acid) brought about a large progress in the treatment of Parkinson's disease and its main symptoms, like tremor and akinesia. Until today, however, no means has been found to stop or slow down the underlying process, a progressive degeneration of the nigrostriatal neurons in the basal ganglia. There is an ongoing search for new neuroprotective strategies: glutamate receptor antagonists (Takei et al., 1994; Kornhuber et al., 1994; Wenk et al., 1994), neurotrophins (Henrich-Noack et al., 1995), the monoamine oxidase B inhibitor L-deprenyl (Przuntek, 1994; LeWitt, 1994), and dopamine receptor agonists of the ergoline type, like bromocryptine or pergolide, have been in the focus of attention. Pergolide, for example, has been shown to preserve the dopaminergic nigrostriatal system during aging in Fischer-344 rats (Felten et al., 1992; Lange et al., 1994).

Apomorphine is an agonist of the dopamine D_1 and D_2 receptors, acting both pre- and postsynaptically (Linazasoro, 1994). Although it has been used in the therapy of Parkinson's disease as early as 1950, it has never been examined for any neuroprotective potential. In the past, a widespread use of apomorphine as a replacement for L-DOPA in the symptomatic therapy of Parkinson's disease was prevented by its short duration of action, the need for subcutaneous application, and several serious side-effects like nausea, vomiting, postural hypotension or sedation (Lees, 1993). Recently, peripherally acting dopamine receptor antagonists helped to overcome many of the side-effects, and novel delivery systems, such as portable infusion pumps, make the application more comfortable for the patient (Frankel et al., 1990). Apomorphine proved especially useful for treating patients in the late stage of the disease, when L-DOPA is decreasingly effective. It helps to reduce on-off oscillations (Stibe et al., 1988), improves the gastrointestinal symptoms and bladder dysfunction

* Corresponding author. Department of Pharmacology, Bruce Rappaport Faculty of Medicine, Technion, PO Box 9649, Haifa 31096, Israel. Tel.: +972 4 8295271; fax: +972 4 8513145.

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characteristic of the off-periods, and reduces L-DOPA need (Gancher et al., 1995).

Today, it is widely accepted that the main factors leading to cell death in the parkinsonian brain are (1) metal-catalyzed or other free radical reactions (Ben-Shachar et al., 1991; Fahn and Cohen, 1992; Youdim et al., 1994), (2) insufficiency of cellular respiration leading to depletion of ATP and generation of free radicals (Löschmann et al., 1994), and (3) Ca^{2+}-mediated excitotoxicity (Kornhuber et al., 1994; Fagni et al., 1994). Compounds with a catechol structure have metal chelating properties and can act as reducing agents (Liu and Mori, 1993), therefore, apomorphine may inhibit metal-catalyzed free radical processes and act as a radical scavenger. As a reducing agent, apomorphine can also contribute to the generation of highly toxic hydroxyl radicals ($HO^{\cdot}$) by maintaining iron in the ferrous state. $HO^{\cdot}$ radicals are most likely responsible for most of the damage caused by free radicals in vivo (Ben-Shachar et al., 1991). The overall manner by which an antioxidant drug affects the level of oxidative stress depends on the balance between radical scavenging and radical activating properties.

A protective effect of a drug can be expressed in terms of decreasing damage to cellular structures, such as membranes or proteins. The experiments described in this paper show the effect of apomorphine on lipid peroxidation and protein carbonyl formation after ascorbate/iron-induced free radical formation in rat brain mitochondrial fractions. We also examined the effects of apomorphine on the mitochondrial respiratory chain, another potentially radical forming pathway. This is a first step to evaluate the anti- or pro-oxidant properties of apomorphine in brain tissue.

2. Materials and methods

2.1. Materials

Pergolide was kindly provided by Lilly, desferrioxamine was a donation by Ciba-Geigy. All other reagents were purchased from Sigma at the highest available purity. 2,4-Dinitrophenylhydrazine was purchased as a preparation containing 30% water for safe transport. Stock solutions of oxidizable compounds (ascorbate, $FeSO_4$, dopamine, desferrioxamine, 6-hydroxydopamine) were prepared daily in water degassed by sonication. Apomorphine, being poorly soluble in water, was dissolved in ethanol. 6-Hydroxydopamine solutions contained 2 mM dithionite as antioxidant and were always stored on ice. The final dithionite concentration in the assay (2–10 μM) did not effect the measurement.

2.2. Mitochondria isolation

Male Sprague-Dawley rats (300–450 g) were killed by decapitation. The brains were immediately extracted and cooled in the ice-cold isotonic 10 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose, 2 M EDTA (sodium salt) and 2% bovine serum albumin free of fatty acids (isolation buffer). A crude mitochondrial fraction was prepared from the brain by differential centrifugation (McCormack and Denton, 1989) and stored at −18°C 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.

For the assay of respiratory activity, the mitochondria were further purified by recentrifugation in 16% isoosmotic Percoll (Garvin et al., 1990). This preparation contained approximately 25–35 mg of protein/ml and aliquots were kept frozen at −20°C for 2 weeks. Each aliquot was thawed and used only once. By this process there was no loss of enzyme activities or their sensitivity to inhibition.

The protein concentration was measured using the biuret reaction.

2.3. Assay of free radical damage

2.3.1. Lipid peroxidation experiments

All experiments were carried out in triplicates. 7.5 μl of mitochondrial preparation (equivalent to 0.25 mg protein) were suspended in 750 μl of 25 mM Tris-HCl (pH 7.4) containing 50 μM ascorbic acid (Takei et al., 1994). Samples of the tested drugs were dissolved in water or ethanol (apomorphine) and added to the suspension. 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 h 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) thiobarbituric acid and heated to 95°C for 30 min. The absorption of thiobarbituric acid 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)

2.3.2. Protein oxidation

One ml samples of mitochondrial suspension containing 1 mg protein were incubated in 100 mM Tris-HCl (pH 7.4) containing 15 mM ascorbic acid, 250 μM $FeSO_4$, and protease inhibitors (0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, 0.5 μg/ml apoprotonin) for 1 h. Two control samples were left without iron. For the assay of radical scavenging activity, a 10 mM stock solution of 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 (Levine et al., 1990). Briefly, four 200 μl 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 1,4-dinitrophenylhydrazine in 2 M HCl, while the remaining aliquot was treated with 2 M HCl. The samples were allowed to stand for 1 h at ambient temperature, mixed with 500 μl of 20% (w/v) trichloroacetic acid and centrifuged for 5 min. The precipitate is washed subsequently with 500 μl of 10% (w/v) trichloroacetic acid and two times with 500 μl ethyl acetate/ethanol (1:1), until the washing solution of the 1,4-dinitrophenylhydrazine-treated samples is 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 1,4-dinitrophenylhydrazine derivatives was monitored at $\lambda = 366$ nm, using the HCl-treated sample as a reference. A molar absorption $\varepsilon = 22000\ M^{-1}\ cm^{-1}$ was used to calculate concentration of derivatized carbonyl sites.

2.3.3. Measurement of apomorphine oxidation

A 10 μM solution of apomorphine in 25 mM Tris-HCl buffer was left at room temperature and exposed to air for 2 h. The spectrum of the solution showed two maxima at $\lambda = 413$ and 619 nm, the latter was used for monitoring the oxidation of apomorphine. For incubations of apomorphine with mitochondria, protein concentration was 1 mg/ml. Digitonin (0.05%) was added before photometric analysis. Analysis by HPLC (high pressure liquid chromatography) was carried out according to a method reported in the literature (Sam et al., 1994).

2.4. Mitochondrial respiration

2.4.1. NADH dehydrogenase (EC 1.6.99.3) activity

The mitochondrial suspension was diluted with 10 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose. The final protein concentration in the assay mixture was 0.07 mg/ml. The buffer system used was 50 mM Tris-HCl, pH 7.4 (assay buffer). All measurements were at 25°C. NADH dehydrogenase activity was measured in the assay system, containing 0.24 mM NADH, 0.1 mM decylubiquinone (a synthetic water-soluble analog of ubiquinone Q) and 3 mM KCN. The reaction was registered as NADH turnover and followed photometrically at 340 nm (Singer, 1974). The reaction was started by the addition of decylubiquinone. Rotenone inhibition was 95%.

Since non-enzymatic oxidation of 6-hydroxydopamine under some experimental conditions may produce significant amounts of colored semi-quinone, the assays routinely were run in parallel with blanks, depleted of NADH, and the final results were corrected for the blank values.

2.4.2. Cytochrome c oxidase (EC 1.9.3.1) activity

The activity of the enzyme was assayed with ferrocytochrome c as a substrate at a final concentration ranging from 0.12 to 0.36 mg/ml, and cytochrome c was reduced by solid dithionite (Tolbert, 1974). According to the method, concentration of ferrocytochrome c was estimated by the ratio of the optical densities of the cytochrome c solution at 550 nm and 565 nm, which was equal to 10. The reaction was started with the addition of cytochrome c and followed by the measurement of the decrease in optical density at 550 nm. The correction for non-enzymatic oxidation of 6-hydroxydopamine was similar to that described for NADH dehydrogenase assay.

2.5. Statistical analysis

All results were obtained at least in triplicate and were presented as mean ± S.E.M. (Student's *t*-test), unless otherwise described.

3. Results

Ascorbate/iron-induced lipid peroxidation in rat brain mitochondrial fraction and inhibition by apomorphine in rat brain mitochondrial fraction was initiated by the addition of ferrous sulfate and monitored by the thiobarbituric acid reaction. The formation of thiobarbituric acid reactive substances was detectable with 0.5 μM Fe^{2+}, strongly increasing between 0.5 and 2.5 μM with saturation at concentrations exceeding 5 μM $FeSO_4$ (data not shown). Consequently, for all the experiments described in this paper, 2.5 and 5 μM iron were used.

The addition of submicromolar concentrations of apomorphine to the incubation buffer induced a marked reduction in the formation of thiobarbituric acid reactive substances as compared with samples containing only ascor-

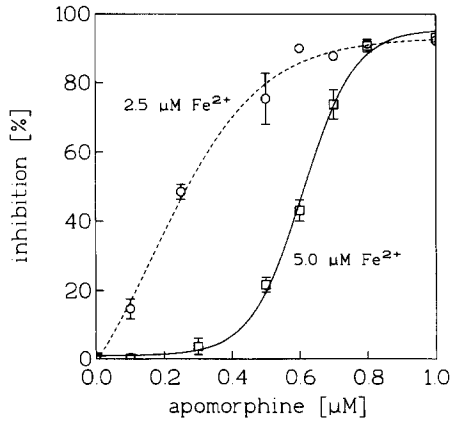

Fig. 1. Inhibition of lipid peroxidation by apomorphine in the presence of 50 mM ascorbic acid and 2.5 mM (circles, dashed line) and 5.0 mM $FeSO_4$ (squares, straight line). Statistical analysis ($n = 3$): 2.5 mM $FeSO_4$: $r = 0.996$, $P < 0.0001$; 5.0 mM $FeSO_4$: $r = 0.996$; $P < 0.0001$.

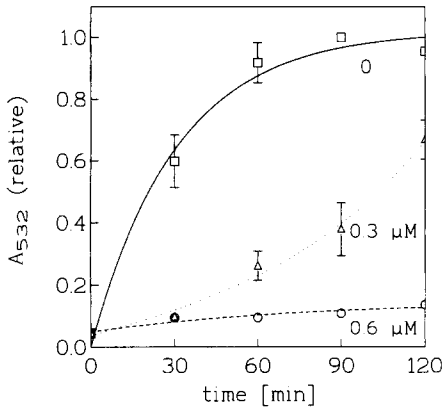

Fig. 2. Time course of thiobarbituric acid reactive substances formation in the presence of 50 μM ascorbic acid and 5.0 μM $FeSO_4$. Controls (squares, straight line) with no apomorphine, 0.3 μM (triangles, dotted line) and 0.6 μM (circles). Statistical analysis ($n = 3$): Control: $r = 0.998$, $P < 0.005$; 0.3 μM apomorphine: $r = 0.997$, $P < 0.005$; 0.6 μM apomorphine: $r = 0.992$, $P < 0.01$.

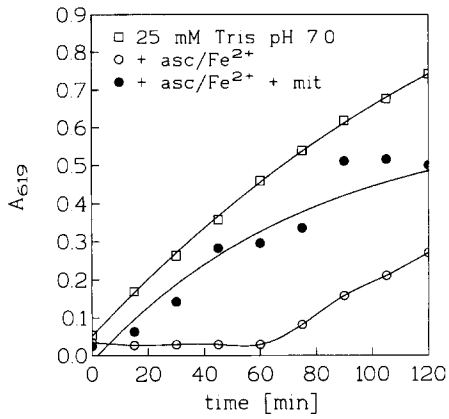

Fig. 3. Time course of apomorphine oxidation (monitored at λ = 619 nm).

bate and iron: As little as 1 μM apomorphine inhibited lipid peroxidation by 87%, even in the presence of 10 μM $FeSO_4$, a 10-fold excess of the metal ion (Fig. 1). The effectiveness of apomorphine inhibition was dependent on the iron concentration; the IC_{50} varied in the range between 0.1 and 1 μM. This influence, however, could be indirect as the overall intensity of free radical formation is also subject to changes in iron concentration. The exact values obtained: IC_{50} was 0.3 μM for 2.5 μM and 0.6 μM for 5 μM iron (Table 1). The sigmoid character of the concentration-response relation for the inhibition was stronger at the higher iron concentration, and the apparent Hill coefficient rose from 1.7 (2.5 μM $FeSO_4$) to 4.2 (5 μM $FeSO_4$, see Table 1). This is consistent with a multistep oxidation of apomorphine with a melanin-like polymer as an end product.

The time course of the formation of thiobarbituric acid reactive substances is shown in Fig. 2. In our assay system 0.3 μM apomorphine almost completely blocked lipid peroxidation at the beginning of the incubation. The increase in thiobarbituric acid reactive substances observed later during the reaction is consistent with the concept of apomorphine as a radical scavenger, which capacities are exhausted by subsequent oxidation.

3.1. Oxidation of apomorphine

Aqueous solutions of apomorphine are relatively stabile at acidic pH, whereas the catachol undergoes rapid oxidation at neutral pH, when approximately 0.1% of the molecules are deprotonated. During oxidation an intensive green chromophore ($\lambda_{max} = 619$ nm) is formed which has been used to monitor the reaction, as there is little interference with other components in the assay. The color formation (Fig. 3) reflects a complicated multistep process with autoxidation of apomorphine itself being only the initial step. The reaction is considerably slowed down by 50 μM ascorbate; addition of 5 μM Fe^{2+} afforded no significant change. In a system containing brain mitochondria, apomorphine is oxidized even in the presence of ascorbate/iron. HPLC analysis of the reaction mixtures showed that 90% of the originally added apomorphine have been consumed after 120 min. We conclude that this reaction reflects the free radical scavenging effect of apomorphine, which leads to a decrease in the formation of thiobarbituric acid reactive substances as demonstrated above.

3.2. Effects of dopamine, pergolide, and desferrioxamine on lipid peroxidation iniciated by ascorbate / iron

For a comparison with apomorphine, we checked the effect of dopamine and pergolide, an ergoline type dopamine receptor agonist selective for the dopamine D_2

Table 1
Inhibition of ascorbate/iron-induced lipid peroxidation by apomorphine, dopamine and desferrioxamine

$FeSO_4$ (μM) [a]	Apomorphine		Dopamine	Desferrioxamine
	2.5	5.0	2.5	2.5
IC_{50} (μM) [b]	0.28 ± 0.02	0.61 ± 0.02	6.59 ± 0.2	0.78 ± 0.04
Maximum inhibition (%) [c]	92 ± 1	93 ± 2	93 ± 1	75 ± 1
Hill coefficient [d]	1.7 ± 0.1	4 ± 0.3	1.0 ± 0.1	0.9 ± 0.15

[a] Concentration of ascorbate was 50 μM in all cases. [b] Obtained from regression data (mean ± S.E., $n = 6$). [c] Maximum inhibition as determined from a triplicate experiment (mean ± S.E.M.). [d] Apparent values (mean ± S.E., $n = 5$), as obtained from the slope of the cooperativity plot (Hill plot), log [apomorphine] vs. log $(I/(I_{max} - I))$.

receptor. It was found that dopamine inhibited the formation of thiobarbituric acid reactive substances in a similar manner as apomorphine, the effective concentrations being twenty times higher (Table 1). Unlike apomorphine and dopamine, pergolide does not have a catecholic structure and showed only a minor effect at concentrations higher than 50 μM (Fig. 4a). The effects of dopamine and apomorphine were found to be additive (Fig. 4b).

Desferrioxamine is a potent iron chelator that is able to inhibit iron catalyzed lipid peroxidation (Sorrenti et al., 1994). We found (Fig. 4a) that in rat brain mitochondrial fraction desferrioxamine inhibited ascorbate/iron-induced formation of thiobarbituric acid reactive substances, if added at concentrations equivalent to those of iron. Desferroxamine was not able to block lipid peroxidation completely. At maximum inhibition, 16% of the activity remained (Table 1).

3.3. Effect of dopamine and apomorphine on lipid peroxidation in the absence of ascorbate

For the induction of metal catalyzed free radical reactions, the presence of a reducing agent, like ascorbate, is required. We found no increase in the formation of thiobarbituric acid reactive substances with respect to controls, if only iron was present in the assay system. As dopamine and apomorphine can also reduce ferric iron in aqueous solution, it could not be ruled out that they stimulate lipid peroxidation in the absence of ascorbate. However, in mitochondrial suspensions containing 2.5 μM Fe^{2+}, the addition of apomorphine or dopamine did not lead to an increase in lipid peroxidation. In some samples, even a slight decrease of control values was found.

3.4. Apomorphine suppresses ascorbate / iron-induced protein carbonyl formation

Measuring protein carbonyl formation is a more specific but less sensitive method to assay free radical damage in a

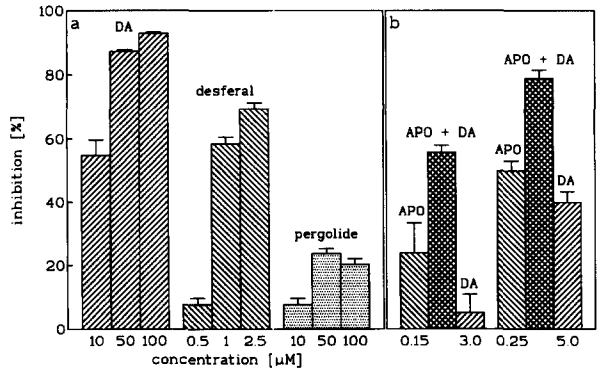

Fig. 4. (a) Inhibition of lipid peroxidation in the presence of 2.5 μM $FeSO_4$ /50 μM ascorbate by dopamine (dopamine, $P < 0.0001$), desferrioxamine ($P < 0.001$), and pergolide ($P < 0.05$). (b) Additive effects of apomorphine (APO) and dopamine (dopamine) at (from left) IC_{50} ($P < 0.0001$) and IC_{50} /2 ($P < 0.001$) ($n = 3$ in all experiments).

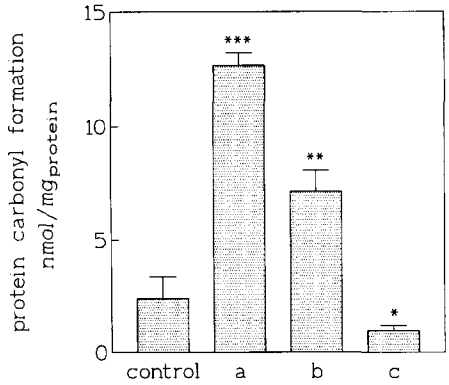

Fig. 5. Protein carbonyl formation in cortical membrane preparation treated with (a) ascorbate (15 mM) and iron (250 μM), (b) ascorbate (15 mM), iron (250 μM) and apomorphine (100 μM). (c) was identical to control, treated with 20 μl of 1 M sodium borohydride in 100 mM NaOH to completely reduce all carbonyl groups after incubation. Statistical analysis ($n = 4$): * $P < 0.5$, ** $P < 0.05$, *** $P < 0.005$. The difference between column a and b is highly significant ($P < 0.005$).

biological system. Under conditions of oxidative stress, proline, arginine, lysine and threonine residues are converted into aldehydes and ketones (Stadtman, 1993), which can be labeled with specific reagents, like 1,4-dinitrophenylhydrazine. We found that protein oxidation required more stringent conditions than lipid peroxidation and increased the concentrations of ascorbate and iron. With 250 μM ferrous iron and 15 mM ascorbic acid, a carbonyl content 12.6 nmol/mg of protein was obtained, a more than 5-fold increase as compared with the controls. After the addition of 100 μM apomorphine, protein oxidation was significantly decreased by 40% (Fig. 5).

3.5. Effect of apomorphine on mitochondrial NADH-coenzyme Q reductase, cytochrome c oxidase and on 6-hydroxydopamine inhibition of these enzymes

Within the concentration range from 0.01 to 1 μM, apomorphine had no effect on the activity of mitochondrial NADH-dehydrogenase (complex I). At 1 μM and above, it inhibited the enzyme by 60–70%. Apomorphine slowly reduced cytochrome c, but did not affect the activity of cytochrome c oxidase (complex IV). This direct interaction between apomorphine and cytochrome c was also reflected by the accelerated formation of an apomorphine-derived melanin-like polymer (data not shown). Inhibition of respiratory enzymes by 6-hydroxydopamine was unchanged by 0.01–1 μM apomorphine for complex I and 1–100 μM for complex IV.

4. Discussion

The different biochemical approaches to neuroprotection in Parkinson's disease reflect the current concepts of the ethiology of the disease. Antioxidant strategies, aiming

at inactivating free radicals or inhibiting their formation, have been in the focus of attention: Allopurinol is a competitive inhibitor of xantine oxidase, an important source of reactive oxygen species (Sorrenti et al., 1994). Inhibitors of monoamine oxidase, like the monoamine oxidase B inhibitor L-deprenyl (LeWitt, 1994; Przuntek, 1994), interrupt the metabolic formation of H_2O_2, while iron chelators, like desferrioxamine, block the formation of $HO^{\cdot}$ radicals via the Fenton reaction. The protective effect of lazaroids and 21-aminosteroids has been originally attributed to iron chelation and prevention of membrane lipid peroxidation (Braughler et al., 1987). The current literature states potent free radical sacavenging activity for this class of drugs (Zhao et al., 1995). Inhibitors of NO (nitric oxide) synthase prevent the formation of NO, which combines with $O_2^{\cdot -}$ to the toxic peroxynitrite ($ONOO^-$). $ONOO^-$ is unstable and its decomposition gives rise to $HO^{\cdot}$ and NO_2 radicals (Olanow, 1993). Classical free radical scavengers, like ascorbate or α-tocopherol, react easily with reactive oxygen species and, thus, protect biological structures from oxidation. However, promising results with vitamin E in vitro could not be reproduced clinically to slow down the progression of Parkinson's disease (LeWitt, 1994).

The neuroprotection by desferrioxamine (Liu and Mori, 1993) can be related to its activation of respiratory chain complex I and protection form 6-hydroxydopamine inhibition (Glinka et al., 1996) or to its property as an iron chelator and radical scavenger (Morel et al., 1992). The inhibition of complex I by 6-hydroxydopamine is reversible and the radical scavenger lipoic acid does not show any protective effect, and a radical mechanism, therefore, appears as unlikely.

In our model system, apomorphine did not show any protective effect on mitochondrial respiration, and at concentrations > 1 μM it inhibited NADH-coenzyme-Q reductase by 70%. Instead, apomorphine acts as a potent antioxidant and protects lipids and proteins from radical damage. This effect most likely depends on its catechol structure. The radical scavenging effect of other catechols has been established (Liu and Mori, 1993) with dopamine = norepinephrine > dihydroxyphenylacetic acid > homovanillic acid. We compared dopamine with apomorphine and found apomorphine to be more potent by a factor of 20 (Table 1). Among the compounds tested, only apomorphine was able to afford full protection from lipid peroxidation induced by ascorbate/Fe^{2+}. It showed maximum activity even if added at much lower concentrations than the metal ion. Consequently, it can be ruled out that chelation of iron contributes to this effect to a significant extent. This interpretation is confirmed by the latest results by Sam and Verbeke (1995), who demonstrated that the $R(-)$ and the $S(+)$ enantiomer of apomorphine, as well as dopamine, inhibit the oxidation of the two polyunsaturated fatty acids cholesteryl linoleate and trilinolein by free radicals, which were generated in an iron-free system through thermal decomposition of 2,2′-azobis(2-methylpropionitrile).

Pergolide without the catechol structural element showed no significant reduction of lipid peroxidation in our assay. The concentrations of apomorphine used in the described experiments (0.1–1 μM) are close to the plasma levels reached during clinical application in Parkinson's disease (50–100 nM) (Hughes et al., 1991).

While apomorphine protects membrane lipids, itself is oxidized by a multistep reaction that eventually leads to a polymeric melanin-like end product. Many of the partially oxidized intermediates may interact with free radicals. This can account for the marked iron-dependent increase in cooperativity, as can be read from the Hill coefficients (see Table 1, Fig. 2). The capacity of dopamine as a radical scavenger is smaller than that of apomorphine and it does not show an iron-dependent change of cooperativity, which may be related to the fact that dopamine melanine acts as as a pro-oxidant (Youdim et al., 1994).

Apomorphine also significantly reduces protein carbonyl formation. As the available methods to assay oxidative damage to proteins are considerably less sensitive than the thiobarbituric acid reaction, lipid peroxidation and protein oxidation can only be compared qualitatively. Much higher concentrations of ascorbate and iron must be employed to measure significant radical damage to proteins (Levine, 1983). Unlike lipid peroxidation, protein oxidation requires metal binding and proteins without metal binding site (like bovine serum albumin) are considerably less sensitive. Modification of amino acid residues can be observed primarily in the close neighborhood of the bound metal. Consequently, these processes are relatively insensitive towards antioxidants (Stadtman, 1993). Therefore, a reduction of protein oxidation by 40%, as afforded by apomorphine, can be regarded as quite relevant.

Radical scavengers have so far been successfully applied in the prevention of reperfusion injury after cerebral ischemia (Hall, 1993; Chan, 1994) and seem to have a high potential for the control of neurodegenerative diseases. From what we have learned in this study regarding the properties of apomorphine, this compound would be an important candidate as a potential neuroprotective agent in the treatment of Parkinson's disease, if indeed oxidative stress is operative.

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