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Research Paper

Brain mitochondria from DJ-1 knockout mice show increased respiration-dependent hydrogen peroxide consumption

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ABSTRACT

Mutations in the DJ-1 gene have been shown to cause a rare autosomal-recessive genetic form of Parkinson's disease (PD). The function of DJ-1 and its role in PD development has been linked to multiple pathways, however its exact role in the development of PD has remained elusive. It is thought that DJ-1 may play a role in regulating reactive oxygen species (ROS) formation and overall oxidative stress in cells through directly scavenging ROS itself, or through the regulation of ROS scavenging systems such as glutathione (GSH) or thioredoxin (Trx) or ROS producing complexes such as complex I of the electron transport chain. Previous work in this laboratory has demonstrated that isolated brain mitochondria consume H_2O_2 predominantly by the Trx/Thioredoxin Reductase (TrxR)/Peroxiredoxin (Prx) system in a respiration dependent manner (Drechsel et al., Journal of Biological Chemistry, 2010). Therefore we wanted to determine if mitochondrial H_2O_2 consumption was altered in brains from DJ-1 deficient mice (DJ-$1^{-/-}$). Surprisingly, DJ-$1^{-/-}$ mice showed an *increase* in mitochondrial respiration-dependent H_2O_2 consumption compared to controls. To determine the basis of the increased H_2O_2 consumption in DJ$1^{-/-}$ mice, the activities of Trx, Thioredoxin Reductase (TrxR), GSH, glutathione disulfide (GSSG) and glutathione reductase (GR) were measured. Compared to control mice, brains from DJ-$1^{-/-}$ mice showed an *increase* in (1) mitochondrial Trx activity, (2) GSH and GSSG levels and (3) mitochondrial glutaredoxin (GRX) activity. Brains from DJ-$1^{-/-}$ mice showed a decrease in mitochondrial GR activity compared to controls. The increase in the enzymatic activities of mitochondrial Trx and total GSH levels may account for the increased H_2O_2 consumption observed in the brain mitochondria in DJ-$1^{-/-}$ mice perhaps as an adaptive response to chronic DJ-1 deficiency.

Introduction

Parkinson's disease (PD) is a neurodegenerative movement disorder which is characterized by a progressive loss of dopaminergic (DA) neurons within the substantia nigra pars compacta (SNpc) [1,2]. There are two main forms of PD, an idiopathic/sporadic form, which affects the majority of people diagnosed with PD, and a genetically linked form which can be autosomal dominant or recessive [2,3]. The precise causes of DA neuronal death in the sporadic form of PD is unknown, but it is hypothesized that the pathogenesis of PD may involve oxidative damage and mitochondrial dysfunction [2,4–7]. This has been supported by postmortem studies in the brains of PD patients which indicated increased levels of oxidative stress within the SNpc, a decrease in complex I activity and an increase in 4-hydroxyl-2-nonenal (4-HNE) [4,8,9]. Of recent interest is the discovery that some of the genetic forms of PD also indicate a role of oxidative stress in the development of PD. Specifically, mutations in Parkin, Pink1 and DJ-1 have been shown to generate early onset PD and their functions have been linked to reactive oxygen species (ROS) formation resulting in oxidative stress, mitochondrial dysfunction and cell death [10,11].

The homozygous mutation in the DJ-1 gene (PARK7) has been shown to cause autosomal recessive early on-set PD [12,13]. DJ-1 has been associated with multiple functions in disease and non-disease states which include, but is not limited to a ROS scavenger, a regulator of glutathione (GSH) levels and complex I activity, a molecular chaperone, and a transcriptional co-activator [7,14–25]. DJ-1 has also been implicated in the thioredoxin/apoptosis signal-regulating

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Abbreviations: PD, Parkinson's disease; DA, dopaminergic; Trx, thioredoxin; TrxR, thioredoxin reductase; Trx1, cytosolic trx; Trx2, mitochondrial trx; TrxR1, cytosolic TrxR; TrxR2, mitochondrial Trx; Prx, peroxiredoxin; H_2O_2, hydrogen peroxide; GSH, reduced glutathione; GSSG, oxidized glutathione; ROS, reactive oxygen species; GRX, glutaredoxin; GR, glutathione reductase; Gpx, glutathione peroxidase; DJ$1^{-/-}$, DJ-1 knockout; 4-HNE, 4-hydroxyl-2-nonenal; SNpc, substantia nigra pars compacta; ASK1, apoptosis signal-regulating kinase 1; Nrf2, nuclear factor erythroid 2-related factor; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; BSA, Bovin Serum Albumin; HEDS, 2-hydroxyethyl disulfide; 6OHDA, 6-hydroxydopamine; PQ, paraquat; Cox IV, complex IV; TH, tyrosine hydroxylase; MEF, mouse embryonic fibroblasts.

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kinase 1 (Trx/Ask1) complex, a regulator of the nuclear factor erythroid 2-related factor (Nrf2) and has been shown to act like an atypical peroxiredoxin-like peroxidase thereby regulating the endogenous ROS scavenging systems [14,18,25]. Mitochondria have long been thought to be major producers of ROS. Recently our laboratory demonstrated that mitochondria consume H_2O_2 in a respiration-dependent manner primarily through the Trx/peroxiredoxin (Prx) system and that knockdown of mitochondrial thioredoxin reductase (TrxR2) decreases the ability of mitochondria and DA cells to consume exogenous H_2O_2 in a respiration dependent manner [26,27]. Furthermore previous literature has shown that decreases in the mitochondrial GSH or Trx/TrxR systems render DA cells more sensitive to oxidative stress and cell death [27–29].

The purpose of this study was to determine if DJ-1 deficiency alters the ability of brain mitochondria to consume and detoxify H_2O_2. Based on previous literature showing DJ-1's role in regulating antioxidant pathways and increasing ROS-dependent sensitivity of DJ-$1^{-/-}$ to PD toxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), we hypothesized that brain mitochondria from DJ-$1^{-/-}$ mice would show decreased respiration dependent H_2O_2 consumption [26]. Surprisingly we found that DJ-$1^{-/-}$ mouse brains had (1) an *increase* in mitochondrial respiration-dependent H_2O_2 consumption, (2) an *increase* in mitochondrial Trx activity and (3) an increase in total glutathione (GSH and GSSG respectively) levels, (4) an increase in mitochondrial glutaredoxin (GRX) activity and a (5) decrease in mitochondrial glutathione reductase (GR) activity. Thus DJ-1 deficiency resulted in increased H_2O_2 consumption likely due to adaptive changes in the mitochondrial GSH and Trx/TrxR systems.

Methods

Chemical reagents

All reagents and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise noted.

Mice

Animal studies were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23). All animal experiments were carried out with approval from the Institutional Animal Care and Use Committee at the University of Colorado Denver Anschutz Medical Campus, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. DJ-1 homozygous knockout mice (DJ-$1^{-/-}$) mice on a C57BL/6 background and C57BL/6 controls (DJ-$1^{+/+}$) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). DJ1 knockout in mice was confirmed by Western blot using a primary antibody from Millipore (Billerica, MA, USA) at 1:500 and secondary antibody purchased from Abcam (Cambridge, MA, USA) at 1:10,000 (data not shown). All experiments were conducted on mice 3–6 months old.

Isolation of pure mouse brain mitochondria

Adult male DJ-$1^{+/+}$ or DJ-$1^{-/-}$ had their brain excised and mitochondria was isolated as previously described through a Percoll density gradient centrifugation [26,30]. Briefly, mouse brain was homogenized in a sucrose isolation buffer and diluted 1:1 in 24% Percoll and centrifuged. After centrifugation the sediment was removed and added to 40%/19% Percoll gradient and centrifuged. Mitochondria from the interface was removed and diluted with isolation buffer and centrifuged. The mitochondrial pellet was re-suspended in isolation buffer containing 1 mg/ml Bovin Serum Albumin (BSA) and centrifuged for final pure mitochondrial pellet.

Isolation of crude mouse brain mitochondria

Adult male DJ-$1^{+/+}$ or DJ-$1^{-/-}$ mouse brains where homogenized in isolation buffer then centrifuged at 3000 RPM for 10 min at 4 °C. The supernatant was removed and centrifuged at 13,000 RPM for 10 min at 4 °C. Supernatant was removed and saved for cytosolic fraction and mitochondrial pellet was re-suspended in isolation buffer.

Exogenous H_2O_2 removal rates by polarographic measurement

H_2O_2 rates were measured in 100 μg of isolated pure mouse brain mitochondria (as determined by Bradford assay) using a 100-μM Clark-type electrode with an Apollo 4000 Free Radical Analyzer (World Precision Instruments, Inc., Sarasota, FL, USA). As described by Drechsel et al. [26] after obtaining a stable baseline for 60 s, 3 μM of H_2O_2 was added to an open thermostatted chamber (30 °C) and allowed to stabilize. Isolated mitochondria, and the respiration substrates malate (2.5 mM) and glutamate (10 μM) were added and a stable H_2O_2 consumption rate was measured for 30–60 s. Isolated pure mitochondria were added to the chamber and H_2O_2 removal rates were calculated based on the linear signal decay after the addition of mitochondria compared to respiration substrates.

TrxR and Trx activity assay

TrxR and Trx activity was measured in crude brain mitochondria using an insulin-reduction assay in the presence of *Escherichia coli* Trx or rat TrxR as previously described by Arnér et al. [31]. Briefly, crude mitochondria were lysed using sonication and protein levels were determined using a Bradford protein assay. 25 μg of isolated mitochondria where plated and Trx, TrxR or water was added. Assay buffer was added (for final volume of 50 μl) and mitochondria where incubated for 1 h. After incubation the number of reduced thiols was determined on a Versamax micro plate reader (Molecular Devices, Sunnyvale, CA, USA).

HPLC to determine glutathione levels

GSH, glutathione disulfide (GSSG) assays were performed with an ESA 5600 CoulArray HPLC equipped with eight electrochemical cells as previously described by Patel et al. [32]. The potentials of the electrochemical cells were set and analyte separation was conducted on a TOSOHAAS (Montgomeryville, PA, USA) reverse-phase ODS 80-TM C-18 analytical column (4.6 mm × 250 cm; 5 μm particle size). A two-component gradient elution system was used with component A of the mobile phase composed of 50 mM NaH_2PO_4, pH 3.2, and component B composed of 50 mM NaH_2PO_4 and 40% methanol, pH 3.2. An aliquot (40 μl) of the supernatant was injected into the HPLC.

Glutaredoxin (GRX) activity assay

GRX activity was measured as previously described by Mesecke et al. with a slight modification [33]. 100 μg isolated crude mitochondria was incubated in reaction buffer (0.1 M Tris/HCl, and 1 mM EDTA, pH 8.0, and contained 0.1 mM NADPH, 0.25 U/ml GR, and 1 mM GSH) and the consumption of NADPH to $NADP^+$ through the addition of 2-hydroxyethyl disulfide (HEDS) was measured at 340 nm every 30 s for 10 min on a Versamax micro plate reader.

Glutathione reductase (GR) activity assay

GR activity was measured spectrophotometrically in 100 μg of isolated crude mitochondria incubated in a reaction buffer containing 50 mM sodium phosphate and 240 mM NADPH (pH 7.6). The reaction was initiated with the addition of 16 mM GSSG and the conversion of

NADPH to NADP$^+$ at 340 nM was measured for 10 min on a Versamax micro plate reader.

Results

Increased H_2O_2 consumption rates in DJ-1 $^{-/-}$ mice compared to DJ-1$^{+/+}$ controls

We initially determined the role of DJ-1 in the ability of brain mitochondria to consume H_2O_2 in the presence of respiration substrates, malate and glutamate. Based on the link between DJ1 and antioxidant functions we hypothesized DJ-1$^{-/-}$ mice would have a decrease in H_2O_2 removal rates compared to DJ-1$^{+/+}$ mice. Pure brain mitochondrion were isolated from DJ-1$^{-/-}$ and DJ-1$^{+/+}$ mice and their ability to consume exogenous H_2O_2 in real-time was measured using the polarographic method with a Clark-type electrode. As summarized in Fig. 1A, a stable baseline was achieved followed by addition of 3 μM H_2O_2. After stabilization, the respiration substrates malate and glutamate were added and the H_2O_2 consumption rates were measured for ˜30 s. After a steady rate had been achieved 0.1 mg/ml of isolated mitochondria from either DJ-1$^{-/-}$ or DJ-1$^{+/+}$ mice was added and the H_2O_2 consumption rates were determined. As shown graphically in Fig. 1A and quantified in Fig. 1B, brain mitochondria from DJ-1$^{-/-}$ mice showed a significant increase in their ability to consume H_2O_2 compared to DJ-1$^{+/+}$ mice.

Increased mitochondrial thioredoxin (Trx2) activity in brains from DJ-1 $^{-/-}$ mice

The surprising finding that brain mitochondria from DJ1$^{-/-}$ mice show increased H_2O_2 consumption prompted us to determine the major enzymatic system underlying this effect. Previous work in our laboratory has shown that inhibiting TrxR activity in isolated rat brain mitochondria dramatically decreases their H_2O_2 consumption rates [26]. Based on the *increase* in H_2O_2 consumption rates in DJ1$^{-/-}$ mice we wanted to determine if there was an increase in Trx2 and/or TrxR2 activity**.** As indicated in Fig. 2, there was a significant increase in the Trx2 activity in DJ-1$^{-/-}$ mice compared to DJ-1$^{+/+}$ mice whereas no change in Trx activity was observed in the cytosolic (Trx1) compartment. No significant differences were observed between the mitochondrial or cytosolic (TrxR1) activity of TrxR in DJ-1$^{-/-}$ vs. DJ-1$^{+/+}$ mice. To ensure the changes in activity were not a result of changes in mitochondria quantity, a Western blot for the mitochondrial marker cytochrome oxidase, subunit IV, (Cox IV) was conducted in whole brain lysate of DJ-1$^{+/+}$ and DJ-1$^{-/-}$ mice. There was no change in Cox IV levels when normalized to β-Actin between DJ-1$^{+/+}$ and DJ-1$^{-/-}$ mice ($n = 4$) (data not shown).

Increased brain GSH and GSSG levels in DJ-1 $^{-/-}$ mice

Previous literature has shown when DJ-1$^{-/-}$ cells are under oxidative stress they are more susceptible to cell death due to a decrease in GSH levels [17,24,34]. Therefore based on the increased activity of Trx2 we wanted to determine the status of the GSH system under baseline conditions. GSH and GSSG levels were measured in the whole brains of DJ-1$^{-/-}$ and DJ-1$^{+/+}$ mice. As indicated in Fig. 3A and B, DJ-1$^{-/-}$ mice showed a significant increase in GSH levels (2148 ± 22.6 vs. 2029 ± 17.3 nmol/g, DJ-1$^{-/-}$ vs. DJ-1$^{+/+}$ respectively) and GSSG (7.51 ± 0.4 vs. 2.86 ± 0.4 nmol/g, DJ-1$^{-/-}$ vs. DJ-1$^{+/+}$ respectively) compared to DJ-1$^{+/+}$ mice. This resulted in the DJ-1$^{-/-}$ mouse brains having significantly more total glutathione (GSH + GSSG) compared to DJ-1$^{+/+}$ mice (Fig. 3C).

Alterations in glutathione peroxidase (gpx), glutathione reductase (GR) and glutaredoxin (GRX) activities in DJ-1 $^{-/-}$ mice

To determine if the changes in GSH and GSSH levels were due in part to changes in enzymatic regulation of the glutathione pathway, we measured the activities of GR, GRX and GPx. GR and GRX activities were measured in cytosolic and mitochondrial fraction from the brains of DJ-1$^{-/-}$ and DJ-1$^{+/+}$ mice. There was no change in the cytosolic GR and GRX activity between the two mice (data not shown). Mitochondrial GR activity was significant decreased in brains from DJ-1$^{-/-}$ compared to DJ-1$^{+/+}$ mice (7.01 ± 0.2 vs. 9.08 ± 0.6 pmol/mg, in DJ-1$^{-/-}$ and DJ-1$^{+/+}$ mice, respectively). A significant increase in GRX activity was observed in DJ-1$^{-/-}$ compared to DJ-1$^{+/+}$ mice (24.71 ± 0.6 vs. 17.63 ± 1.8 pmol/mg, in DJ-1$^{-/-}$ and DJ-1$^{+/+}$ respectively). Interestingly, there was no change in the brain mitochondrial GPx activity in DJ-1$^{-/-}$ compared to DJ-1$^{+/+}$ mice (Fig. 4A). This data suggests that despite an increase in GSH levels, decreased GR activity results in an increased GSSG level leading to increased total glutathione levels (GSH + GSSG) in the brains of DJ1$^{-/-}$ mice. Increased GSSG can favor glutathionylation reactions resulting in increased GRX activity.

Discussion

DJ-1 function has been implicated in a multitude of functions including a ROS scavenger, a regulator of mitochondrial function, a regulator of GSH levels, a molecular chaperone, and a transcriptional co-activator [14–25,35]. DJ-1 is ubiquitously expressed throughout the body including the brains of normal and idiopathic PD patients; however point mutations or large deletion in the kinase region of the DJ-1 gene is sufficient to cause early on-set autosomal recessive PD [12,36]. It has been demonstrated that DJ-1 deficiency renders both cells and animals more susceptible to MPTP, paraquat (PQ), rotenone, and 6-hydroxydopamine (6OHDA) toxicities which can be rescued with its over expression [18,22,23,37–43]. It has been hypothesized that the increased sensitivity to oxidative stress in DJ-1$^{-/-}$ mice and cells is related to a decrease in ROS scavenging arising from decreased peroxidase-like scavenging, deficient Nrf2 transcriptional factors and increased mitochondrial dysfunction due to complex I deficiency [15,16,18,20,24]. Indeed deletion of DJ-1 in tyrosine hydroxylase (TH) positive neurons of the midbrain resulted in higher mitochondrial oxidant stress assessed by increased fluorescence of ro-GFP levels in TH$^+$ neurons in the SNpc compared to control animals [17]. Interestingly, there was no change in ro-GFP levels between neurons in the ventral trigeminal area indicating the loss of DJ-1 in mice increases mitochondrial oxidant stress in the neuronal population associated with PD etiology [17].

Whereas decreases in cytosolic thioredoxin (Trx1) mRNA and protein levels has been observed due to DJ-1 deficiency in SH-SY5Y cells and mouse embryonic fibroblasts (MEF) [14], to our knowledge, the role of the mitochondrial Trx (Trx2) has not been studied in the context of DJ-1 deficiency. Our previous studies have demonstrated that pharmacological inhibition or deletion of Trx/TrxR activity in isolated mitochondria result in decreased respiration dependent H_2O_2 consumption by isolated mitochondria and render DA cells more susceptible to subtoxic concentrations of PQ and 6OHDA [26,27]. Thus, we sought to determine the antioxidant function of DJ-1 i.e. brain mitochondrial H_2O_2 consumption and detoxification in DJ-1$^{-/-}$ mice [26,27]. We hypothesized that DJ-1$^{-/-}$ mice would show decreased H_2O_2 removal rates due to its role in oxidative stress and Trx1 levels. Surprisingly, our study indicated that brain mitochondria from DJ-1$^{-/-}$ mice showed *increased* rates of H_2O_2 consumption which was correlated with an increase in Trx2 activity. Interestingly, we found no change in Trx1 activity in DJ-1$^{-/-}$ mice compared to mock controls as previously reported which may be due to the use of whole animal brain (our study) vs. MEFs [14].

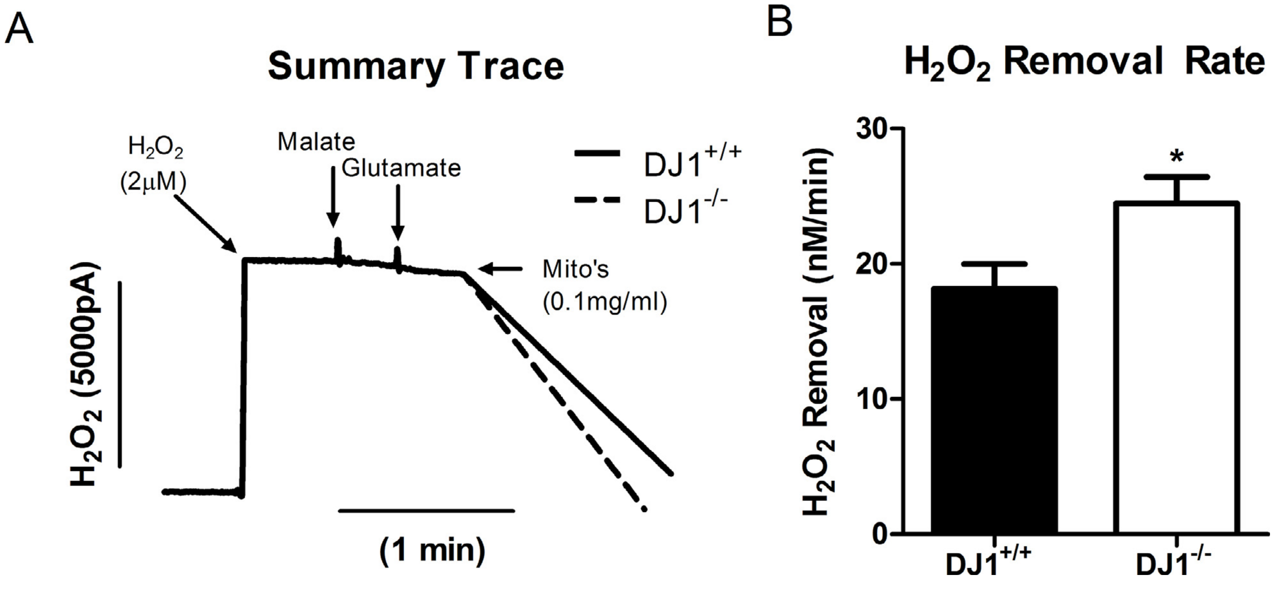

Fig. 1. DJ-1$^{-/-}$ mice show increased mitochondrial H_2O_2 consumption rates compared to DJ-1$^{+/+}$ mice. Pure mitochondria was isolated from the whole brain of DJ-1$^{-/-}$ and DJ-1$^{+/+}$ mice and their H_2O_2 consumption rates was determined as depicted in (A). As quantified in (B), actively respiring mitochondria from DJ-1$^{-/-}$ mice had a significant increase in their consumption rates compared to DJ-1$^{+/+}$ mice ($n = 7$–8 mice; 2–4 runs for each mouse). $^*p < 0.05$ as determined by Student's *t*-test. Bars represent mean ± SEM.

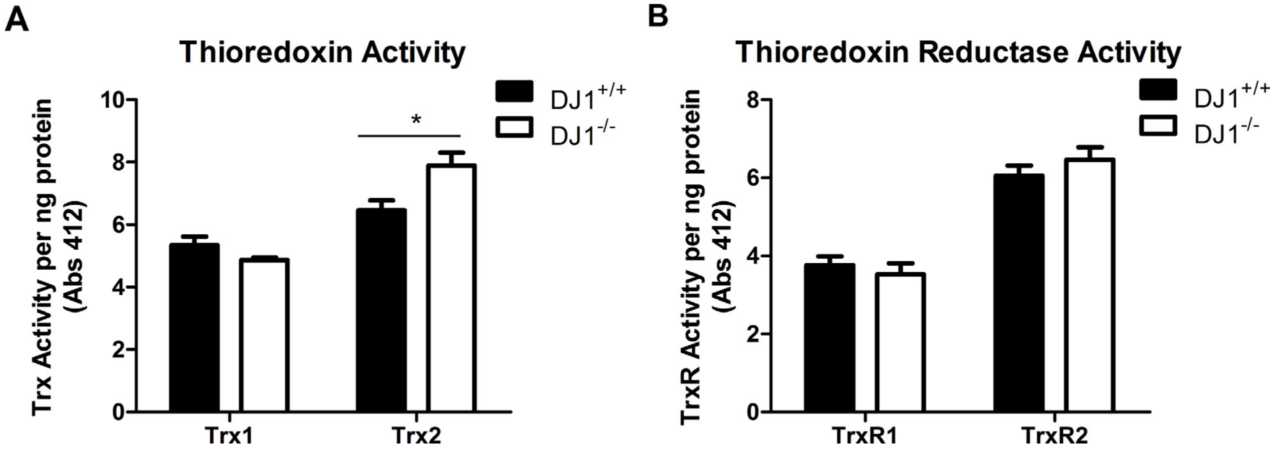

Fig. 2. Increased mitochondrial Trx activity in brains from DJ-1$^{-/-}$ mice. Isolated mitochondrial and cytosolic fractions from DJ-1$^{+/+}$ and DJ-1$^{-/-}$ mice were analyzed for Trx (A) and TrxR (B) activities. There was a significant increase in the mitochondrial (Trx2) but not cytosolic (Trx1) or TrxR1 or TrxR2 activity in DJ-1$^{-/-}$ compared to DJ-1$^{+/+}$ mice. $^*p < 0.05$ as determined by 2way ANOVA ($n = 4$–5 mice with 2–3 runs for each mouse). Bars represent mean ± SEM.

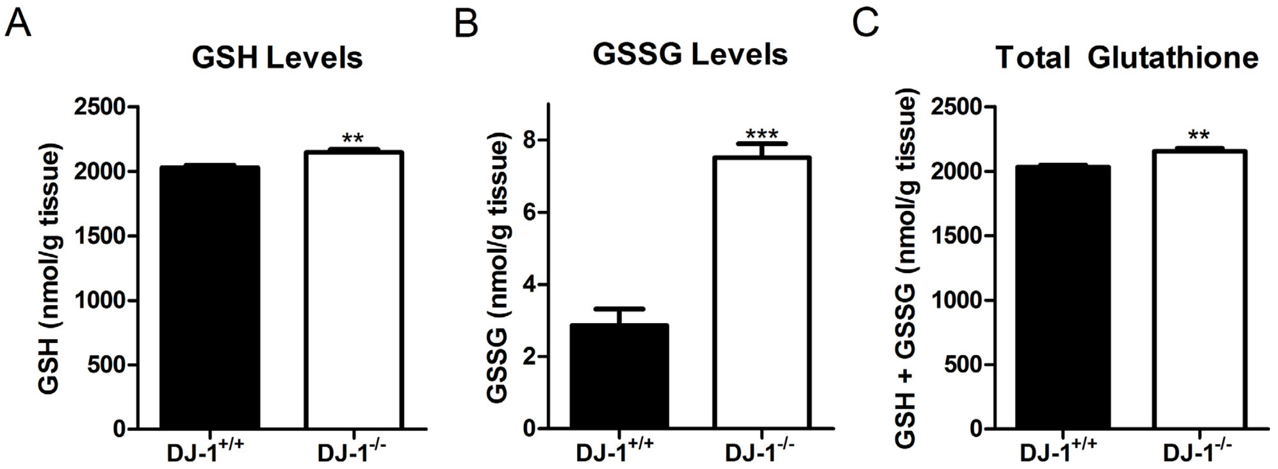

Fig. 3. DJ-1$^{-/-}$ mice have higher GSH and GSSG levels compared to DJ-1$^{+/+}$ mice. GSH (A) and GSSG (B) levels were measured in DJ-1$^{+/+}$ mice and DJ-1$^{-/-}$ mice via HPLC. There was a significant increase in GSH and GSSG level in DJ-1$^{-/-}$ mice which resulted in a significant increase in total glutathione (GSH + GSSG). $^{**}p < 0.01$, $^{***}p < 0.005$ as determined by Student's *t*-test. All bars represent mean ± SEM ($n = 4$–5).

Fig. 4. DJ-$1^{-/-}$ mice show alterations in GR and GRX activity compared to DJ-$1^{+/+}$. Crude mitochondria was isolated from the whole brain of DJ-$1^{-/-}$ and DJ$^{+/+}$ mice and different activities of the GSH system were measured. There was no change in Gpx activity (A) however there was a significant decrease in GR (B) and a significant increase in GRX activity (C) in DJ-$1^{-/-}$ mice compared to DJ-$1^{+/+}$ mice. $^*p < 0.05$; $^{**}p < 0.01$ as determined by Students t-test. Bars represent mean ± SEM ($n = 5$).

The finding that DJ-$1^{-/-}$ mice had an *increase* in H_2O_2 consumption rates which correlated with an increase in the Trx2 activity is noteworthy due to the conflicting results regarding the lack of basal dopaminergic neurodegeneration in DJ-$1^{-/-}$ mice. Humans with homozygous deficiency of DJ-1 develop early–onset PD, however there are contradictory studies regarding whether or not mice lacking DJ-1 develop baseline neuronal degeneration of dopamine cells or motor dysfunctions [18,37,44,45]. Our data suggests that increased Trx2 activity may be one compensatory mechanism utilized by the DJ-$1^{-/-}$ mice to prevent long-term oxidative stress thus limiting the amount of neuronal cell death. The effect of increased Trx2 activity may be mitigated with exposure to pro-oxidant and PD linked chemicals such as MPTP, rotenone, and PQ. As previously shown in the literature, DJ-$1^{-/-}$ mice are more sensitive to MPTP toxicity than DJ-$1^{+/+}$ mice [38]. Increased Trx2 activity in DJ-$1^{-/-}$ mice may limit DA cell death under basal conditions, however when exposed to a large oxidative stress, such as MPTP, this compensatory mechanism may no longer be effective. Thus, under larger levels of oxidative stress DJ-1 may play a critical role in maintaining DA neuronal health while under a controlled environment it may have other functions. This could also partly explain the difference in phenotypes between human populations, which are constantly exposed to environmental factors, and mice, which live in a controlled environment.

It is widely believed the GSH system is the primary antioxidant system in ROS detoxification in the brain due to GSH being present in mM concentrations and thus the most abundant intracellular thiol-based system [46,47]. GSH has been reported to be present both in astrocytes and neurons within the SNpc but with higher concentrations in astrocytes compared to neurons [47]. Measurement of GSH and GSSG levels in postmortem tissue of PD patients has revealed significantly lower GSH levels (̃40%) and slight but insignificant increase in GSSG levels (̃29%) in the SNc compared to age matched controls [48]. Previous literature has indicated that knockout of DJ-1 in cell-based models results in increased susceptibility to ROS due to a decrease in GSH levels which can be rescued with over-expression of DJ-1 through increased activity of glutamate cysteine ligase [24,34]. The finding that GSSG levels were increased in DJ-$1^{-/-}$ mice together with decreased GR activity suggested that an inability to recycle GSSG back to GSH. Of interest, there was also a small but significant *increase* in GSH levels most likely arising due the inability to maintain reduced GSH in the face of decreased GR coupled with the efflux of accumulated GSSG from the cell.

Based on the results of this study, we hypothesize that increased mitochondrial H_2O_2 consumption rates in the brains of DJ-$1^{-/-}$ mice is most likely due to increased Trx activity protecting brain mitochondria from the increased baseline ROS production. In corroboration with previous literature, DJ-$1^{-/-}$ mice had a decrease in GR activity leading to the increase in GSSG level, resulting in an increase in glutathionylation and thus an increase in GRX activity. Based on the increased H_2O_2 consumption and mitochondrial Trx activity, it leads one to speculate that upregulation of Trx, TrxR or Prx would be a beneficial target for the treatment of PD.

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References

[1] D.B. Calne, J.W. Langston, Aetiology of Parkinson's disease, Lancet 2 (1983) 1457–1459, 6140548.

[2] A.E. Lang, A.M. Lozano, Parkinson's disease. First of two parts, New England Journal of Medicine 339 (1998) 1044–1053. http://dx.doi.org/10.1056/NEJM199810083391506, 9761807.

[3] W.K. Scott, M.A. Nance, R.L. Watts, J.P. Hubble, W.C. Koller, K. Lyons, et al, Complete genomic screen in Parkinson disease: evidence for multiple genes, Journal of the American Medical Association 286 (2001) 2239–2244. http://dx.doi.org/10.1001/jama.286.18.2239, 11710888.

[4] W.D. Parker Jr., J.K. Parks, R.H. Swerdlow, Complex I deficiency in Parkinson's disease frontal cortex, Brain Research 1189 (2008) 215–218. http://dx.doi.org/10.1016/j.brainres.2007.10.061, 18061150.

[5] P. Jenner, C.W. Olanow, The pathogenesis of cell death in Parkinson's disease, Neurology 66 (2006) S24–S36. http://dx.doi.org/10.1212/WNL.66.10_suppl_4.S24, 16717250.

[6] P. Jenner, Oxidative stress in Parkinson's disease and other neurodegenerative disorders, Pathologie–Biologie 44 (1996) 57–64.

[7] Z. Wang, J. Liu, S. Chen, Y. Wang, L. Cao, Y. Zhang, et al, DJ-1 modulates the expression of Cu/Zn-superoxide dismutase-1 through the Erk1/2-Elk1 pathway in neuroprotection, Annals of Neurology 70 (2011) 591–599. http://dx.doi.org/10.1002/ana.22514, 21796667.

[8] E. Floor, M.G. Wetzel, Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay, Journal of Neurochemistry 70 (1998) 268–275, 9422371.

[9] A. Yoritaka, N. Hattori, K. Uchida, M. Tanaka, E.R. Stadtman, Y. Mizuno, Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease, Proceedings of the National Academy of Sciences of the United States of America 93 (1996) 2696–2701. http://dx.doi.org/10.1073/pnas.93.7.2696, 8610103.

[10] V. Dias, E. Junn, M.M. Mouradian, The role of oxidative stress in Parkinson's disease, Journal of Parkinson's Disease 3 (2013) 461–491, 24252804.

[11] M.R. Cookson, O. Bandmann, Parkinson's disease: insights from pathways, Human Molecular Genetics 19 (2010) R21–R27. http://dx.doi.org/10.1093/hmg/ddq167, 20421364.

[12] V. Bonifati, P. Rizzu, F. Squitieri, E. Krieger, N. Vanacore, J.C. van Swieten, et al, DJ-1(PARK7), a novel gene for autosomal recessive, early onset Parkinsonism, Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology 24 (2003) 159–160. http://dx.doi.org/10.1007/s10072-003-0108-0, 14598065.

[13] V. Bonifati, P. Rizzu, M.J. van Baren, O. Schaap, G.J. Breedveld, E. Krieger, et al, Mutations in the DJ-1 gene associated with autosomal recessive early-onset Parkinsonism, Science 299 (2003) 256–259. http://dx.doi.org/10.1126/science.1077209, 12446870.

[14] J.Y. Im, K.W. Lee, J.M. Woo, E. Junn, M.M. Mouradian, DJ-1 induces thioredoxin 1 expression through the Nrf2 pathway, Human Molecular Genetics 21 (2012) 3013–3024. http://dx.doi.org/10.1093/hmg/dds131, 22492997.

[15] J.Y. Heo, J.H. Park, S.J. Kim, K.S. Seo, J.S. Han, S.H. Lee, et al, DJ-1 null dopaminergic neuronal cells exhibit defects in mitochondrial function and structure: involvement of mitochondrial complex I assembly, PloS One 7 (2012) e32629. http://dx.doi.org/10.1371/journal.pone.0032629, 22403686.

[16] I. Irrcher, H. Aleyasin, E.L. Seifert, S.J. Hewitt, S. Chhabra, M. Phillips, et al, Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics, Human Molecular Genetics 19 (2010) 3734–3746. http://dx.doi.org/10.1093/hmg/ddq288, 20639397.

[17] J.N. Guzman, J. Sanchez-Padilla, D. Wokosin, J. Kondapalli, E. Ilijic, P.T. Schumacker, et al, Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1, Nature 468 (2010) 696–700. http://dx.doi.org/10.1038/nature09536, 21068725.

[18] E. Andres-Mateos, C. Perier, L. Zhang, B. Blanchard-Fillion, T.M. Greco, B. Thomas, et al, DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase, Proceedings of the National Academy of Sciences of the United States of America 104 (2007) 14807–14812. http://dx.doi.org/10.1073/pnas.0703219104, 17766438.

[19] R.M. Canet-Aviles, M.A. Wilson, D.W. Miller, R. Ahmad, C. McLendon, S. Bandyopadhyay, et al, The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 9103–9108. http://dx.doi.org/10.1073/pnas.0402959101, 15181200.

[20] E. Junn, W.H. Jang, X. Zhao, B.S. Jeong, M.M. Mouradian, Mitochondrial localization of DJ-1 leads to enhanced neuroprotection, Journal of Neuroscience Research 87 (2009) 123–129. http://dx.doi.org/10.1002/jnr.21831, 18711745.

[21] H.M. Li, T. Niki, T. Taira, S.M. Iguchi-Ariga, H. Ariga, Association of DJ-1 with chaperones and enhanced association and colocalization with mitochondrial Hsp70 by oxidative stress, Free Radical Research 39 (2005) 1091–1099. http://dx.doi.org/10.1080/10715760500260348, 16298734.

[22] H.J. Kwon, J.Y. Heo, J.H. Shim, J.H. Park, K.S. Seo, M.J. Ryu, et al, DJ-1 mediates paraquat-induced dopaminergic neuronal cell death, Toxicology Letters 202 (2011) 85–92. http://dx.doi.org/10.1016/j.toxlet.2011.01.018, 21300143.

[23] W. Zhou, K. Bercury, J. Cummiskey, N. Luong, J. Lebin, C.R. Freed, Phenylbutyrate up-regulates the DJ-1 protein and protects neurons in cell culture and in animal models of Parkinson disease, Journal of Biological Chemistry 286 (2011) 14941–14951. http://dx.doi.org/10.1074/jbc.M110.211029, 21372141.

[24] W. Zhou, C.R. Freed, DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T alpha-synuclein toxicity, Journal of Biological Chemistry 280 (2005) 43150–43158, 16227205.

[25] C.M. Clements, R.S. McNally, B.J. Conti, T.W. Mak, J.P. Ting, DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2, Proceedings of the National Academy of Sciences of the United States of America 103 (2006) 15091–15096. http://dx.doi.org/10.1073/pnas.0607260103, 17015834.

[26] D.A. Drechsel, M. Patel, Respiration-dependent H_2O_2 removal in brain mitochondria via the thioredoxin/peroxiredoxin system, Journal of Biological Chemistry 285 (2010) 27850–27858, 20558743.

[27] P. Lopert, B.J. Day, M. Patel, Thioredoxin reductase deficiency potentiates oxidative stress, mitochondrial dysfunction and cell death in dopaminergic cells, PloS One 7 (2012) e50683. http://dx.doi.org/10.1371/journal.pone.0050683, 23226354.

[28] L.P. Liang, T.J. Kavanagh, M. Patel, Glutathione deficiency in Gclm null mice results in complex I inhibition and dopamine depletion following paraquat administration, Toxicological Sciences: An Official Journal of the Society of Toxicology 134 (2013) 366–373. http://dx.doi.org/10.1093/toxsci/kft112, 23704229.

[29] J. Martensson, A. Jain, E. Stole, W. Frayer, P.A. Auld, A. Meister, Inhibition of glutathione synthesis in the newborn rat:a model for endogenously produced oxidative stress, Proceedings of the National Academy of Sciences of the United States of America 88 (1991) 9360–9364. http://dx.doi.org/10.1073/pnas.88.20.9360, 1681551.

[30] P.R. Castello, D.A. Drechsel, M. Patel, Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain, Journal of Biological Chemistry 282 (2007) 14186–14193. http://dx.doi.org/10.1074/jbc.M700827200, 17389593.

[31] E.S. Arnér, A. Holmgren, Measurement of thioredoxin and thioredoxin reductase, in: Current Protocols in Toxicology, 2001 (Chapter 7, Unit 7 4).

[32] L.P. Liang, M. Patel, Seizure-induced changes in mitochondrial redox status, Free Radical Biologyand Medicine 40 (2006) 316–322. http://dx.doi.org/10.1016/j.freeradbiomed.2005.08.026, 16413413.

[33] N. Mesecke, A. Spang, M. Deponte, J.M. Herrmann, A novel group of glutaredoxins in the cis-Golgi critical for oxidative stress resistance, Molecular Biology of the Cell 19 (2008) 2673–2680. http://dx.doi.org/10.1091/mbc.E07-09-0896, 18400945.

[34] F. Liu, J.L. Nguyen, J.D. Hulleman, L. Li, J.C. Rochet, Mechanisms of DJ-1 neuroprotection in a cellular model of Parkinson's disease, Journal of Neurochemistry 105 (2008) 2435–2453. http://dx.doi.org/10.1111/j.1471-4159.2008.05333.x, 18331584.

[35] J.Y. Im, K.W. Lee, E. Junn, M.M. Mouradian, DJ-1 protects against oxidative damage by regulating the thioredoxin/ASK1 complex, Neuroscience Research 67 (2010) 203–208. http://dx.doi.org/10.1016/j.neures.2010.04.002, 20385180.

[36] R. Bandopadhyay, A.E. Kingsbury, M.R. Cookson, A.R. Reid, I.M. Evans, A.D. Hope, et al, The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease, Brain:A Journal of Neurology 127 (2004) 420–430, 14662519.

[37] L. Chen, B. Cagniard, T. Mathews, S. Jones, H.C. Koh, Y. Ding, et al, Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice, Journal of Biological Chemistry 280 (2005) 21418–21426, 15799973.

[38] R.H. Kim, P.D. Smith, H. Aleyasin, S. Hayley, M.P. Mount, S. Pownall, et al, Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress, Proceedings of the National Academy of Sciences of the United States of America 102 (2005) 5215–5220, 15784737.

[39] S.J. Mullett, D.A. Hinkle, DJ-1 knock-down in astrocytes impairs astrocyte-mediated neuroprotection against rotenone, Neurobiology of Disease 33 (2009) 28–36, 18930142.

[40] T. Yokota, K. Sugawara, K. Ito, R. Takahashi, H. Ariga, H. Mizusawa, Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition, Biochemical and Biophysical Research Communications 312 (2003) 1342–1348, 14652021.

[41] M. Inden, Y. Kitamura, K. Takahashi, K. Takata, N. Ito, R. Niwa, et al, Protection against dopaminergic neurodegeneration in Parkinson's disease-model animals by a modulator of the oxidized form of DJ-1, a wild-type of familial Parkinson's disease-linked PARK7, Journal of Pharmacological Sciences 117 (2011) 189–203, 22041943.

[42] S.J. Kim, Y.J. Park, I.Y. Hwang, M.B. Youdim, K.S. Park, Y.J. Oh, Nuclear translocation of DJ-1 during oxidative stress-induced neuronal cell death, Free Radical Biologyand Medicine 53 (2012) 936–950, 22683601.

[43] S.Y. Sun, C.N. An, X.P. Pu, DJ-1 protein protects dopaminergic neurons against 6-OHDA/MG-132-induced neurotoxicity in rats, Brain Research Bulletin 88 (2012) 609–616, 22664331.

[44] M.W. Rousseaux, P.C. Marcogliese, D. Qu, S.J. Hewitt, S. Seang, R.H. Kim, et al, Progressive dopaminergic cell loss with unilateral-to-bilateral progression in a genetic model of Parkinson disease, Proceedings of the National Academy of Sciences of the United States of America 109 (2012) 15918–15923, 23019375.

[45] H. Yamaguchi, J. Shen, Absence of dopaminergic neuronal degeneration and oxidative damage in aged DJ-1-deficient mice, Molecular Neurodegeneration 2 (2007) 10, 17535435.

[46] I.G. Kirkinezos, C.T. Moraes, Reactive oxygen species and mitochondrial diseases, Seminars in Cell and Developmental Biology 12 (2001) 449–457, 11735379.

[47] R. Dringen, J.M. Gutterer, J. Hirrlinger, Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species, European Journal of Biochemistry/FEBS 267 (2000) 4912–4916, 10931173.

[48] J. Sian, D.T. Dexter, A.J. Lees, S. Daniel, Y. Agid, F. Javoy-Agid, et al, Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia, Annals of Neurology 36 (1994) 348–355, 8080242.