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A Potential Role for Cyclized Quinones Derived from Dopamine, DOPA, and 3,4-Dihydroxyphenylacetic Acid in Proteasomal Inhibition

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ABSTRACT

We examined the ability of oxidation products of dopamine, DOPA, and 3,4-dihydroxyphenylacetic acid (DOPAC) to inhibit proteasomal activity. Dopamine, DOPA, and DOPAC underwent tyrosinase-catalyzed oxidation to generate aminochrome, dopachrome, and furanoquinone, respectively. In these studies, the oxidation of dopamine by tyrosinase generated product(s) that inhibited the proteasome, and proteasomal inhibition correlated with the presence of the UV-visible spectrum of aminochrome. The addition of superoxide dismutase and catalase did not prevent proteasomal inhibition. The addition of NADH and the quinone reductase NAD(P)H:quinone oxidoreductase 1 (NQO1) protected against aminochrome-induced proteasome inhibition. Although NQO1 protected against dopamine-induced proteasomal inhibition, the metabolism of aminochrome by NQO1 led to oxygen uptake because of the generation of a redox-labile cyclized hydroquinone, further demonstrating the lack of involvement of oxygen radicals in proteasomal inhibition. DOPA underwent tyrosinase-catalyzed oxidation to form dopachrome, and similar to aminochrome, proteasomal inhibition correlated with the presence of a dopachrome UV-visible spectrum. The inclusion of NQO1 did not protect against proteasomal inhibition induced by dopachrome. Oxidation of DOPAC by tyrosinase generated furanoquinone, which was a poor proteasome inhibitor. These studies demonstrate that oxidation products, including cyclized quinones derived from dopamine and related compounds, rather than oxygen radicals have the ability to inhibit the proteasome. They also suggest an important protective role for NQO1 in protecting against dopamine-induced proteasomal inhibition. The ability of endogenous intermediates formed during dopaminergic metabolism to cause proteasomal inhibition provides a potential basis for the selectivity of dopaminergic neuron damage in Parkinson's disease.

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by destruction of dopamine containing neurons in the substantia nigra pars compacta coupled with the formation of neuronal cytoplasmic inclusions known as Lewy bodies (Olanow and Tatton, 1999). Several lines of evidence have implicated failure of the ubiquitin proteasomal system (UPS) as central in the pathogenesis of PD, and a number of excellent, recent reviews have summarized the evidence linking defects in the UPS to both familial and sporadic PD (McNaught et al., 2001; Halliwell, 2002; Chung et al., 2003; Dawson and Dawson, 2003; McNaught and Olanow, 2003, 2005). Inhibition or failure of the UPS leads to the accumulation and aggregation of proteins, Lewy body formation, and dopaminergic cell death (McNaught et al., 2002b). It is noteworthy that the association of genetic mutations in familial PD has provided important clues to the role of a frustrated UPS and proteolytic stress in PD. Genetic mutations that have been associated with PD include α-synuclein, parkin, and UCH-L1, and all of these have been associated with impaired UPS activity (McNaught et al., 2001; Chung et al., 2003; McNaught and Olanow, 2003, 2005). α-Synuclein mutations have been suggested to result in protein misfolding, aggregation, and proteasomal impairment; parkin is a ubiquitin ligase, and UCH-L1 is a deubiquinating enzyme (McNaught et al., 2001; Chung et al., 2003; McNaught and Olanow, 2003, 2005).

In addition to genetic evidence, there have been a number of important biochemical findings that have linked an impaired UPS system to both familial and sporadic PD. The evidence for involvement of an inhibited UPS system in PD includes a loss of UPS activity in the substantia nigra of

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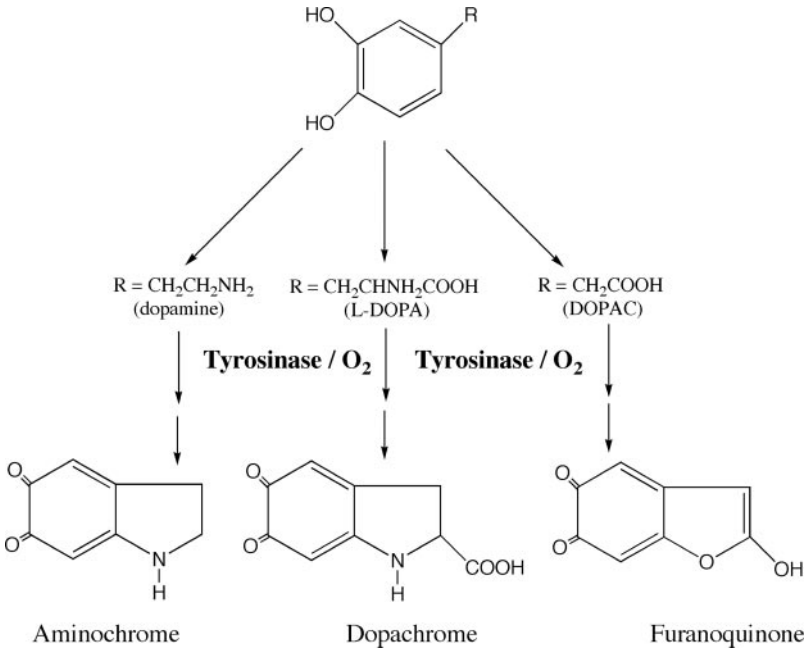
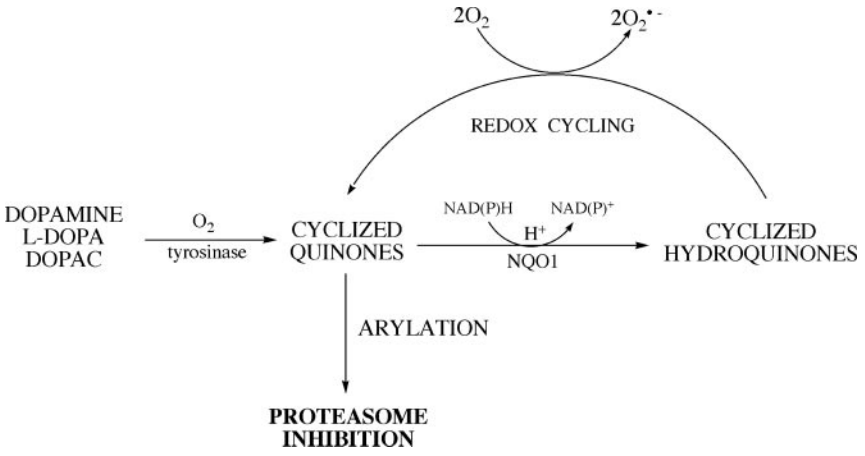
ABBREVIATIONS: PD, Parkinson's disease; DOPAC, 3,4-dihydroxyphenylacetic acid; SOD, superoxide dismutase; NQO1, NAD(P)H:quinone oxidoreductase 1; UPS, ubiquitin proteasomal system; RRL, rabbit reticulocyte lysate; MG132, *N*-benzoyloxycarbonyl (*Z*)-Leu-Leu-leucinal.

patients with PD relative to those without PD (McNaught and Jenner, 2001; McNaught et al., 2001) and immunocytochemical evidence for the presence of UPS protein residues in Lewy bodies in patients with PD (Ii et al., 1997; Andersen, 2000). More recently, defects and impairment of the 26/20S proteasomes have been detected in the substantia nigra pars compacta in PD (McNaught et al., 2002a), and importantly, dosing of proteasome inhibitors to rats caused a progressive model of PD accompanied by dopaminergic cell death, apoptosis, and the formation of α-synuclein/ubiquitin-containing inclusion bodies resembling Lewy bodies (McNaught et al., 2004).

Dopamine is known to undergo a very complex series of metabolic events in dopaminergic cells involving the tyrosinase-mediated generation of dopamine *o*-quinone and at physiological pH cyclization to leukoaminochrome, subsequent oxidation to the cyclized *o*-quinone aminochrome (Scheme 1) with eventual polymerization to melanins (Graham, 1978; Graham et al., 1978). There has been some debate regarding the expression of tyrosinase in the substantia nigra (Xu et al., 1997; Gimenez et al., 2003), but other enzymes such as peroxidases, including prostaglandin H synthase, can also catalyze the oxidation of dopamine to its quinone derivatives (Hastings, 1995; Mattammal et al., 1995). In addition to the generation of reactive quinone metabolites, autoxidation of many of the intermediates in this pathway is also possible with concomitant generation of reactive oxygen species (Graham, 1978; Graham et al., 1978; Segura-Aguilar et al., 1998). Similar pathways exist for DOPA, norepinephrine, epinephrine, and 3,4-dihydroxyphenylacetic acid (DOPAC), generating dopachrome, noradrenochrome, adrenochrome (Graham, 1978), and furanoquinone (Sugumaran et al., 1999), respectively. Simplified pathways are shown for dopamine, DOPA, and DOPAC in Scheme 1. The generation of *o*-quinones from these molecules during metabolism produces reactive species capable of arylating cellular nucleophiles (Graham, 1978; Graham et al., 1978; Khan et al., 2001) Thus, metabolic intermediates in the dopaminergic pathway are capable of both arylation and inducing oxidative stress (Scheme 2). Dopaminergic quinoid intermediates formed from dopamine may therefore represent endogenous toxic compounds and provide a potential basis for the selective loss of dopaminergic neurons in PD. Given the potential importance of proteasomal impairment in the



Scheme 1. Chemical structures of dopamine, DOPA, and DOPAC and their corresponding cyclized *o*-quinone oxidation products.



Scheme 2. Proposed pathway for the generation of cyclized *o*-quinones and the role of NQO1 in protection against proteasome inhibition.

pathogenesis of PD, in this work, we examined whether cyclized quinones generated during the tyrosinase-mediated metabolism of dopamine, DOPA, and DOPAC are capable of directly inhibiting the proteasome.

Materials and Methods

Reagents. Dopamine HCl, L-DOPA, DOPAC, tyrosinase, NADH, and catalase were obtained from Sigma Chemical Co (St. Louis, MO). Untreated rabbit reticulocyte lysate (RRL) was obtained from Promega (Madison, WI). Fluorescently labeled proteasome substrate Suc-Leu-Leu-Val-Tyr-AMC was obtained from Bachem (Torrance, CA). Superoxide dismutase (SOD) was purchased from Roche (Indianapolis, IN). MG132 was obtained from Biomol International (Plymouth Meeting, PA). Recombinant human NAD(P)H:quinone oxidoreductase 1 (NQO1) was purified from *Escherichia coli* using cibacron blue affinity chromatography as described previously (Beall et al., 1994).

Formation of Aminochrome, Dopachrome, and Furanoquinone during Tyrosinase-Mediated Metabolism of Dopamine, DOPA, and DOPAC. In the case of dopamine, reactions (60 μl, 30°C) contained 3.3 mM dopamine and 100 μg of tyrosinase in 8.3 mM Tris-HCl buffer, pH 7.4. To limit further tyrosinase-catalyzed oxidative reactions, after 3 min, the reaction mixture was centrifuged (13,000 rpm for 7 min at 4°C) through a 100-kDa molecular mass cutoff membrane filter (Microcon; Millipore Corporation, Bedford, MA), and the filtrate was collected and stored on ice. To quantify the amount of aminochrome generated, a 5-μl sample of filtrate was removed and added to 995 μl of 25 mM Tris-HCl, pH 7.4, and the UV-visible spectrum was collected (200–800 nm). The aminochrome concentration was determined at 474 nm using a molar extinction coefficient of 3058 (Baez et al., 1997). Under these conditions, the aminochrome concentration was approximately 2.7 mM (Baez et al., 1997). Metabolism of DOPA by tyrosinase was performed under identical conditions. The dopachrome concentration was determined at 474 nm using a molar extinction coefficient of 4770 (Baez et al., 1997). Under these conditions, the dopachrome concentration was approximately 1.9 mM. Tyrosinase-catalyzed oxidation of DOPAC was performed as described for dopamine and DOPA.

Inhibition of Proteasome Activity. Proteasomal activity was measured in RRL after incubation with aminochrome, dopachrome, and furanoquinone. RRL was used as a model system because it is a robust source of proteasome and is void of NQO1 activity. Reactions (100 μl, 30°C) contained 10 mM Tris-HCl, pH 7.4, 250 mM sucrose, 5 mM $MgCl_2$, 2 mM ATP, and 10 μl (1.3 mg) of RRL. After a 5-min incubation of RRL with either aminochrome, dopachrome, or furanoquinone in the absence or presence of antioxidant enzymes, the proteasome activity was determined by measuring the remaining chymotrypsin peptidase activity (Chu-Ping et al., 1992). Labeled peptide (50 μM; Suc-Leu-Leu-Val-Tyr-AMC) was added to the RRL reaction for an additional 30 min at 30°C. Reactions were terminated by the addition of 200 μl of ice-cold ethanol, centrifuged (13,000 rpm for 2 min), and 200 μl of supernatant was transferred to a 96-well plate, and the fluorescence was determined (excitation, 380 nm; emission, 460 nm) using a microplate reader at 30°C. The proteasome inhibitor MG132 (100 μM) was included as a positive control. In control experiments, no significant quenching of the hydrolyzed fluorophore by oxidation products of dopamine, DOPA, and DOPAC was observed.

Oxygen Consumption by Cyclized Quinones. Oxygen consumption was measured in stirred 3-ml reactions at 37°C using a Clark electrode. Reactions included 25 mM Tris-HCl, pH 7.4, 0.2 mM NADH, recombinant human NQO1 (3 or 50 μg), and cyclized quinone (15 or 30 μl). Oxygen consumption was measured over 20 min, and linear rates were calculated over 5 min.

Statistical Analysis. One-way analysis of variance with Tukey post test for multiple comparisons was used for statistical analysis in these studies. Statistical analysis were performed using Prism software (GraphPad Software Inc., San Diego, CA).

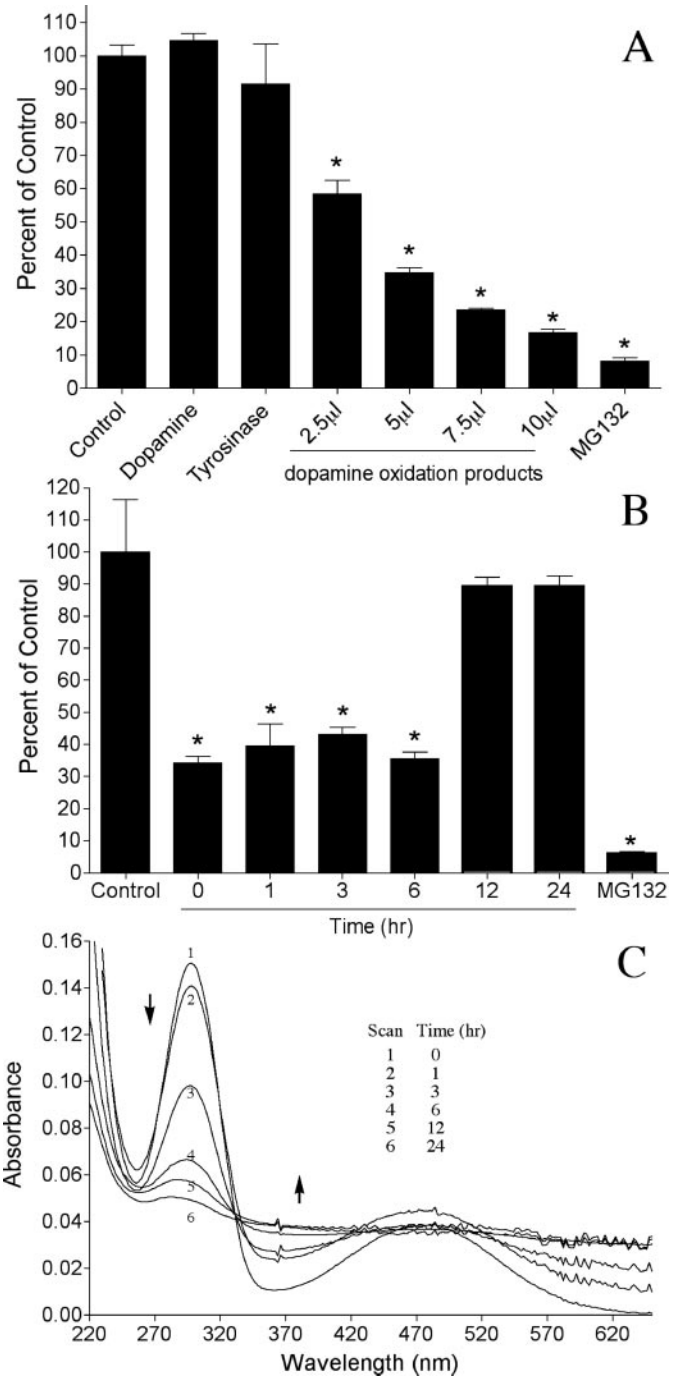


Fig. 1. The inhibition of proteasomal activity by dopamine oxidation products. A, proteasomal activity was measured in RRL after exposure to increasing concentrations of freshly prepared oxidation products. B, proteasomal activity was measured in RRL after exposure to 5 μl of freshly prepared oxidation products. For these experiments, after generation of oxidation products by tyrosinase, the enzyme was removed by centrifugation through a membrane filter, and the metabolites were then incubated at 30°C for the indicated times before the treatment of RRL. C, spectrophotometric analysis of aminochrome in solution at pH 7.4 for the indicated times. For these experiments, dopamine oxidation products were prepared as in B. Bars represent the mean ± S.D. of three to four determinations. *, $p < 0.001$ significantly different from tyrosinase-only control (A) or significantly different from control (B).

Results

We examined the inhibition of proteasomal activity in RRL during tyrosinase-mediated metabolism of dopamine, DOPA, and DOPAC. Aminochrome, dopachrome, and furanoquinone were generated by tyrosinase-catalyzed oxidation of dopamine, DOPA, and DOPAC, respectively, and the subsequent removal of tyrosinase greatly slowed any further oxidation into higher molecular weight polymers. The formation of aminochrome, dopachrome, and furanoquinone was confirmed by UV-visible spectroscopy, and the absorbance of these compounds was identical with spectra reported previously (Graham and Jeffs, 1977; Graham, 1978; Graham et al., 1978; Sugumaran et al., 1999). In these studies, oxidation by tyrosinase was used as a model system to generate reactive intermediates from dopamine, DOPA, and DOPAC. The treatment of RRL with increasing quantities of tyrosinase-catalyzed dopamine oxidation products resulted in a corresponding decrease in proteasome activity (Fig. 1A). No significant proteasome inhibition was observed in control incubations containing dopamine or tyrosinase alone. Once generated, dopamine oxidation products caused effective proteasomal inhibition for at least 6 h in buffer at 30°C (Fig. 1B). In these studies, proteasomal inhibition could be directly correlated with the presence of a UV-visible spectrum for aminochrome. After 12 and 24 h in buffer, aminochrome had lost its characteristic UV-visible absorbance and had begun to form a brown insoluble precipitate (Fig. 1C). No proteasome inhibition could be detected when RRL was added to these samples (12 and 24 h), suggesting that aminochrome or derivatives other than polymerization products were responsible for proteasome inhibition. To determine whether reactive oxygen species may be responsible for the observed proteasome inhibition, we examined the ability of the aminochrome solution to consume O_2 using a Clark electrode. Very low levels of oxygen consumption were detected when aminochrome was placed into buffer (Table 1). In addition, the inclusion of SOD and/or catalase did not prevent proteasome inhibition by aminochrome (Fig. 2A), suggesting that superoxide and hydrogen peroxide were not responsible for proteasome inhibition. The ability of aminochrome to inhibit proteasome activity could be prevented if NADH and NQO1 were included in the incubation (Fig. 2B). Previous work has shown that aminochrome could be reduced by NQO1 in the presence of NAD(P)H and that the resultant hydroquinone was unstable to O_2 and underwent rapid redox cycling (Segura-Aguilar and Lind, 1989). We confirmed these data using our experimental conditions and observed a substantial decrease in the absorbance of aminochrome at 475 nm upon the addition of NADH and NQO1 (data not shown). In addition, we measured a high rate of oxygen consumption in reactions with aminochrome, NADH, and NQO1 (Table 1). It is interesting that although the addition of NADH and NQO1 resulted in more oxygen consumption and redox cycling reactions, they protected against dopamine-induced proteasomal inhibition. These findings are in agreement with the lack of effect of SOD and catalase on dopamine-induced proteasomal inhibition (Fig. 2A) and confirm that reactive oxygen species generated during dopamine metabolism are not responsible for proteasomal blockade.

The ability of metabolites generated during tyrosinase-

TABLE 1

Oxygen consumption by cyclized quinones

Values are presented as mean ± S.D. of three separate determinations.

Treatment	Oxygen Consumption	
	nmol O_2/min	*nmol O_2/min/μg NQO1*
Aminochrome (15 μl)		
Buffer	3.0 ± 0.2	
NADH	4.9 ± 0.2	
NADH, NQO1 (3.3 μg)	29.8 ± 2.4	9.0 ± 0.7
Dopachrome (30 μl)		
Buffer	1.2 ± 0.2	
NADH	3.2 ± 0.2	
NADH, NQO1 (50 μg)	9.5 ± 0.8	0.2 ± 0.02
Furanoquinone (30 μl)		
Buffer	3.7 ± 0.1	
NADH	8.4 ± 0.8	
NADH, NQO1 (50 μg)	23.8 ± 3.5	0.5 ± 0.07

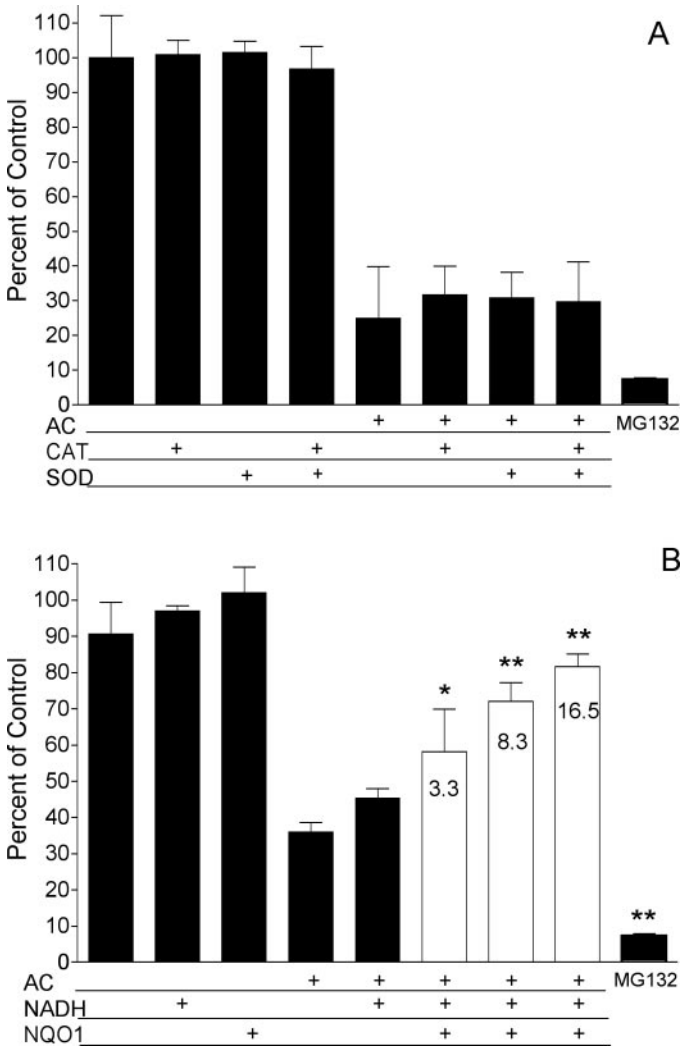


Fig. 2. The effect of antioxidant enzymes on the inhibition of proteasomal activity by aminochrome. A, proteasomal activity was measured in RRL supplemented with SOD (5 μg) and catalase (5 μg) and then exposed to freshly prepared aminochrome (5 μl). B, proteasomal activity was measured in RRL supplemented with NADH and increasing quantities of NQO1 and then exposed to freshly prepared aminochrome (5 μl). Values shown in open bars indicate the quantity (in micrograms) of NQO1 (AC, aminochrome). Bars represent the mean ± S.D. of three to four determinations. Treatment with antioxidant enzymes did not have a significant effect on proteasome inhibition compared with aminochrome alone. *, $p < 0.01$, **, $p < 0.001$ significantly different from aminochrome plus NADH control.

catalyzed oxidation of DOPA to inhibit RRL proteasome activity was also measured. A similar concentration-dependent decrease in RRL proteasomal activity was induced by metabolites formed by tyrosinase-mediated oxidation of DOPA (Fig. 3A), and these metabolites lost the ability to significantly inhibit proteasomal activity after only 3 h in buffer at 30°C (Fig. 3B). Proteasomal inhibition correlated with the formation of the characteristic absorption spectrum of dopachrome (Fig. 3C). The broadening of the characteristic dopachrome spectrum (λ_{max}, 474 nm) as a function of time in buffer indicated the formation of insoluble polymeric oxidation products. As the dopachrome spectrum was lost, the efficiency of proteasomal inhibition was decreased (Fig. 3C). Spectral changes at later time points were consistent with oxidative decarboxylation of dopachrome to form the water-insoluble product 5,6-dihydroxy-indole dihydroxy-indole (Vachtenheim et al., 1985), and proteasomal inhibitory potency was lost. The ability of NQO1 to protect against dopachrome-induced proteasome inhibition was examined. The inclusion of NADH had a small but significant protective effect on dopachrome-induced proteasome inhibition (Fig. 4), whereas the addition of NQO1 did not result in further protection (Fig. 4). Dopachrome generated via tyrosinase-catalyzed oxidation of DOPA did not generate a high rate of oxygen consumption when placed into buffer (Table 1). The addition of NADH and NQO1 resulted in only a small amount of additional O_2 consumption despite using very high quantities of NQO1 (Table 1). This confirms previous data that although dopachrome is a substrate for NQO1, it is relatively inefficient, and high concentrations of NQO1 are needed for metabolism (Baez et al., 1994).

Tyrosinase-generated metabolites of DOPAC induced only a small decrease in proteasomal activity at the highest concentration tested (Fig. 5A). A small but significant decrease in proteasomal activity was observed when metabolites were immediately incubated with RRL, but no significant proteasomal inhibition was observed if DOPAC metabolites remained in buffer for 3 h at 30°C before exposure to RRL (Fig. 5B) Furanoquinone was generated rapidly during tyrosinase-mediated metabolism of DOPAC, and further oxidation led to the formation of insoluble polymeric products (data not shown). The addition of NADH and high concentrations of

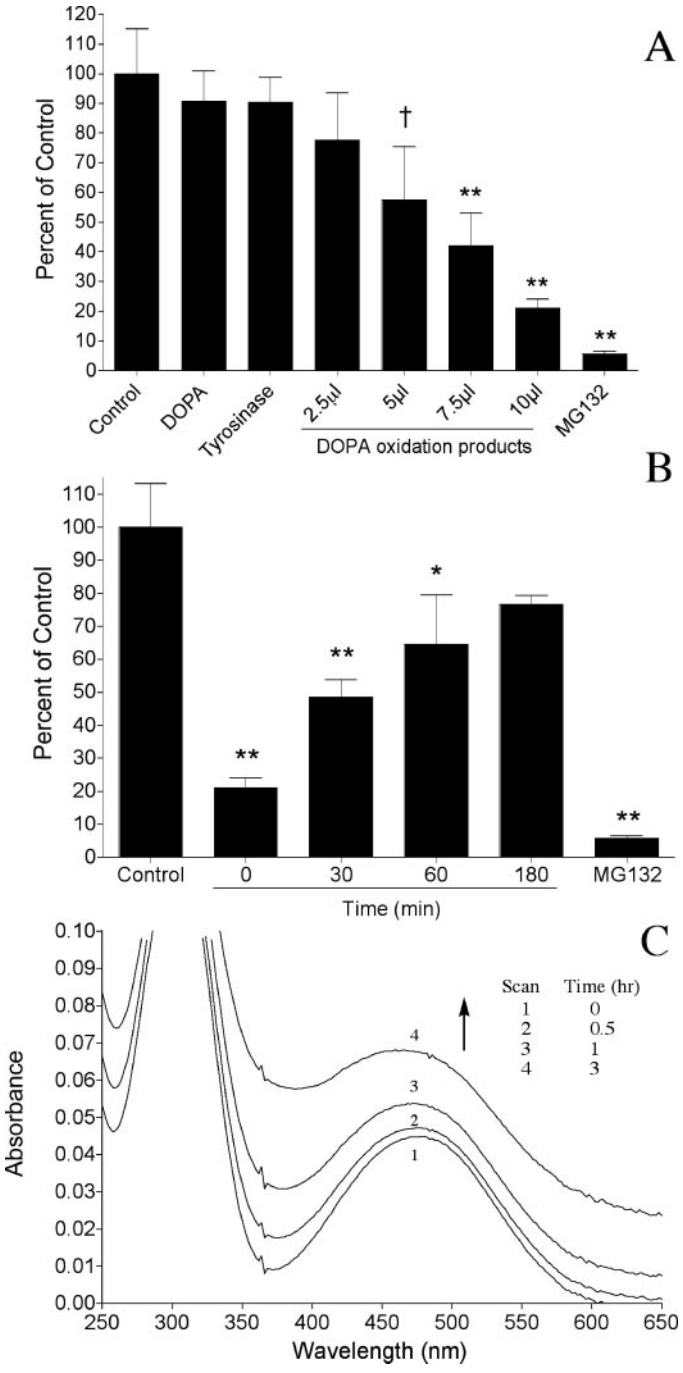


Fig. 3. The inhibition of proteasomal activity by DOPA oxidation products. A, proteasomal activity was measured in RRL after exposure to increasing concentrations of freshly prepared DOPA oxidation products. B, proteasomal activity was measured in RRL after exposure to 10 μl of DOPA oxidation products. For these experiments, after generation of oxidation products by tyrosinase, the enzyme was removed by centrifugation through a membrane filter, and the metabolites were then incubated at 30°C for the indicated times before the treatment of RRL. C, spectrophotometric analysis of dopachrome in solution at pH 7.4 for the indicated times. For these experiments, DOPA oxidation products were prepared as in B. Bars represent mean ± S.D. of three to four determinations; †, $p < 0.05$, *; $p < 0.01$; **, $p < 0.001$, significantly different from tyrosinase-only control (A) or significantly different from control (B).

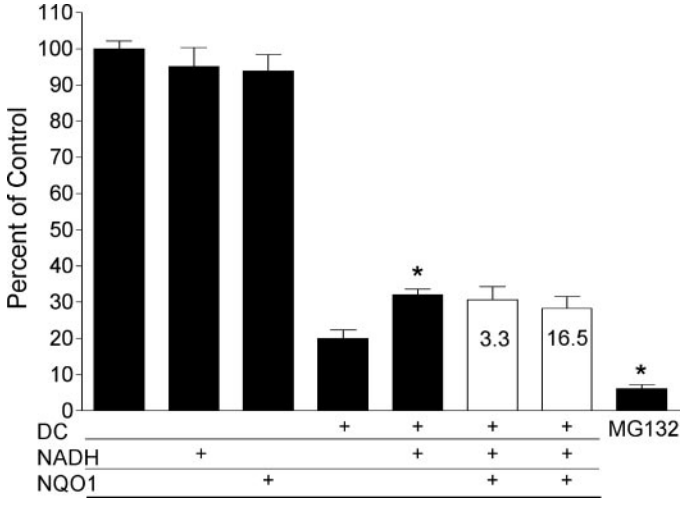


Fig. 4. The effect of NQO1 on the inhibition of proteasomal activity by dopachrome. Proteasome activity was measured in RRL supplemented with NADH and increasing amounts of NQO1 and then exposed to freshly prepared dopachrome (10 μl). Values shown in open bars indicate the quantity (in micrograms) of NQO1 (DC, dopachrome). Bars represent the mean ± S.D. of three to four determinations. *, $p < 0.01$, significantly different from dopachrome only.

NQO1, in contrast to the results found with dopamine, resulted in a small but significant protection against proteasomal inhibition (Fig. 6). DOPAC metabolites did not generate a high rate of oxygen consumption when placed into buffer (Table 1), but incubation with NADH resulted in some O_2 consumption, whereas the addition of NQO1 at high concentrations resulted in only a small additional increase in O_2 consumption (Table 1). These data suggest that furanoquinone generated during tyrosinase-mediated oxidation of DOPAC is not an efficient substrate for human NQO1 and is more similar in substrate efficiency to dopachrome. Based on the oxygen consumption data (Table 1), aminochrome was by far the best substrate for human NQO1 of the three cyclized quinones and is also the most potent and long-lasting cyclized quinone in terms of its ability to induce proteasomal inhibition (compare Figs. 1, 3, and 5).

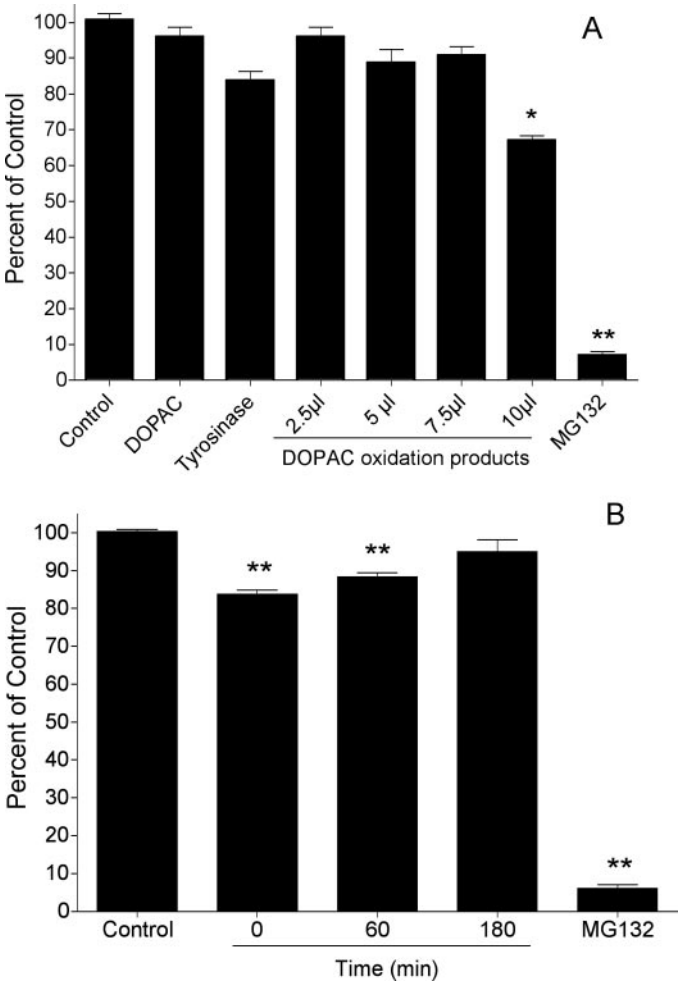


Fig. 5. The inhibition of proteasomal activity by DOPAC oxidation products. A, proteasomal activity was measured in RRL after exposure to increasing concentrations of freshly prepared DOPAC oxidation products. B, proteasomal activity was measured in RRL after exposure to 10 μl of DOPAC oxidation products. For these experiments, after generation of oxidation products by tyrosinase, the enzyme was removed by centrifugation through a membrane filter, and the metabolites were then incubated at 30°C for the indicated times before the treatment of RRL. Bars represent the mean ± S.D. of three to four determinations. *, $p < 0.01$; **; $p < 0.001$, significantly different from tyrosinase only (A) or significantly different from control (B).

Discussion

The major observation from this study is that endogenous intermediates formed during the metabolism of dopamine, DOPA, and DOPAC result in proteasomal impairment. Our data suggest that cyclized quinones generated during the tyrosinase-mediated oxidation of dopamine, DOPA, and DOPAC are capable of inhibiting proteasomal activity. Given the importance of proteasomal impairment to the pathogenesis of PD, this provides a potential basis for the selectivity of destruction of dopaminergic neurons in PD. It is noteworthy that dopamine can cause proteasomal impairment in dopaminergic neural cell lines in culture (Keller et al., 2000; Zafar et al., 2006).

Metabolism of catecholamines in dopaminergic cells is complex and involves the generation of reactive oxygen species, quinonoid metabolites, and polymeric products. Unequivocal characterization of the chemical species responsible for proteasomal inhibition in such a system is difficult. However, our data suggest that at least in this RRL-containing cell-free system, cyclized quinones or metabolites generated from them and not reactive oxygen species are responsible for proteasomal inhibition. In the case of dopamine, proteasomal inhibition correlated temporally with the optimal formation of the cyclized quinone (aminochrome) chromophore, indicating an important role for aminochrome in proteasomal inhibition. Another piece of evidence strongly linking the dopamine metabolite aminochrome to proteasomal inhibition was the protective effect of the quinone reductase NQO1. These experiments demonstrate that aminochrome, either as a result of direct reactions or via secondary reactions to generate additional reactive species, plays an important role in proteasomal inhibition. Likewise, our results suggested that the corresponding cyclized quinone dopachrome derived from DOPA was capable of causing proteasomal inhibition. Oxidation products of DOPAC were less potent at inducing proteasomal inhibition, but temporal experiments were consistent with furanoquinone playing a

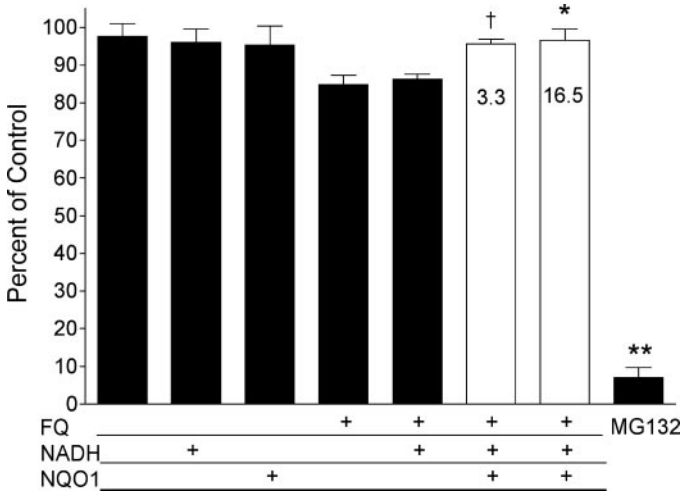


Fig. 6. The effect of NQO1 on the inhibition of proteasomal activity by furanoquinone. Proteasomal activity was measured in RRL supplemented with NADH and increasing amounts of NQO1 and then exposed to freshly prepared furanoquinone (10 μl). Values shown in open bars indicate the quantity (in micrograms) of NQO1 (FQ, furanoquinone). Bars represent the mean ± S.D. of three to four determinations. †, $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$, significantly different from furanoquinone and NADH control.

potential role in proteasomal blockade. Both aminochrome and dopachrome, when injected into the rat substantia nigra, have marked motor and behavioral effects consistent with effects on the nigrostriatal dopamine system (Diaz-Veliz et al., 2004).

Reactive oxygen species did not seem to be responsible for dopamine-induced proteasomal inhibition in our experiments. The evidence supporting this conclusion includes a lack of effect of SOD and catalase on dopamine-induced proteasomal inhibition and the fact that NQO1-mediated metabolism of aminochrome results in an increase in the generation of reactive oxygen species as a result of the redox instability of the hydroquinone generated, but it actually protects against dopamine-induced proteasomal inhibition.

Although the later products of oxidative metabolism such as polymeric melanin like products do not seem to play a role in proteasomal blockade, it remains a possibility that metabolites downstream of the cyclized quinones may be responsible for proteasomal inhibition. The situation is made more complex by the suggestion that additional reactive intermediates may be formed in the dopaminergic metabolic cascade such as reactive quinone methides (Sugumaran et al., 1999). It is interesting to note that the interaction of dopamine-derived aminochrome with α-synuclein, which has been proposed to cause accumulation of pathogenic protofibrils (Conway et al., 2001), has been recently demonstrated to occur via a conformational change in the protein rather than a covalent modification (Norris et al., 2005). Thus, quinonoid species formed during dopaminergic metabolism may have additional noncovalent mechanisms of interaction with proteins that might underlie pathogenesis. Unequivocal definition of the reactive metabolite(s) responsible for proteasomal impairment and the mechanism underlying inhibition should be a direction for future research.

The control of quinone concentrations in dopaminergic neurons will not only depend on their rate of generation but on other parameters including the levels of cellular thiols such as glutathione and enzymes capable of quinone metabolism such as NQO1. Thiols will interact with quinones generated during dopaminergic metabolism either directly or via glutathione transferase-mediated reactions (Graham et al., 1978; Baez et al., 1997; Xu et al., 1998; Stokes et al., 1999, 2000; Drukarch and van Muiswinkel, 2000). It is noteworthy that glutathione transferase isozyme GST M2-2 is known to catalyze the conjugation of glutathione with cyclized quinones extremely efficiently (Baez et al., 1997; Segura-Aguilar et al., 1997), and levels of this enzyme are likely to be important in the ultimate disposition of any quinones generated. One of the significant findings in this study was that NQO1 protected against dopamine-induced proteasomal impairment. NQO1 is known to metabolize aminochrome and dopachrome (Segura-Aguilar and Lind, 1989; Baez et al., 1994), has been located in both rat (Schultzberg et al., 1988) and human mesencephalic tissue (van Muiswinkel et al., 2004), and has also been found to be elevated in the substantia nigra pars compacta of parkinsonian brains (van Muiswinkel et al., 2004). A neuroprotective role for NQO1 against aminochrome-dependent toxicity is supported by previous work in catecholaminergic cell lines (Paris et al., 2001, 2005; Arriagada et al., 2004) and in vivo in rats (Diaz-Veliz et al., 2002; Segura-Aguilar et al., 2004). There is conflicting evidence regarding the relationship of NQO1 polymorphisms to the incidence of PD (Harada et al., 2001; Shao et al., 2001), but the elevation of enzyme levels in the target cells for PD in parkinsonian brains suggested that it may play a protective role (van Muiswinkel et al., 2004). However, van Muiswinkel et al. (2004) pointed out that NQO1 may also contribute to dopamine-induced pathology as a result of the generation of redox unstable hydroquinones, which can redox cycle (Segura-Aguilar and Lind, 1989; Baez et al., 1994). At least with respect to proteasomal inhibition, our data suggest that NQO1 plays a protective role against dopamine-derived quinones, and this conclusion is strengthened by the recent observation that NQO1 also protects against dopamine-induced apoptosis (Inayat-Hussain et al., 2005) .

In summary, our data implicate cyclized *o*-quinones from dopamine, DOPA, and DOPAC, or reactive species derived from these quinones, in the inhibition of proteasomal activity. Reactive oxygen species do not seem to be involved in dopamine-induced proteasomal inhibition. It is noteworthy that the quinone reductase NQO1 is capable of abrogating dopamine-induced proteasomal inhibition by efficiently reducing aminochrome. The ability of cyclized *o*-quinones generated during dopaminergic metabolism to cause proteasomal impairment provides a potential basis for the selectivity of dopaminergic neuron damage in PD.

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