Dopamine and L-dopa as Selective Endogenous Neurotoxins

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Abstract

Selective neurotoxins have the ability to exert their neurotoxic effects in some specific neuronal systems. In dopaminergic neurons, the selectivity of exogenous neurotoxins depends on their affinity to the dopamine transporter. However, dopamine and 3,4-L-dihydroxyphenylalanine (L-dopa) are synthesized in dopaminergic neurons and are likewise able to induce neurotoxicity. The possible molecular mechanisms involved in dopamine and L-dopa neurotoxicity in dopaminergic neurons are discussed. Dopamine seems to be neurotoxic in dopaminergic neurons by undergoing oxidation to aminochrome, which is the precursor to neuromelanin. However,

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aminochrome can be neurotoxic when it forms adducts with proteins such as alpha-synuclein, parkin, mitochondrial complexes I and III, actin, tubulin, and the dopamine transporter, or when aminochrome is one-electron reduced by flavoenzymes that use NADH, generating redox cycling with the concomitant depletion of energy and the formation of reactive oxygen species. L-dopa is also neurotoxic in cell cultures after oxidizing to a quinone species, but L-dopa seems to be a transient precursor of dopamine in that it is not able to induce neurotoxicity in vivo due to the efficient decarboxylation to dopamine catalyzed by amino acid decarboxylase. In fact, the only metabolite found in vivo is L-3-*o*-methyldopa, as detected in microdialysis experiments in animals treated with L-dopa. L-dopa is used in Parkinson's disease treatment, and it is still questionable whether L-dopa accelerates the degeneration of remaining dopaminergic neurons. It seems that L-dopa itself does not accelerate dopaminergic neuron degeneration because L-dopa is efficiently converted to dopamine, both in the peripheral and the central nervous systems. However, L-dopa induces dyskinesias in approximately 40 % patients with 4–6 years of treatment, and although the mechanism for L-dopa-induced dyskinesias is very complex, the rapid oscillation of striatal dopamine during L-dopa treatment has been found to be required for the induction of dyskinesias. The remaining dopaminergic neurons convert L-dopa to dopamine and release dopamine to the striatum under regulated conditions, but the majority of dopamine release to the striatum is mediated by serotonergic neurons without regulation, resulting in dyskinesias.

Keywords

Aminochrome • Dopamine • Dyskinesia • L-dopa • Metabolism • Neurotoxicity • Neurotoxins • Orthoquinones • Oxidation • Parkinson's disease

List of Abbreviations

AADC	Aromatic amino acid decarboxylase
COMT	Catechol ortho-methyltransferase
DA	Dopamine
GST M2-2	Glutathione S-transferase M2-2
L-dopa	L-dihydroxyphenylalanine
MAO	Monoamine oxidases
TH	Tyrosine hydroxylase
VMAT-2	Vesicular monoaminergic transporter-2

1 Synthesis of L-dopa and Dopamine

De novo synthesis of dopamine is mediated by two enzymes using the amino acid tyrosine as precursor. The first step is catalyzed by the enzyme tyrosine hydroxylase (TH), in which tyrosine is used to form L-dihydroxyphenylalanine (L-dopa). L-dopa is decarboxylated by the enzyme aromatic amino acid decarboxylase (AADC) to generate dopamine and CO_2. This dopamine is stored in monoaminergic

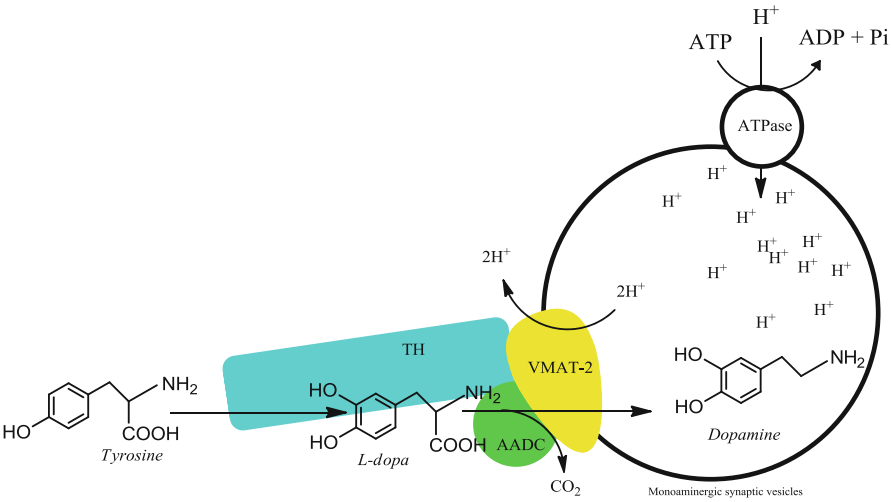
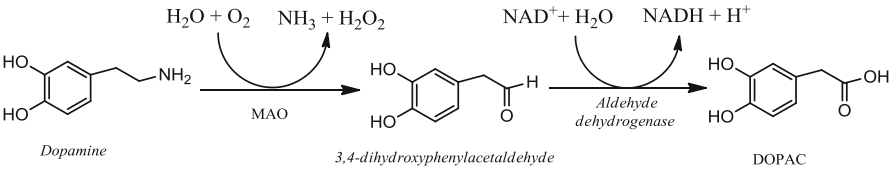


Fig. 1 *Dopamine synthesis*. Dopamine synthesis from tyrosine is catalyzed by tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase (AADC), and uptake into monoaminergic synaptic vesicles is mediated by VMAT-2

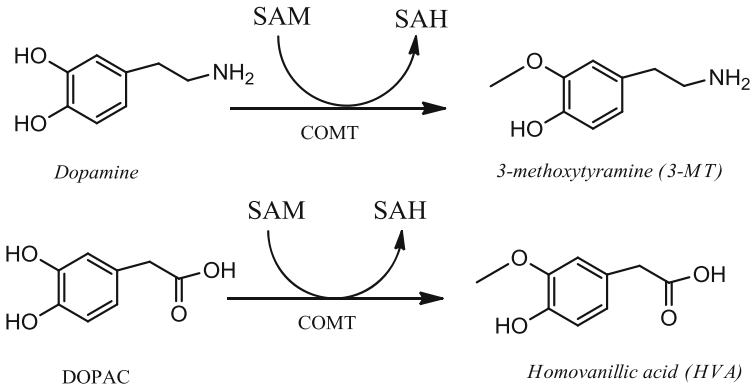
vesicles with a weak acid pH that prevents dopamine oxidation to *o*-quinone species, as dopamine autoxidizes at physiological pH due to the dissociation of the protons of hydroxyl groups. Dopamine oxidation to *o*-quinones after the synthesis of L-dopa and dopamine is prevented by the association of the enzymes TH and AADC with the vesicular monoaminergic transporter-2 (VMAT-2), which is present in the membranes of monoaminergic synaptic vesicles, generating a kind of complex that prevents free cytosolic dopamine (Cartier et al. 2010). Dopamine inside monoaminergic synaptic vesicles is hard protonated, preventing the oxidation of catechol groups to *o*-quinone. Monoaminergic synaptic vesicles express a vesicular ATPase that hydrolyzes ATP to ADP plus inorganic phosphate with one proton (H^+) translocation into the vesicles, creating a proton gradient with a weak acid pH inside the vesicles (Guillot and Miller 2009; Fig. 1).

1.1 Dopamine Degradation

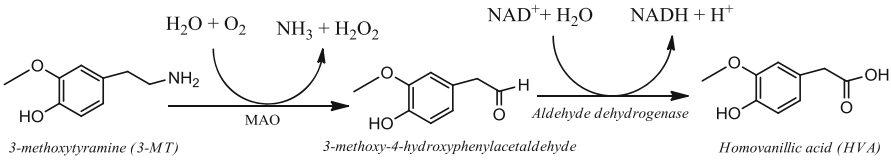
Dopamine accumulates inside monoaminergic synaptic vesicles for neurotransmission, which is an essential process in the control of movements. However, the catechol group of free cytosolic dopamine can be oxidized to neurotoxic *o*-quinones (Arriagada et al. 2004; Paris et al. 2010, 2011). To prevent this effect, dopamine participates in a neurotoxic reaction, and there are two types of enzymes that degrade dopamine. The first is the monoamine oxidases (MAO, E.C. 1.4.3.4), which catalyze the oxidative deamination of the dopamine amino group to 3,4-dihydroxyphenylacetaldehyde with concomitant formation of ammonia and hydrogen peroxide. Aldehyde dehydrogenase then catalyzes the oxidation of 3,4-dihydroxyphenylacetaldehyde to 3,4-dihydroxyphenylacetic acid (DOPAC) with the formation of a molecule of NADH.



The MAO enzymes have multiple isoforms (A and B forms) that are 70 % identical and are localized to the outer membranes of the mitochondria in neurons, glial cells, and other cells (Weyler et al. 1990; Shih et al. 1997). MAO-B is found in histaminergic and serotonergic neurons, as well as in astrocytes, while MAO-A is mainly found in catecholaminergic neurons (Westlund et al. 1988; Saura et al. 1994). MAO-A uses the compounds dopamine, noradrenaline, adrenaline, and serotonin as substrates, while MAO-B uses compounds such as phenylethylamine and tyramine (Strolin-Benedetti et al. 1992). The second enzyme that degrades dopamine is catechol ortho-methyltransferase (COMT; EC 2.1.1.6), which catalyzes the methylation of dopamine by using S-adenosylmethionine (SAM) as a cofactor to form 3-methoxytyramine and S-adenosylhomocysteine (SAH). COMT also plays a role in MAO-catalyzed degradation of dopamine, as DOPAC is methylated to homovanillic acid (HVA).



MAO also participates in dopamine degradation catalyzed by COMT because 3-methoxytyramine is a substrate for MAO, which catalyzes the oxidative deamination of 3-methoxytyramine to 3-methoxy-4-hydroxyphenylacetaldehyde. Aldehyde dehydrogenase catalyzes the oxidation of 3-methoxy-4-hydroxyphenylacetaldehyde to homovanillic acid with the concomitant formation of NADH.



COMT is expressed in pyramidal neurons, striatal spiny neurons, cerebellar Purkinje, and granular cells (Myöhänen et al. 2010), and it has two isoforms – a soluble form (S-COMT) and a membrane-bound form (MB-COMT). Inhibition of COMT by entacapone has been used in the treatment of Parkinson's disease, prolonging the half-life of L-dopa (Marin and Obeso 2010).

2 Dopamine Neurotoxicity

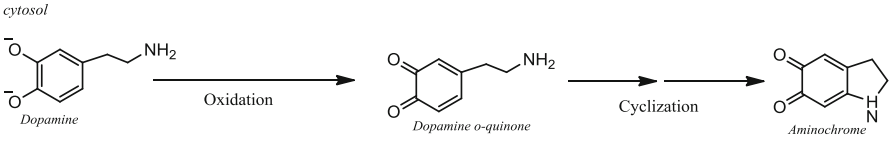
Dopamine exposure at different cell cultures or cell lines resulted in the induction of cell death, suggesting a neurotoxic role for dopamine (Hoyt et al. 1997; Jeon et al. 2010). Dopamine-dependent cell death has been reported to be dependent on an apoptotic cell death mechanism (Ziv et al. 2001). Dopamine exposure of human neuroblastoma NMB cells, which are able to take up dopamine, induces morphological changes, such as cell shrinkage, apoptosis-like atrophy, accumulation of apoptotic particles, DNA fragmentation, and protein synthesis-dependent cell death (Simantov et al. 1996). A study conducted with SH-SY5Y neuroblastoma cells displayed caspase-9 and caspase-3 activation, cleavage of poly(ADP-ribose) polymerase, and nuclear condensation, accompanied by the activation of p38 mitogen-activated protein kinase. The presence of the antioxidant N-acetyl-L-cysteine prevents dopamine-induced p38 kinase activation, caspase-9 and caspase-3 cleavage, and subsequent apoptosis (Junn and Mouradian 2001). Overexpression of Bcl-2, which blocks physiological apoptosis, in PC-12 cells results in a marked resistance to dopamine-induced cell death (Offen et al. 1997). The activation of the JNK pathway precedes dopamine-induced apoptosis and is persistently sustained during the process of apoptosis. Overexpression of a dominant negative mutant SEK1, an upstream kinase of JNK, inhibits both dopamine-induced JNK activation and apoptosis (Luo et al. 1998). Another study reported that dopamine activates SAPK/JNK and p38, but not MEK or ERK/MAPK (Gómez-Santos et al. 2003). It has been reported that dopamine oxidation activates the DNA-binding activity of NF-kappaB and the suppression of NF-kappaB transcriptional activity in PC-12 cells, inducing apoptotic cell death during dopamine oxidation (Lee et al. 2001b). The formation of adducts between alpha-synuclein and the presynaptic human dopamine transporter facilitates the membrane clustering of the dopamine transporter (DAT), thereby accelerating cellular dopamine uptake and dopamine-induced cellular apoptosis (Lee et al. 2001a). Interestingly, parkin prevents dopamine-induced alpha-synuclein-dependent cell death by blocking DAT-mediated dopamine uptake, which is accelerated by alpha-synuclein (Moszczynska et al. 2007). Another study showed that parkin protects against dopamine toxicity by decreasing oxidative stress and the subsequent activation of the JNK/caspase pathway (Jiang et al. 2004). The apoptosis induced by dopamine in PC-12 cells was found to be accompanied by an impairment of mitochondrial bioenergetic functions (Jana et al. 2011). The treatment of human SH-SY5Y cells with dopamine induced an early increase in the expression of hypoxia-inducible factor-1alpha (HIF-1alpha) followed by increases in p53, Puma, and Bnip3, in

which caspase-3, caspase-7, and PARP were activated after 12 h (Giménez-Xavier et al. 2009). The agonist of protein kinase A, forskolin, stimulated dopamine uptake in SK-N-SH cells and blocked dopamine-induced apoptosis (Liu et al. 2001). Dopamine-induced cell death was found to be preceded by a decrease in proteasome activity (Keller et al. 2000). Antioxidants were found to protect against dopamine-induced cell death (Junn and Mouradian 2001; Jana et al. 2011), and the glutathione-depleting compound L-buthionine sulfoximine enhanced dopamine-induced cell death (Stokes et al. 2000).

Why does dopamine induce neurotoxicity when it is an essential neurotransmitter for the control of movement? A possible explanation is that dopamine inside monoaminergic synaptic vesicles is completely inert because the protons of dopamine groups are hardbound to the oxygen as a consequence of the weak acid pH inside the vesicles, which is 2–2.4 pH units lower than the pH in the cytosol (Guillot and Miller 2009). However, the protons of dopamine hydroxyl groups are dissociated at cytosolic pH and can oxidize in the presence of oxygen, even in the absence of metal catalysis (Linert et al. 1996). The dopamine catechol structure oxidizes to orthoquinones, which can induce neurotoxicity.

3 Dopamine Oxidation to Orthoquinones

The protons of dopamine hydroxyl groups are dissociated when dopamine is in the cytosol at physiological pH and spontaneously oxidizes in the presence of oxygen and in the absence of metal traces (Linert et al. 1996). Dopamine oxidizes to dopamine *o*-quinone, which cyclizes to form aminochrome at physiological pH. Dopamine *o*-quinone is not stable at physiological pH because the amino group of dopamine *o*-quinone spontaneously rearranges and undergoes cyclization; thus, dopamine *o*-quinone is only stable at a pH below 2.0 (Segura-Aguilar and Lind 1989).



Dopamine oxidation can also be catalyzed by metals such as manganese(III), copper sulfate(II), iron chloride(III), and sodium periodate (Segura-Aguilar and Lind 1989; Paris et al. 2001, 2005a; Graham et al. 1978) or by enzymes such as prostaglandin H synthase, cytochrome P450, xanthine oxidase, lactoperoxidase, tyrosinase, and dopamine β-monooxygenase (Galzigna et al. 2000; Thompson et al. 2000; Segura-Aguilar 1996; Foppoli et al. 1997; Hastings 1995; Segura-Aguilar et al. 1998; Jimenez et al. 1984). It is important to remember that aminochrome formation is dependent on the presence of free cytosolic dopamine that can oxidize to aminochrome, and VMAT-2 and MAO prevent dopamine oxidation, as VMAT-2 mediates dopamine accumulation in monoaminergic synaptic vesicles and MAO catalyzes the degradation of dopamine.

4 Aminochrome Metabolism

Aminochrome is able to participate in five different reactions, including (i) the polymerization of aminochrome to neuromelanin. Neuromelanin is a pigment present in dopaminergic neurons, which is localized in the substantia nigra and accumulates with age (Zecca et al. 2002) in healthy individuals; it is also dramatically decreased in patients with Parkinson's disease. Therefore, neuromelanin seems to play a protective role in dopaminergic neurons because it chelates metals and binds proteins such as alpha-synuclein (Gerlach et al. 2003; Hong and Simon 2007; Fasano et al. 2006). The localization of neuromelanin in double-membrane vesicles seems to be very important for its protective role, as free neuromelanin has been found to be neurotoxic in cell cultures (Naoi et al. 2008) and extracellular neuromelanin induces microglial activation in the substantia nigra (Zhang et al. 2011; Fig. 2). (ii) Aminochrome also forms adducts with proteins. Aminochrome induces and stabilizes the formation of neurotoxic protofibrils of alpha-synuclein (Conway et al. 2001; Norris et al. 2005). Aminochrome forms adducts with other proteins, such as actin and α- and β-tubulin, and it disrupts the architecture of the cytoskeleton and complexes I and III of the mitochondria (Paris et al. 2010; Van Laar et al. 2009). Dopamine *o*-quinone, the transient precursor of aminochrome at physiological pH, forms adducts with and inactivates parkin, which is a ubiquitin ligase of the proteasomal system (LaVoie et al. 2005); tyrosine hydroxylase; the human dopamine transporter; and tryptophan hydroxylase (Xu et al. 1998; Whitehead et al. 2001; Kuhn and Arthur 1998). (iii) Aminochrome can be one-electron reduced, catalyzed by flavoenzymes that transfer one electron by using NADH or NADPH ions. The leukoaminochrome *o*-semiquinone radical is extremely reactive under aerobic conditions, generating a redox cycle between the leukoaminochrome *o*-semiquinone radical and aminochrome (Baez et al. 1995; Segura-Aguilar et al. 1998). This redox cycling depletes the NADH and O_2 required for ATP production in the mitochondria or the NADPH required for the reduction of oxidized glutathione, which is an important antioxidant. There is significant evidence that supports the neurotoxic role of the one-electron reduction of aminochrome (Paris et al. 2001, 2005a, b, 2009, 2010, 2011; Arriagada et al. 2004; Fuentes et al. 2007; Díaz-Véliz et al. 2008; Muñoz et al. 2012a, b; Fig. 2). (iv) Aminochrome can be two-electron reduced to leukoaminochrome, catalyzed by DT-diaphorase (EC.1.6.99.2), which is a flavoenzyme that uses both NADH and NADPH as electron donors. DT-diaphorase has been proposed to play a protective role in aminochrome metabolism because this enzyme prevents the neurotoxic reactions of aminochrome, including the one-electron reduction of aminochrome and the formation of adducts with proteins (Arriagada et al. 2004; Lozano et al. 2010; Paris et al. 2011; Muñoz et al. 2012a, b; Segura-Aguilar et al. 2006; Cardenas et al. 2008; Paris et al. 2010; Fig. 2). (v) Aminochrome can be glutathione-conjugated by glutathione S-transferase M2-2 (GST M2-2) to 4-*S*-glutathionyl-5,6-dihydroxyindoline, which is a stable molecule that is resistant to biological oxidizing agents (Segura-Aguilar, et al. 1997; Baez et al. 1997). GST M2-2 also conjugates the precursor of aminochrome dopamine *o*-quinone to 5-glutathionyl-dopamine, preventing the

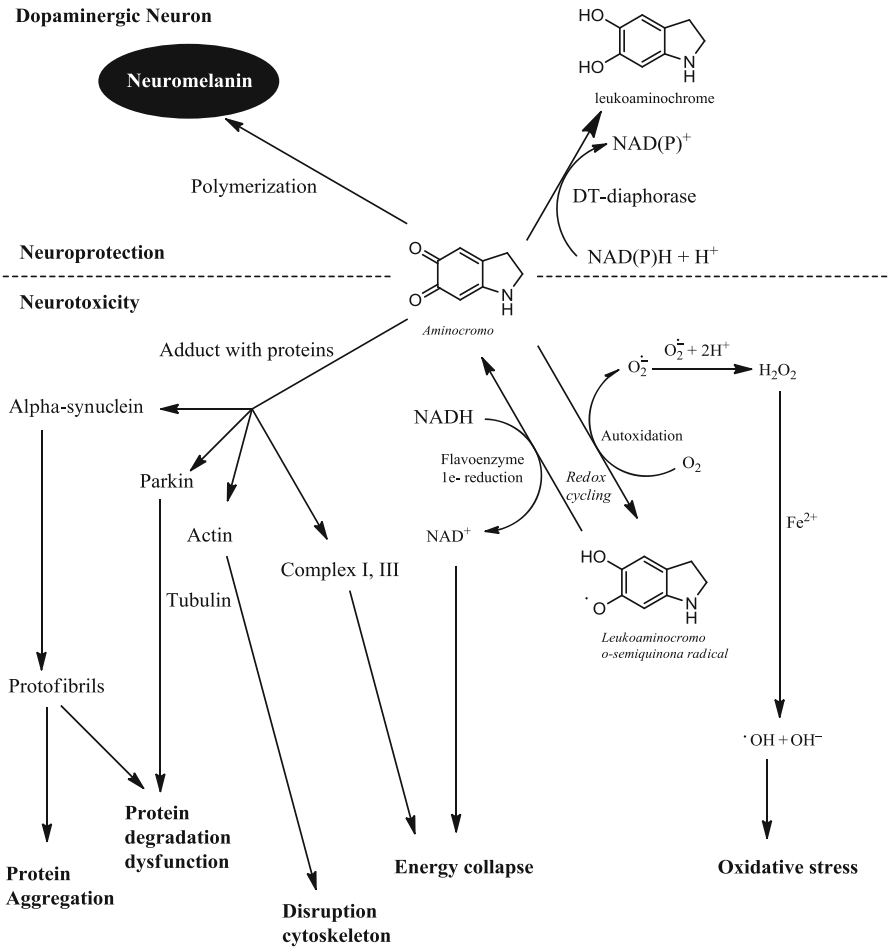


Fig. 2 *Possible metabolism of aminochrome in dopaminergic neurons*. Aminochrome is able to participate in two neurotoxic reactions, such as the formation of aminochrome adducts with alpha-synuclein, parkin, actin, tubulin, and mitochondrial complexes I and III, along with aminochrome one-electron reduction to the leukoaminochrome *o*-semiquinone radical. However, aminochrome can polymerize into neuromelanin, or it can be two-electron reduced to leukoaminochrome, catalyzed by DT-diaphorase, a reaction that prevents aminochrome-induced neurotoxicity

formation of aminochrome (Dagnino-Subiabre et al. 2000). The 5-glutathionyl-dopamine is finally converted to 5-cysteinyl dopamine (Shen et al. 1996), which has been detected in the cerebrospinal fluid of Parkinson's disease patients and in dopamine-rich brain regions such as the caudate nucleus, putamen, globus pallidus, and substantia nigra, as well as in neuromelanin (Cheng et al. 1996; Rosengren et al. 1985; Carstam et al. 1991). Therefore, the conjugation of glutathione has been proposed to be a protective reaction against aminochrome neurotoxicity in astrocytes (Fig. 3).

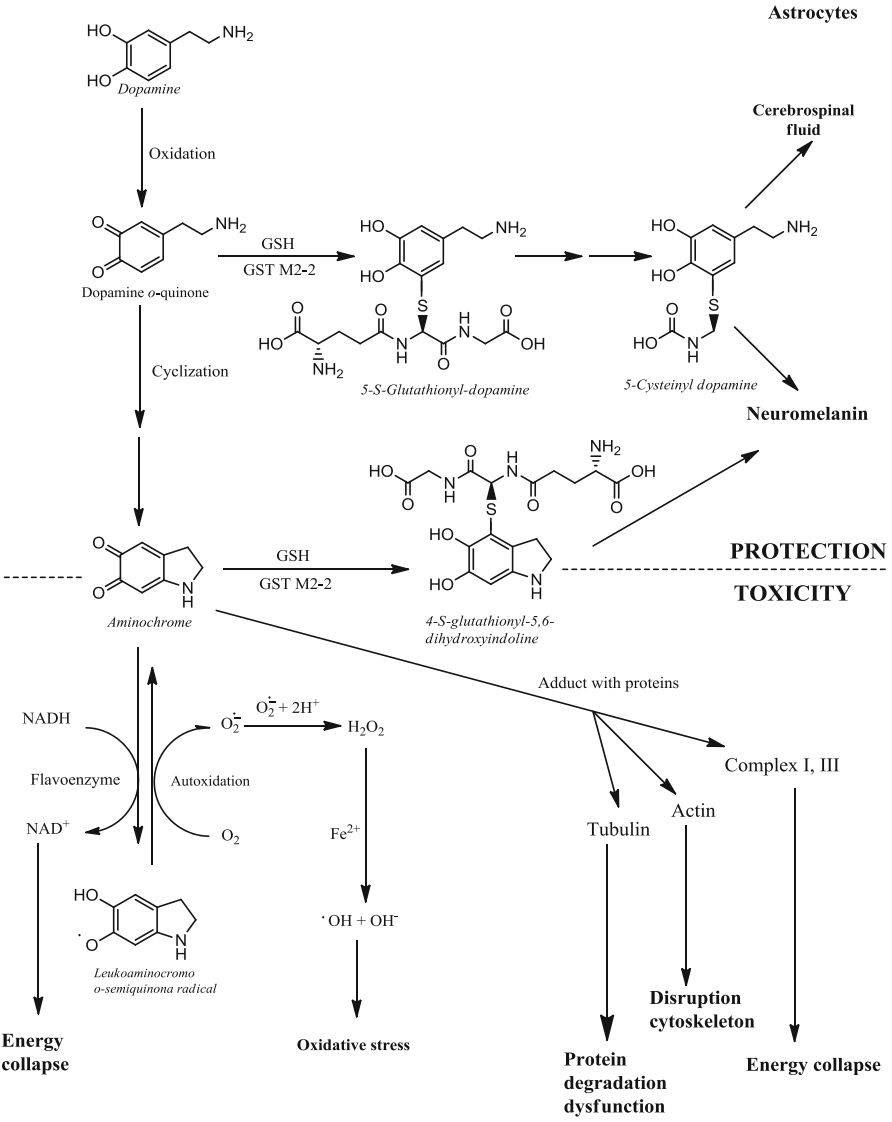


Fig. 3 *Possible metabolism of aminochrome in astrocytes*. Dopamine is taken up into astrocytes and is able to oxidize into dopamine *o*-quinone. GST M2-2 is able to conjugate this compound with glutathione to form 5-glutathionyl-dopamine, which is degraded to 5-S-cysteinyl dopamine, a compound that is found in the cerebrospinal fluid and in neuromelanin. Dopamine o-quinone cyclizes to aminochrome, which can also be conjugated with GSH by GST M2-2 to 4-S-glutathionyl-5,6-dihydroxyindoline, which is resistant to biological oxidizing agents. Both conjugations prevent aminochrome-induced neurotoxicity

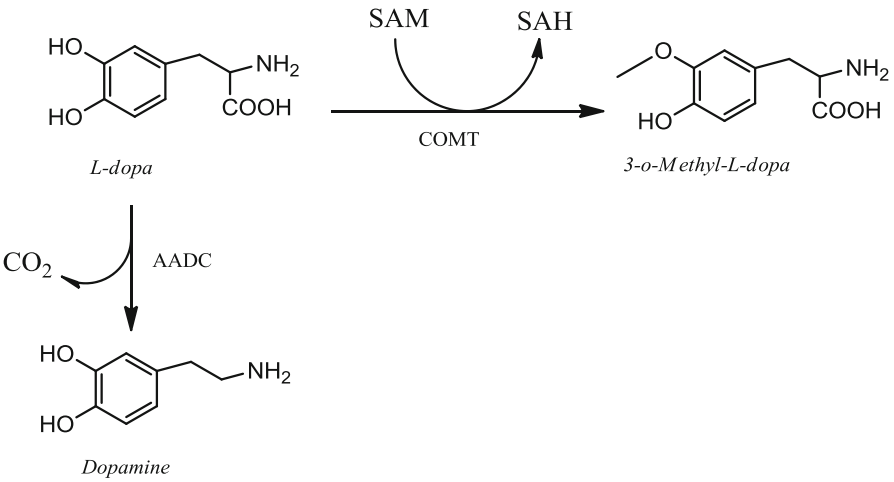
5 Aminochrome and Parkinson's Disease

The progressive loss of nigral dopaminergic neurons containing neuromelanin in the substantia nigra most likely begins long before the symptomatic phases of Parkinson's disease are present (Braak et al. 2004). Intensive research has been conducted over the past few decades to understand the molecular mechanism of the neurodegeneration of neuromelanin-containing dopaminergic neurons, with the discovery of mutations associated with Parkinson's disease. It is generally accepted that the mechanism that results in the loss of dopaminergic neurons in the substantia nigra involves the aggregation of alpha-synuclein to neurotoxic protofibrils, a dysfunction of protein degradation, mitochondrial dysfunction, oxidative stress, and neuroinflammation (Schapira 2011; Conway et al. 2001; McNaught et al. 2004; Cuervo et al. 2010; Schapira and Jenner 2011). There is growing evidence that supports the role of aminochrome in the degeneration of dopaminergic neurons that contain neuromelanin, including the following findings: (i) Aminochrome forms adducts and inactivates complexes I and III and isocitrate dehydrogenase, an enzyme of the citric acid cycle in the mitochondria (Van Laar et al. 2009). One-electron reduction of aminochrome depletes NADH, thereby decreasing ATP production in cell culture (Muñoz et al. 2012b). (ii) Aminochrome was found to induce the formation and stabilization of alpha-synuclein protofibrils (Norris et al. 2005). (iii) Aminochrome inactivates the proteasomal system of degradation of proteins by forming adducts with parkin (Zafar et al. 2006; La Voie et al. 2005). Interestingly, alpha-synuclein protofibrils inhibit chaperone-mediated autophagy (Xilouri et al. 2009) and the 26S proteasomal system (Zhang et al. 2008). Aminochrome forms adducts with tubulin (Van Laar et al. 2009), and one-electron reduction of aminochrome induces the aggregation of α- and β-tubulin, disrupting the cytoskeletal structure (Paris et al. 2010; Muñoz et al. 2012a). Tubulin aggregation prevents the microtubule formation required for the fusion of autophagocytic vacuoles and lysosomes (Monastyrska et al. 2009). (iv) Aminochrome one-electron reduction induces oxidative stress (Arriagada et al. 2004). Interestingly, aminochrome is involved in four of the five mechanisms that are generally accepted to be involved in the degeneration of neuromelanin-containing dopaminergic neurons in Parkinson's disease.

6 L-dopa Metabolism

The amino acid tyrosine is used as a substrate in the production of L-dopa, a reaction catalyzed by tyrosine hydroxylase in dopaminergic neurons. However, L-dopa is immediately converted to dopamine in a reaction catalyzed by AADC. It has been reported that the enzymes TH and AADC are associated with the membrane-bound VMAT-2, forming a kind of complex to prevent L-dopa and dopamine from remaining free in the cytosol (Cartier et al. 2010). It is unknown whether free L-dopa in the cytosol is able to undergo metabolism under

normal conditions because in Parkinson's patients, L-dopa undergoes metabolism, an important part of treatment. L-dopa can be decarboxylated to dopamine even outside the central nervous system, and L-dopa can be converted to L-3-*o*-methyldopa by COMT (Okada et al. 2011).



7 L-dopa Neurotoxicity In Vitro

Experiments in vitro with cultured neurons exposed to L-dopa resulted in apoptosis that included cell shrinkage, membrane blebbing, and nuclear and DNA fragmentation (Melamed et al. 1998; Walkinshaw and Waters 1995). L-dopa was found to be neurotoxic when it increased the expression of cytosolic cytochrome c, cleaved caspase-3, and decreased phosphorylated Akt (Ser473), phosphorylated glycogen synthase kinase-3beta (GSK-3beta) (Ser9), and heat shock transcription factor-1. The activation of PI3K protects the cells against L-dopa neurotoxicity (Park et al. 2009). L-dopa has been reported to induce cell death in SH-SY-5Y cells with the concomitant deactivation of glutaredoxin. This compound was deactivated in a dose-dependent manner, suggesting an irreversible adduction of L-dopachrome to its nucleophilic active-site Cys-22. L-dopa also decreases thioredoxin and thioredoxin reductase activity and protein content. Knockdown of glutaredoxin increases L-dopa-induced apoptosis (Sabens et al. 2010). L-dopa activates apoptosis signaling kinase 1 (ASK1), as indicated by the phosphorylation of its downstream mitogen-activated protein kinases (MAPK), p38 and JNK. The inhibition of either p38 or JNK or the knockdown of ASK1 provides protection against L-dopa-induced apoptotic neuronal cell death in the SH-SY-5Y cell line (Liedhegner et al. 2011). L-dopa has also been found to impair proteasome activity through the D_1 dopamine receptor (Berthet et al. 2012). The ability of L-dopa to be neurotoxic is dependent on its ability to oxidize to L-dopa *o*-quinone, which

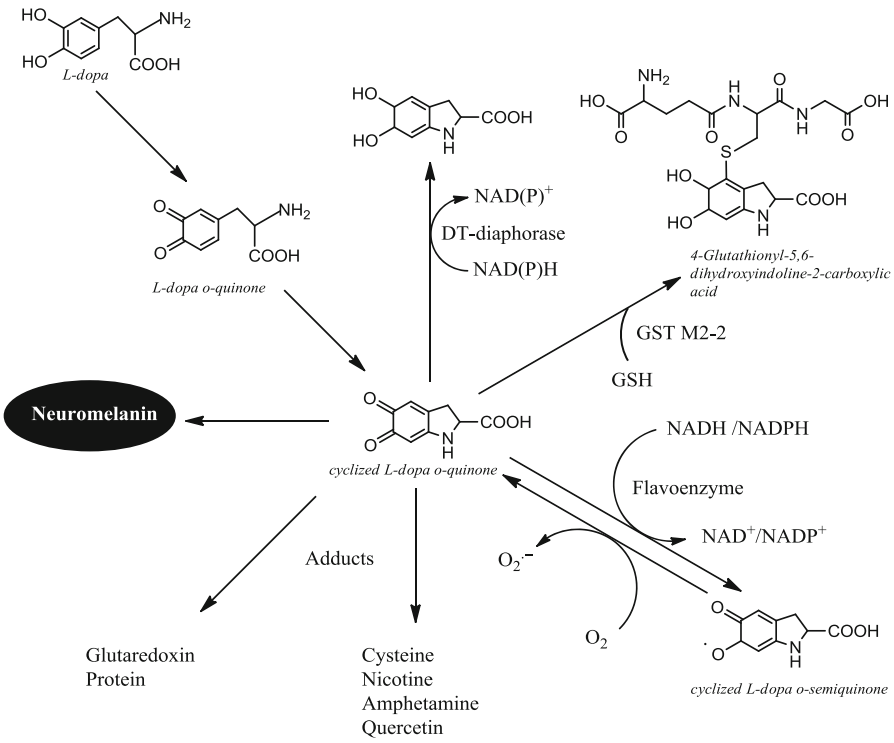


Fig. 4 *Possible metabolism of cyclized L-dopa o-quinone*. L-dopa oxidizes to L-dopa o-quinone, which cyclizes to cyclized L-dopa o-quinone. This compound is able to (i) polymerize to form neuromelanin, (ii) form adducts with proteins, (iii) form adducts with biomolecules, (iv) be one-electron reduced to the cyclized L-dopa o-semiquinone radical, (v) be conjugated by GSH to 4-glutathionyl-5,6-dihydroxyindoline-2-carboxylic acid, or (vi) be two-electron reduced, catalyzed by DT-diaphorase

spontaneously cyclizes to cyclized L-dopa *o*-quinone at physiological pH (Baez et al. 1994; Takeshima et al. 2011; Kostrzewa et al. 2002).

The cyclized L-dopa *o*-quinone is able to participate in several reactions, such as (i) polymerization to melanin (for review, see Prota 1995) and (ii) the formation of adducts with proteins such as glutaredoxin (Sabens et al. 2010). A significant increase in cell death and the formation of quinoproteins were observed when CATH.a cells were incubated with L-dopa (Asanuma et al. 2012). (iii) The formation of adducts with molecules such as cysteine, nicotine, amphetamine, and quercetin has also been reported (Dehn et al. 2001; Claffey and Ruth 2001; Kubo et al. 2007; Caudle et al. 2007; Müller and Muhlack 2012). (iv) L-dopa can be one-electron reduced with flavoenzymes that transfer one electron and use NADH or NADPH. (iv) L-dopa can also be two-electron reduced by DT-diaphorase (Baez et al. 1994), and (v) it can be conjugated by glutathione transferase M2-2 (Baez et al. 1997) (Fig. 4).

8 L-dopa-Induced Dyskinesia

L-dopa induces neurotoxicity in cell lines that are used as a model for in vitro studies of Parkinson's disease or primary cell cultures (Melamed et al. 1998; Walkinshaw and Waters 1995; Park et al. 2009; Sabens et al. 2010; Liedhegner et al. 2011). However, these neurotoxic effects in dopaminergic neurons are not observed in animal models of L-dopa, most likely as a consequence of the efficient conversion of L-dopa to dopamine. In fact, only one L-dopa metabolite is found in the literature (L-3-*o*-methyldopa), supporting the idea that the major route of metabolism of L-dopa is its conversion to dopamine and that under normal conditions, L-dopa is not found, free or accumulated. Although L-dopa does not induce neurotoxicity of dopaminergic neurons, as observed in cell cultures, L-dopa induces dyskinesia in approximately 40 % patients with 4–6 years of treatment (Ahlskog & Muenter 2001). The mechanism for L-dopa-induced dyskinesias is very complex, but a role for serotonergic neurons has been proposed. The rapid oscillation of striatal dopamine during L-dopa treatment has been found to be required for the induction of dyskinesias (Meissner et al. 2006; Pavese et al. 2006). In L-dopa treatment of a Parkinson's disease patient, the dopaminergic neurons that are still functioning convert L-dopa to dopamine, which is stored in monoaminergic synaptic vesicles. Dopamine is released by the axon terminal, which is regulated by D_2 dopamine receptors and the dopamine transporter (DAT). However, serotonergic neurons express both AADC and VMAT-2 and release dopamine, but without the regulation of the D_2 receptor and DAT (for review, see Cheshire and Williams 2012). Studies in animals with experimental Parkinsonism showed that serotonergic neurons are responsible for 80 % of dopamine release during L-dopa treatment (Tanaka et al. 1999)

9 Dopamine and L-dopa as Selective Neurotoxins

Dopamine is an essential neurotransmitter in dopaminergic neurons, accumulating inside monoaminergic synaptic vesicles for use as a neurotransmitter. Dopamine released into the intersynaptic space is recaptured by dopaminergic neurons with DAT, whereas cytosol dopamine can be (i) accumulated into monoaminergic synaptic vesicles mediated by VAMT-2, (ii) degraded by MAO and COMT, or (iii) autoxidized into aminochrome. However, dopamine induces neurotoxicity when undergoing oxidation to aminochrome, which can be neurotoxic by (i) forming adducts with proteins, such as alpha-synuclein, parkin, mitochondrial complexes I and III, isocitrate dehydrogenase, actin, tubulin, or the dopamine transporter or by (ii) being one-electron reduced by flavoenzymes that use NADH or NADPH as an electron donor. It was proposed that aminochrome is the endogenous neurotoxin that is responsible for the neurodegenerative process of neuromelanin-containing dopaminergic neurons (Lozano et al. 2010; Paris et al. 2010, 2011; Muñoz et al. 2012 a, b). Aminochrome is a selective neurotoxin, as it is formed inside a single dopaminergic neuron. Aminochrome can induce

neurotoxicity, resulting in a focalized neurotoxic event, which is in line with the slow neurodegeneration that occurs in neuromelanin-containing dopaminergic neurons, which takes years.

Experiments with cell cultures demonstrate the potential neurotoxic effects of L-dopa, but depending on the cellular model used for these experiments, it is possible that a part or all of the L-dopa added to the cell cultures was decarboxylated to dopamine before it induced neurotoxicity. It seems plausible that L-dopa is only a transient precursor in the synthesis of dopamine because the synthesis of dopamine is performed in a type of complex with TH and AADC, which are associated with VMAT-2, localized in monoaminergic synaptic vesicles. This suggests that L-dopa is not free in the cytosol because free L-dopa is immediately converted to dopamine and transported into the vesicles (Cartier et al. 2010). In Parkinson's therapy, L-dopa also acts as dopamine, as the decarboxylation of L-dopa and the unregulated release of dopamine from serotonergic neurons occur in both the peripheral and central nervous system, a process that seems to be involved in L-dopa-induced dyskinesias (Cheshire and Williams 2012).

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References

Ahlskog, J. E., & Muenter, M. D. (2001). Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Movement Disorders, 16*, 448–458.

Arriagada, A., Paris, I., Matas MJ, S. d. l., Martinez-Alvarado, P., Cardenas, S., Castañeda, P., Graumann, R., Perez-Pastene, C., Olea-Azar, C., Couve, E., Herrero, M. T., Caviedes, P., & Segura-Aguilar, J. (2004). On the neurotoxicity of leukoaminochrome o-semiquinone radical derived of dopamine oxidation: Mitochondria damage, necrosis and hydroxyl radical formation. *Neurobiology of Disease, 16*, 468–477.

Asanuma, M., Miyazaki, I., Kikkawa, Y., Kimoto, N., Takeshima, M., Murakami, S., & Miyoshi, K. (2012). Cyclooxygenase-independent neuroprotective effects of aspirin against dopamine quinone-induced neurotoxicity. *Neurochemical Research, 37*, 1944–1951.

Baez, S., Linderson, Y., & Segura-Aguilar, J. (1994). Superoxide dismutase and catalase prevent the formation of reactive oxygen species during reduction of cyclized dopa ortho-quinone by DT-diaphorase. *Chemico-Biological Interactions, 93*, 103–116.

Baez, S., Linderson, Y., & Segura-Aguilar, J. (1995). Superoxide dismutase and catalase enhance autoxidation during one-electron reduction of aminochrome by NADPH-cytochrome P-450 reductase. *Biochemical and Molecular Medicine, 54*, 12–18.

Baez, S., Segura-Aguilar, J., Widersten, M., Johansson, A. S., & Mannervik, B. (1997). Glutathione transferases catalyse the detoxication of oxidized metabolites (o-quinones) of catecholamines and may serve as an antioxidant system preventing degenerative cellular processes. *The Biochemical Journal, 324*, 25–28.

Berthet, A., Bezard, E., Porras, G., Fasano, S., Barroso-Chinea, P., Dehay, B., Martinez, A., Thiolat, M. L., Nosten-Bertrand, M., Giros, B., Baufreton, J., Li, Q., Bloch, B., & Martin-Negrier, M. L. (2012). L-dopa impairs proteasome activity in parkinsonism through D1 dopamine receptor. *The Journal of Neuroscience, 32*, 681–691.

Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., & Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell and Tissue Research, 318*, 121–134.

Cardenas, S. P., Perez-Pastene, C., Couve, E., & Segura-Aguilar, J. (2008). The DT-diaphorase prevents the aggregation of α-synuclein induced by aminochrome. *Neurotoxicity Research, 13*, 136.

Carstam, R., Brinck, C., Hindemith-Augustsson, A., Rorsman, H., & Rosengren, E. (1991). The neuromelanin of the human substantia nigra. *Biochimica et Biophysica Acta, 1097*, 152–160.

Cartier, E. A., Parra, L. A., Baust, T. B., Quiroz, M., Salazar, G., Faundez, V., Egaña, L., & Torres, G. E. (2010). A biochemical and functional protein complex involving dopamine synthesis and transport into synaptic vesicles. *The Journal of Biological Chemistry, 151*, 957–666.

Caudle, W. M., Richardson, J. R., Wang, M. Z., Taylor, T. N., Guillot, T. S., McCormack, A. L., Colebrooke, R. E., Di Monte, D. A., Emson, P. C., & Miller, G. W. (2007). Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *The Journal of Neuroscience, 27*, 8138–8148.

Cheng, F. C., Kuo, J. S., Chia, L. G., & Dryhurst, G. (1996). Elevated 5-S-cysteinyldopamine/homovanillic acid ratio and reduced homovanillic acid in cerebrospinal fluid: Possible markers for and potential insights into the pathoetiology of Parkinson's disease. *Journal of Neural Transmission, 103*, 433–446.

Cheshire, P. A., & Williams, D. R. (2012). Serotonergic involvement in levodopa-induced dyskinesias in Parkinson's disease. *Journal of Clinical Neuroscience, 19*, 343–348.

Claffey, D. J., & Ruth, J. A. (2001). Amphetamine adducts of melanin intermediates demonstrated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Chemical Research in Toxicology, 14*, 1339–1344.

Conway, K. A., Rochet, J. C., Bieganski, R. M., & Lansbury, P. T., Jr. (2001). Kinetic stabilization of the a-synuclein protofibril by a dopamine- a-synuclein adduct. *Science, 294*, 1346–1349.

Cuervo, A. M., Wong, E. S., & Martinez-Vicente, M. (2010). Protein degradation, aggregation, and misfolding. *Movement Disorders, 25*(Suppl 1), S49–S54.

Dagnino-Subiabre, A., Cassels, B. K., Baez, S., Johansson, A. S., Mannervik, B., & Segura-Aguilar, J. (2000). Glutathione transferase M2-2 catalyzes conjugation of dopamine and dopa *o*-quinones. *Biochemical and Biophysical Research Communications, 274*, 32–36.

Dehn, D. L., Claffey, D. J., Duncan, M. W., & Ruth, J. A. (2001). Nicotine and cotinine adducts of a melanin intermediate demonstrated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Chemical Research in Toxicology, 14*, 275–279.

Díaz-Véliz, G., Paris, I., Mora, S., Raisman-Vozari, R., & Segura-Aguilar, J. (2008). Copper neurotoxicity in rat substantia nigra and striatum is dependent on DT-diaphorase inhibition. *Chemical Research in Toxicology, 21*, 1180–1185.

Fasano, M., Bergamasco, B., & Lopiano, L. (2006). Is neuromelanin changed in Parkinson's disease? Investigations by magnetic spectroscopies. J. *Neural Transm, 113*, 769–774.

Foppoli, C., Coccia, R., Cini, C., & Rosei, M. A. (1997). Catecholamines oxidation by xanthine oxidase. *Biochimica et Biophysica Acta, 1334*, 200–206.

Fuentes, P., Paris, I., Nassif, M., Caviedes, P., & Segura-Aguilar, J. (2007). Inhibition of VMAT-2 and DT-diaphorase induce cell death in a substantia nigra-derived cell line–an experimental cell model for dopamine toxicity studies. *Chemical Research in Toxicology, 20*, 776–783.

Galzigna, L., De Iuliis, A., & Zanatta, L. (2000). Enzymatic dopamine peroxidation in substantia nigra of human brain. *Clinica Chimica Acta, 300*, 131–138.

Gerlach, M., Double, K. L., Ben-Shachar, D., Zecca, L., Youdim, M. B., & Riederer, P. (2003). Neuromelanin and its interaction with iron as a potential risk factor for dopaminergic neurodegeneration underlying Parkinson's disease. *Neurotoxicity Research, 5*, 35–44.

Giménez-Xavier, P., Francisco, R., Santidrián, A. F., Gil, J., & Ambrosio, S. (2009). Effects of dopamine on LC3-II activation as a marker of autophagy in a neuroblastoma cell model. *Neurotoxicology, 30*, 658–665.

Gómez-Santos, C., Ferrer, I., Santidrián, A. F., Barrachina, M., Gil, J., & Ambrosio, S. (2003). Dopamine induces autophagic cell death and alpha-synuclein increase in human neuroblastoma SH-SY5Y cells. *Journal of Neuroscience Research, 73*, 341–350.

Graham, D. G., Tiffany, S. M., Bell, W. R., Jr., & Gutknecht, W. F. (1978). Autoxidation versus covalent binding of Autoxidation versus covalent binding of quinones as the mechanism of

toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Molecular Pharmacology, 14*, 644–653.

Guillot, T. S., & Miller, G. W. (2009). Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. *Molecular Neurobiology, 39*, 149–170.

Hastings, T. G. (1995). Enzymatic oxidation of dopamine: The role of prostaglandin H synthase. *Journal of Neurochemistry, 64*, 919–924.

Hong, L., & Simon, J. D. (2007). Current understanding of the binding sites, capacity, affinity, and biological significance of metals in melanin. *The Journal of Physical Chemistry. B, 111*, 7938–4797.

Hoyt, K. R., Reynolds, I. J., & Hastings, T. G. (1997). Mechanisms of dopamine-induced cell death in cultured rat forebrain neurons: Interactions with and differences from glutamate-induced cell death. *Experimental Neurology, 143*, 269–281.

Jana, S., Sinha, M., Chanda, D., Roy, T., Banerjee, K., Munshi, S., Patro, B. S., & Chakrabarti, S. (2011). Mitochondrial dysfunction mediated by quinone oxidation products of dopamine: Implications in dopamine cytotoxicity and pathogenesis of Parkinson's disease. *Biochimica et Biophysica Acta, 1812*, 663–673.

Jeon, S. M., Cheon, S. M., Bae, H. R., Kim, J. W., & Kim, S. U. (2010). Selective susceptibility of human dopaminergic neural stem cells to dopamine-induced apoptosis. *Experimental Neurobiology, 19*, 155–164.

Jiang, H., Ren, Y., Zhao, J., & Feng, J. (2004). Parkin protects human dopaminergic neuroblastoma cells against dopamine-induced apoptosis. *Human Molecular Genetics, 13*, 1745–1754. 15.

Jimenez, M., Garcia-Carmona, F., Garcia-Canovas, F., Iborra, J. L., Lozano, J. A., & Martinez, F. (1984). Chemical intermediates in dopamine oxidation by tyrosinase, and kinetic studies of the process. *Archives of Biochemistry and Biophysics, 235*, 438–448.

Junn, E., & Mouradian, M. M. (2001). Apoptotic signaling in dopamine-induced cell death: The role of oxidative stress, p38 mitogen-activated protein kinase, cytochrome c and caspases. *Journal of Neurochemistry, 78*, 374–383.

Keller, J. N., Huang, F. F., Dimayuga, E. R., & Maragos, W. F. (2000). Dopamine induces proteasome inhibition in neural PC12 cell line. *Free Radical Biology & Medicine, 29*, 1037–1042.

Kostrzewa, R. M., Kostrzewa, J. P., & Brus, R. (2002). Neuroprotective and neurotoxic roles of levodopa (L-dopa) in neurodegenerative disorders relating to Parkinson's disease. *Amino Acids, 23*, 57–63.

Kubo, I., Nitoda, T., & Nihei, K. (2007). Effects of quercetin on mushroom tyrosinase and B16-F10 melanoma cells. *Molecules, 12*, 1045–1056.

Kuhn, D. M., & Arthur, R., Jr. (1998). Dopamine inactivates tryptophan hydroxylase and forms a redox-cycling quinoprotein: Possible endogenous toxin to serotonin neurons. *The Journal of Neuroscience, 18*, 7111–7117.

LaVoie, M. J., Ostaszewski, B. L., Weihofen, A., Schlossmacher, M. G., & Selkoe, D. J. (2005). Dopamine covalently modifies and functionally inactivates parkin. *Nature Medicine, 11*, 1159–1161.

Lee, F. J., Liu, F., Pristupa, Z. B., & Niznik, H. B. (2001a). Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *The FASEB Journal B, 15*, 916–926.

Lee, H. J., Kim, S. H., Kim, K. W., Um, J. H., Lee, H. W., Chung, B. S., & Kang, C. D. (2001b). Antiapoptotic role of NF-kappaB in the auto-oxidized dopamine-induced apoptosis of PC12 cells. *Journal of Neurochemistry, 76*, 602–609.

Liedhegner, E. A., Steller, K. M., & Mieyal, J. J. (2011). Levodopa activates apoptosis signaling kinase 1 (ASK1) and promotes apoptosis in a neuronal model: Implications for the treatment of Parkinson's disease. *Chemical Research in Toxicology, 24*, 1644–1652.

Linert, W., Herlinger, E., Jameson, R. F., Kienzl, E., Jellinger, K., & Youdim, M. B. (1996). Dopamine, 6-hydroxydopamine, iron, and dioxygen–their mutual interactions and possible

implication in the development of Parkinson's disease. *Biochimica et Biophysica Acta, 1316*, 160–168.

Liu, Z., Zhang, J., Fei, J., & Guo, L. (2001). A novel mechanism of dopamine neurotoxicity involving the peripheral extracellular and the plasma membrane dopamine transporter. *Neuroreport, 12*, 3293–3297.

Lozano, J., Muñoz, P., Nore, B. F., Ledoux, S., & Segura-Aguilar, J. (2010). Stable expression of short interfering RNA for DT-diaphorase induces neurotoxicity. *Chemical Research in Toxicology, 23*, 1492–1496.

Luo, Y., Umegaki, H., Wang, X., Abe, R., & Roth, G. S. (1998). Dopamine induces apoptosis through an oxidation-involved SAPK/JNK activation pathway. *The Journal of Biological Chemistry, 273*, 3756–3764.

Marin, C., & Obeso, J. A. (2010). Catechol-O-methyltransferase inhibitors in preclinical models as adjuncts of L-dopa treatment. *International Review of Neurobiology, 95*, 191–205.

McNaught, K. S., Perl, D. P., Brownell, A. L., & Olanow, C. W. (2004). Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Annals of Neurology, 56*, 149–162.

Meissner, W., Ravenscroft, P., Reese, R., Harnack, D., Morgenstern, R., Kupsch, A., Klitgaard, H., Bioulac, B., Gross, C. E., Bezard, E., & Boraud, T. (2006). Increased slow oscillatory activity in substantia nigra pars reticulata triggers abnormal involuntary movements in the 6-OHDA-lesioned rat in the presence of excessive extracellular striatal dopamine. *Neurobiol, 22*, 586–598. Epub 2006 Mar 10.

Melamed, E., Offen, D., Shirvan, A., Djaldetti, R., Barzilai, A., & Ziv, I. (1998). Levodopa toxicity and apoptosis. *Annals of Neurology, 44*, S149–S154.

Monastyrska, I., Rieter, E., Klionsky, D. J., & Reggiori, F. (2009). Multiple roles of the cytoskeleton in autophagy. *Biological Reviews of the Cambridge Philosophical Society, 84*, 431–448.

Moszczynska, A., Saleh, J., Zhang, H., Vukusic, B., Lee, F. J., & Liu, F. (2007). Parkin disrupts the alpha-synuclein/dopamine transporter interaction: Consequences toward dopamine-induced toxicity. *Journal of Molecular Neuroscience, 32*, 217–227.

Müller, T., & Muhlack, S. (2012). Cysteine decrease following acute Levodopa intake in patients with Parkinson's disease. *Neuroscience Letters, 521*, 37–39.

Muñoz, P., Huenchuguala, S., Paris, I., Cuevas, C., Villa, M., Caviedes, P., Segura-Aguilar, J., & Tizabi, Y. (2012a). Protective effects of nicotine against aminochrome-induced toxicity in substantia nigra derived cells: Implications for Parkinson's disease. *Neurotoxicity Research, 22*, 177–180.

Muñoz, P., Paris, I., Sanders, L. H., Greenamyre, J. T., & Segura-Aguilar, J. (2012b). Overexpression of VMAT-2 and DT-diaphorase protects substantia nigra-derived cells against aminochrome neurotoxicity. *Biochimica et Biophysica Acta, 1822*, 1125–1136.

Myöhänen, T. T., Schendzielorz, N., & Männistö, P. T. (2010). Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. *Journal of Neurochemistry, 113*, 1632–1643.

Naoi, M., Maruyama, W., Yi, H., Yamaoka, Y., Shamoto-Nagai, M., Akao, Y., Gerlach, M., Tanaka, M., & Riederer, P. (2008). Neuromelanin selectively induces apoptosis in dopaminergic SH-SY5Y cells by deglutathionylation in mitochondria: Involvement of the protein and melanin component. *Journal of Neurochemistry, 105*, 2489–2500.

Norris, E. H., Giasson, B. I., Hodara, R., Xu, S., Trojanowski, J. Q., Ischiropoulos, H., & Lee, V. M. (2005). Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome – mediated conformational alterations. *The Journal of Biological Chemistry, 280*, 21212–21219.

Offen, D., Ziv, I., Panet, H., Wasserman, L., Stein, R., Melamed, E., & Barzilai, A. (1997). Dopamine-induced apoptosis is inhibited in PC12 cells expressing Bcl-2. *Cellular and Molecular Neurobiology, 17*, 289–304.

Okada, M., Nakao, R., Hosoi, R., Zhang, M. R., Fukumura, T., Suzuki, K., & Inoue, O. (2011). Microdialysis with radiometric monitoring of L-[β-^{11}C]DOPA to assess dopaminergic metabolism: Effect of inhibitors of L-amino acid decarboxylase, monoamine oxidase, and

catechol-O-methyltransferase on rat striatal dialysate. *Journal of Cerebral Blood Flow and Metabolism, 31*, 124–131.

Paris, I., Dagnino-Subiabre, A., Marcelain, K., Bennett, L. B., Caviedes, P., Caviedes, R., Olea-Azar, C., & Segura-Aguilar, J. (2001). Copper neurotoxicity is dependent on dopamine-mediated copper uptake and one-electron reduction of aminochrome in a rat substantia nigra neuronal cell line. *Journal of Neurochemistry, 77*, 519–529.

Paris, I., Martinez-Alvarado, P., Cardenas, S., Perez-Pastene, C., Graumann, R., Fuentes, P., Olea-Azar, C., Caviedes, P., & Segura-Aguilar, J. (2005a). Dopamine-dependent iron toxicity in cells derived from rat hypothalamus. *Chemical Research in Toxicology, 18*, 415–419.

Paris, I., Martinez-Alvarado, P., Perez-Pastene, C., Vieira, M. N., Olea-Azar, C., Raisman-Vozari, R., Cardenas, S., Graumann, R., Caviedes, P., & Segura-Aguilar, J. (2005b). Monoamine transporter inhibitors and norepinephrine reduce dopamine-dependent iron dependent iron toxicity in cells derived from the substantia nigra. *Journal of Neurochemistry, 92*, 1021–1032.

Paris, I., Perez-Pastene, C., Couve, E., Caviedes, P., Ledoux, S., & Segura-Aguilar, J. (2009). Copper dopamine complex induces mitochondrial autophagy preceding caspase-independent apoptotic cell death. *The Journal of Biological Chemistry, 284*, 13306–13315.

Paris, I., Perez-Pastene, C., Cardenas, S., Iturriaga-Vasquez, P., Muñoz, P., Couve, E., Caviedes, P., & Segura-Aguilar, J. (2010). Aminochrome induces disruption of actin, alpha-, and beta-tubulin cytoskeleton networks in substantia-nigra-derived cell line. *Neurotoxicity Research, 18*, 82–92.

Paris, I., Muñoz, P., Huenchuguala, S., Couve, E., Sanders, L. H., Greenamyre, J. T., Caviedes, P., & Segura-Aguilar, J. (2011). Autophagy protects against aminochrome-induced cell death in substantia nigra-derived cell line. *Toxicological Sciences, 121*, 376–388.

Park, H. H., Lee, K. Y., Kim, S. H., Lee, Y. J., & Koh, S. H. (2009). L-dopa-induced neurotoxicity is reduced by the activation of the PI3K signaling pathway. *Toxicology, 265*, 80–86. 30.

Pavese, N., Evans, A. H., Tai, Y. F., Hotton, G., Brooks, D. J., Lees, A. J., & Piccini, P. (2006). Clinical correlates of levodopa-induced dopamine release in Parkinson disease: A PET study. *Neurology, 67*, 1612–1617.

Prota, G. (1995). The chemistry of melanins and melanogenesis. *Fortschritte der Chemie Organischer Naturstoffe, 64*, 93–148.

Rosengren, E., Linder-Eliasson, E., & Carlsson, A. (1985). Detection of 5-S-cysteinyldopamine in human brain. *Journal of Neural Transmission, 63*, 247–253.

Sabens, E. A., Distler, A. M., & Mieyal, J. J. (2010). Levodopa deactivates enzymes that regulate thiol-disulfide homeostasis and promotes neuronal cell death: Implications for therapy of Parkinson's disease. *Biochemistry, 49*, 2715–2724.

Saura, J., Luque, J. M., Cesura, A. M., Da Prada, M., Chan-Palay, V., Huber, G., Loffler, J., & Richards, J. G. (1994). Increased monoamine oxidase B activity in plaque-associated astrocytes of alzheimer brains revealed by quantitative enzyme radioautography. *Neuroscience, 62*, 15–30.

Schapira, A. H. (2011). Mitochondrial pathology in Parkinson's disease. *The Mount Sinai Journal of Medicine, 78*, 872–881.

Schapira, A. H., & Jenner, P. (2011). Etiology and pathogenesis of Parkinson's disease. *Movement Disorders, 26*, 1049–1055.

Segura-Aguilar, J. (1996). Peroxidase activity of liver microsomal vitamin D 25-hydroxylase and cytochrome P450 1A2 catalyzes 25-hydroxylation of vitamin D3 and oxidation of dopamine to aminochrome. *Biochemical and Molecular Medicine, 58*, 122–129.

Segura-Aguilar, J., & Lind, C. (1989). On the mechanism of Mn^{3+} induced neurotoxicity of dopamine: Prevention of quinone derived oxygen toxicity by DT-diaphorase and superoxide dismutase. *Chemico-Biological Interactions, 72*, 309–324.

Segura-Aguilar, J., Baez, S., Widersten, M., Welch, C. J., & Mannervik, B. (1997). Human class Mu glutathione transferases, in particular isoenzyme M2-2, catalyze detoxication of the dopamine metabolite aminochrome. *The Journal of Biological Chemistry, 272*, 5727–5731.

Segura-Aguilar, J., Metodiewa, D., & Welch, C. (1998). Metabolic activation of dopamine o-quinones to o-semiquinones by NADPH cytochrome P450 reductase may play an important role in oxidative stress and apoptotic effects. *Biochimica et Biophysica Acta, 1381*, 1–6.

Segura-Aguilar, J., Cardenas, S., Riveros, A., Fuentes-Bravo, P., Lozano, J., Graumann, R., Paris, I., Nassif, M., & Caviedes, P. (2006). DT-diaphorase prevents the formation of alpha-synuclein adducts with aminochrome. *Soc Neurosci Abstr, 824*, 17.

Shen, X. M., Xia, B., Wrona, M. Z., & Dryhurst, G. (1996). Synthesis, redox properties, in vivo formation, and neurobehavioral effects of N-acetylcysteinyl conjugates of dopamine: Possible metabolites of relevance to Parkinson's disease. *Chemical Research in Toxicology, 9*, 1117–1126.

Shih, J. C., Grimsby, J., & Chen, K. (1997). Molecular biology of monoamine oxidase A and B: Their role in the degradation of serotonin. In H. G. Baumgarten & M. Gothert (Eds.), *Handbook of experimental pharmacology, vol 129, Serotoninergic neurons and 5-HT receptors in the CNS* (pp. 655–670). Berlin: Springer.

Simantov, R., Blinder, E., Ratovitski, T., Tauber, M., Gabbay, M., & Porat, S. (1996). Dopamine-induced apoptosis in human neuronal cells: Inhibition by nucleic acids antisense to the dopamine transporter. *Neuroscience, 74*, 39–50.

Stokes, A. H., Lewis, D. Y., Lash, L. H., Jerome, W. G., 3rd, Grant, K. W., Aschner, M., & Vrana, K. E. (2000). Dopamine toxicity in neuroblastoma cells: Role of glutathione depletion by L-BSO and apoptosis. *Brain Research, 858*, 1–8.

Strolin-Benedetti, M., Dostert, P., & Tipton, K. F. (1992). Developmental aspects of the monoamine-degrading enzyme monoamine oxidase. *Developmental Pharmacology and Therapeutics, 18*, 191–200.

Takeshima, M., Murata, M., Urasoe, N., Murakami, S., Miyazaki, I., Asanuma, M., & Kita, T. (2011). Protective effects of baicalein against excess L-dopa-induced dopamine quinone neurotoxicity. *Neurological Research, 33*, 1050–1056.

Tanaka, H., Kannari, K., Maeda, T., Tomiyama, M., Suda, T., & Matsunaga, M. (1999). Role of serotonergic neurons in L-dopa-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport, 10*, 631–634. 25.

Thompson, M., Capdevila, J. H., & Strobel, H. W. (2000). Recombinant cytochrome P450 2D18 metabolism of dopamine and arachidonic acid. *The Journal of Pharmacology and Experimental Therapeutics, 294*, 1120–1130.

Van Laar, V. S., Mishizen, A. J., Cascio, M., & Hastings, T. G. (2009). Proteomic identification of dopamine-conjugated proteins from isolated rat brain mitochondria and SH-SY5Y cells. *Neurobiology of Disease, 34*, 487–500.

Walkinshaw, G., & Waters, C. M. (1995). Induction of apoptosis in catecholaminergic PC12 cells by L-dopa. Implications for the treatment of Parkinson's disease. *The Journal of Clinical Investigation, 95*, 2458–2464.

Westlund, K. N., Denney, R. M., Rose, R. M., & Abell, C. W. (1988). Localization of distinct monoamine oxidase A and monoamine oxidase B cell populations in human brainstem. *Neuroscience, 25*, 439–456.

Weyler, W., Hsu, Y. P., & Breakefield, X. O. (1990). Biochemistry and genetics of monoamine oxidase. *Pharmacology and Therapeutics, 47*, 391–417.

Whitehead, R. E., Ferrer, J. V., Javitch, J. A., & Justice, J. B. (2001). Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. *Journal of Neurochemistry, 76*, 1242–1251.

Xilouri, M., Vogiatzi, T., Vekrellis, K., Park, D., & Stefanis, L. (2009). Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. *PLoS One, 4*, e5515.

Xu, Y., Stokes, A. H., Roskoski, R., Jr., & Vrana, K. E. (1998). Dopamine, in the presence of tyrosinase, covalently modifies and inactivates tyrosine hydroxylase. *Journal of Neuroscience Research, 54*, 691–697.

Zafar, K. S., Siegel, D., & Ross, D. (2006). A potential role for cyclized quinones derived from dopamine, DOPA, and 3,4-dihydroxyphenylacetic acid in proteasomal inhibition. *Molecular Pharmacology, 70*, 1079–1086.

Zecca, L., Fariello, R., Riederer, P., Sulzer, D., Gatti, A., & Tampellini, D. (2002). The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. *FEBS Letters, 510*, 216–220.

Zhang, N. Y., Tang, Z., & Liu, C. W. (2008). Alpha-Synuclein protofibrils inhibit 26 S proteasome-mediated protein degradation: Understanding the cytotoxicity of protein protofibrils in neurodegenerative disease pathogenesis. *The Journal of Biological Chemistry, 283*, 20288–20298.

Zhang, W., Phillips, K., Wielgus, A. R., Liu, J., Albertini, A., Zucca, F. A., Faust, R., Qian, S. Y., Miller, D. S., Chignell, C. F., Wilson, B., Jackson-Lewis, V., Przedborski, S., Joset, D., Loike, J., Hong, J. S., Sulzer, D., & Zecca, L. (2011). Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: Implications for progression of Parkinson's disease. *Neurotoxicity Research, 19*, 63–72.

Ziv, I., Shirvan, A., Offen, D., Barzilai, A., & Melamed, E. (2001). Molecular biology of dopamine-induced apoptosis: Possible implications for Parkinson's disease. *Methods in Molecular Medicine, 62*, 73–87.