J Bioenerg Biomembr (2009) 41:469–472
DOI 10.1007/s10863-009-9257-z

The role of dopamine oxidation in mitochondrial dysfunction: implications for Parkinson's disease

Teresa G. Hastings

Published online: 5 December 2009

Abstract The etiology of sporadic Parkinson's disease (PD) is unknown, although mitochondrial dysfunction and oxidative stress have been implicated in the mechanisms associated with PD pathogenesis. Dopamine (DA) neurons of the substantia nigra pars compacta have been shown to degenerate to a greater extent in PD than other neurons suggesting the possibility that DA itself may be contributing to the neurodegenerative process. This review discusses our work on the effects of DA oxidation and reactive DA quinones on mitochondrial function and protein modification and the potential for exacerbating toxicity associated with mitochondrial dysfunction in PD.

Keywords Parkinson's disease · Mitochondria · Dopamine quinone · Oxidative stress

Introduction

Parkinson's disease (PD) affects 1–2% of the population over the age of 65 and is the second most common neurodegenerative disorder. Parkinson's disease is pathologically characterized by the loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and the presence of Lewy bodies. In a small percentage of cases, genetic mutations in proteins α-synuclein, DJ-1, parkin, UCH-L1, PINK1, LRRK2, and others have been linked to the familial form of PD (Lesage and Brice 2009; Bueler 2009; Gasser 2009). However, in the vast majority of PD patients, the cause of sporadic Parkinson's disease remains unknown. Although multiple factors such as gene-environment interactions are likely involved, increasing evidence implicates mitochondrial dysfunction and oxidative stress in the pathological degeneration in PD (Greenamyre and Hastings 2004).

T. G. Hastings (✉)
Pittsburgh Institute for Neurodegenerative Diseases,
3501 Fifth Avenue, 7038 Biomedical Science Tower 3,
Pittsburgh, PA 15261, USA
e-mail: hastingst@upmc.edu

T. G. Hastings
Department of Neurology,
University of Pittsburgh School of Medicine,
Pittsburgh, PA 15261, USA

Mitochondrial dysfunction in Parkinson's disease

Mitochondria have been shown to play a role in multiple forms of cell death. Mitochondrial dysfunction in PD has been identified as a systemic deficiency of the electron transport chain Complex I activity. A deficiency or partial inhibition of Complex I has been shown to result in increased mitochondrial reactive oxygen species (ROS) production and subsequently increased oxidative stress. Oxidative stress in PD has been indicated by multiple observations of oxidative damage to macromolecules such as proteins, lipids, and DNA, as well as decreased GSH levels. As the major reducing agent in the cell, the reduction in GSH is particularly important, especially because it has been reported to be significantly decreased early in PD (Martin and Teismann 2009). Mitochondrial dysfunction and oxidative stress have been increasingly implicated in the pathology associated with genetic models of familial PD (Banerjee et al. 2009; Narendra et al. 2008; Grunewald et al. 2009). Mitochondrial toxins MPTP and rotenone, acting as inhibitors of complex I, have been used to generate animal and cellular models of PD. Chronic systemic administration of rotenone in rats results in the selective loss of SN dopaminergic neurons and recapitulates many of the pathological features observed in PD (Betarbet et al. 2000;

Betarbet et al. 2006; Cannon et al. 2009). Despite the ubiquitous nature of the rotenone-induced Complex I inhibition, DA neurons were the most vulnerable to degeneration which mimics the loss of DA neurons in PD (Betarbet et al. 2000; Betarbet et al. 2006; Cannon et al. 2009).

DA as an endogenous toxin

Even though neuronal alterations observed in PD are not restricted to DA neurons, the DA neurons of the nigrostriatal pathway are the major site of neuronal degeneration in PD (Sulzer 2007). The presence of mitochondrial dysfunction and oxidative stress, however, does not explain the heightened vulnerability of dopaminergic neurons. The fact that SN DA neurons degenerate in PD to a greater extent than other neurons may be due to the presence of DA itself. Under conditions of oxidative stress, the unique chemical structure of DA has the potential to form an endogenous toxin that will contribute further to mitochondrial dysfunction and oxidative damage, accelerating dopaminergic cell death in PD.

Under normal conditions, DA is synthesized from tyrosine via the enzymatic activities of tyrosine hydroxylase and aromatic amino acid decarboxylase. Once formed, DA is safely stored in high millimolar concentrations in synaptic vesicles following uptake by the vesicular monoamine transporter (VMAT2) (Staal et al. 2004). Problems develop when vesicular storage of DA is altered by the presence of α-synuclein protofibrils, oxidative stress, and weak base compounds such as methamphetamine as recently reviewed in (Caudle et al. 2008). If vesicular storage is disrupted and DA levels increase in the cytoplasm, DA has the ability to form reactive metabolites by two distinct pathways. The first pathway utilizes the enzymes monoamine oxidase located on the outer surface of mitochondria and aldehyde dehydrogenase to metabolize DA to dihydroxyphenylacetic acid (DOPAC) and forming H_2O_2 in the process. The second pathway involves oxidation of the catechol ring of DA to form ROS and the electron-deficient DA quinone. The autoxidation of DA may be facilitated by the presence of transition metal ions or DA may be oxidized enzymatically acting as a co-substrate in catalyzed reaction (Hastings 1995).

Electron-deficient DA quinones will react readily with cellular nucleophiles, such as the reduced sulfhydryl group on small peptide and protein cysteinyl residues (Graham et al. 1978; Hastings and Zigmond 1994; Hastings et al. 1996). Thus, the DA quinone will bind accessible protein thiols, covalently modifying protein structure. Because cysteinyl residues are often at the active site of proteins, covalent modification by DA quinones often results in inactivation of protein function. Depending upon the critical function of these proteins, protein modification may be very detrimental to the survival of the cell. It is possible that this mechanism plays a contributing role to the degenerative process in PD (Stokes et al. 1999).

The toxicity of DA has been demonstrated in a variety of in vitro models. Our laboratory has demonstrated the selective toxicity of DA in vivo. Following intrastriatal injections, exogenous DA caused selective toxicity to DA terminals (Hastings et al. 1996; Rabinovic et al. 2000) as well as the loss of DA neurons in SN (Hastings and Rabinovic, unpublished observations). The extent of the DA-induced damage to DA terminals was dependent on intracellular DA oxidation, directly related to the amount of quinone modification of proteins, and was reduced by the presence of antioxidants (Hastings et al. 1996; Rabinovic et al. 2000). The toxicity of cytoplasmic DA was recently demonstrated in vivo in two separate mouse models (Caudle et al. 2007; Chen et al. 2008). Particularly noteworthy was the observation that mice expressing a low level of VMAT2, the protein responsible for sequestering DA into vesicles, showed evidence of DA oxidation and the loss of SN DA neurons upon aging, presumably due to chronic increases in cytoplasmic DA (Caudle et al. 2007). Likewise, striatal neurons expressing a functional DA transporter to increase cytoplasmic DA levels in GABA neurons also resulted in neuronal toxicity (Chen et al. 2008). Combined, these observations strongly suggest that without the ability to sequester intracellular DA and maintain low cytoplasmic levels, reactive metabolites of DA will cause toxicity in vivo. In addition, evidence of in vivo DA oxidation in human SN is shown by the accumulation of the DA oxidation product neuromelanin (Zecca et al. 2003). Increased DA oxidation in postmortem PD brain SN as compared to age-matched controls was indicated by higher levels of cysteinyl-catechol derivatives (Spencer et al. 1998). Other factors implicated in PD pathogenesis such as reduced GSH levels and iron accumulation will further promote DA oxidation. Thus, DA oxidation is likely to contribute to the vulnerability of DA neurons in PD.

DA oxidation and mitochondria

Because of the defined role of mitochondrial dysfunction in PD, the Hastings laboratory also examined the effects of DA and DA quinone exposure on mitochondrial function (Berman and Hastings 1999). Exposure of isolated, well-coupled rat brain mitochondria to the DA oxidation product DA quinones resulted in a dramatic increase in State 4 resting respiration. Significant increases in State 4 rates to levels similar to State 3 respiration were indicative of a quinone-induced uncoupling of mitochondria where utilization of substrate occurs without the production of ATP,

most likely due to increased proton permeability across the inner mitochondrial membrane. The DA quinone-induced increase in State 4 respiration was completely blocked by the presence of the reducing agent GSH, whereas co-incubation with catalase and superoxide dismutase to eliminate ROS had no effect. These results suggested that GSH is acting as a thiol-dependent quinone scavenger preventing the reactive DA quinone from interacting with key mitochondrial proteins. DA quinone exposure of intact, isolated mitochondria also resulted in mitochondrial swelling that was completely prevented in the presence of cyclosporin A, suggesting opening of a permeability transition pore. Similar to the protective effect on respiration, the addition of GSH was also able to block quinone-induced mitochondrial swelling (Berman and Hastings 1999). We also reported on the important observation that permeability transition in isolated brain and liver mitochondria were strikingly different, indicating the need to perform experiments on brain mitochondria to model what may be occurring in neurodegenerative diseases (Berman et al. 2000).

Proteomic analyses following DA quinone exposure

In subsequent experiments, we sought to identify which mitochondrial proteins were altered following exposure to DA quinones. Using the unbiased proteomic technique of 2-D difference-in-gel electrophoresis (2D-DIGE), we examined changes in abundance of mitochondrial proteins following exposure of isolated brain mitochondria to DA quinones (Van Laar et al. 2008). Results showed a rapid loss of a subset of mitochondrial proteins following DA quinone exposure including the 75 kDa subunit of NADH dehydrogenase of Complex I, mitochondrial creatine kinase, mortalin/GRP75, mitofilin, voltage-dependent anion channel 2 (VDAC2) as well as several other proteins (Van Laar et al. 2008). Because the majority of mitochondrial proteins were unaffected, the findings suggest a subset of mitochondrial proteins are highly susceptible to either degradation and/or aggregation following oxidative modification. The degradation of select proteins may be catalyzed by mitochondrial proteases, which also have been implicated recently in neurodegeneration (Martinelli and Rugarli 2009).

To examine the direct targets of DA quinone modification in the mitochondrial proteome, we exposed intact brain mitochondria to radiolabeled DA quinone (Van Laar *et al.* 2009). Following separation by 2D gel electrophoresis, DA quinone-modified protein spots were analyzed by autoradiography, mass spectrometry, and peptide mass fingerprinting. Several of the proteins identified as directly modified by the DA quinone were also observed to be decreased in abundance in the previous study. These proteins included the 75 kDa subunit of NADH dehydrogenase of Complex I, mitochondrial creatine kinase, mortalin/GRP75, mitofilin, isocitrate dehydrogenase and succinate Co A ligase. Some additional proteins modified by DA quinone included HSP60, aldehyde dehydrogenase, peroxiredoxin 3, VDAC1, and NADH oxidoreductase 30 kDa subunit of Complex I (Van Laar et al. 2009). Mortalin/GRP75 was also identified as a DA quinone-modified protein in SH-SY5Y cells exposed to radiolabeled DA along with two separate protein disulfide isomerases (Van Laar et al. 2009).

The mitochondrial proteins shown to be affected by DA oxidation serve a variety of critical mitochondrial functions (Van Laar et al. 2009). DA quinone modification of protein subunits of Complex I (75 kDa and 30 kDa) and complex III (ubiquinol-cytochrome c reductase core protein 1) of the electron transport chain may affect mitochondrial respiration and ROS production. Alterations of mitochondrial chaperone proteins HSP60 and mortalin/GRP75 are likely to affect protein folding, protein import and Fe-S protein assembly. Mitofilin has been shown to interact with mitochondrial import proteins (Xie et al. 2007) and to play a role in maintaining cristae morphology (John et al. 2005), and thus the integrity of the mitochondria.

Interestingly, in our study, two familial PD-associated proteins DJ-1 and UCH-L1 were shown to be DA quinone-modified in both DA-exposed SH-SY5Y cells and isolated brain mitochondria (Van Laar et al. 2009). DA quinone modification of PD-associated proteins α-synuclein and parkin have been reported by others (Conway et al. 2000; LaVoie et al. 2005). In most cases, DA quinone modification has resulted in alterations of protein function (Rochet *et al.* 2004; Martinez-Vicente et al. 2008; LaVoie et al. 2005) (Mishizen and Hastings, unpublished results}. DA quinone modification of α-synuclein was shown to inhibit the normal degradation of the protein by chaperone-mediated autophagy (Martinez-Vicente et al. 2008). The presence of the DA quinone modification blocked the LAMP2A-mediated transport of α-synuclein into the lysosome for degradation, and continued to block CMA-mediated degradation of other proteins, thus contributing to the accumulation of α-synuclein and other proteins (Martinez-Vicente et al. 2008). Whether altered protein degradation or protein solubility/aggregation is a common effect of other DA quinone modified proteins remains to be determined.

Endoplasmic reticulum (ER) proteins involved in protein folding such as ER-60/GRP58/ERp57 and protein disulfide isomerase-5 were shown to be targets of DA quinone modification in differentiated SH-SY5Y cells (Van Laar et al. 2009). Aberrant protein folding and protein aggregation that occurs in PD are likely to be potentiated by DA oxidation and oxidative modification of proteins. Proteomic analysis by 2D-DIGE of mitochondrial-enriched fractions isolated from differentiated PC12 cells exposed to exoge-

nous DA showed increased expression of ER chaperone proteins. Significant increases in GRP78, GRP58/ER-60, GRP94 and calreticulin indicated that intracellular DA oxidation initiated an unfolded protein and ER stress response (Dukes et al. 2008). Oxidative stress and mitochondrial deficits have been linked to the initiation of ER stress (Xu et al. 2004; Holtz et al. 2006). Functional interactions between mitochondria and the ER are critically important in maintaining intracellular calcium homeostasis and preventing cell death (Giorgi et al. 2009). DA oxidation appears to target proteins in both organelles (Dukes et al. 2008; Van Laar et al. 2009).

Summary

Mitochondrial dysfunction and oxidative stress have been clearly linked to the pathogenic mechanisms causing PD. Nigrostriatal DA neurons show an enhanced vulnerability to degeneration in PD, which may be related to the potential for DA oxidation products to exacerbate mitochondrial dysfunction in these neurons. Exposure of mitochondria to oxidized DA resulted in uncoupling of mitochondrial respiration and mitochondrial swelling. Electron-deficient DA quinones will readily bind thiol groups on proteins, often resulting in inactivation of the protein function. The effect of DA quinone modification on the activity of the identified mitochondrial proteins still needs to be investigated, as well as the impact of the modified proteins such as Complex I subunits on overall mitochondrial function. In a subset of mitochondrial proteins, DA quinone modification appears to target the protein for degradation. The exact mechanism by which this occurs is not known, although mitochondrial proteases are likely to play a role. Targeted degradation of modified proteins ensures the loss of protein function, which may have detrimental effects on mitochondrial integrity and neuronal viability. DA oxidation also contributes to other factors implicated in PD such as protein aggregation, ER stress, and proteasomal and/or lysosomal dysfunction. Thus, it is highly likely that the combination of mitochondrial dysfunction, oxidative stress, and DA oxidation increases the vulnerability of dopaminergic neurons to degeneration in PD (Greenamyre and Hastings 2004).

Acknowledgements This work was supported in part by NIH grants NS044076 and NS059806. The author is grateful to all trainees, colleagues, and collaborators that participated in the studies cited in this article.

References

Banerjee R, Starkov AA, Beal MF, Thomas B (2009) Biochim Biophys Acta 1792:651–663

Berman SB, Hastings TG (1999) J Neurochem 73:1127–1137

Berman SB, Watkins SC, Hastings TG (2000) Exp Neurol 164:415–425

Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (2000) Nat Neurosci 3:1301–1306

Betarbet R, Canet-Aviles RM, Sherer TB et al (2006) Neurobiol Dis 22:404–420

Bueler H (2009) Exp Neurol 218:235–246

Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT (2009) Neurobiol Dis 34:279–290

Caudle WM, Richardson JR, Wang MZ et al (2007) J Neurosci 27:8138–8148

Caudle WM, Colebrooke RE, Emson PC, Miller GW (2008) Trends Neurosci 31:303–308

Chen L, Ding Y, Cagniard B, Van Laar AD, Mortimer A, Chi W, Hastings TG, Kang UJ, Zhuang X (2008) J Neurosci 28:425–433

Conway KA, Lee SJ, Rochet JC, Ding TT, Harper JD, Williamson RE, Lansbury PT Jr (2000) Ann N Y Acad Sci 920:42–45

Dukes AA, Van Laar VS, Cascio M, Hastings TG (2008) J Neurochem 106:333–346

Gasser T (2009) Biochim Biophys Acta 1792:587–596

Giorgi C, De Stefani D, Bononi A, Rizzuto R, Pinton P (2009) Int J Biochem Cell Biol 41:1817–1827

Graham DG, Tiffany SM, Bell WR Jr, Gutknecht WF (1978) Mol Pharmacol 14:644–653

Greenamyre JT, Hastings TG (2004) Science 304:1120–1122

Grunewald A, Gegg ME, Taanman JW, King RH, Kock N, Klein C, Schapira AH (2009) Exp Neurol 219:266–273

Hastings TG (1995) J Neurochem 64:919–924

Hastings TG, Zigmond MJ (1994) J Neurochem 63:1126–1132

Hastings TG, Lewis DA, Zigmond MJ (1996) Proc Natl Acad Sci USA 93:1956–1961

Holtz WA, Turetzky JM, Jong YJ, O'Malley KL (2006) J Neurochem 99:54–69

John GB, Shang Y, Li L, Renken C, Mannella CA, Selker JM, Rangell L, Bennett MJ, Zha J (2005) Mol Biol Cell 16:1543–1554

LaVoie MJ, Ostaszewski BL, Weihofen A, Schlossmacher MG, Selkoe DJ (2005) Nat Med 11:1214–1221

Lesage S, Brice A (2009) Hum Mol Genet 18:R48–59

Martin HL, Teismann P (2009) FASEB J 23:3263–3272

Martinelli P, Rugarli EI (2009) Biochim Biophys Acta, (In Press)

Martinez-Vicente M, Talloczy Z, Kaushik S et al (2008) J Clin Invest 118:777–788

Narendra D, Tanaka A, Suen DF, Youle RJ (2008) J Cell Biol 183:795–803

Rabinovic AD, Lewis DA, Hastings TG (2000) Neuroscience 101:67–76

Rochet JC, Outeiro TF, Conway KA, Ding TT, Volles MJ, Lashuel HA, Bieganski RM, Lindquist SL, Lansbury PT (2004) J Mol Neurosci 23:23–34

Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B (1998) J Neurochem 71:2112–2122

Staal RG, Mosharov EV, Sulzer D (2004) Nat Neurosci 7:341–346

Stokes AH, Hastings TG, Vrana KE (1999) J Neurosci Res 55:659–665

Sulzer D (2007) Trends Neurosci 30:244–250

Van Laar VS, Dukes AA, Cascio M, Hastings TG (2008) Neurobiol Dis 29:477–489

Van Laar VS, Mishizen AJ, Cascio M, Hastings TG (2009) Neurobiol Dis 34:487–500

Xie J, Marusich MF, Souda P, Whitelegge J, Capaldi RA (2007) FEBS Lett 581:3545–3549

Xu W, Liu L, Charles IG, Moncada S (2004) Nat Cell Biol 6:1129–1134

Zecca L, Zucca FA, Wilms H, Sulzer D (2003) Trends Neurosci 26:578–580