Impaired hepatic function and central dopaminergic denervation in a rodent model of Parkinson's disease: A self-perpetuating crosstalk?

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A B S T R A C T

In Parkinson’s disease (PD), aside from the central lesion, involvement of visceral organs has been proposed as part of the complex clinical picture of the disease. The issue is still poorly understood and relatively unexplored. In this study we used a classic rodent model of nigrostriatal degeneration, induced by the intrastriatal injection of 6-hydroxydopamine (6-OHDA), to investigate whether and how a PD-like central dopaminergic denervation may influence hepatic functions. Rats received an intrastriatal injection of 6-OHDA or saline (sham), and blood, cerebrospinal fluid, liver and brain samples were obtained for up to 8 weeks after surgery. Specimens were analyzed for changes in cytokine and thyroid hormone levels, as well as liver mitochondrial alterations. Hepatic mitochondria isolated from animals bearing extended nigrostriatal lesion displayed increased ROS production, while membrane potential (ΔΨ) and ATP production were significantly decreased. Reduced ATP production correlated with nigral neuronal loss. Thyroid hormone levels were significantly increased in serum of PD rats compared to sham animals while steady expression of selected cytokines was detected in all groups. Hepatic enzyme functions were comparable in all animals. Our study indicates for the first time that in a rodent model of PD, hepatic mitochondrial dysfunctions arise as a consequence of nigrostriatal degeneration, and that thyroid hormone represents a key interface in this CNS-liver interaction. Liver plays a fundamental detoxifying function and a better understanding of PD-related hepatic mitochondrial alterations, which might further promote neurodegeneration, may represent an important step for the development of novel therapeutic strategies.

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1. Introduction

Parkinson’s disease (PD) is one of the most common and devastating neurological disorders in the elderly, and is principally characterized by the degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNc) projecting to the striatum. Although PD is the prototypical movement disorder, numerous non-motor functions are also affected in PD patients or PD animal models [1,2]. The splanchnic district, in particular, is significantly involved; gastrointestinal dysfunctions [3–5] represent one of the most common PD non-motor symptoms. In this frame, a potential involvement of the liver in the cascade of events triggered by the central dopaminergic deficit has been occasionally proposed [6], but the issue has never been fully investigated.

The liver is responsible for the body’s mainstay protein metabolism, synthesis and degradation, and possesses fundamental detoxifying functions. It is highly connected with the CNS and evidence indicates that the autonomic system plays an important role in regulating hepatic functions [7]. Alterations of liver detoxification capacity and mitochondrial oxidative phosphorylation have been observed in PD [8]. Moreover, detoxification of medications and toxins is less efficient in PD patients compared to healthy individuals, thus possibly reflecting altered liver function [9]. Impaired capacity of hepatic P450 subsystems have been observed in late but not early onset PD.

Abbreviations: PD, Parkinson’s disease; 6-OHDA, 6-hydroxydopamine; CSF, cerebrospinal fluid; ROS, reactive oxygen species; ΔΨ, mitochondrial membrane potential; SNc, substantia nigra pars compacta; TH, Tyrosine Hydroxylase; DA, dopamine; DAT, dopamine transporter; ATP, adenosine triphosphate; HPLC, high-performance liquid chromatography; LDH, lactate dehydrogenase; HPA, hypothalamic–pituitary–adrenal pathway; AST, aspartate aminotransferase; ALT, transaminases–alanine aminotransferase; AP, alkaline phosphatase; CNS, central nervous system; HPA, hypothalamic–pituitary–adrenal; T3, triiodothyronine; GSH/GSSH, glutathione/glutathione disulfide

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patients [10,11] suggesting that hepatic alterations are not involved in the etiology of the disease but may emerge as a consequence of central neurodegenerative processes. Recent data indicate that brain dopaminergic systems represent an important center regulating hepatic cytochrome P450 activity [6,12,13]. Complex interactions may, therefore, link liver function to the efficiency of the nigrostriatal system, which represents the main source of dopamine in the CNS.

The development of animal models of PD has considerably improved our understanding of the cellular and biochemical mechanisms involved in the onset and progression of the disease. In particular, loss of nigrostriatal dopaminergic neurons can be induced by the intracerebral administration of the neurotoxin 6-OHDA in rats. Six-OHDA is a hydroxylated analog of dopamine and its neurotoxicity largely depends on its incorporation in dopaminergic neurons via selectively and actively uptake by the dopamine transporter (DAT) [14,15]. In this model unilateral injection of 6-OHDA in the rat striatum results in extensive loss of striatal dopaminergic terminals and a progressive retrograde degeneration of dopaminergic neurons in the ipsilateral SNc [16]. The unilateral 6-OHDA model has been extensively used to investigate various aspects of PD pathophysiology, as well as to test innovative therapeutic strategies [16]. We have recently shown that a massive reduction in fecal output, reminiscent of the constipation seen in PD patients, is present in unilateral 6-OHDA-lesioned animals [17], thereby supporting the use of the “hemi-parkinsonian rat” as a model to assess PD-symptoms affecting the splanchic district. The central aim of this study was, therefore, to evaluate whether and how changes in hepatic mitochondrial function occur in the presence of on-going nigrostriatal degeneration caused by intrastratial injection of 6-OHDA.

2. Materials and methods

2.1. Animals, surgery, sacrifice and tissue processing

Male Sprague–Dawley rats (Charles River), weighing 200–225 g at the beginning of the experiment, were housed two per cage, at 20–22 °C on a 12-h light–dark cycle, with food and water ad libitum. Animals were left in the housing facilities for at least one week, before the beginning of the experiments. All procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the local Animal Care Committee.

Rats (n = 46) were anesthetized with sodium-thiopental (50 mg/kg) and placed in a stereotaxic frame (Stoeling), with the incisor bar positioned 3.3 mm below the interaural line. Animals received an intrastriatal injection of 6-OHDA as described before [18] and were sacrificed at different time points (24 h, 4 and 8 weeks) after the neurotoxic insult. At the time of sacrifice animals were anesthetized (50 mg/kg of sodium-thiopental) and 100–200 μL of cerebrospinal fluid (CSF) was withdrawn from the cisterna magna. Rats were then decapitated, trunk blood was collected and serum aliquots stored in liquid nitrogen. Brains were rapidly removed, and separated in two portions containing the striatum or SNc using a cold Coronal Brain Matrix (2Biological Instruments). Striata from both hemispheres were dissected out, weighted and stored, separately, at –80 °C, while brain coronal portions containing the SNc were stored at –80 °C. At the time of sacrifice, liver from each animal was also removed immediately and processed for isolation of mitochondrial fraction.

2.1.1. Western blot

Striatal and liver samples were homogenized in lysis buffer (Cel-Lytic, Sigma; 1:10, weight:volume) containing protease inhibitors (Roche). Homogenates were centrifuged at 16,000 g for 10 min and protein-containing supernatants aliquoted and stored at –80 °C for successive assays. Protein content was quantified using the colorimetric Bicinchoninic Acid Protein method (Sigma).

Striatal and liver homogenates were separated on a poly-acrylamide gel (4–12% NuPAGE Novex, Invitrogen) and transferred to a nitrocellulose membrane (Invitrogen). Membranes were blocked using a commercial buffer ( Odyssey Blocking Buffer, LiCor BioSciences) and incubated overnight at 4 °C with specific primary antibodies against: tyrosine hydroxylase (TH) (Chemicon), β-actin (Santa Cruz) or dopamine transporter (DAT). IRDye800 and IRD700 goat anti-rabbit or anti-mouse secondary antibodies were used for detection (LiCor Biosciences).

2.1.2. Striatal dopamine levels

Quantitative determination of dopamine (DA) was carried out on striatal homogenates using a commercially available competitive ELISA kit (IBL-Hamburg) according to the manufacturer’s procedure. All samples were analyzed in duplicates using a spectrophotometer (Elx 808, Biotek) at 450 nm.

2.1.3. Immunohistochemistry

Serial coronal sections (25 μm) were cut throughout the SNc using a cryostat (Leica) picked up on polylysine-coated slides (Thermo Scientific) and stored at –80 °C. Immunohistochemical staining for TH (Chemicon) was carried out as described before [18]. Image analyses were performed by a blinded investigator using an AxioSkop2 microscope (Zeiss) and a computerized image analysis system (Axiocam MRC5, Zeiss) equipped with a dedicated software (AxioVision Rel 4.2, Zeiss). The number of TH-positive neurons was counted bilaterally on every fourth section throughout the SNc. Neuronal survival was expressed as the percentage of TH-positive neurons on the ipsilateral (lesioned) side with respect to the contralateral (intact) side. This approach avoided possible bias due to inter-individual differences. Loss of TH-positive neurons was determined as 100 minus the percentage of surviving neurons.

2.2. Isolation of liver mitochondria

Whole livers (9 g) were washed with ice-cold saline and processed immediately for mitochondria isolation by standard techniques using differential centrifugation [19]. Briefly, minced tissue was homogenized in ice cold medium containing 0.25 M sucrose, 1 mM EDTA, 5 mM HEPES (pH 7.2; all from Sigma, Italy) using a teflon/glass Potter homogenizer (Sartorius). The homogenate was centrifuged at 1000 g for 10 min. The supernatant taken up and centrifuged again 10 min at 10,000 g. The resulting pellet, resuspended in medium containing 0.25 M sucrose, 5 mM HEPES and centrifuged again 10 min at 10,000 g, was kept on ice and used immediately for subsequent determination (see below). Single mitochondrial preparations were obtained for each individual animal (n = 4–6) and protein concentration was determined using the Lowry method [20].

2.2.1. Determination of hepatic mitochondrial ATP production

ATP production was measured using the Perkin Elmer ATPLite kit according to the manufacturer’s instructions. Bioenergetics experiments were performed in state 3 in the presence of ADP. Briefly, hepatic mitochondria pellets, obtained from single animals, were re-suspended in phosphate buffer (250 mM sucrose, 5 mM KH2PO4, 1 μM rotenone, pH 7.2) and 25 μM ADP and 6 mM succinate were added. After two minutes suspensions were lysed and ATP production measured [21]. Results represent mean ± S.E of ATP (nmol) produced per mg of protein. Luminescence changes were monitored using a Perkin Elmer Victor 2 luminometer.

2.2.2. Determination of hepatic mitochondrial membrane potential

Mitochondrial membrane potential (ΔΨ) was assessed by measuring the uptake of the fluorescent dye rhodamine 123 [22]. Measurements were taken from single hepatic mitochondrial preparations.
obtained from individual animals and resuspended in 2 mL of phosphate buffer (250 mM sucrose, 5 mM KH2PO4, 1 μM rotenone, pH 7.2 at 25 °C) containing 6 mM succinate, and 0.3 mM rhodamine 123. The fluorescence of rhodamine 123 was monitored using a Perkin Elmer LS 50B fluorescence spectrometer. Emission and excitation wavelengths were 503 and 527 nm, respectively. \( \Delta \Psi \) was calculated according to the following relationship: \( \Delta \Psi = \frac{\log (\text{rhodamine 123})_{\text{in}}}{\log (\text{rhodamine 123})_{\text{out}}} \), assuming that the distribution of rhodamine 123 between mitochondria and medium follows the Nernst equation.

2.2.3. Determination of hepatic mitochondrial reactive oxygen species

Generation of reactive oxygen species (ROS) by single hepatic mitochondria preparation, obtained from individual animals, was followed by the conversion of 2,7'-dichlorofluorescein diacetate (H2DCFDA) to fluorescent 2,7'-dichlorofluorescein (DCF). freshly isolated rat mitochondria were resuspended in 2 mL of phosphate buffer (250 mM sucrose, 5 mM KH2PO4, 1 μM rotenone, pH 7.2 at 25 °C) and incubated for 10 min at 37 °C with 6 mM succinate and 5 mM H2DCFDA (Molecular Probes Inc.). Production of the fluorescent derivative DCF as a function of time (minutes) was measured using a microplate reader (Perkin Elmer Life Science, Monza, Italy).

2.3. Treatment of isolated hepatocytes with 6-OHDA

Isolated rat hepatocytes were prepared by liver perfusion with collagenase (Roche) as previously described [23]. Cell viability, estimated at the beginning of the experiments, ranged between 85 and 90%. Cells were suspended (10^6 cells/mL) in Krebs–Henseleit–HEPES buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.3 mM CaCl2, 25 mM NaHCO3, 20 mM HEPES, pH 7.4) and incubated at 37 °C in 50-mL glass bottles under continuous flushing with 95% O2 and 5% CO2. Increasing concentrations of 6-OHDA (12.5, 25, 50, 100 and 200 μM) were added to isolated hepatocytes and cell viability was monitored at various time points (30, 60, 90 and 120 min) after addition of the toxin by measuring the activity of lactate dehydrogenase (LDH) released into the medium as described before [24]. Maximal LDH release was determined after exposing the cells to 10% of triton-X-100 (50 μL into 1 mL of cell suspension). Hepatocyte ATP content was determined 120 min after 6-OHDA-treatment using an ATP Bioluminescence Assay Kit (Perkin Elmer, Italy), following the manufacturer’s instructions.

2.4. Serum levels of hepatic enzymes and triiodothyronine (T3)

Evaluation of liver transaminases – alanine aminotransferase (ALT), aspartate aminotransferase (AST) – and alkaline phosphatase (AP) was performed on rat serum using an automated Hitachi 747 analyzer (Roche/Hitachi).

Total T3 levels in serum were measured by using a time-resolved fluororimmunoassay (AutoDELFIA, Wallac, Finland). Total serum T3 levels were measured by using a time-resolved fluororimmunoassay (AutoDELFIA, Wallac, Finland).

2.5. Serum and CSF levels of cytokines

Cytokines levels were determined using an infrared Multiplex ELISA kit (Quansys Biosciences), according to the manufacturer’s instructions, and data were quantified using an Odyssey Infrared Imaging System (Li-Cor Biosciences). Minimal detection levels were: IL-1α: 2 pg/mL; IL-6, IL-10 and IL-12p70: IL-1α: 50 pg/mL; IL-2: 4.5 pg/mL; IL-4: 2.5 pg/mL; IFN-γ: 7.5 pg/mL and TNF-α: 3 pg/mL.

2.6. Plasma levels of 6-OHDA

Rats (n = 8) received an intrastriatal injection of 6-OHDA or vehicle, as described above (Surgery). Blood was drawn from the portal vein 10, 30 min, 60 min and 24 h after toxin injection and plasma from each animal was aliquoted and stored at −80 °C. Evaluation of 6-OHDA plasma level was determined by High Liquid pressure Chromatography (HPLC) on a reverse-phase ion-pairing system (Younglin Instrument) using a C18 analytical column. The mobile phase composition was 50 mM citric Acid, 100 mM sodium phosphate, 100 mg/L EDTA and 50 mg/L octylsulfate, pH 3.0, and flow rate was 1 mL/min. Electrodetection was performed at +0.35 V at a range of 0.5 nA.

Stock solution of 6-OHDA (1 mg/mL) was prepared freshly in 0.1 M perchloric acid and 0.1% ascorbic acid. For analysis 0.5 mL aliquots of rat plasma, complemented with dimtroxybenzylamine (3 ng/mL) as internal standard, were extracted using a catecholamines extraction kit (Chromsystems Diagnostics). Precision and accuracy of the HPLC analysis were determined using a pool of rat plasma spiked with different concentration of 6-OHDA (diluted from stock solution) freshly prepared in perchloric acid 0.1 M and 0.1% ascorbic acid. Linearity was obtained for concentrations of up to 1 mg/mL. The Limit of Detection and Limit of Quantification were determined as 2.27 and 1.59 ng/mL, respectively. The precision intra-day and inter-day were of 3.7% and 6.0%, respectively.

2.7. Image analysis

Protein expression in the western blotting analysis was quantified using an Odyssey Infrared Imaging System (Li-Cor Biosciences), all samples being normalized to actin levels. Results represent the percentage of protein expression in the injected striatum with respect to the intact, contralateral striatum.

Immunohistochemical image analysis was performed by an investigator, unaware of group distribution, using an Axioskope II microscope (Zeiss, Oberkochen, Germany) connected to a computerized image analysis system (Zeiss). TH-positive cells in the SNc of both lesioned and intact hemispheres were counted in every fifth section on comparable sections for all the subgroups of treatment throughout the entire nucleus. Results represent the percentage of the total number of TH-positive neurons in lesioned (right) SNc vs intact side (left) (neuron survival). In the absence of a stereological count, such approach was chosen to avoid methodological biases due to inter-individual differences and has been previously used to assess the extent of 6-OHDA-induced lesion in the SNc.

2.8. Statistical analysis

All values are expressed as mean ± S.E. Comparisons between groups were carried out using the analysis of variance (ANOVA) coupled with the Tukey’s post-hoc or Bonferroni’s correction using a dedicated software (Prism 3 software, GraphPad Software). Correlation among variables was assessed by the Pearson’s correlation coefficient (r). Minimum level of statistical significance was set at p < 0.05.

3. Results

3.1. 6-OHDA-induced nigrostriatal degeneration

Intrastriatal injection of 6-OHDA induced loss of dopaminergic terminals in the lesioned striatum compared with the intact side, as detected by the reduced TH expression (Fig. 1A and B). This reduction was minimal 24 h after injection of the neurotoxin, reached a significant (60–70%) level at 4 weeks and remained stable until 8 weeks after surgery (Fig. 1A and B). Similarly, dopamine levels diminished slightly after 24 h and were reduced by almost 80%, 4 and 8 weeks after 6-OHDA injection.
Toxic-induced nigrostriatal degeneration. A. Representative western blot analysis of TH expression in the left (contra-lateral, C) and injected (ipsi-lateral, I) striatum observed 24 h, 4 and 8 weeks after unilateral injection of 6-OHDA or vehicle (sham). M: molecular weight marker. Bands corresponding to TH (green) and actin (red) are indicated. B. Results (n=4–6) indicate the percentage of TH and DA expression in the right (R) vs left (L) striatum evaluated 24 h, 4 and 8 weeks after unilateral 6-OHDA injection or vehicle (sham). Black and white bars represent striatal TH expression and dopamine (DA) levels, respectively. C. Results (n=4–6) indicate the percentage of TH-positive neurons in the right (I) vs left (C) SNc detected 24 h, 4 and 8 weeks after unilateral injection of 6-OHDA or vehicle (sham). * and # p<0.001 vs sham.

The striatal injection of 6-OHDA caused a 50–60% depletion of TH-positive neurons in the ipsilateral SNc (Fig. 1C). Cell loss was observed at 4 weeks after surgery and remained stable until 8 weeks after the neurotoxin infusion. No cell loss was detectable after 24 h.

3.2. Nigrostriatal degeneration and liver mitochondria dysfunction

Liver mitochondria obtained from animals sacrificed 4 or 8 weeks after 6-OHDA injection exhibited a significant decrease in ATP production, compared with the respective vehicle-treated rats. Interestingly, ATP reduction correlated with the extent of neuronal loss (Fig. 2A and B). Similarly, the measurement of membrane potential showed a statistically significant decrease 8 weeks after infusion of the toxin, with respect to mitochondria obtained from control animals (Fig. 2C). This decrease was associated with a higher production of reactive oxygen species (ROS) of hepatic mitochondria obtained from animals 8 weeks after 6-OHDA-treatment (Fig. 2D and E). No alterations were detected in hepatic mitochondria isolated 24-hours after the toxic insult (Fig. 2A and C).

To exclude a direct effect of the toxin on liver, blood was obtained from 6-OHDA and sham animals 10, 30, 60 min and 24 h after intrastriatal injection of 6-OHDA. No 6-OHDA, at any time point considered, was detected in plasma of animals following HPLC analysis (data not shown).

DAT expression, required for cellular uptake and 6-OHDA-toxicity, was not detected in liver extracts (Fig. 3A).

When suspensions of isolated hepatocytes were challenged with increasing concentration – 12.5, 25, 50, 100 and 200 μM – of 6-OHDA, no changes were observed either in LDH release, evaluated as an index of cell viability (Fig. 3B), or ATP content (Fig. 3C), as measured for up to 120 min.

3.3. Effect of 6-OHDA-induced nigrostriatal degeneration on levels of thyroid hormone, cytokine expression and liver enzyme activities

Thyroid hormones have been recognized as crucial modulators of hepatic mitochondrial efficiency. Interestingly, serum levels of triiodothyronine (T3) were significantly increased in PD animals 4 and 8 weeks after 6-OHDA injection when compared to sham animals (Table 1).

The concentrations of cytokine levels that may potentially affect hepatic mitochondrial function were measured in both serum and CSF. Levels were generally low or not detectable in sham animals and remained unchanged following 6-OHDA injection, at all time points considered in this study (Table 2A and 2B).

Similarly, serum level of AST, ALT and AP—index of liver viability/function—observed from animals sacrificed 24 h, 4 weeks and 8 weeks after 6-OHDA injection were not modified with respect to those obtained from sham animals (Fig. 3D).

4. Discussion

PD is considered primarily a movement disorder, but non-motor dysfunctions are prominent and well-recognized features of the disease. Previous data have suggested that altered hepatic enzyme functions may be associated with PD [8]. However, in view of the long preclinical phase of the disorder, it is unclear whether such dysfunctions may be a cause or a consequence of the loss of dopaminergic neurons, which starts well before PD symptoms become overt. While defective liver activity may cause forms of hepatic-derived encephalopathies which starts well before PD symptoms become overt, the influence of PD on hepatic activity is still uncertain. While extrapyramidal symptoms [25,26] and neurological symptoms [27] through activation of the liver–brain axis, to date no studies have directly addressed the biochemical, pathological and functional aspects of hepatic dysfunction consequent to lesion of central dopaminergic pathways. Our data indicate, for the first time, an association between nigrostriatal degeneration and the subsequent emergence of hepatic mitochondrial alterations.

4.1. Hepatic mitochondrial activity is reduced in rats with nigrostriatal lesion

In this study, we used a well-characterized rodent PD model [16] relying on the unilateral intrastriatal injection of 6-OHDA, which prompts rapid damage to striatal dopaminergic terminals followed by delayed, progressive, and retrograde loss of nigral cell bodies. Bilateral injections of the neurotoxin are usually avoided because associated with marked aphagia, adipsia and high mortality rate [28]. Bilateral lesioning would therefore require intense nursing care, including administration of high-caloric liquid diet to maintain body mass [29] that might bias any evaluation within peripheral systems.

Characteristic time-dependent nigrostriatal degeneration [30] was observed with modest reduction of striatal dopamine and TH
expression and no loss of nigral neurons detectable 24 h after 6-OHDA injection, while extended neurodegeneration was observed in both nuclei after 4 weeks and remained stable for up to 8 weeks. Analysis of hepatic mitochondria activity revealed no differences in mitochondrial ΔΨ, as well as ATP and ROS production in lesioned compared to control animals 24 h after toxin injection. Conversely, hepatic mitochondria isolated from rats bearing an extended 6-OHDA-induced lesion (4 and 8 weeks), displayed significantly reduced energy production capacity and ΔΨ while their propensity to generate ROS was significantly increased, clearly indicating the presence of hepatic mitochondrial dysfunctions in PD animals. Importantly, reduction of mitochondrial ATP release in liver correlated with the percentage of neuronal cell loss in the SNc.

We excluded the possibility that the toxin may have in some way directly affected liver function, by leaking outside the CNS and entering the bloodstream based on a number of substantial evidence. First of all, 6-OHDA is very unstable and readily oxidizes at the injection site [31]; it is therefore unlikely that, following intra-cerebral injection, the toxin might have leaked into the bloodstream and acted on a peripheral organ. Accordingly, we demonstrate that no trace of 6-OHDA could be detected in plasma of lesioned-rats following intra-cerebral injection, even at very early time points (10 or 30 min post-injection). In addition, 6-OHDA-toxicity is mostly linked to the presence of DAT, that is required for intracellular transport of the toxin [15], although at high toxin concentration, auto-oxidation may cause unspecific death of any cell type. We show that hepatocytes do not express any DAT and are fully resistant to 6-OHDA-treatment in vitro, even at high toxin concentrations.

Our data unequivocally demonstrate that hepatic alterations are not detected at the onset of the toxin-induced neurodegenerative process but emerge as a consequence of extended and stable degeneration of nigrostriatal dopaminergic neurons.
4.2. Neuroinflammation and hepatic mitochondrial function

A crucial question arising from our results is how the selective nigrostriatal denervation may affect mitochondrial function in the liver. A potential explanation may lie in the neuroinflammatory response associated with nigrostriatal damage [32,33]. A previous study has demonstrated that unilateral intrastriatal injection of 6-OHDA and consequent DA depletion in mice compromise both the humoral and the immune systems with repercussions in peripheral organs, such as the spleen and mainly the liver [34]. The authors show that in lesioned animals under stress conditions, such as a bacterial infection, the liver is significantly more vulnerable while global morphology and functional integrity of the organ are preserved. Similarly, we show that significant changes in hepatocyte mitochondrial physiology are triggered by a central dopaminergic lesion, while gross liver functions are not affected.

Fig. 3. A. Expression of the dopamine transporter (DAT) in liver. Increasing concentrations of liver extracts were separated on gel and western blot analysis was performed. Ctr: striatal extract that expresses high levels of DAT, as indicated by the arrow, was used as a positive control. M: molecular weight marker. B. Hepatocytes, isolated from liver of control rats, were incubated with increasing concentration of 6-OHDA (0, 12.5, 25, 50, 100 and 200 μM) and % lactate dehydrogenase (LDH) release, as an index of cell viability, was evaluated after 30, 60, 90 and 120 min. C. Isolated hepatocytes were incubated with increasing concentration of 6-OHDA (0, 12.5, 25, 50, 100 and 200 μM) and ATP content was evaluated after 120 min. Each measurement represents the mean ± S.E. of 4–6 separate hepatocyte preparations. D. Serum levels of hepatic enzymes. Values (mean ± SD) are given in U/ mL and were measured 24 h, and 4 and 8 weeks after intrastriatal injection of 6-OHDA or vehicle (Sham). AST: alanine aminotransferase; ALT: aspartate aminotransferase; AP: alkaline phosphatase.
cerebroventricular injection of endotoxin lipopolysaccharide can, for example, cause massive inflammatory reaction in brain tissues and affect hepatic levels of P450 isozymes [40]. Conversely, we did not detect any significant changes in cytokines levels, in either plasma or CSF, of lesioned compared to control animals. Cytokine release is a rapid and short-lived phenomenon; we cannot exclude that acute, transient changes that occurred as an early event, possibly triggered a signaling cascade, leading to delayed hepatic mitochondrial alterations in lesioned animals. A more extensive time course analysis, taking into consideration earlier time points, would be, therefore, required.

Alteration of central cytokine levels could also intervene on autonomic and endocrine systems [41] that bi-directionally connect liver to CNS and play an important role in hepatic function and regulation [7]. The hypothalamus, that sends parasympathetic and sympathetic to CNS and play an important role in hepatic function and regulation was not available.

4.3. Central dopaminergic denervation and hepatic mitochondrial function

The importance of dopaminergic systems in liver regulation has been previously suggested [6,12,13] and while precise mechanisms remain elusive a crucial role of increased T3 levels has been suggested. It has been shown that central treatment with dopaminergic drugs can modulate peripheral levels of T3 by affecting the tuberoinfundibular and mesolimbic pathways [13]. Our study shows a significant increase of serum T3 levels in PD animals, 4 and 8 weeks after 6-OHDA injection, and further sustains an important role for this hormone in the delayed emergence of hepatic mitochondrial dysfunction detected in rats bearing a PD-like lesion of nigrostriatal tract. Thyroid hormones have been recognized as crucial modulators of mitochondrial efficiency [49] and high affinity receptors for thyroid hormone have been detected in liver mitochondria [50]. In vivo studies showed that T3-treatment causes decreased hepatic mitochondrial ΔΨ. We have also previously demonstrated that in rats with increased T3 levels, similar to those observed in the present study, a significant reduction of hepatic ATP levels could be detected compared with levels in euthyroid rats [51]. In addition, livers from hyperthyroid rats, showed a GSH/GSSG ratio significantly lower than euthyroid rats, suggesting higher hepatic susceptibility to free radical formation. The expression of several nuclear genes encoding mitochondrial proteins is T3-regulated, as shown for beta-F1ATPase and several sub-units of the respiratory chain [52]. Recently, proteomic characterization has indicated that elevated peripheral T3 levels can cause the differential expression of liver proteins involved in energy metabolism and response to oxidative stress, as well as alteration of hepatic mitochondrial protein [53].

Clinical studies assessing thyroid function in PD patients also showed significant abnormalities in the thyroid laboratory tests and a “subclinical” hyperthyroidism was found to be prevalent in the PD patients when compared to age-matched controls [54]. Altered thyroid hormone levels could derive from HPA dysfunctions, knowingly present in PD patients [44] and 6-OHDA-lesioned animals [34,45–48].

4.4. Hepatic mitochondria dysfunction and neurodegeneration

The metabolically active liver requires continuous ATP synthesis, thus hepatocytes contain a relatively high density of mitochondria compared to other cell types. Hepatic manifestations of mitochondrial disorders range from steatosis, cholestasis and chronic liver disease [56]. Mitochondrial dysfunction is the commitment step in hepatocytes cell death, and hepatocytes cell death is dependent on mitochondria. Notably, mitochondrial impairments and permeabilization can affect cell viability and cause cell death leading hepatocytes down the apoptotic or necrotic pathway [57]. Mitochondrial dysfunctions also represent the major determinant of hepatotoxicity and could be the major mechanism of drug-induced liver disease [58].
Our data indicate that severe hepatic injury was not present in parkinsonian animals, as evidenced by normal serum hepatic enzyme values. This is not entirely surprising when considering, for example, that despite the ultra-structural changes observed in the hepatic mitochondria of some patients with Amyotrophic Lateral Sclerosis (ALS), decreased albumin level, a marker of hepatic synthetic ability has been observed, suggesting some altered hepatic functions.

5. Conclusion

The groundwork evidence that interestingly emerges for the first time from our work is that the liver can be, directly or indirectly, affected by a primary central dopaminergic defect. Although the precise mechanisms still need to be clarified, this phenomenon likely occurs through the activation of signaling pathways and/or release of soluble mediators, including hormones, generated in response to the neurodegenerative process.

A very recent report [64] indicates that, after stroke, altered noradrenergic innervation in the liver can signal specialized hepatic immune cells (iNKT cells) to promote systemic immunosuppression suggesting that such signaling pathways involving neurotransmitters might also be involved in neurodegenerative diseases. This study sustains our data and further demonstrates that the CNS communicates with peripheral systems and organs, through signaling pathways involving neurotransmitters, to regulate immune and inflammatory response and even regulate the central nervous system itself through feedback mechanisms.

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