Uncoupling protein-2 promotes nigrostriatal dopamine neuronal function

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Abstract

Uncoupling protein 2 (UCP2) is known to promote neuroprotection in many forms of neurological pathologies including Parkinson’s disease. Here, we examined the hypothesis that UCP2 also mediates aspects of normal nigrostriatal dopamine (DA) function. Mice lacking UCP2 exhibited reduced dopamine turnover in the striatum as measured by the 3,4-dihydroxyphenylacetic acid/dopamine (DOPAC/DA) ratio, reduced tyrosine hydroxylase immunoreactivity (TH IR) in the substantia nigra pars compacta (SNc) and reticulata, striatum and nucleus accumbens. UCP2-knockout (KO) mice also had reduced dopamine transporter immunoreactivity (DAT IR) in the SNc but not other brain regions examined. In order to determine if these biochemical deficits are transcribed into behavioural deficits, we examined locomotor function in UCP2-KO mice compared to wild-type (WT) controls. UCP2-KO mice exhibited significantly reduced total movement distance, movement velocity and increased rest time compared to wild-type controls. These results suggest that UCP2 is an important mitochondrial protein that helps to maintain normal nigrostriatal dopamine neuronal function and a reduction in UCP2 levels may predispose individuals to environmental causes of Parkinson’s disease.

Introduction

Neuronal mitochondria are essential organelles as they provide neurons with the energy required to mediate diverse functions such as synaptic plasticity (Li et al., 2004), neuronal survival (Nicholls & Budd, 2000), neurogenesis and proliferation (Limoli et al., 2004), calcium regulation (Leo et al., 2005), synaptic neurotransmission (Hollenbeck, 2005) and synaptic neurotransmission (Richter, 1988; Fuxe et al., 2005; Rivera et al., 2006). Thus, it is not surprising that mitochondrial dysfunction lies at the heart of many neurological pathologies and the ageing process (Beal, 2005). In order to serve their essential role in energy generation, mitochondria rely on a host of both nuclear and mitochondrial genes. The recently identified mitochondrial protein, uncoupling protein 2 (UCP2), encoded from nuclear DNA, has received a great deal of research attention in the CNS due to its potentially important role in promoting neuronal function and prevent neurological disease (Diano et al., 2003; Mattiasson et al., 2003; Sullivan et al., 2003; Andrews et al., 2005a; Andrews et al., 2005b; Conti et al., 2005). Activation of UCP2 allows protons to enter the mitochondrial matrix without entering the ATP synthase and as such uncouples mitochondrial respiration from ATP production. This uncoupling activity reduces mitochondrial reactive oxygen species (ROS) production and promotes ATP production through increased mitochondrial biogenesis (Diano et al., 2003; Andrews et al., 2005a). Through these mechanisms, UCP2 may contribute to important neuronal functions such as synaptic plasticity and neurotransmission.

Indeed, perturbed striatal dopamine (DA) neurotransmission and nigral DA loss cell is a hallmark of Parkinson’s disease (Dauer & Przedborski, 2003). In particular, UCP2 prevents DA cell loss after 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) treatment indicating UCP2 helps to restrict the pathogenesis of Parkinson’s disease (Andrews et al., 2005b; Conti et al., 2005). Although striatal DA levels are not decreased in unchallenged UCP2 knockout (KO) mice, these mice are more susceptible to striatal DA loss after MPTP injection (Andrews et al., 2005b). This suggests that UCP2 is important to maintain normal striatal DA neurotransmission. Further, UCP2-KO mice possess fewer mitochondria in nigral DA neurons compared to wild-type (WT) controls (Andrews et al., 2005b), highlighting a potential for nigrostriatal DA dysfunction. Our current understanding suggests that the pathogenesis of Parkinson’s disease is 90% sporadic and 10% familial (Dauer & Przedborski, 2003). Thus, genetic/alleric variation of UCP2 in humans may underlie an inherent predisposition to nigrostriatal DAergic dysfunction and lower the threshold of cell loss after exposure to deleterious environmental conditions. The purpose of these studies was to establish aspects of normal nigrostriatal DA function in UCP2-KO mice compared to wild-type controls, in order to determine if UCP2 affects this predisposition.

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Materials and methods

All male mice used in the following experiments were aged between 12 and 15 weeks at the time of killing. All procedures were approved by the Institutional Animal Care and Use Committee of Yale University. All mice were maintained under standard laboratory conditions with water and food freely available; lights were maintained on a 12-h light : 12-h dark cycle. UCP2 KO and human UCP2 expressing transgenic lines were generated as described previously (Andrews et al., 2005b).

Striatal dopamine turnover

At the time of killing each dorsal striatal sample was dissected, transferred to 400 μL ice-cold perchloric acid, sonicated and centrifuged for 10 min at 10 000 g. The pellet was saved for protein determination according to the spectrophotometric method of Lowry. 3,4-dihydroxyphenylacetic acid (DOPAC) and DA were assayed as previously reported (Andrews et al., 2005b).

Immunohistochemistry

Animals were killed under ether anaesthesia and perfused transcardially with 0.1 M phosphate-buffered saline, pH 7.4 (PBS) followed by 4% paraformaldehyde (w/v) in 0.1 M phosphate buffer, pH 7.4 (PB). Tyrosine hydroxylase (TH) was detected using a mouse monoclonal antibody (DiaSorin, Stillwater, MN, USA) diluted at 1 : 10 000 in PBS containing 0.2% Triton X-100 (PBS-TX) and 0.1% sodium azide. Dopamine transporter (DAT) was detected using a rabbit polyclonal antibody (Chemicon International, Temecula, CA, USA) diluted at 1 : 5000. Details of the immunohistochemical processing procedure are published elsewhere (Rivera et al., 2006).

Semi-quantitative analysis of optical density (OD) of TH immunoreactivity (IR) and DAT IR was performed using the imaging analysing system NIH image (http://rsb.info.nih.gov/nih-image/). The intensity of TH and DAT IR was expressed as the OD and the value was corrected with the OD from an immunonegative area (Agnati et al., 1984). Two to six sample areas (200 μm²) from six sections of each mouse (WT n = 6; KO, n = 6) were measured.

Behavioural studies

Spontaneous locomotor activity was examined in the same UCP2-KO (n = 5) and WT (n = 5) mice at 2 and 5 months of age. Activity measures were assessed in a 16 inch, square plexiglass arena with photobeams and sensors spaced at intervals sufficient to provide a spatial resolution of animal movement of 1.27 cm in the X-Y dimension (Coulbourn Instruments TruScan, Bilaney Consultants Ltd, Sevenoaks, UK). The arena sensors were computer coupled and sampled every 100 ms using Coulbourn Instruments Truscan 99 software. Activity measures were recorded over a 15-min period and statistical comparisons between KO and WT groups on measures of total distance traveled, average movement velocity and the amount of rest time (time spent not moving) spent during a 15-min session were made using Student’s t-tests.

Potential alterations in gross motor coordination and motor learning were assessed using an accelerating Rota Rod. Five-month-old UCP2-KO and WT mice were placed on a rotating cylinder (AccuScan Instruments, Columbus, OH, USA) and the amount of time they were able to walk on the cylinder without falling was recorded for each of six sessions spaced 5 min apart on two consecutive days. The rotation speed of the cylinder accelerated from 0 to 40 r.p.m. over a 200-s period and the speed remained constant upon reaching 200 r.p.m.

Results

UCP2-KO mice exhibit reduced striatal DA turnover

The DOPAC/DA ratio was used to estimate DA turnover as it measures the coupled neurochemical relationship between the synthesis of DA and the levels of its primary metabolite, DOPAC, which is considered to be proportional to the amount of released DA in terminal axon fields. We found that UCP2-KO mice had significantly reduced DA turnover in comparison to WT controls (Fig. 1, DOPAC/DA ratio 0.065 ± 0.001 vs. 0.099 ± 0.008, respectively, P < 0.002, Student’s t-test). No differences in striatal DA turnover in UCP2 overexpressing transgenic mice were found (data not shown).

UCP2-KO mice exhibit a diminution of TH IR

We analysed TH and DAT IR in the main nigrostriatal and mesolimbic DAergic projection fields in both UCP2-KO and UCP2 overexpressing transgenic mice. TH and DAT IR were also measured in the DAergic nerve cell groups of substantia nigra pars compacta (SNc) and reticulata (SNr). We observed a significant decrease of TH IR in the caudate putamen by 13%, nucleus accumbens by 33%, SNc by 31% and SNr by 39% of UCP2-KO mice as compared with the WT (Fig. 2A–E). No differences were observed in the same areas in the UCP2 overexpressing transgenic mice (data not shown). DAT IR was significantly reduced by 27% in the SNc but no other statistically significant differences were observed in the caudate putamen, nucleus accumbens or SNr (Fig. 1F).

UCP2-KO mice display locomotor impairments

In order to determine if the observed decline in striatal DA turnover and TH IR results in functional deficits, we examined behavioural...
parameters in UCP2-KO compared to WT mice. Comparison of open field activity between UCP2-KO and WT littermates revealed significant differences on all three measures sampled. At both 2 and 5 months of age the UCP2-KO mice had significantly reduced total movement, reduced movement velocity and spent significantly more time resting (Table 1).

In contrast to the significant differences described above, UCP2-KO mice showed no significant gross motor abnormalities when compared to WT littermates on the accelerating Rota Rod task at 5 months of age. Both groups also showed session to session increases in the time spent on the cylinder indicating no impairment of simple motor learning for this task.

Discussion

In this study, we examined biochemical and behavioural indices of nigrostriatal DAergic function in untreated UCP2-KO mice compared to WT controls. The purpose was to establish whether UCP2 plays an important role in maintaining normal DAergic cell function. We discovered that UCP2-KO mice have reduced dorsal striatal DA turnover, reduced striatal and nigral TH immunoreactivity, and reduced SNc DAT immunoreactivity. These deficits culminate in reduced indices of locomotor activity and thereby further implicate UCP2 in preventing the pathogenesis of Parkinson’s disease.

The loss of striatal DA as seen after nigrostriatal DA neurodegeneration leads to rigidity, tremor at rest, slowness or absence of voluntary movement, postural instability and freezing (Dauer & Przedborski, 2003), broadly similar with the observed locomotor deficits such as decreased movement velocity, total movement distance and increased rest time in this study. In Parkinson’s disease, physical motor symptoms supposedly only manifest once striatal DA concentration and nigral DA cell number reaches 20% and 40%, respectively, of original levels. Previously we reported UCP2-KO mice have similar striatal DA and nigral cell number to WT mice in untreated conditions (Andrews et al., 2005b), leading to the question; why do UCP2-KO exhibit locomotor deficits? The answer probably relates to a functional rather than an anatomical loss of DA transmission. Rotenone, a mitochondrial complex I inhibitor that recapitulates features of Parkinson’s disease (Betarbet et al., 2000), causes parkinsonian motor deficits but produces only minimal damage to the nigrostriatal DA system (Hoglinger et al., 2003; Fleming et al., 2004). In an attempt to reconcile these discrepancies, Bao et al. (2005) showed that partial mitochondrial inhibition by rotenone caused a functional suppression of DA release via H2O2 and subsequent activation of ATP-sensitive potassium channels (KATP channels; Avshalumov et al., 2005) leading to hyperpolarization without DA or ATP depletion. While we previously observed no difference in striatal DA in UCP2-KO mice...
compared to WT mice (Andrews et al., 2005b), in this study we witnessed a decrease in DA turnover as estimated by the DOPAC/DA ratio. Thus, the absence of UCP2 affects DA release, but not total DA content in the striatum, which in turn may underlie the observed locomotor deficits. Interestingly, H$_2$O$_2$ is responsible for mitochondrial inhibition that leads to suppression of DA release but not depletion after rotenone exposure (Bao et al., 2005) and the primary function of UCP2 is to limit ROS, including H$_2$O$_2$. Moreover, the ability of UCP2 to buffer ROS in nigral TH cell in vivo provides neuroprotection against MPTP (Andrews et al., 2005b). The same mechanisms are proposed to occur in the remaining DAergic terminals expressing UCP2 after a partial 6-OHDA lesion of DAergic nigrostrial and mesostriatal pathways (Rivera et al., 2006). UCP2 cannot limit ROS production in KO mice, and as such ROS negatively affects DA release as measured by the decrease in dorsal striatal DA turnover. This collectively culminates in motor deficits similar to those witnessed in Parkinson’s disease.

We observed decreased TH IR in SNC and striatum suggesting UCP2 is important in TH production. This is most likely because UCP2 mediates mitochondrial biogenesis and consequently increases ATP in neuronal tissue (Diano et al., 2003; Andrews et al., 2005a). Further, a reduction in mitochondria and overall cell energy production due to diminished ATP levels in UCP2-KO mice compromises the ability to release DA at the nerve terminal via activation of K$_{ATP}$ channels. Moreover, the activation of K$_{ATP}$ channels reduces the firing rate of DA cells in the SNC, further contributing to the reduction of striatal DA release (Liss & Roeppe, 2001) and to an unexpected promotion of degeneration of the nigrostrial DA system in chronic disease (Liss et al., 2005). Indeed, mitochondrial number is decreased in TH cells of the SNC (Andrews et al., 2005b) and VTA (Z. B. Andrews and T. L. Horvath unpublished observations), supporting the idea that reduced ATP levels in UCP2-KO mice compromised the cell’s ability to produce TH protein. TH is the rate limiting step in DA synthesis and despite reduced TH IR, striatal DA is not different between UCP2-KO and WT mice (Andrews et al., 2005b). We suggest that although TH IR is reduced, there are sufficient levels to maintain normal striatal DA concentrations. However, after toxic insults mitochondrial dysfunction in the UCP2-KO mice render these neurons more vulnerable to cell death and the reduced TH IR contributes to the further reduction of DA transmission and degeneration in the nigrostrial dopaminergic system. Indeed, MPTP administration produces greater nigral TH cell loss and striatal dopamine depletion in UCP2-KO mice compared to WT controls (Andrews et al., 2005b).

We also observed a significant decrease in DAT IR in only the SNCs, while the reason for this is unknown we suggest a decrease in TH and DA at the cell body results in a down-regulation in DAT, as MPTP is known to reduce DA and TH and a concurrent decrease in DAT binding (D’Aoust et al., 2004; Jourdain et al., 2005). Moreover, the selective loss of DAT in the SNCs may be the result of greater ROS production and decreased ATP in the cell body due a larger population of mitochondria in the cell body.

In conclusion, UCP2-KO mice exhibited deficits in indices of nigrostrial DA function including reduced striatal DA turnover, reduced striatal and substantia nigral TH IR and reduced nigral DAT IR. Together, these deficits led to locomotor impairments similar to those in Parkinson’s disease. The present results support the growing body of literature that suggests neuronal UCPS are neuroprotective (Andrews et al., 2005a). More specifically, UCP2 is an important mitochondrial protein that helps to maintain normal nigrostrial DA neuronal function and a reduction in UCP2 levels may predispose individuals to environmental causes of Parkinson’s disease.

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Abbreviations

DA, dopamine; DAT, dopamine transporter; DOPAC, IR, immunoreactivity; KO, knock out; 3,4-dihydroxyphenylacetic acid; MPTP, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine; OD, optical density; ROS, reactive oxygen species; SNC, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; TH, tyrosine hydroxylase; UCP2, uncoupling protein 2; WT, wild-type.

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