Neurobiology of Disease

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Neurobiology of Disease 23 (2006) 54 – 60

Enhanced sensitivity of DJ-1-deficient dopaminergic neurons to energy metabolism impairment: Role of Na^+/K^+ ATPase

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Received 4 January 2006; revised 6 February 2006; accepted 8 February 2006
Available online 19 April 2006

DJ-1 gene mutations lead to an inherited form of early-onset parkinsonism. The function of DJ-1 is unclear, though a neuroprotective role has been postulated. Electrophysiological recordings were made of striatal and dopaminergic nigral neurons both of wild-type (WT) and DJ-1-knockout (*DJ-1*$^{-/-}$) mice. We assessed the responses of dopaminergic cells to combined oxygen and glucose deprivation (OGD), and to the mitochondrial toxin rotenone. OGD induced a membrane hyperpolarization in nigral neurons from WT mice. Similarly, rotenone hyperpolarized neurons and then a depolarization occurred. In *DJ-1*$^{-/-}$ mice, the OGD-induced hyperpolarization was significantly enhanced. Moreover, rotenone caused a shorter hyperpolarization followed by an irreversible depolarization. To evaluate the involvement of Na^+/K^+ ATPase, we tested ouabain, a Na^+/K^+ ATPase inhibitor, on two distinct neuronal subtypes. Compared to WT mice, in dopaminergic neurons from *DJ-1*$^{-/-}$ mice, ouabain induced rapid and irreversible membrane potential changes. Notably, this effect was observed at concentrations that were unable to produce membrane potential shifts on striatal spiny neurons, both from WT and *DJ-1*$^{-/-}$ mice. These findings suggest that DJ-1 loss-of-function enhances vulnerability to energy metabolism alterations, and that nigral neurons are particularly sensitive to Na^+/K^+ ATPase impairment.
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Keywords: DJ-1; Parkinson's disease; Electrophysiology; Substantia nigra; Dopamine; Sodium–potassium pump

Introduction

Recessively inherited loss-of-function mutations in the DJ-1 gene cause early-onset, familial Parkinson's disease (PD) (Bonifati et al., 2003). The nature of the physiological function of DJ-1 has not been clarified, although compelling evidence supports a potential neuroprotective role of DJ-1 in oxidative stress, protein folding and degradation pathways (Canet-Aviles et al., 2004; Martinat et al., 2004; Kim et al., 2005; Ved et al., 2005; Zhang et al., 2005). Such indication is consistent with the notion that mitochondrial dysfunction, oxidative stress and proteasome failure play a central role in the pathogenesis of neuronal loss in PD (Dawson and Dawson, 2003; Sherer et al., 2003; Shen and Cookson, 2004; Betarbet et al., 2005). Several animal models have been extensively characterized in attempt to reproduce oxidative stress and mitochondrial impairment. This effort has certainly improved our understanding of the molecular basis of neuronal damage. For instance, the use of the meperidine analogue, MPTP has established the involvement of mitochondrial respiratory chain complex I in dopaminergic neuronal damage in PD (Ramsay et al., 1986).

More recently, generation of DJ-1 null mice has provided an opportunity to gain further insights in the cellular pathways involved in neuroprotection and degeneration. Cortical and midbrain neurons from DJ-1-deficient mice have been reported to exhibit an enhanced vulnerability to MPTP and to oxidative stress. Notably, restoration of DJ-1 expression was able to mitigate this increased sensitivity (Kim et al., 2005). Yet, DJ-1 has been proposed as an oxidative stress sensor, signaled by a key cysteine residue, C106. Acidification of C106 would drive its translocation to the mitochondrial outer membrane, where DJ-1 might exert a protective action (Canet-Aviles et al., 2004). However, conflicting results have been reported on the precise intracellular localization of DJ-1 protein and its possible translocation. By means of electron microscopy and cell fractionation, Zhang et al. (2005) reported that DJ-1 is an integral protein of the mitochondrial matrix and of the inter-membrane space, likely to play a central role in mitochondria function.

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2006.02.001

We took advantage of the recently generated mouse model lacking the DJ-1 gene and performed electrophysiological recordings from slices of striatal and nigral dopaminergic neurons (Goldberg et al., 2005). Our native slice preparation represents a valid tool to monitor, in real time, the electrophysiological properties of single neurons, allowing to detect non-physiological responses. Evidence is provided that dopaminergic neurons from substantia nigra pars compacta (SNpc) exhibit a significantly higher sensitivity to energy metabolism impairment compared to controls. Moreover, compared to striatal neurons, nigral dopaminergic neurons show an increased susceptibility to the block of the Na^+/K^+ ATPase.

Materials and methods

Brain slice preparation

DJ-1$^{-/-}$ mice (6–8 weeks of age) were generated as described previously (Goldberg et al., 2005). The experimental protocols utilized are in accordance to the guidelines of the European Union Council (86/609/EU) and to the Animal Act (1986). All efforts were made to minimize the number of animals utilized and to reduce their suffering. Mice were sacrificed under ether anesthesia by cervical dislocation, the brain was quickly removed and corticostriatal coronal and nigral horizontal slices from SNpc (230–300 μm) were prepared from tissue blocks of mouse brain with the use of a vibratome in an ice-cold (0°C) Krebs' solution (Calabresi et al., 1995a; Mercuri et al., 1997; Goldberg et al., 2005). The composition of the control solution was (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 Glucose, 25 $NaHCO_3$. One slice was then placed into a submerged chamber (0.5–1 ml volume) mounted on an upright microscope and constantly superfused with Krebs solution (33°C, 2–3 ml/min) gassed with 95% O_2–5% CO_2. To induce combined oxygen and glucose deprivation (OGD), glucose was removed from the perfusate and substituted with sucrose to balance osmolarity. This solution was gassed with a mixture of 95% N_2–5% CO_2. Total replacement of the medium in the chamber occurred within 1 min.

Electrophysiology

Sharp microelectrodes were filled with 2 M KCl (30–60 MΩ). An Axoclamp 2 B amplifier (Axon Instruments, USA) was utilized for recordings. Dopaminergic neurons from the SNpc were distinguished from those of ventral tegmental area (VTA) according to both anatomical and electrophysiological criteria (Liss et al., 2005). By visual guidance, the tip of the recording electrode was advanced into the SNpc. VTA and SNpc were identified as the regions medial (VTA), rostral and caudal (SNpc) to the medial terminal nucleus of the accessory optic tract (Marinelli et al., 2000; Mercuri et al., 1994a,b, 1997). Traces were displayed on an oscilloscope (Gould, USA), stored both on a high-gain chart recorder (Gould RS 3400) and on pClamp9 (Axon Instruments) running on a PC for off-line analysis, performed with Clampfit. Cell input resistance was measured by applying small electrotonic hyperpolarizing current steps before and during drug application. Values given in the figures and text are mean ± SEM. Student's *t* test (unpaired) was used to compare the means.

Drugs were bath-applied by switching the solution to one containing known concentrations of drugs. Drugs solutions entered the chamber within 1 min after turning a three ways tap on. Rotenone and ouabain were from Sigma (Italy).

Results

Characterization of dopaminergic neurons

Intracellular recordings of dopaminergic neurons were performed from SNpc both of WT and *DJ-1*$^{-/-}$ mice ($n = 32$). All of these neurons fired spontaneously action potentials at a mean rate of ~2–4 Hz. Spontaneous, rhythmic firing activity of SNpc neurons in *DJ-1*$^{-/-}$ mice was similar compared to their WT littermates. Hyperpolarizing current pulses evoked a prominent sag that has been attributed to an hyperpolarization-activated cation current (I_h) (Mercuri et al., 1995). Both maximal firing rate and sag amplitude were considered as distinguishing features between VTA and SNpc dopaminergic neurons (Liss et al., 2005). As recently reported (Goldberg et al., 2005), no significant change was observed in the resting membrane potential (RMP) or input resistance of the recorded cells. The results shown in the present study were obtained from neurons with these electrophysiological characteristics.

A pharmacological test was performed on nigral neurons both from WT and *DJ-1*$^{-/-}$ mice. A hyperpolarizing response to DA is typical for DA-containing nigral neurons (Mercuri et al., 1994a,b). Indeed, DA (100 μM, 30 s) induced a reversible membrane hyperpolarization and inhibited cell firing (Figs. 1A and 3) in slices obtained from WT mice. In agreement with our previous findings, the response to DA was significantly shorter in neurons from knockout mice, compared to their WT littermates (Goldberg et al., 2005).

Response of dopaminergic neurons to OGD challenge and to mitochondrial complex I inhibition

Previous reports demonstrated that dopaminergic neurons from SNpc are reversibly hyperpolarized by a brief exposure to OGD (Mercuri et al., 1994a,b). We compared the effects of OGD in WT and *DJ-1*$^{-/-}$ mice, to test whether neurons from *DJ-1*$^{-/-}$ exhibited a different sensitivity to energy deprivation. In response to a brief OGD challenge (2 min), dopaminergic neurons from WT mice hyperpolarized and firing activity ceased (Figs. 1B, C; 15 ± 4 mV; $n = 5$). Shortly after washout, the membrane returned to control levels and firing activity resumed. In nigral neurons from *DJ-1*$^{-/-}$ mice, a similar exposure to OGD induced a membrane hyperpolarization of significantly larger amplitude (Figs. 1B, C; 30 ± 7.5 mV; $n = 6$; $P < 0.05$). In some of the recorded neurons, a biphasic hyperpolarization was observed, characterized by an initial trend to return to baseline, followed by a further shift in hyperpolarizing direction. This response has been also observed in rat dopaminergic neurons exposed to anoxia or aglycemia (Mercuri et al., 1994a; Marinelli et al., 2000). In all of the recorded cells, the hyperpolarization was followed by a small depolarization (4 ± 1.5 mV). Then, after a long washout, firing activity resumed.

The observation of a higher sensitivity to a generic energetic stress, such as OGD, prompted us to investigate whether a selective mitochondrial impairment could generate abnormal responses in DJ-1-deficient mice.

Thus, we tested the effects of the selective mitochondrial complex I inhibitor, rotenone, on dopaminergic cells. In WT mice, perfusion with rotenone (3 μM, 3 min) blocked the

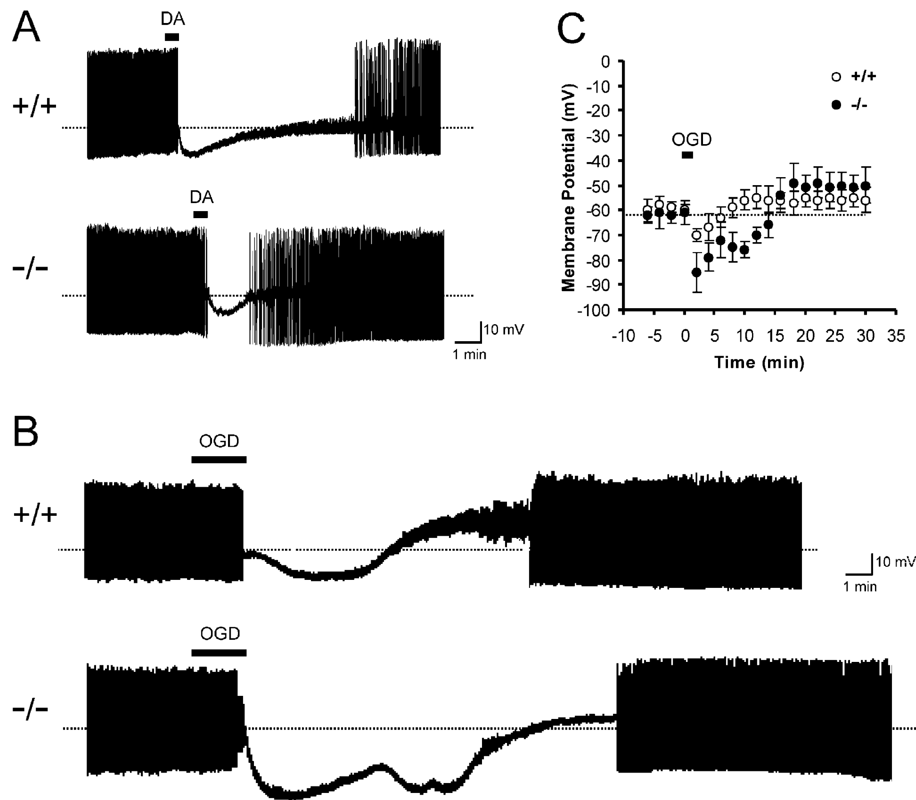

Fig. 1. Enhanced sensitivity of dopaminergic neurons from DJ-1$^{-/-}$ mice to OGD. (A) Characterization of recorded nigral neurons. In slices from WT mice (upper trace, +/+), bath-application of dopamine (DA 100 μM, 30 s) hyperpolarized the cell, and blocked firing activity (resting membrane potential, RMP = −55 mV). Upon drug washout, the cell returned to resting values and firing activity resumed. The lower trace shows a typical response of a nigral neuron to DA (100 μM, 30 s) from a DJ-1-deficient mouse (−/−). Note that the response was significantly shorter than in WT mice, and both resting potential and firing activity rapidly returned to control levels (RMP = −57 mV). (B) The upper trace shows a dopaminergic nigral neuron recorded from WT mice. A brief exposition to OGD (2 min) caused a membrane hyperpolarization and suppressed the spontaneous firing activity. Upon washout, the membrane repolarized and action potential discharge returned to control levels (RMP = −57 mV). In a nigral neuron recorded from *DJ-1*$^{-/-}$ mice (lower trace), OGD rapidly abolished firing discharge, inducing a steep, long-lasting, biphasic membrane hyperpolarization. After OGD washout, the membrane slowly returned to resting levels and to action potential discharge (RMP = −59 mV). The acquisition on chart recorder truncated action potential height. (C) The graph shows the mean time-course of nigral neurons from WT (open circles, +/+) and *DJ-1*$^{-/-}$ (filled circles, −/−) mice in response to OGD. Each data point is the mean ± SEM of 5 independent recordings.

spontaneous firing activity and elicited a membrane hyperpolarization (Fig. 2, 14 ± 6 mV; n = 4). Upon drug washout, the membrane slowly returned to baseline and then a depolarization occurred (30 ± 5.5 mV). None of the recorded neurons returned to resting levels for the whole duration of the recording session (up to 60 min). Strikingly, in SNpc neurons from *DJ-1*$^{-/-}$ mice, rotenone induced an early membrane hyperpolarization followed by a rapid and irreversible shift in depolarizing direction (13 ± 5.6 mV; $P > 0.05$, and 59 ± 6 mV; $P < 0.05$ for the hyperpolarizing and depolarizing response, respectively; n = 6). This larger membrane depolarization was irreversible in all the recorded cells from *DJ-1*$^{-/-}$ mice.

Effects of ouabain

In order to test whether a collapse of the Na^+/K^+ ATPase is involved in the enhanced neuronal sensitivity of *DJ-1*$^{-/-}$ mice, a set of experiments was performed to compare the responses both of nigral and striatal neurons from WT and DJ-1-deficient mice.

Bath application of 3 μM ouabain did not cause detectable effects in the recorded neurons from WT mice (not shown). A higher ouabain concentration (10 μM, 2 min) induced a moderate, persistent membrane depolarization and increased the firing frequency (Fig. 3A; n = 5). Raising the concentration up to 30 μM caused an early depolarizing phase coupled to an increased firing discharge, followed by hyperpolarization in all dopaminergic neurons recorded from WT mice. This hyperpolarization reached a steady-state level, and then an irreversible membrane depolarization occurred (Fig. 3A).

Intriguingly, in SNpc neurons from *DJ-1*$^{-/-}$ mice perfusion with 10 μM ouabain resulted in a dramatic response. In fact, compared to controls, at this concentration, ouabain depolarized nigral neurons, inducing an increase in firing activity rate, accompanied by a decrease in afterhyperpolarization amplitude. This effect was followed by a hyperpolarizing phase and subsequently by a steep, irreversible membrane depolarization (Fig. 3B). As application of 10 μM ouabain already provoked irreversible membrane potential changes of *DJ-1*$^{-/-}$ neurons, experiments at higher concentrations were not performed.

Striatal medium spiny neurons have been shown to tolerate concentrations of ouabain up to 10 μM, without significant effects on their resting membrane potential (Calabresi et al., 1995b). Accordingly, bath-applied ouabain (10 μM, 2 min, n = 3) was unable to determine significant membrane responses in WT mice

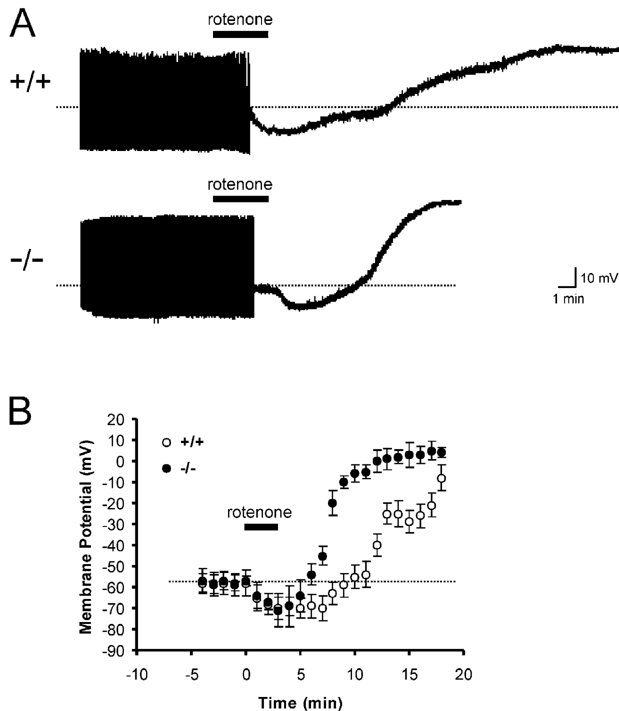

Fig. 2. Higher susceptibility of nigral neurons to rotenone. (A) Representative recordings showing the effect of rotenone on dopaminergic neurons obtained from WT mice (upper trace, RMP = −61 mV) and *DJ-1*$^{-/-}$ mice (lower trace, RMP = −62 mV). Brief bath-application of rotenone (3 μM, 3 min) hyperpolarized the cell membrane of WT neurons, and blocked the firing activity. After rotenone washout, a slow and progressive membrane depolarization was recorded. In dopaminergic neurons recorded from *DJ-1*$^{-/-}$ mice, rotenone abolished firing activity and caused a membrane hyperpolarization of shorter duration. Then, a rapid and irreversible membrane depolarization occurred. The acquisition on chart recorder truncated action potential height. (B) Time-course of the response to rotenone in dopaminergic cells recorded from WT mice (open circle, +/+) or from *DJ-1*$^{-/-}$ mice (filled circles, −/−). Each data point in the graph represents the mean ± SEM of at least 4 independent observations.

(data not shown). Similarly, in spiny neurons from *DJ-1*$^{-/-}$, ouabain (10 μM) did not significantly alter resting membrane potential. A higher ouabain concentration (30 μM, 5 min) caused a membrane depolarization coupled to a fall (n = 3) or to an increase (n = 2) in input resistance in WT mice (Fig. 4; n = 5, 22 ± 7 mV). Upon washout, the recorded cells did not return to baseline levels. Interestingly, in spiny neurons from *DJ-1*$^{-/-}$ mice, a similar ouabain application (30 μM, 5 min) generated a rapid and irreversible depolarization (Fig. 4; n = 6).

Remarkably, the range of ouabain concentrations used in this study was similar to that employed to characterize the effects of ouabain during anoxia in a variety of neuronal subtypes from slice preparations (Fujiwara et al., 1987; Mercuri et al., 1994a,b; Calabresi et al., 1995b).

Discussion

The present work provides the first electrophysiological evidence for an enhanced vulnerability of SNpc dopaminergic neurons from *DJ-1*$^{-/-}$ mice to energy metabolism impairment. Notably, dopaminergic nigral neurons exhibited an increased sensitivity to blockade of the Na^+/K^+ ATPase compared to other neuronal subtypes, such as striatal medium spiny neurons.

Compelling evidence obtained from brain samples of PD patients, as well as data derived from studies conducted on reliable animal models have demonstrated the existence of an altered metabolic state, intimately linked to an abnormal functioning of Complex I of the mitochondrial respiratory chain (Schapira et al., 1990; Betarbet et al., 2000, 2005; Palacino et al., 2004; Shen and Cookson, 2004; Panov et al., 2005). Generation of different lines of mice bearing mutations for distinct forms of recessive parkinsonism has provided a means to confirm the previous assumptions, or even to formulate new hypotheses on this issue.

Mutations in the DJ-1 gene are recessively inherited and include a large truncation deletion and a missense mutation that

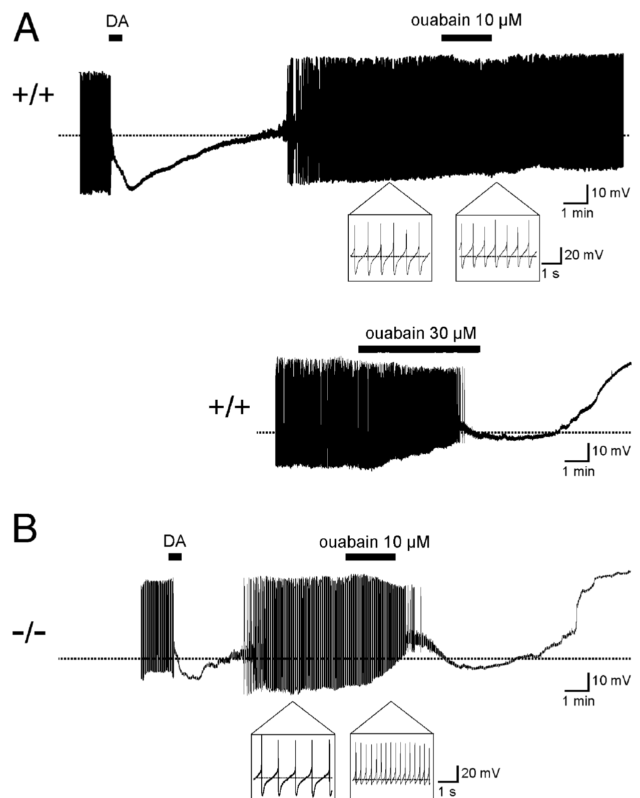

Fig. 3. Sensitivity of dopaminergic neurons to Na^+/K^+ ATPase blockade. (A) In slices from WT mice (upper trace, RMP = −52 mV), brief bath-application of dopamine (DA, 100 μM, 30 s) hyperpolarized the membrane, and suppressed the action potential discharge. After DA washout, the membrane slowly recovered and action potential discharge returned to control levels. In the same neuron, bath-applied ouabain (10 μM, 2 min) slightly depolarized the cell membrane and increased the firing activity rate (inset). Higher ouabain concentration depolarized nigral neurons from WT mice and increased firing activity (30 μM, 5 min, lower trace, RMP = −49 mV). This effect was followed by a hyperpolarizing phase and subsequently by an irreversible membrane depolarization. (B) Compared to controls, DA caused a membrane hyperpolarization of smaller amplitude and shorter duration in nigral neurons from *DJ-1*$^{-/-}$ mice (RMP = −58 mV). Bath-application of ouabain (10 μM, 2 min) caused an early increase in firing activity, coupled to a membrane depolarization. After firing cessation, a membrane hyperpolarization was recorded. This membrane hyperpolarization was then followed by an abrupt and irreversible depolarization. The acquisition on PC at 1 kHz truncated action potential height. The insets show spontaneous firing activity at higher time resolution (see calibration bar).

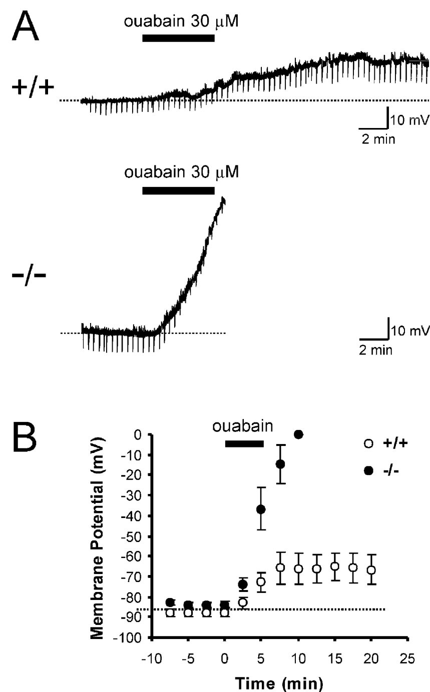

Fig. 4. Response of striatal medium spiny neurons to Na^+/K^+ ATPase blockade. (A) In a medium spiny neuron from WT mice (upper trace, RMP = −89 mV), bath-application of ouabain (30 μM, 5 min) induced a slow and progressive membrane depolarization, coupled to a small increase in input resistance. In neurons from *DJ-1*$^{-/-}$ mice (lower trace, RMP: −83 mV) ouabain caused a much steeper depolarization. Downward deflections represent hyperpolarizing electrotonic potentials evoked by current pulses to monitor input resistance (+/+: 150 pA; −/−: 200 pA, 40 ms). (B) Time-course of the response to rotenone in striatal medium spiny cells recorded from WT mice (open circle, +/+) and from *DJ-1*$^{-/-}$ mice (filled circles, −/−). Each data point in the graph represents the mean ± SEM of at least 5 independent observations.

destabilizes the protein, suggesting that these mutations cause parkinsonism via a loss-of-function mechanism. DJ-1-deficient mice display significant motor abnormalities and functional deficits, consistent with an altered nigrostriatal dopaminergic function, though the number and morphology of dopaminergic neurons are normal (Goldberg et al., 2005). DJ-1 immunoreactivity is present throughout the brain. Both SNpc and pars reticulata are stained for DJ-1, as well as striatal medium spiny neurons (Zhang et al., 2005). Several lines of evidence implicate DJ-1 as an antioxidant with neuroprotective activity in physiological conditions (Yokota et al., 2003; Canet-Aviles et al., 2004; Taira et al., 2004). Accordingly, *DJ-1*$^{-/-}$ mice showed increased sensitivity to oxidative stress produced by the complex I inhibitor MPTP, an effect mitigated by restoration of DJ-1 expression (Kim et al., 2005).

In our study, we found an enhanced sensitivity both to OGD and rotenone in neurons from *DJ-1*$^{-/-}$ mice. Previous reports have demonstrated that either oxygen or glucose deprivation in dopamine-containing neurons of the SNpc results in a membrane hyperpolarization mediated by an increase in potassium conductances. The pharmacological characterization of the conductance, demonstrated that ATP-sensitive potassium (K-ATP) channels play a major role in such a response (Guatteo et al., 1998). Similarly, inhibition of mitochondrial metabolism by rotenone has been shown to elicit a membrane hyperpolarization in dopaminergic neurons in the SNpc, and, accordingly, this current was primarily mediated with sulphonylureas-sensitive potassium currents (Roper and Ashcroft, 1995).

Our recordings from nigral neurons show that the hyperpolarizing response to OGD was larger both in amplitude and duration in DJ-1-deficient mice, suggesting a robust recruitment of potassium conductances. K-ATP channels are, at least in part, the preferred candidate for such a response. These channels are activated by a fall in ATP content, which occurs in conditions of energetic stress. The degree of K-ATP channel activation depends upon the availability of ATP and reflects activity-dependent fluctuations in the ATP concentration within submembrane domains. Furthermore, an ischemia-induced increase of intracellular sodium and calcium is an additional factor that activates potassium currents (Seutin et al., 1996). Both these ions have been shown to generate a potassium conductance in nigral neurons (Mercuri et al., 1994a,b). Indeed, increased intracellular sodium levels were able to induce a potassium current that was selectively blocked by sulphonylureas (Seutin et al., 1996). Accordingly, the anoxia-induced hyperpolarization was reduced significantly in a low-sodium- or in a low-calcium-containing solution (Mercuri et al., 1994a,b).

Rotenone has been shown to produce a hyperpolarization and to inhibit electrical activity in acutely dissociated rat SNpc neurons, an effect fully prevented by the K-ATP channel blockers tolbutamide and glibenclamide (Roper and Ashcroft, 1995). Although both OGD and rotenone are indeed able to hyperpolarize nigral neurons by opening K-ATP channels, in our recordings such responses differed significantly. Indeed, the amplitude of the hyperpolarizing phase did not increase in DJ-1-deficient mice. Conversely, the duration of the membrane hyperpolarization was significantly shorter, ultimately resulting in an irreversible, rapid depolarizing shift. The evidence of a more rapid and irreversible depolarization in nigral neurons from *DJ-1*$^{-/-}$ mice suggests an early increase in cation conductances, observed neither during OGD nor during rotenone application in controls and indicates a higher vulnerability of dopaminergic neurons from *DJ-1*$^{-/-}$ mice.

In principle, either the response to OGD or the effect of rotenone might be attributed to a self-protective response to a metabolic stress. Indeed, maintaining the membrane potential far from firing threshold would considerably reduce oxygen consumption. This is consistent with the observation that dopaminergic neurons from SNpc tolerate a protracted period of OGD, remaining in a hyperpolarized state for several minutes (Mercuri et al., 1994a,b). Furthermore, a neuroprotective role of K-ATP channels has been documented in a variety of experimental models of brain damage (Hernandez-Sanchez et al., 2001; Yamada et al., 2001; Ballanyi, 2004). However, this view has been recently challenged by the observation that K-ATP channels are involved in promoting selective neurodegeneration of nigral neurons. Indeed, genetic ablation of these channels resulted in a selective rescue of SNpc neurons in two different mouse models of parkinsonism (Liss et al., 2005).

It is conceivable that the different duration of the hyperpolarizing phase preceding the depolarizing shift following OGD or rotenone, might be attributed to the specificity and extent of metabolic insult. OGD would favor the hyperpolarizing response by opening K-ATP channels, whereas rotenone would rapidly disrupt ion homeostasis, causing the opening both of potassium channels but also of cation conductances. This interpretation fits

with the hypothesis that K-ATP channel opening might be beneficial during short-term metabolic demands, whereas they could promote neuronal death in course of long-term neurodegenerative processes (Liss et al., 2005). It remains to be established how metabolic insults could trigger distinct mechanisms leading to opposite outcomes.

The Na^+/K^+ ATPase pump is central to neuronal survival. A large amount of ATP supply is devoted to maintenance of ion homeostasis in neurons. In nigral neurons exposed to anoxia, the Na^+/K^+ ATPase pump has been shown to play a key role in reestablishing ion homeostasis upon reoxygenation. As previously reported, the biphasic hyperpolarizing response to OGD in nigral neurons is secondary to the activation of the Na^+/K^+ pump (Mercuri et al., 1994a). Accordingly, the OGD-induced response was significantly worsened by ouabain (Mercuri et al., 1994a,b). Thus, during a profound energy depletion, the loss of intracellular ATP would not allow the Na^+/K^+ ATPase pump to prevent the ensuing depolarization.

Blockade of the pump activity by ouabain in nigral neurons from DJ-1-deficient mice was able to induce a significant membrane depolarization, that was in turn able to trigger a hyperpolarization. Such response might be mediated by the opening of sodium- or calcium-dependent potassium channels, and could be interpreted as an attempt to restore ion balance. The subsequent irreversible membrane depolarization is likely the net result of a complete breakdown of cellular homeostasis.

These results are not surprising considering the central role of the Na^+/K^+ ATPase pump in maintaining resting membrane potential and influencing the pattern of firing activity of nigral neurons (Shen and Johnson, 1998). In physiological conditions, dopamine neurons generate high density pump-dependent currents, compared to other neuronal subtypes. Moreover, Na^+/K^+ ATPase pump is capable of increasing its activity in response to intracellular sodium loading. On the other hand, in conditions of energy depletion, this capacity to generate high-density sodium currents predisposes nigral neurons to sodium accumulation, which could, in turn, favor calcium influx. Another central issue that deserves consideration is the sensitivity of nigral cells to ouabain. First, dopaminergic neurons from WT mice tolerated ouabain concentrations up to 30 μM, whereas neurons from DJ-1-deficient mice underwent irreversible electrical changes with a much lower dose. Secondly, the sensitivity of nigral neurons to ouabain compared to striatal neurons was remarkably different. Striatal medium spiny neurons are among the most susceptible neuronal subtypes. This was further supported by the observation that also in striatal neurons from DJ-$1^{-/-}$ mice, OGD caused a twofold increase in the membrane depolarization recorded in WT mice (Pisani et al., unpublished data). Nonetheless, in our recordings from *DJ-1*$^{-/-}$ mice, the concentration of ouabain required to elicit a response in striatal cells was three times higher than the dose capable of inducing irreversible changes in nigral neurons.

Both in situ hybridization study and pharmacological analysis demonstrated that SNpc neurons express the α3 subunit (Hieber et al., 1991; Shen and Johnson, 1998). This appears of particular interest also in light of the recent evidence that Rapid-Onset Dystonia Parkinsonism (RDP), an autosomal-dominant movement disorder characterized by sudden onset of dystonia and parkinsonism, is associated to mutations in the gene encoding for the Na^+/K^+ ATPase α3 subunit (de Carvalho Aguiar et al., 2004).

Though the downstream events linking mutations in DJ-1 with Na^+/K^+ ATPase activity were not addressed in the present work, we can speculate that the lack of DJ-1 could impair the pump activity. Given that Na^+/K^+ ATPase regulates basal firing activity of SNpc neurons, and that intrinsic properties are not different from WT neurons, it is likely that only under metabolic constraint, the pump activity is altered. Indeed, the fall in ATP would increase intracellular sodium levels that could impair, by means of exchange systems, cytosolic and mitochondrial calcium dynamics, triggering a cascade of events leading to cell death. It will be relevant to address possible changes in the expression of distinct α subunit isoforms as well as their phosphorylation state in DJ-1-deficient mice.

Future work is required to clarify the molecular determinants linking DJ-1 to neuronal vulnerability.

Acknowledgments

This work was supported by grants from: MIUR (FIRB), Ministero Salute (Prog. Finalizzato) to A.P. and P.C., and from Dystonia Medical Research Foundation to A.P.

We thank Mr. M. Tolu for his excellent technical support.

References

Ballanyi, K., 2004. Protective role of neuronal KATP channels in brain hypoxia. J. Exp. Biol. 207, 3201–3212.

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J.T., 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. 3, 1301–1306.

Betarbet, R., Sherer, T.B., Greenamyre, J.T., 2005. Ubiquitin–proteasome system and Parkinson's diseases. Exp. Neurol. 191 (Suppl. 1), S17–S27.

Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J.W., Vanacore, N., van Swieten, J.C., Brice, A., Meco, G., van Duijn, C.M., Oostra, B.A., Heutink, P., 2003. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299, 256–259.

Calabresi, P., Pisani, A., Mercuri, N.B., Bernardi, G., 1995a. On the mechanisms underlying hypoxia-induced membrane depolarization in striatal neurons. Brain 118 (Pt. 4), 1027–1038.

Calabresi, P., De Murtas, M., Pisani, A., Stefani, A., Sancesario, G., Mercuri, N.B., Bernardi, G., 1995b. Vulnerability of medium spiny striatal neurons to glutamate: role of Na+/K+ ATPase. Eur. J. Neurosci. 7, 1674–1683.

Canet-Aviles, R.M., Wilson, M.A., Miller, D.W., Ahmad, R., McLendon, C., Bandyopadhyay, S., Baptista, M.J., Ringe, D., Petsko, G.A., Cookson, M.R., 2004. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. Proc. Natl. Acad. Sci. U. S. A. 101, 9103–9108.

Dawson, T.M., Dawson, V.L., 2003. Molecular pathways of neurodegeneration in Parkinson's disease. Science 302, 819–822.

de Carvalho Aguiar, P., Sweadner, K.J., Penniston, J.T., Zaremba, J., Liu, L., Caton, M., Linazasoro, G., Borg, M., Tijssen, M.A., Bressman, S.B., Dobyns, W.B., Brashear, A., Ozelius, L.J., 2004. Mutations in the Na+/K+-ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. Neuron 43, 169–175.

Fujiwara, N., Higashi, H., Shimoji, K., Yoshimura, M., 1987. Effects of hypoxia on rat hippocampal neurones in vitro. J. Physiol. 384, 131–151.

Goldberg, M.S., Pisani, A., Haburcak, M., Vortherms, T.A., Kitada, T., Costa, C., Tong, Y., Martella, G., Tscherter, A., Martins, A., Bernardi, G., Roth, B.L., Pothos, E.N., Calabresi, P., Shen, J., 2005. Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. Neuron 45, 489–496.

Guatteo, E., Federici, M., Siniscalchi, A., Knopfel, T., Mercuri, N.B., Bernardi, G., 1998. Whole cell patch-clamp recordings of rat midbrain dopaminergic neurons isolate a sulphonylurea- and ATP-sensitive component of potassium currents activated by hypoxia. J. Neurophysiol. 79, 1239–1245.

Hernandez-Sanchez, C., Basile, A.S., Fedorova, I., Arima, H., Stannard, B., Fernandez, A.M., Ito, Y., LeRoith, D., 2001. Mice transgenically overexpressing sulfonylurea receptor 1 in forebrain resist seizure induction and excitotoxic neuron death. Proc. Natl. Acad. Sci. U. S. A. 98, 3549–3554.

Hieber, V., Siegel, G.J., Fink, D.J., Beaty, M.W., Mata, M., 1991. Differential distribution of (Na, K)-ATPase alpha isoforms in the central nervous system. Cell. Mol. Neurobiol. 11, 253–262.

Kim, R.H., Smith, P.D., Aleyasin, H., Hayley, S., Mount, M.P., Pownall, S., Wakeham, A., You-Ten, A.J., Kalia, S.K., Horne, P., Westaway, D., Lozano, A.M., Anisman, H., Park, D.S., Mak, T.W., 2005. Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. Proc. Natl. Acad. Sci. U. S. A. 102, 5215–5220.

Liss, B., Haeckel, O., Wildmann, J., Miki, T., Seino, S., Roeper, J., 2005. K-ATP channels promote the differential degeneration of dopaminergic midbrain neurons. Nat. Neurosci. 8, 1742–1751.

Marinelli, S., Bernardi, G., Giacomini, P., Mercuri, N.B., 2000. Pharmacological identification of the K(+) currents mediating the hypoglycemic hyperpolarization of rat midbrain dopaminergic neurones. Neuropharmacology 39, 1021–1028.

Martinat, C., Shendelman, S., Jonason, A., Leete, T., Beal, M.F., Yang, L., Floss, T., Abeliovich, A., 2004. Sensitivity to oxidative stress in DJ-1-deficient dopamine neurons: an ES-derived cell model of primary parkinsonism. PLoS Biol. 2, e327.

Mercuri, N.B., Bonci, A., Calabresi, P., Stratta, F., Bernardi, G., 1994a. Responses of rat mesencephalic dopaminergic neurons to a prolonged period of oxygen deprivation. Neuroscience 63, 757–764.

Mercuri, N.B., Bonci, A., Johnson, S.W., Stratta, F., Calabresi, P., Bernardi, G., 1994b. Effects of anoxia on rat midbrain dopamine neurons. J. Neurophysiol. 71, 1165–1173.

Mercuri, N.B., Bonci, A., Calabresi, P., Stefani, A., Bernardi, G., 1995. Properties of the hyperpolarization-activated cation current Ih in rat midbrain dopaminergic neurons. Eur. J. Neurosci. 7, 462–469.

Mercuri, N.B., Saiardi, A., Bonci, A., Picetti, R., Calabresi, P., Bernardi, G., Borrelli, E., 1997. Loss of autoreceptor function in dopaminergic neurons from dopamine D2 receptor deficient mice. Neuroscience 79, 323–327.

Palacino, J.J., Sagi, D., Goldberg, M.S., Krauss, S., Motz, C., Wacker, M., Klose, J., Shen, J., 2004. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J. Biol. Chem. 279, 18614–18622.

Panov, A., Dikalov, S., Shalbuyeva, N., Taylor, G., Sherer, T., Greenamyre, J.T., 2005. Rotenone model of Parkinson disease: multiple brain mitochondria dysfunctions after short term systemic rotenone intoxication. J. Biol. Chem. 280, 42026–42035.

Ramsay, R.R., Salach, J.I., Dadgar, J., Singer, T.P., 1986. Inhibition of mitochondrial NADH dehydrogenase by pyridine derivatives and its possible relation to experimental and idiopathic parkinsonism. Biochem. Biophys. Res. Commun. 135, 269–275.

Roper, J., Ashcroft, F.M., 1995. Metabolic inhibition and low internal ATP activate K-ATP channels in rat dopaminergic substantia nigra neurones. Pflugers Arch. 430, 44–54.

Schapira, A.H., Cooper, J.M., Dexter, D., Clark, J.B., Jenner, P., Marsden, C.D., 1990. Mitochondrial complex I deficiency in Parkinson's disease. J. Neurochem. 54, 823–827.

Seutin, V., Shen, K.Z., North, R.A., Johnson, S.W., 1996. Sulfonylurea-sensitive potassium current evoked by sodium-loading in rat midbrain dopamine neurons. Neuroscience 71, 709–719.

Shen, J., Cookson, M.R., 2004. Mitochondria and dopamine: new insights into recessive parkinsonism. Neuron 43, 301–304.

Shen, K.Z., Johnson, S.W., 1998. Sodium pump evokes high density pump currents in rat midbrain dopamine neurons. J. Physiol. 512, 449–457.

Sherer, T.B., Betarbet, R., Testa, C.M., Seo, B.B., Richardson, J.R., Kim, J.H., Miller, G.W., Yagi, T., Matsuno-Yagi, A., Greenamyre, J.T., 2003. Mechanism of toxicity in rotenone models of Parkinson's disease. J. Neurosci. 23, 10756–10764.

Taira, T., Saito, Y., Niki, T., Iguchi-Ariga, S.M., Takahashi, K., Ariga, H., 2004. DJ-1 has a role in antioxidative stress to prevent cell death. EMBO Rep. 5, 213–218.

Ved, R., Saha, S., Westlund, B., Perier, C., Burnam, L., Sluder, A., Hoener, M., Rodrigues, C.M., Alfonso, A., Steer, C., Liu, L., Przedborski, S., Wolozin, B., 2005. Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of {alpha}-synuclein, parkin, and DJ-1 in *Caenorhabditis elegans*. J. Biol. Chem. 280, 42655–42668.

Yamada, K., Ji, J.J., Yuan, H., Miki, T., Sato, S., Horimoto, N., Shimizu, T., Seino, S., Inagaki, N., 2001. Protective role of ATP-sensitive potassium channels in hypoxia-induced generalized seizure. Science 292, 1543–1546.

Yokota, T., Sugawara, K., Ito, K., Takahashi, R., Ariga, H., Mizusawa, H., 2003. Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. Biochem. Biophys. Res. Commun. 312, 1342–1348.

Zhang, L., Shimoji, M., Thomas, B., Moore, D.J., Yu, S.W., Marupudi, N.I., Torp, R., Torgner, I.A., Ottersen, O.P., Dawson, T.M., Dawson, V.L., 2005. Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. Hum. Mol. Genet. 14, 2063–2073.