

Mitochondrial toxins and neurodegenerative diseases

Antonio Ayala, Jose L. Venero, Josefina Cano and Alberto Machado

Departamento de Bioquímica, Bromatología, Toxicología y Medicina Legal. Facultad de Farmacia University of Seville, Spain

TABLE OF CONTENTS

1. Abstract
2. Mitochondrial toxin involved in neurodegeneration
3. Toxin that produced a neurodegeneration like that found in parkinson's disease
 - 3.1. 1. Methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)
 - 3.2. Tetrahydroisoquinoline derivatives as possible endogenous neurotoxins
 - 3.2.1. 1. Methyl-1,2,3,4-tetrahydroisoquinoline as neuroprotector of dopaminergic systems
 - 3.3. Rotenone
 - 3.3.1. In vivo rotenone models of Parkinson's disease
 - 3.3.2. Rotenone and oxidative stress
 - 3.3.3. Rotenone and the proteasome.
 - 3.3.4. Rotenone and apoptosis
 - 3.3.5. Rotenone and the dopaminergic system
 - 3.4. Other inhibitors of Complex I
4. Toxins that produced a neurodegeneration like that found in Huntington disease: Inhibitor of succinate dehydrogenase
 - 4.1. Pathways of cells death induced by 3-NPA.
 - 4.1.1. Energy depletion
 - 4.1.2. Excitotoxicity
 - 4.1.3. Oxidative stress
 - 4.2. Specificity of the 3-NPA toxicity.
 - 4.3. CNS regional vulnerability to toxins
 - 4.4. Mechanism of neuronal cell death
 - 4.5. Other inhibitors of complex II
5. Conclusions
6. Perspective
7. Acknowledgments
8. References

1. ABSTRACT

The selective loss of a particular subset of neurons is a common feature of neurodegenerative disorders. A failure in respiratory chain complex activities in mitochondria seems to be a causative factor. The aim of this review is to describe the most important toxins affecting the mitochondrial function, which could be involved in the incidence of some of these diseases: MPTP, rotenone and 3-nitropropionic (3-NPA).

2. MITOCHONDRIAL TOXIN INVOLVED IN NEURODEGENERATION

Neurodegenerative disorders are characterized clinically by insidious onset and a slow progressive course. Pathologically, these diseases share a common feature: the selective loss of a particular subset of neurons for unknown reasons, such as cerebral cortical neurons in Alzheimer's disease (AD), substantia nigra neurons in Parkinson's disease (PD), spinal motoneurons in Amyotrophic Lateral Sclerosis (ALS), and medium-sized striatal neurons in Huntington's disease (HD).

Like many other age-related diseases, they are frequently hereditary, showing some familial association. Therefore, a genetic basis can be assumed. However, their etiology is to a large extent unknown. Metabolic defects, produced by failure in respiratory chain complex activities in mitochondria, are thought to underlie defects in energy metabolism and seem to be a causative factor in several of these neurodegenerative diseases (1,2). Evidence for mitochondria being a damage site in neurodegenerative disorders is partially based on the impairments in respiratory function found in some of these diseases.

Small subsets of PD patients have hereditary characteristics including Mendelian pedigrees. There are different kinds of PD and some of them are better known than others: one is related to the gene encoding *PINK1* (PTEN-induced kinase) protein, a kinase localized in mitochondria (3); another is related to the gene encoding *parkin*, a E3 ubiquitin ligase (4); and a rare autosomal dominant form due to mutations in alpha-synuclein (5). In these cases, mitochondrial dysfunction has been described.

Mitochondria and neurodegenerative diseases

Genetic inactivation of *parkin* in mice resulted in nigrostriatal defects associated with a decrease in several mitochondrial proteins including some subunits of pyruvate dehydrogenase, complex I and complex IV with a general decrease in mitochondrial state 3 respiration. Expression of alpha-synuclein mutants in various cell systems has resulted in altered mitochondrial function (6-8). Moreover, in idiopathic PD patients, reduced complex I activity was found in autopsied substantia nigra (9-12) and in platelets (13,14).

Similarly, early onset of familial AD has been associated with mutations in the Abeta precursor protein (APP) and the presenilin-containing APP peptide processing complexes (15). Abeta peptide toxicity might also be related to mitochondrial dysfunction. The Abeta peptide has been reported entering the mitochondria, binding to mitochondrial alcohol dehydrogenase, and increasing mitochondrial ROS production (16,17). In addition, incubation of rat brain mitochondria with the Abeta 25-35 fragment results in a selective and dose independent inhibition of complex IV (18). Mitochondrial oxidative phosphorylation defects have also been detected in a variety of tissues from late-onset AD patients (19,20). Moreover, in familial cases, ALS is caused by mutations in the Cu,Zn-SOD (*Sod1*) (21). Transgenic mice that express a human mutant Cu,Zn-SOD develop a motor neuron disease associated with vacuolated mitochondria containing high concentrations of Cu,Zn-SOD (22). Mitochondrial dysfunction has also been implicated in the etiology of Huntington disease (23,24); mtDNA rearrangements are elevated in the basal ganglia of patients with this disease (25).

Recently, Wallace (26) has pointed out that these age-related degenerative diseases are increasing to epidemic proportions in all industrialized countries. Today, AD affects about 4.5 million Americans, and calculations say it will reach 11-16 million by 2050 (27). Parkinson's disease afflicts approximately one million persons in the United States today, 60,000 new cases are diagnosed every year, and the incidence is expected to quadruple by 2040 (28). Wallace (29) also indicated that the nature and frequency of genetic variants in the human population has not changed significantly in the past 50 years, even though the incidence of these diseases has climbed continuously. Mitochondria are the only human genetic system that embodies the features necessary to explain common features observed in these age related diseases. Environment seems to be the most important factor in continuous change, diet being the most dynamic change. One of the most important factors included in both environment and diet is the presence of many toxins, some of them producing mitochondrial inhibition. These toxins could be involved in the incidence increase of some of these degenerative diseases; environmental toxins and lack of dietary vitamin E have also been suggested as causing ALS disease (30,31).

In this review our aim is to describe the most important toxins affecting the mitochondrial function, which could be involved in the incidence of some

neurodegenerative diseases. Animal models were used for studying these diseases.

3. TOXIN THAT PRODUCED A NEURODEGENERATION LIKE THAT FOUND IN PARKINSON'S DISEASE

3.1. 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)

Parkinson's disease is characterized by the progressive loss of dopamine (DA) in the caudate nucleus, putamen, and substantia nigra (SN) upon the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), resulting in cardinal motor symptoms such as tremor at rest, bradikinesia, muscular rigidity, stooped posture, and instability. PD is believed to be caused both by genetic and environmental factors (32). However, and in spite of the fact that familial PD correlates with mutations of genes that encode several proteins, including alpha-synuclein, parkin and ubiquitin C terminal hydrolase-L1 (33), the etiology of idiopathic PD, which accounts for more than 90% of PD, is still not fully understood. It seems to be multifactorial (34-36). It is well documented that there is an epidemiological link between PD and people who are associated with rural living and working in agriculture. In particular, people exposed to various herbicides and insecticides in an agricultural setting showed an increased risk of developing PD (37).

The possibility that environmental neurotoxins played an important role in PD came to light in the early 1980s when several drug users in Northern California developed an acute state of akinesia (initially confused with catatonia) following the intravenous injection of a street preparation of 1-methyl-4-phenyl-4-propionpiperidine (MPPP), an analog of the narcotic meperidine. Also, the finding that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which was inadvertently produced during the illicit synthesis of MPPP led to Parkinsonism (38) shifted the focus toward environmental factors as potential PD initiators or contributors.

The administration of MPTP produces extensive destruction of nigrostriatal dopaminergic neurons. Indeed, this compound is able to produce a severe parkinsonian-like syndrome in humans and in primates (38,39). The neurotoxic role of MPTP is critically dependent on its metabolite MPP⁺, formed in glial cells in the reaction catalyzed by the mitochondrial monoamine oxidase B (MAOB). MPP⁺ then accumulates inside the mitochondria, where it binds to Complex I (40-44) causing an inhibition of NAD-linked mitochondrial respiration. Human mitochondrial complex I (NADH:ubiquinone oxidoreductase; EC: 1.6.5.3) is a large multisubunit assembly comprising 39 nuclear encoded and 7 mitochondrially encoded subunits (45).

Therefore, the cellular damage caused by MPP⁺ is due primarily to energy depletion caused by the specific binding to Complex I (46). This may then lead to neuronal depolarization and secondary excitotoxicity (47). A consequence of this is an influx of Ca²⁺ into neurons, which accumulates in mitochondria.

Mitochondria and neurodegenerative diseases

A secondary effect of complex I inhibition is the damage caused by free radical production (48-51). O_2^- formation by Complex I appears to be the primary source of O_2^- in the brain under normal conditions and in a variety of pathological scenarios ranging from aging to Parkinson's disease (48,52). During electron transfer, electrons from NADH are passed to ubiquinone in a quinone-reducing site via the flavin (FMN) and iron-sulfur centers (49). Ubisemiquinone (UQ10) species generated in the course of electron transfer reactions in the respiratory chain donate electrons to O_2 and provides a constant source of O_2^- (53-55). The resulting semiquinone can also be reduced by an electron to form QH₂. But in the presence of complex I inhibitors such as rotenone and piericidin this reduction step is strongly slowed, so the semiquinone accumulates and O_2^- production increases (56,57).

Inhibition of mitochondrial complex I opens mitochondrial permeability transition pore and may trigger apoptotic cell death (32). Inhibition of complex I creates an environment of oxidative stress that ultimately leads to aggregation of alpha-synuclein and the subsequent death of DA neurons (56). It also makes neurons vulnerable to glutamate excitotoxicity. All these results show that impairments in complex I may be central to the pathogenesis of DA neuronal demise in sporadic PD (59).

Reduced complex I activity may predispose to excitotoxicity by altering ATP levels, by impairing Ca^{2+} homeostasis, or both (60). Reduced ATP levels decrease the activity of the plasma membrane Na^+/K^+ ATPase, resulting in partial neuronal depolarization. Neuronal depolarization decreases the voltage dependent Mg^{2+} blockade of the *N*-methyl- D-aspartate (NMDA) glutamate receptor. Under these conditions, even normal levels of extracellular glutamate may cause excitotoxic activation of NMDA receptors and elevation of intracellular Ca^{2+} . Moreover, chronic complex I defects have been shown to disrupt normal Ca^{2+} signalling in neural cells (61).

The most important argument against the animal model of PD produced by the toxicity of MPTP is that it was unable to reproduce the neuronal inclusions called Lewy bodies described in PD (62,63). However, recently Fornai *et al.* (64) have developed a mouse Parkinson's disease model that is based on continuous MPTP administration with an osmotic minipump and mimics many features of the human disease, severe striatal dopamine depletion, nigral cell loss along with the formation of nigral inclusions immunoreactive for ubiquitin and alpha-synuclein. Moreover, this model also caused long-lasting activation of glucose uptake and inhibition of the ubiquitin-proteasome system. These data suggest that continuous low-level exposure of mice to MPTP causes a Parkinson-like syndrome in an alpha-synuclein dependent manner supporting the probable implication of toxicity in parkinsonism.

3.2. Tetrahydroisoquinoline derivatives as possible endogenous neurotoxins

Since the discovery of MPTP toxicity particular attention has been focused on the possible role of different

structurally MPTP-like endogenous amines. Two of the more significant families of compounds with specific neurotoxic effects on the dopaminergic system producing parkinsonism in experimental animals, as does MPTP, are the tetrahydroisoquinolines (TIQs) and beta-carbolines (65,66). Beta-carbolines have structures similar to those of MPTP/MPP⁺, and may be synthesized *in vivo* from tryptophan via tryptamine (32). Like MPTP, beta-carbolines may be precursor neurotoxins that are *N*-methylated and oxidized by MAO-B to form, in their case, beta-carbolinium ions, which may produce neuronal death and PD symptoms. Various TIQs have also been found in both normal and PD brains as endogenous amines by gas chromatography-mass spectrometry (32). TIQ, 1MeTIQ, *N*-Me-TIQ, *N*-Me-6,7-(OH)2-TIQ (*N*-Me-norsalsolinol), 1,*N*-(Me)2-6,7-(OH)2-TIQ (*N*-Me-salsolinol), 1-phenyl-TIQ, *N*-Me-1-phenyl-TIQ, and 1-benzyl-TIQ (1-Bn-TIQ). Isoquinoline derivatives have been found in foods (67). 1,2,3,4-Tetrahydroisoquinoline is present in flour (0.52 ng/g), banana (2.2 ng/g), cheese (5.2 ng/g), broiled sardine (0.96 ng/g), broiled beef (1.3 ng/g), and the yolk (1.8 ng/g) and white (2.2 ng/g) of boiled eggs and in various alcoholic beverages. They are produced in the CNS by condensation of biogenic amines (e.g. catecholamines and phenylethylamines) with aldehydes (or with alpha-keto acids followed by decarboxylation) by the Pickett-Spengler reaction.

Isoquinoline derivatives are metabolized by various enzymes including *N*-methyltransferase and MAO (but very slow compared with MPTP) to produce *N*-methylated derivatives and isoquinolinium cations, respectively (68,69). Isoquinoline derivatives are also metabolized in brain and liver by cytochrome P450 isoenzymes to produce 4-hydroxylated products (70).

Physiologically, these compounds may be involved in the regulation of monoamine function through reversible and competitive inhibition of enzymes involved in monoamine synthesis and metabolism (e.g. tyrosine hydroxylase, l-aromatic amino acid decarboxylase, catechol-*O*-methyltransferase, and MAO-A and -B) (65). Their methylation in the reaction catalyzed by *N*-methyltransferase and oxidation with MAO participation leads, similarly as in the case of MPTP, to the formation of *N*-methylisoquinoline ions, exhibiting neurotoxic properties, which however are much weaker (71).

Tetrahydroisoquinolines are complex I inhibitors after their transformation into *N*-methylisoquinoline ions. Neurotoxic effects and inhibitory influence on the activity of mitochondria are attributed mostly to *N*-methylisoquinoline ions, whose formation, contrary to MPTP, requires initial *N*-methylation, catalyzed by *N*-methyltransferase, in addition to MAOB action. Low availability of this enzyme can explain much lower neurotoxicity of tetrahydroisoquinolines (72) in comparison with MPTP.

Whether a relationship exists between the structure and the potency to inhibit mitochondrial respiration and complex I has been studied. Presence of a

phenyl ring at or near the C1 position augmented the inhibitory potency against NADH-linked respiration and complex I activity (71). N-methylation had little effect on the inhibition of NADH-linked respiration and complex I activity; however, oxidation of N-methylated isoquinoline into N-methylisoquinolinium ion augmented the potency to inhibit NADH-linked respiration. The order of potency of the isoquinoline derivatives is isoquinolinium cations > isoquinolines > dihydroisoquinolines > 1,2,3,4-tetrahydroisoquinolines showing that pyridinium cations are more potent than their corresponding neutral congeners in inhibiting mitochondrial respiration (73).

Experimental studies indicated that isoquinolines (e.g. N-methylsalsolinol) could initiate apoptotic process in dopaminergic neurons by the generation of free radicals. Isoquinoline and 1,2,3,4-tetrahydroisoquinoline induced apoptotic cell death in PC12 cells and SK-N-MC dopaminergic cell lines by a mechanism involving generation of free radicals, perhaps secondary to inhibition of the mitochondrial respiratory chain (74).

These endogenous and exogenous heterocyclic molecules, which are structurally related to MPTP/ MPP⁺, such as isoquinolines and beta-carbolines, also have the specific similar toxic properties on DA cells, which are also conferred by their uptake by the DAT (44). This shows that DAT might be responsible for the selectivity of DA cell death in these PD models.

3.2.1. 1-methyl-1,2,3,4-tetrahydroisoquinoline as neuroprotector of dopaminergic systems

Other isoquinolines, e.g. 1-methyl-1,2,3,4-tetrahydroisoquinoline (1Me-TIQ) can exhibit neuroprotective activity. This compound differs in one methyl group from TIQ. The amount of TIQ seems to increase in the Parkinson brain (75). It is toxic to nigro-striatal dopaminergic neurons (76). However, 1MeTIQ decreases in Parkinson's disease and also seems to decrease with age in humans (77).

An occurrence of TIQ and MeTIQ has been reported in two areas especially influenced by aging and Parkinson's disease-substantia nigra (SN) and striatum (ST) in rats of different ages (78). SN has the greater content of both compounds: 3-4-fold more than ST of the young animals. During the aging process there is a significant decrease (50 %) of 1MeTIQ concentration in SN. However, 1MeTIQ seems to increase in ST although this change is not significant. The TIQ levels change in the same directions as 1MeTIQ in both structures and ages, but these changes are not statistically significant. Because the ratio 1MeTIQ/TIQ could be important, it was suggested that the protective action of MeTIQ could be related to its possible action against other endogenous compounds with neurotoxic actions, such as TIQ. In fact, one possible cause of the major incidence of PD during aging could be due to a decrease in the levels of protective compounds such as 1MeTIQ which would produce an increased vulnerability to environmental neurotoxins.

1MeTIQ in rats prevents the extensive degeneration of dopaminergic terminals induced by MPP⁺

injection into the striatum (79). In hepatic mitochondria, 1MeTIQ (5 mM) totally prevents complex I inhibition by 20 mM MPP⁺. 1MeTIQ could protect the activity of this enzyme, avoiding the interaction between MPP⁺ and complex I (80). A similar phenomenon between a weak and a strong inhibitor of complex I has been previously described (81).

1MeTIQ has been described as being enzymatically formed in the brain by the 1MeTIQ synthesizing enzyme (1MeTIQse) (82). The activity is spread throughout the brain, the highest activity being in the dopaminergic areas (striatum and substantia nigra) and in the cortex. During aging there is a 1MeTIQse activity reduction (50%) in the areas implicated in the etiology of Parkinson disease (substantia nigra, striatum) and in the cerebral cortex.

3.3. Rotenone

Dopaminergic toxicity of the MPTP active metabolite MPP⁺ is mediated by the DAT through accumulation in DA neurons, where it inhibits mitochondrial complex I activity. Moreover, various endogenous and exogenous heterocyclic molecules, which are structurally related to MPTP/ MPP⁺, such as isoquinolines and beta-carbolines, have been reported to exhibit similar toxic properties on DA cells, which are conferred by their DAT uptake. Taken together, there is a large body of evidence from morphological, molecular biological and toxicological studies indicating that the DAT might be responsible for the selectivity of DA cell death in PD. Recently, rotenone has given new clues to our current understanding of PD pathogenesis. Rotenone is a classical, high affinity inhibitor of complex I, which has been widely used to know the specific activity of the complex. Unlike MPP⁺, rotenone is extremely lipophilic, by which it freely crosses the blood brain barrier and biological membranes, thus reaching the brain rapidly (83).

3.3.1. *In vivo* rotenone models of Parkinson's disease

Strikingly, *in vivo* rotenone administrations to rodents have mimicked typical histopathological features of PD, including the formation of Lewy bodies. Heikkila and colleagues had already reported that acute stereotaxic injection of rotenone into the rat median forebrain bundle induced striatal DA depletion (84). Subsequent studies administered rotenone orally (85) and subcutaneously (86) without selective damage to the nigrostriatal dopaminergic system. Ferrante *et al* (87) demonstrated that a high-dose intravenous infusion of rotenone for 7–9 days in rats resulted in selective tissue damage in the striatum and globus pallidus, but not in the substantia nigra (87). Using minipumps for chronic intravenous rotenone infusion for 7–35 days, Betarbet and colleagues clearly demonstrated selective pathologic and biochemical changes in the nigrostriatal dopaminergic system (88). In this study, rotenone partially inhibited complex I uniformly throughout the brain accompanied by selective neurodegeneration of the nigrostriatal dopaminergic system. The optimal dose for inducing parkinsonian-like features was determined to be 2-3 mg/kg per day in Lewis rats. A principal limitation of this model is the difficulty associated with jugular vein

Mitochondria and neurodegenerative diseases

administration of rotenone. Sherer *et al* (89) described a simpler but efficient way of administering rotenone by means of chronic subcutaneous exposure to 2.0-3.0 mg/kg/day of rotenone with osmotic minipumps for 28 days. According to the authors, chronic subcutaneous exposure to rotenone induced selective nigro-striatal dopaminergic degeneration and formation of alpha-synuclein-positive cytoplasmic aggregates with no signs of degeneration in other brain areas (89). Chronic administration of rotenone has also been performed by intraperitoneal injections (1.5-2.5 mg/kg/day for 2 months) in Sprague-Dawley rats (90). In this study, there was a dopamine depletion in striatum and prefrontal cortex along with catalepsy. However, high variability observed among different species and the exact nature of the mechanism by which rotenone induces cell death remain to be established.

If behavioural impairment in response to rotenone is due to striatal dopaminergic denervation, then L-DOPA administration should be able to improve motor deficits. Few reports have addressed this issue. Chronic exposure of *Drosophila* to sublethal doses of rotenone is accompanied by selective loss of dopaminergic neurons inducing locomotor deficits (91). In this study, L-DOPA administration prevented locomotor deficits but not neuronal degeneration, while melatonin treatment prevented both locomotor deficits and neuron degeneration, thus suggesting that rotenone-induced cell death, at least in *Drosophila*, results from an oxidative process, and that locomotor deficits are dopamine-dependent. Furthermore, Alam and Schmidt, (90) reported that hypokinetic behaviour, which mimics parkinsonian behavioural symptoms, *e.g.* akinesia and rigidity (catalepsy) in rats, was reversed by L-DOPA treatment. Similar results were found by Alam *et al* (92) in response to bilateral injection of 3 microg of rotenone into the medial forebrain bundle. Under these conditions, rotenone induced a 50 % reduction in striatal dopamine levels along with motor deficits.

With all this a pair of questions arise in the rotenone model of PD: a) what is the mechanism(s) of the rotenone-induced cell death?; b) what makes the nigrostriatal dopaminergic more susceptible to rotenone neurotoxicity ?

3.3.2. Rotenone and oxidative stress

Sherer *et al* (93,94) investigated potential mechanisms of rotenone toxicity. In the first study (93), they used a chronic *in vitro* model based on treating neuroblastoma at low concentrations of rotenone with the aim of partially inhibiting complex I activity to mimic that seen in PD. They found that oxidative damage correlated temporally with alpha-synuclein aggregation and importantly, chronic rotenone treatment sensitized cells to further oxidative challenge. This study would highlight the role of the rotenone-induced oxidative damage to further induce cell death. In keeping with this view, in a different study, Sherer *et al* (94) concluded that rotenone toxicity did not result solely from a bioenergetic defect but rather from oxidative damage. Firstly, in neuroblastoma cells, rotenone caused dose-dependent oxidative damage, and rotenone toxicity was prevented by two antioxidants, alpha-

tocopherol and coenzyme Q₁₀ (61). Secondly, using a chronic midbrain slice model, rotenone-induced oxidative damage and toxicity to mature dopaminergic neurons were also attenuated by antioxidant treatment (61). Thirdly, rotenone infusion caused relatively selective oxidative damage to certain dopamine-rich brain regions known to degenerate in PD, thus suggesting that free radicals originated by rotenone and dopamine metabolism synergistically interact to induce cell death (94). In addition, micromolar Ca²⁺ concentrations in the presence of rotenone strongly increase the detection of ROS production in isolated rat forebrain mitochondria (95). Ca²⁺-stimulated mitochondrial ROS release could thus participate as an important link between the partial mitochondrial complex I inhibition and oxidative damage observed in PD. Sapos *et al* (96) reported that a moderate level of complex I inhibition by rotenone leads to significant ROS formation. Since a partial inhibition of complex I is obtained in response to chronic *in vivo* administration of rotenone, the finding by Sapos *et al* (96) would justify the intense oxidative stress in the rotenone model of PD.

A low dose of rotenone at 1.5 mg kg⁻¹ in Sprague-Dawley rats enhanced NO generation in different brain areas including striatum and frontal cortex along with lipid peroxidation-like products (97), thus suggesting that NO-derived products may participate in the toxicity of rotenone. In keeping with this view, He *et al* (86) found that following the low dose of rotenone (1.5 mg kg⁻¹ i.p.) there is a significant increase of neuronal NOS activity along with increased generation of peroxynitrite in the striatum, which may explain the possible involvement of NO in the induction of selective nigro-striatal dopaminergic damage by rotenone. In fact, administration of the neuronal NOS inhibitor 7-nitroindazole significantly attenuated the increased NOS activity and 3-NT production, and provided significant protection against rotenone-induced nigro-striatal injury (98).

An endoplasmic reticulum oxidative stress response to rotenone which may induce cell death was suggested (99). Accumulation of damaged oxidized proteins could interfere with the cellular protein degradation machinery, thereby causing the ER to retain unfolded proteins (99).

Although it is clear that the main mechanism of rotenone-induced cell death is associated with inhibition of complex I, it should be considered the potential role of activated microglia. Reactive microglia are seen in the basal ganglia following rotenone treatment, even before development of nigrostriatal dopaminergic lesions (100,101). Gao *et al* (98,100) observed the deleterious role of microglial NADPH oxidase in the rotenone model of PD. These studies were conducted in primary neuron-enriched and neuron/glia cultures from rat mesencephalon (102) or NADPH oxidase—null (gp91^{phox-/-}) mice (100). It is yet to be established if a similar effect is seen in adult animals under *in vivo* conditions. NADPH oxidase generates O₂⁻ towards the extracellular space, the effect being toxic to DA neuron. In addition, the increase of intracellular ROS in microglia is critical in the

Mitochondria and neurodegenerative diseases

proinflammatory signalling in professional phagocytes and serves to amplify the production of neurotoxic proinflammatory factors, such as TNF- α (103).

Lipopolysaccharide (LPS) is the active immunostimulant of the cell wall of Gram-negative bacteria that is responsible for triggering the cascade of events following bacterial infection (104,105). A single intranigral injection of LPS induces loss of nigral tyrosine hydroxylase (TH)-immunopositive neurons with concomitant decreases of DA and its metabolites in the nigrostriatal system (106). Interestingly, the SN is especially susceptible to LPS-induced neurotoxicity as compared with other brain areas (107). The neurotoxic effect of LPS has been observed in mesencephalic cell cultures (108-110). Gao *et al* (111) tested the possibility that inflammation and inhibition of complex I may act synergistically to further induce cell death. They used primary mesencephalic dopaminergic cultures that were exposed to non-toxic doses of rotenone and the inflammogen LPS and found that they acted synergistically to induce dopaminergic degeneration (111). NADPH oxidase-mediated production of O₂⁻ in rotenone and LPS-activated microglia appears as an important mediator of the synergistic neurotoxicity (111).

3.3.3. Rotenone and the proteasome.

Two major features of parkinsonian brains are decreased complex I activity and reduced activity of the ubiquitin proteasomal system (UPS). Formation of proteinaceous inclusions is a well-established feature in the rotenone model of PD (88,112). Microinfusion of low doses of the proteasome inhibitors, lactacystin and epoxymycin, in the striatum resulted in the loss of striatal TH-immunoreactive terminals along with retrograde degeneration of substantia nigra dopaminergic neurons and formation of neuronal inclusions reactive to alpha-synuclein (113). With these precedents, Hoglinger *et al*. (114) tested the possibility that inhibition of complex I and reduced activity of the UPS could be related events. Interestingly, the authors found that reduced activity of the UPS increases the vulnerability of mesencephalic neurones *in vitro* to rotenone and that complex I inhibition can decrease proteasomal activity, suggesting a possible mechanism by which the UPS might be impaired in sporadic PD. Further evidence in favour of a relationship between inhibition of complex I activity and dysfunction of the UPS was obtained by Shamoto-Nagai *et al*. (115) that observed that rotenone greatly inhibited the UPS in neuroblastoma SH-SY5Y cells. The reduction in activity was neither due to ATP depletion nor to reduction of the protein level, suggesting that accumulation of oxidized proteins in response to rotenone treatment may underlie the subsequent inhibition of the proteasome (115). Finally, Betarbet *et al*. (116) examined the 20S proteasomal activity in rotenone-infused rats, and found a significant reduction in ventral midbrain regions.

3.3.4. Rotenone and apoptosis

Ahmadi *et al* (117) reported that low concentrations of rotenone induce caspase-dependent apoptosis in mesencephalic dopaminergic neurons, a

process which was significantly inhibited by the caspase-3 inhibitor, DEVD. Rotenone has been shown to induce apoptosis in human dopaminergic SH-SY5Y cells, a process that was demonstrated to be mitochondrial caspase-dependent (118-121). Caspase-12 is an endoplasmic reticulum-specific caspase which is known to participate in endoplasmic reticulum stress-induced apoptosis. Kitamura *et al*. (118) found that rotenone induced the degradation of procaspases-12 before DNA fragmentation and cell death, thus suggesting that endoplasmic reticulum stress is also involved in the rotenone-induced apoptosis. As pointed out earlier, Ryu *et al*. (99) suggested that oxidative stress caused by rotenone may be responsible for inducing endoplasmic reticulum stress. In keeping with this view, formation of ROS has been shown to precede apoptotic events such as caspase-3 activation and poly(ADP-ribose) polymerase cleavage in SH-SY5Y cells (120). Different studies have attempted to establish the apoptotic pathways triggered by rotenone, especially in SH-SY5Y cells. The apoptotic death of dopaminergic cells was preceded by the activation of NF-kappaB (122), p38 and JNK but not ERK 1/2 signalling pathways were reported to participate in the caspase 3 activation in response to rotenone (121). In addition, rotenone was reported to induce Bad dephosphorylation, a pro-apoptotic member of the bcl-2 family, which precedes further activation of caspase 9 and apoptosis (123).

Since apoptosis is a remarkable feature of dopaminergic cells in response to rotenone, different studies tested the potential antiapoptotic properties of different compounds. Thus, treatment of SH-SY5Y cells with different antioxidants including myricetin, fraxetin and N-acetylcysteine prevented the rotenone-induced caspase 3 activation and cell death (120). Cyclopentenone prostaglandin A₁, an inducer of heat shock proteins, dramatically increased cell survival, attenuating both the apoptotic and the necrotic cell deaths of SH-SY5Y in response to rotenone. Search for compounds with neuroprotective properties are encouraging with the aim of finding potential neuroprotective pharmacological strategies to treat Parkinson's disease.

Activation of K_{ATP} channels in PC12 cells has been shown to confer protection against mitochondrial complex-I inhibition-induced cell death (124,125). However, it awaits further elucidation of the signal transduction elements up- and downstream of mitochondrial K_{ATP} channels.

3.3.5. Rotenone and the dopaminergic system

The higher toxicity of rotenone towards the dopaminergic neurons is still under investigation, taking into account that some data suggest more generalized damage. Lapointe *et al*. (126) subjected male Lewis rats to systemic treatment of rotenone via subcutaneous delivery at a dose of 2.5 mg/kg/day and found that 75% of the animals showed signs of illness that required sacrifice. Significantly, autopsy analysis demonstrated peripheral damage to many organs that might be related to hypokinesia seen in different reports. Another major issue dealing with the rotenone model of PD is the high rate of

Mitochondria and neurodegenerative diseases

lethality in response to low doses of rotenone ranging between 2-2.5 mg/kg/day. In the study by Lapointe *et al* (126), less than 20% of all rotenone-treated animals showed loss of striatal dopaminergic denervation. Similar results were found by Fleming *et al* (127). Hoglinger *et al* (112) investigated the differential vulnerability of various brain structures to generalized complex I inhibition induced by intravenously infusion with rotenone (2.5 mg/kg/day) for 28 days in male Lewis rats. The authors found a pronounced loss of spontaneous locomotor activity that did not correlate with a moderate loss of only 55% of striatal dopaminergic fibres, thus suggesting that factors other than dopaminergic denervation may be involved in the behavioural changes induced by rotenone. In keeping with this view, the study of Hoglinger *et al* (112) also found degeneration of non-dopaminergic systems, including striatal dopaminoceptive DARPP-32-immunoreactive projection neurones (dopamine- and cAMP-regulated phosphoprotein) and cholinergic interneurons. In addition, the study of Hoglinger *et al* (112) found degeneration not only in basal ganglia but in different brain stem nuclei as well, concluding that substantia nigra is not more susceptible to generalized inhibition of complex I than other basal ganglia or brain stem nuclei.

However, a specific vulnerability of the dopaminergic system to the inhibition of complex I seems to be unquestionable. Likely the more diffuse damages could be related to the variability observed among different cell types along with different ways of administration and used concentrations.

The highest susceptibility of dopaminergic neurons to rotenone has been described in different types of cells. In culture, rotenone killed more immortalized midbrain dopaminergic differentiated (MN9D) cells than non-dopaminergic differentiated (MN9X) cells (128). Oxidative stress played an important role in rotenone-induced neurodegeneration of MN9X cells. In contrast, disruption of energy supply played a more important role in MN9D cells: rotenone decreased mitochondrial membrane potential and ATP levels in MN9D cells more than in MN9X cells (128). In the study by Betarbet *et al*. (88), rotenone (2-3 mg/kg per day in Lewis rats) partially inhibited complex I uniformly throughout the brain accompanied by selective neurodegeneration of the nigrostriatal dopaminergic system. However, the question that arises deals with the primary target of rotenone action; *i.e.* the cell body region in substantia nigra or the nerve ending region in striatum. Zhu *et al*. (129) have tested the effect of chronic (3 weeks) subcutaneous rotenone infusion (2 mg/kg) on the integrity of the nigrostriatal system in adult male Lewis rats and found that only half of the animals treated with rotenone presented a diffuse reduction in striatal TH-IR staining without significant loss of nigral DA neurons, thus suggesting an earlier effect of rotenone on DA nerve terminals rather than on DA cell bodies. This study would suggest that the striatum is the primary target of rotenone that would precede a subsequent retrograde degeneration of the DA neurons, as initially suggested by Betarbet *et al*. (88). In keeping with this view, longer exposure (4 weeks) has been shown to induce both a

diffuse reduction in striatal TH-IR and a significant loss of DA cell bodies in the substantia nigra (89,112).

The endogenous levels of dopamine have been suggested to contribute to dopaminergic cells selective vulnerability. Sakka *et al*. (130) reported that treatment of mesencephalic cultures with alpha-methyl-p-tyrosine, a tyrosine hydroxylase inhibitor, protected dopaminergic neurons against the chronic treatment with low-concentration of rotenone. Further evidence that dopamine itself contributes to rotenone toxicity is that inhibition of dopamine metabolism significantly reduced rotenone toxicity (131). Maragos *et al*. (132) studied the effect of mitochondrial inhibitors on the striatal DA uptake activity. They found that in spite of all of the toxins inhibiting (³H)DA uptake, there was a large variation in their inhibitory potencies, the rank order being rotenone >> cyanide > azide > 3-NPA >> malonate. This suggests that the mechanism by which extracellular DA is increased by mitochondrial toxins involves factor other than mitochondrial ATP production or oxidative stress. A similar study was carried out by Marey-Semper *et al*. (133) that compared the inhibitory effects of rotenone on the uptake of dopamine, serotonin, noradrenaline and GABA in mouse striatal synaptosomes, and of dopamine, serotonin and GABA in cultured mesencephalic neurons. In both preparations, the uptake of dopamine was much more affected by rotenone than that of other neurotransmitters. Apparently, intrinsic metabolic properties of the nigrostriatal dopaminergic neurons explain the strong inhibition by rotenone of striatal dopamine uptake. We studied the possible constitutive metabolic deficiency that could account, at least in part, for the selective vulnerability of the dopaminergic terminals to the action of rotenone (134) by studying the effect of inhibitors of the respiratory chain (rotenone, antimycin and KCN) on the maximal respiratory rate of synaptosomes and isolated synaptosomal mitochondria from different brain areas, *i.e.* cortex, hippocampus and striatum, and in isolated liver mitochondria. The results showed no differences in the effect of the inhibitors in isolated mitochondria. In contrast, a greater inhibition was found in striatal synaptosomes than in cortical or hippocampal synaptosomes when rotenone was used. Moreover, nomifensine or GBR-12909, inhibitors of the dopamine uptake system, had a protective effect on rotenone inhibition. This study indicates the great importance of the dopamine uptake system in the vulnerability of the striatal dopaminergic system. We reached similar conclusions when the rotenone effect on the nigrostriatal system was tested by microdialysis (135).

A hypothesis, which would justify the selective vulnerability of nigral dopaminergic neurons to the neurotoxic effect of rotenone, relies on the microtubule-depolymerizing activity of rotenone (131). In this study, rotenone-induced microtubule depolymerization on midbrain dopaminergic neurons in culture disrupted vesicular transport along microtubules and caused the accumulation of dopamine vesicles in the soma. This led to increased oxidative stress due to oxidation of cytosolic dopamine leaked from vesicles. Supporting the view that the microtubule-depolymerizing activity of rotenone may

Table 1. Inhibitors of complex I

Pesticides	Natural compounds
Benzimidazole	Acetogenins from Annonaceae plants (custard apple, paw)
Bullatacin	Antibiotics from <i>Myxobacteria</i>
6-Chloro-benzothiadiazole	Piericidins ¹ from <i>Streptomyces strains</i>
Cyhalothrin	Rhein from <i>Rhubarb</i>
Fenazaquin	Rotenoids from <i>Leguminosae plants</i>
Fenpyroximate	
Hoe 110779	
Pyridaben	
Pyrimidifen	
Sandoz 548A	
Tebufenpyrad	
Thiangazole	

¹More potent than Rotenone. References: 134,135.

be involved in the selectivity of rotenone towards the dopaminergic system, toxicity was significantly decreased by the microtubule-stabilizing drug taxol and mimicked by microtubule-depolymerizing agents such as colchicine or nocodazole (131).

3.4.6. Other inhibitors of complex I.

Actually there are some pesticides and natural compounds that are inhibitors of complex I and some of them are used in agriculture (136,137) (Table 1). Some of these compounds could contaminate food, air, etc. and could be involved in the slow degeneration of the dopaminergic system. Its concentration could be especially related to its selectivity. Moreover, this could also depend on the age of the receptor along with genetic susceptibility.

4. TOXINS THAT PRODUCED A NEURODEGENERATION LIKE THAT FOUND IN HUNTINGTON DISEASE: INHIBITION OF SUCCINATE DEHYDROGENASE

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder characterized by involuntary choreiform movements, along with memory disturbances, psychiatric manifestations, frontal type cognitive abnormalities, decreased verbal fluency, and poor recall of recently learned information (138-141). HD patients show progressive cell loss in brain regions belonging to the basal ganglia, particularly the striatum (142,143). The genetic mutation associated with HD has been localized in the gene named *IT15*, on the short arm of chromosome 4, and consists of an enlarged repeat of CAG triplets in the 5' coding region (144). This genetic defect seems to lead to an impairment of oxidative phosphorylation has been proved *in vivo* with the detection, by nuclear magnetic resonance spectroscopy, of an increased concentration of lactate in the cerebral cortex and in the striatum in HD patients (145). Moreover, several studies have reported decreased striatal glucose metabolism (146-151), and abnormalities in the mitochondrial electron transfer chain (152-155).

Ludolph *et al.* (156) showed that contaminant, toxin-generating fungus *Arthrinium spp.*, was the culprit of encephalopathy and the late onset of dystonia appearing in children after the ingestion of sugarcane described in The People's Republic of China. Children seem especially vulnerable to the toxin which produces sudden-onset nausea, vomiting, abdominal pain, and diarrhea; afterward, they develop double vision, somnolence, nystagmus, convulsions, decerebrate rigidity and coma. Computerized tomography of the disabled children reveals bilateral hypodensity of the putamen and, to a lesser extent, the globus pallidum (157,158). The mycotoxin 3-nitropropionic acid (3-NPA) from *Arthrinium* has been reported as the cause of mildewed sugarcane intoxication (156). 3-NPA is an irreversible inhibitor of the Krebs' cycle enzyme succinate dehydrogenase (SDH), localized in the inner mitochondrial membrane and responsible for the oxidation of succinate to fumarate (159,160). The 3-NPA inhibits Complex II of the respiratory chain as well as Krebs' cycle (159).

Animals chronically treated with 3-NPA develop a HD-like syndrome. Chronic systemic administration of low doses of 3-NPA produces excitotoxic-like lesions regionally restricted to striatum in rats (161-164) and non-human primates (165,166). Histologically, these 3-NPA lesions are characterized by marked loss of medium-sized spiny neurons, astrogliosis, dopaminergic axon sparing, preservation of nicotinamide adenine nucleotide phosphate (NADPH)-diaphorase positive striatal interneurons and alterations in the dendritic morphology of medium spiny neurons (163,167). These changes are strikingly similar to those described in HD striatum (168-170). Moreover, animals with 3-NPA lesions also develop behavioral characteristics of HD. Rats chronically treated with low concentration of 3-NPA showed spontaneous motor symptoms including mild dystonia, bradikinesia and gait abnormalities. In these animals, the degree of striatal neuronal loss was significantly correlated to the severity of spontaneous motor abnormalities, as is the case for HD (171). In addition, Palfi *et al.* (166) showed that chronic 3-NPA treatment in primates replicates the basic histological and pathophysiological triad of HD, including frontostriatal syndrome of cognitive impairment.

4.1. Pathways of cells death induced by 3-NPA

The most generally accepted hypothesis for the neuronal cell death induced by 3-NPA has, at least, three different components: a) depletion of ATP levels, produced by a deficit in energy metabolism as a consequence of Complex II inhibition; b) excitotoxicity produced by energy impairment and membrane depolarization along with NMDA toxicity via the release of a voltage-dependent Mg²⁺ block, leading to the influx of Ca²⁺ and subsequent activation of a "cell-death" pathways (167,172-175), and c) oxidative stress produced by the formation of reactive oxygen and nitrogen species, also as a consequence of impaired energy metabolism (176).

4.1.1. Energy depletion

SDH activity was reduced by 75% in whole brain mitochondrial suspensions by the injection of lethal doses

of 3-NPA (177). Similarly, SDH activity in brain showed a profound decrease 30 min after the injection of sublethal doses of 3-NPA, shown by qualitative SDH histochemistry. Brouillet *et al.* (178) demonstrated that the threshold of irreversible inhibition by 3-NPA *in vivo* in the brain to produce neurotoxicity was 50-60% of the control levels. In addition, Pang and Geddes, (179), also described that ATP depletion was an early event in the 3-NPA-induced neuronal degeneration, and that decline in ATP was exacerbated by glutamate in primary cultures of rat hippocampal neurons. Erecinska and Nelson (180), using synaptosomal preparations, described the time course of events produced by 3-NPA toxicity. First of all, there was a decrease in the phosphocreatine/creatine ratio along with an increase in the lactate/pyruvate ratio. Afterward, severe decreases in ATP/ADP and GTP/GDP ratios were produced. Moreover, the administration of either creatine or cyclocreatine leads to increase in the brain concentrations of phosphocreatine and phosphocyclocreatine respectively, with a significant protection against malonate (MA) and 3-NPA neurotoxicity without depletion of ATP (181).

4.1.2. Excitotoxicity

It is well accepted that impairment in energy metabolism, that depletes ATP, causes the failure of ATP-dependent ion pumps and channels which results in depolarization of the cell (182). As glutamatergic neurons depolarize, the Mg^{2+} block in the NMDA receptor channel is released, thus allowing even physiological concentrations of glutamate to activate the receptor channel (163). This activation of NMDA receptors resulting from partial energy impairment could also be, at least partially, independent of the effect of the Mg^{2+} block (175). Activation of the NMDA receptor channel leads to Ca^{2+} influx (183), which cannot be reversed due to the lack of ATP to run the ion antiporter pump. Thus, intracellular Ca^{2+} overload leads to neurotoxicity. Consequently, compromised energy metabolism leads to secondary excitotoxicity and neurodegeneration in glutamate target regions (184), since decortication removing the cortico-striatal glutamatergic input attenuates the lesions (161,162). In addition, cell loss can be prevented by pretreatment with NMDA receptor antagonists. Significant prevention of the neuronal death induced by 3-NPA toxicity *in vitro* and *in vivo*, in spite of the ATP concentration continuing to be severely depleted, has been described (185). Blockade of excitatory transmission by NMDA glutamate receptor antagonists such as MK801 largely prevents striatal damage due to systemic 3-NPA or intrastriatal MA (161,186,187). This shows that the selective blockade of the NMDA receptor is able to reduce neuronal death induced by 3-NPA toxicity (185).

4.1.3. Oxidative stress

This stress is due to the actions of reactive oxygen species (ROS) such as O_2^- and H_2O_2 produced in excess by the mitochondrial respiratory chain. Secondary activation of Ca^{2+} -dependent enzymes such as phospholipase A_2 (production of inflammatory mediators) and nitric oxide synthase (production of the second messenger, NO) also promotes the production of ROS

(188,189). Another risk factor is the excessive release of dopamine, which generates H_2O_2 when metabolized (190).

In vivo lesioning by 3-NPA or MA causes increases of various parameters of oxidative stress, such as 3-nitrotyrosine, 8-hydroxy-2-deoxyguanosine and malondialdehyde (16,191,192). Moreover, 3-NPA induced *in vivo* protein oxidation in striatal and cortical synaptosomes (193). Schulz *et al.* (194) showed that the systemic administration of 3-NPA increased the production of hydroxyl radicals (HO \cdot) in striatum measured by the salicylate assay. Free radicals scavengers, free radical spin trap DMPO and nitric oxide synthase inhibitors significantly attenuated both MA and 3-NPA neurotoxicity (192,194). The selective lesions in the basal ganglia produced by 3-NPA were attenuated in mice, overexpressing Cu,Zn-SOD (193) and in Bcl-2 overexpressing mice, in which the conversion of 4-hydroxybenzoic acid to 3,4-dihydroxybenzoic acid (as a measure of free radical production) was completely blocked (196). In addition, Calkins *et al.* (197), pointed out the implication of nuclear factor erythroid 2-related factor 2, involved in the antioxidant response element, as an essential inducible factor in the protection against complex II inhibitor-mediated neurotoxicity.

4.2. Specificity of the 3-NPA toxicity

There are two different kinds of specificity: the brain structure and the kind of cell mainly affected. The striatum is the most affected brain region (161,162,198-200), and the GABAergic neurons, which make up the bulk of the neurons in the striatum, the cells which are preferentially vulnerable to 3-NPA toxicity, with relative sparing of NADPH-diaphorase and cholinergic neurons (171,199). Glial mitochondria are morphologically well preserved after 3-NPA toxicity (201), and the selective inhibition of tricarboxylic acid cycle of GABAergic neurons after 3-NPA was seen *in vivo*, while the tricarboxylic acid cycle of glial cells remained uninhibited (197). Astrocytes or endothelial cells may be the first to fall, which could be involved in 3-NPA-induced blood-brain barrier disruption (179,202,203).

The excitotoxic nature of these lesions could explain the specific vulnerability of GABAergic neurons and the relative sparing of cholinergic and NADPH-diaphorase positive interneurons. In addition, their deleterious effects could be prevented by prior administration of NMDA-receptor antagonists or removal of glutamatergic striatal afferents. Furthermore, subtoxic doses of the mitochondrial inhibitor malonate, has been shown to potentiate the excitotoxicity of NMDA (204). Both striatum and hippocampus receive glutamatergic inputs which predisposes them to excitotoxic mechanisms. In addition, SDH inhibitors produce long-term potentiation of the NMDA-mediated corticostriatal excitation in spiny neurons, but not in cholinergic interneurons (205). Saule *et al.* (206) have demonstrated that inhibition of mitochondrial complex II produces opposite membrane potential changes in striatal spiny neurons and in cholinergic interneurons. These different effects may well account for the observed differential neuronal vulnerability

Mitochondria and neurodegenerative diseases

of the two cell subtypes following inhibition of mitochondrial complex II. Moreover, the excitotoxic nature of the 3-NPA lesions could also be explained taking into account the similarity between the neurotoxic effect of 3-NPA and quinolinic acid (QA), an agonist of the NMDA receptor. Intrastratial injections of QA as well as systemic administration of 3-NPA have been shown to produce an HD-like pattern of neurodegeneration, including loss of striatal projection neurons and a relative sparing of cholinergic and NADPH-diaphorase positive interneurons (207-209).

4.3. CNS regional vulnerability to toxins

Insults that cause metabolic compromise, such as ischemia, hypoglycemia and respiratory poisons, often lead to striatal and/or hippocampal damage within a few days (for review see 187). Mitochondrial poisons, such as cyanide, carbon monoxide, MPTP, 3-NPA, manganese and rotenone, produce neuropathologies selectively in the basal ganglia after peripheral exposure despite widespread collapse of energy metabolism throughout the entire brain and body. In addition, damage in basal ganglia structures also occurred in humans who attempt suicide by ingesting cyanide (210) or by inhaling carbon monoxide (209), both of which block complex IV of the mitochondrial respiratory chain, which causes prolonged energy impairment. This regional vulnerability is not understood; however, various circumstances could be involved in it.

Blood-brain-barrier (BBB) disruption may play an important role in the regional vulnerability to toxins. Portal of the BBB within the striatum have been found to be especially vulnerable to systemic 3-NPA administration since there is an increased extravasation of IgG into the lesioned striatum (212-214). 3-NPA is first toxic to astrocytes then to neurons both *in vivo* (214) and *in vitro* (215). In addition, the early expression and activation of metalloproteinase-9 by ROS seems to be involved in early BBB disruption and progressive striatal damage after 3-NPA treatment (216). These facts have not been described for other toxins. The existence of specific carrier process should not be forgotten. Paraquat is possibly taken up into the brain by the neutral amino acid transport system, and then transported into brain cells in a Na⁺-dependent manner (217).

Metabolic circumstances. The differences in metabolic activity levels between brain regions could explain selective regional vulnerabilities. Regional glucose utilization in the two most vulnerable areas, striatum and hippocampus, is moderate compared to other brain areas such as the cortex and cerebellum (218). Moreover, while striatum has one of the highest levels of SDH activity in the rat brain, CA1 hippocampus has one of the lowest (219,220), yet both are preferentially vulnerable to systemic 3-NPA.

Glutamatergic target. Gould and Gustine (198) proposed that the vulnerability of both structures is due to secondary excitotoxicity. Striatum is a glutamatergic target field. GABAergic neurons are vulnerable to 3-NPA; however, glutamatergic neurons were not affected. Both

striatum and hippocampus receive glutamatergic inputs which predisposes them to excitotoxic mechanisms.

Dopamine level. Several reports indicate that the neurotransmitter DA contributes to striatal damage induced by impaired energy metabolism. The factor that makes the striatum unique is its high concentration of dopamine, a neurotransmitter that has been implicated in neuronal toxicity (214,221,222). Indeed, removal of DA afferents or depletion of striatal dopamine stores by pretreatment with reserpine (an inhibitor of the vesicular monoamine transporter type-2) in combination with alpha-methyl-p-tyrosine significantly reduces the severity of striatal lesions caused by systemic administration of 3-NPA or intrastratial malonate (190,223,224). Systemic or intrastratial application of L-DOPA or dopamine, respectively, reconstituted malonate toxicity (224). Addition of DA to cultured striatal neurons potentiated apoptotic cell death, caused by methyl malonate (222). Extracellular dopamine concentrations in striatum rise 100 to 300-fold during intraparenchymal infusion of malonate (225,226) and after systemic 3-NPA, and both dopamine and 3-NPA caused an increase in intracellular Ca²⁺ concentrations in cultured astrocytes, indicating a potential for Ca²⁺ overload and subsequent cell death (214). In the striatum of animals chronically treated with 3-NPA/MA an enhanced DA oxidation by MAO is produced (161) with the consequent increases in H₂O₂, as measured by microdialysis (224). This increase in ROS production is one of the dopamine effects that enhanced the neurodegeneration induced by the complex II inhibition. In addition, DA itself enhances neurodegeneration through D2 receptor, since this is protected by sulpiride, a D2 antagonist. Besides, the D2 receptor agonist lisuride, but not the D1 receptor agonist SKF38393, partially restored malonate toxicity in 6-OHDA lesioned rats (224).

Therefore, BBB disruption, metabolic specificity, glutamatergic afferences and dopamine may help to render the striatum selectively vulnerable to the metabolic impairment produced by complex II inhibition.

4.4. Mechanism of neuronal cell death

Cell death produced by 3-NPA *in vitro* and *in vivo* involves apoptosis and necrosis. The excitotoxic necrosis results from excessive NMDA receptor activation and is blocked by the NMDA-receptor antagonist MK801. Delayed apoptosis, that is NMDA receptor-independent, is prevented by a protein synthesis inhibitor, cycloheximide, in striatum and in hippocampus (179,202,227-229), which is also involved in HD (230,231). Interestingly, both excitotoxic and apoptotic features have also been reported for glutamate-induced toxicity (230).

3-NPA/MA induced apoptosis, probably as a consequence of a Ca²⁺ release produced by rapid mitochondrial potential collapse, is not affected by neither thapsigargin nor free- Ca²⁺ medium, along with the increase in ROS generation that overwhelms mitochondrial antioxidant capacity and produces permeability transition (PTP) opening. Interestingly, these effects were attenuated by pre- and cotreatment with vitamin E (232,233). The

Mitochondria and neurodegenerative diseases

increase in Ca^{2+} also justifies the activation of some proteases that are involved in cell death also through apoptosis, such as caspase-9 and 3 (228). In addition, activation of calpain was also described, suggesting that it is essential in 3-NP-induced striatal cell death (234). Peptidyl broad-spectrum caspase inhibitor Q-VD-OPH significantly reduced striatal lesions produced by malonate and 3-NPA (235). Calcineurin has been implicated in neuronal apoptosis induced by insults that elevate cytosolic Ca^{2+} (119,236,237), through dephosphorylation of Bad (a proapoptotic member of the Bcl-2 family) (119). Exposure to FK506, a selective inhibitor of calcineurin (or protein phosphatase 2B) (238,239), decreased the number of apoptotic neurons, cytochrome c release and caspase-3-like activity induced by 3-NPA, along with a decrease in mitochondrial Bax and an increase in mitochondrial Bcl-2 levels (240). Elevations in both bcl-xl and bax, resulting in a detrimental bax/bcl-xl ratio, along with the cytochrome c translocation from the mitochondria to the cytosol, has been reported in the apoptosis process produced by 3-NPA treatment (241). Moreover, activation (by phosphorylation) of the c-Jun N-terminal kinase, followed by its translocation to the nucleus and activation of the transcription factor c-Jun has been shown involved in the induction of striatal degeneration by 3-NPA (242). Yu *et al.* (243) reported that mice lacking the p50 subunit of NF-kappaB exhibit increased damage to striatal neurons following administration of 3-NP. The neuronal death occurs by apoptosis as indicated by increased caspase activation and DNA fragmentation into oligonucleosomes. Cultured striatal neurons from p50^{-/-} mice exhibited enhanced oxidative stress, perturbed Ca^{2+} regulation, and increased cell death following exposure to 3-NP, suggesting a direct adverse effect for the impossibility of activating NF-kappaB.

4.5. Other inhibitors of complexII

L-methylmalonic acid. This is the metabolite accumulated in methylmalonic acidemias, metabolic disorders caused by a severe deficiency of methylmalonyl-CoA mutase activity. L-methylmalonic acid is an inhibitor of SDH (222,244,245) which is one of the main causes of the toxicity.

Glutaric acid (GA). GA type I, is an inborn error of organic acid metabolism associated with acute neurological crises with necrosis of the putamen (246) that resembles the acute putaminal neurodegeneration produced by 3-NP. Moreover, GA type I is caused by the deficiency of glutaryl CoA dehydrogenase. Mitochondrial damage may be mediated by metabolites accumulating proximal to the deficient enzyme like glutaric, 3-hydroxyglutaric and glutaconic acids.

1,3-Dinitrobenzene (DNB), an intermediate in the explosive, dye and plastic industries, induce characteristic lesions of acute energy deprivation syndromes. Both histochemical and spectrophotometric assays confirmed significant inhibition of SDH activity in brainstem and cortical astrocytes (247,248).

3-Nitropropanol, a precursor of 3-NPA present in *Astragalus indogofera* is responsible for acute

encephalopathy in cattle in the western regions of North America (156).

5. CONCLUSIONS

The possible implication of several toxins in neurodegenerative disorders seems to be real, especially taking into account that chronic administration of low concentrations of rotenone and MPTP in rodents produces histopathological changes highly reminiscent of that seen in PD, including the neuronal inclusions called Lewy bodies characteristic of PD. However, the mechanisms that make the dopaminergic neurons sensitive to complex I inhibition are still under investigation, in spite that the phenomenon is especially evident in the rotenone model of PD. Unlike MPP⁺, rotenone is extremely lipophilic and therefore accessible for most cellular systems. One may suggest that neuronal mitochondria could be different or that some endogenous compounds, such as dopamine, synergistically interact with mitochondrial toxins to lead to neurodegeneration only on dopaminergic neurons. The latter is supported by experimental data; *i.e.* the proinflammatory LPS enhances rotenone (111) and MPTP effects (249). Moreover, corticosterone, the primary glucocorticoid in the rat, acts synergistically with sodium azide to inhibit cytochrome oxidase activity producing the potentiation of the sodium azide-induced learning deficit (250).

6. PERSPECTIVES

The results presented in this review show the importance of certain potential endogenous and/or exogenous neurotoxins as risk factors in neurodegeneration. This knowledge of predisposing risk factors may help to establish efficient prophylactic precautions to reduce the incidence of these pathologies.

7. ACKNOWLEDGEMENTS

Financial support from Ministerio de Sanidad FIS PI 031233 and Ministerio de Educación y Ciencia. SAF 203-01996. SAF2004-06601, SAF2005-02847. Spain

8. REFERENCES

1. Bowling AC, E.M.Mutisya, L.C. Walker, D.L. Price, L.C. Cork & M.F. Beal: Age-dependent impairment of mitochondrial function in primate brain. *J Neurochem* 60, 1964-7 (1993)
2. Bowling AC & M.F. Beal: Bioenergetic and oxidative stress in neurodegenerative diseases. *Life Sci* 56, 1151-71 (1995)
3. Valente EM, P.M. Abou-Sleiman, V. Caputo, M.M. Muqit, K. Harvey, S. Gispert, Z. Ali, D. Del Turco, A.R. Bentivoglio, D.G. Healy, A. Albanese, R. Nussbaum, R. Gonzalez-Maldonado, T. Deller, S. Salvi, P. Cortelli, W.P. Gilks, D.S. Latchman, R.J. Harvey, B. Dallapiccola, G. Auburger & N.W. Wood: Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304:1158-60 (2004)

Mitochondria and neurodegenerative diseases

4. Vaughan JR, M.B- Davis & N.W. Wood: Genetics of Parkinsonism: a review. *Ann Hum Genet* 65, 111-126 (2001)
5. Polymeropoulos MH, C. Lavedan, E. Leroy, S.E. Ide, A. Dehejia A, *et al.*: Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276, 2045-47 (1997)
6. Hsu LJ, Y. Sagara, A. Arroyo, E. Rockenstein, A. Sisk, M. Mallory, J. Wong, T. Takenouchi, M. Hashimoto & E. Masliah: Alpha-synuclein promotes mitochondrial deficit and oxidative stress. *Am J Pathol* 157, 401-10 (2000)
7. Lee HJ, S.Y. Shin, C. Choi, Y.H. Lee & S.J. Lee: Formation and removal of alpha-synuclein aggregates in cells exposed to mitochondrial inhibitors. *J Biol Chem* 277, 5411-17 (2002)
8. Orth M, S.J. Tabrizi, A.H. Schapira & J.M. Cooper: Alpha-synuclein expression in HEK293 cells enhances the mitochondrial sensitivity to rotenone. *Neurosci Lett.* 351, 29-32 (2003)
9. Mizuno Y, S. Ohta, M. Tanaka, S. Takamiya, K. Suzuki, T. Sato, H. Oya, T. Ozawa & Y. Kagawa: Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. *Biochem Biophys Res Commun* 163, 1450-5 (1989)
10. Schapira AH, J.M. Cooper, D. Dexter, P. Jenner, J.B. Clark & C.D. Marsden: Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* 1(8649), 1269 (1989)
11. Schapira AH, J.M. Cooper, D. Dexter, J.B. Clark, P. Jenner & C.D. Marsden: Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* 54, 823-7 (1990)
12. Schapira AH, V.M. Mann, J.M. Cooper, D. Dexter, S.E. Daniel, P. Jenner, J.B. Clark & C.D. Marsden: Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. *J Neurochem* 55, 2142-5 (1990)
13. Parker WD Jr, S.J. Boyson & J.K. Parks: Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Ann Neurol.* 26, 719-23 (1989)
14. Mann VM, J.M. Cooper, D. Krige, S.E. Daniel, A.H. Schapira & C.D. Marsden: Brain, skeletal muscle and platelet homogenate mitochondrial function in Parkinson's disease. *Brain* 115, 333-42 (1992)
15. Tandon A, Rogaeva E, Mullan M, St George-Hyslop PH. 2000. Molecular genetics of Alzheimer's disease: the role of beta-amyloid and the presenilins. *Curr Opin Neurol.* 13, 377-84 (2000)
16. Cottrell DA, G. M. Borthwick, M.A. Johnson, P.G. Ince & D.M. Turnbull: The role of cytochrome c oxidase deficient hippocampal neurones in Alzheimer's disease. *Neuropathol Appl Neurobiol* 28, 390-6 (2002)
17. Lustbader JW, M. Cirilli, C. Lin, H.W. Xu, K. Takuma, N. Wang, C. Caspersen, X. Chen, S. Pollak, M. Chaney, F. Trinchese, S. Liu, F. Gunn-Moore, L.F. Lue, D.G. Walker, P. Kuppusamy, Z.L. Zewier, O. Arancio, D. Stern, S.S. Yan & H. Wu: ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* 304, 448-52 (2004)
18. Canevari L, J.B. Clark & T.E. Bates: Beta-Amyloid fragment 25-35 selectively decreases complex IV activity in isolated mitochondria. *FEBS Lett* 457,131-34 (1999)
19. Valla J, J.D. Berndt & F. Gonzalez-Lima: Energy hypometabolism in posterior cingulate cortex of Alzheimer's patients: superficial laminar cytochrome oxidase associated with disease duration. *J Neurosci* 21, 4923-30 (2001)
20. Trimmer PA, P.M. Keeney, M.K. Borland, F.A. Simon, J. Almeida, R.H. Swerdlow, J.P. Parks, W.D.Jr Parker & J.P. Jr Bennett: Mitochondrial abnormalities in cybrid cell models of sporadic Alzheimer's disease worsen with passage in culture. *Neurobiol Dis* 15, 29-39 (2004)
21. Rowland LP & N.A. Shneider NA: Amyotrophic lateral sclerosis. *N Engl J Med* 344, 1688-700 (2001)
22. Jaarsma D, F. Rognoni, W. van Duijn, H.W. Verspaget, E.D. Haasdijk & J.C. Holstege: CuZn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. *Acta Neuropathol (Berl)* 102, 293-305 (2001)
23. Sawa A, G.W. Wiegand, J. Cooper, R.L. Margolis, A.H. Sharp, J.F. Jr Lawler, J.T. Greenamyre, S.H. Snyder & C.A. Ross: Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. *Na Med* 5, 1194-98 (1999)
24. Panov AV, C.A. Gutekunst, B.R. Leavitt, M.R. Hayden, J.R. Burke, W.J. Strittmatter & J.T. Greenamyre: Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci* 5, 731-36 (2002)
25. Horton TM, B.H. Graham, M. Corral-Debrinski, J.M. Shoffner, A.E. Kaufman, M.F. Beal & D.C Wallace: Marked increase in mitochondrial DNA deletion levels in the cerebral cortex of Huntington's disease patients. *Neurology* 45, 1879-83 (1995)
26. Wallace DC: A Mitochondrial Paradigm of Metabolic and Degenerative Diseases, Aging, and Cancer: A Dawn for Evolutionary Medicine. *Annu Rev Genet* 39, 359-407 (2005)
27. Hebert LE, P.A. Scherr, J.L. Bienias, D.A. Bennett DA & D.A. Evans: Alzheimer disease in the US population:

Mitochondria and neurodegenerative diseases

- prevalence estimates using the 2000 census. *Arch Neurol* 60, 1119–22 (2003)
28. Obeso JA, C.W. Olanow & J.G. Nutt: Levodopa motor complications in Parkinson's disease. *Trends Neurosci* 23, S2–7 (2000)
29. Wallace DC: Mitochondrial diseases in man and mouse. *Science* 283, 1482–88 (1999)
30. De la Rúa-Domenech R, H.O. Mohammed, J.F. Cummings, T.J. Divers, A. De Lahunta & B.A. Summers: Association between plasma vitamin E concentration and the risk of equine motor neuron disease. *Vet J* 154, 203-13 (1997)
31. Cox PA, S.A. Banack & S.J. Murch: Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. *Proc Natl Acad Sci U S A* 100, 13380-3 (2003)
32. Nagatsu T: Amine-related neurotoxins in Parkinson's disease: past, present, and future. *Neurotoxicol Teratol* 24, 565-9 (2002)
33. Mouradian MM: Recent advances in the genetics and pathogenesis of Parkinson disease. *Neurology* 58, 179-85 (2002)
34. Paganini-Hill A: Risk factors for Parkinson's disease: the leisure world cohort study. *Neuroepidemiology* 20, 118-24 (2001)
35. Siderowf A: Parkinson's disease: clinical features, epidemiology and genetics. *Neurol Clin* 19, 565-78 (2001)
36. Zhang Y, V.L. Dawson & T.M. Dawson: Oxidative stress and genetics in the pathogenesis of Parkinson's disease. *Neurobiol Dis* 7, 240-50 (2000)
37. Gorell JM, C.C. Johnson, B.A. Rybicki, E.L. Peterson & R.J. Richardson: The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. *Neurology* 50, 1346-50 (1998)
38. Langston JW, P. Ballard, J.W. Tetrud & I. Irwin: Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219, 979–980 (1983)
39. Burns RS, C.C. Chiueh, S.P. Markey, M.H. Ebert, D.M. Jacobowitz & I.J. Kopin: A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci USA* 80, 4546-4550 (1983)
40. Javitch JA, R.J. D'Amato, S.M. Strittmatter & S.H. Snyder: Parkinsonism inducing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite 1-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci USA* 82, 2173-2177 (1985)
41. Ramsay RR, J.I. Salach, J. Dadgar & T.P. Singer: Inhibition of mitochondrial NADH dehydrogenase by pyridine derivatives and its possible relation to experimental and idiopathic parkinsonism. *Biochem Biophys Res Commun* 135, 269-75 (1986)
42. Nicklas WJ, S.K. Youngster, M.V. Kindt & R.E. Heikkila: MPTP, MPP⁺ and mitochondrial function. *Life Sci.* 40, 721-729 (1987)
43. Davey GP, K.F. Tipton & M.P. Murphy: Uptake and accumulation of 1-methyl-4-phenylpyridinium by rat liver mitochondria measured using an ion -selective electrode. *Biochem J* 288, 439-433 (1992)
44. Storch A, A.C. Ludolph & J. Schwarz: Dopamine transporter: involvement in selective dopaminergic neurotoxicity and degeneration. *J Neural Transm* 111, 1267–1286 (2004)
45. Hirst J, J. Carroll, I.M. Fearnley, R.J. Shannon & J.E. Walker: The nuclear encoded subunits of complex I from bovine heart mitochondria. *Biochim Biophys Acta* 1604, 135-50 (2003)
46. Fallon J, R.T. Matthews, B.T. Hyman & M.F. Beal: MPP⁺ produces progressive neuronal degeneration which is mediated by oxidative stress. *Exp Neurol* 144, 193-8 (1997)
47. Beal MF: Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Ann Neurol* 31, 119–130 (1992)
48. Turrens JF: Mitochondrial formation of reactive oxygen species. *J Physiol* 552, 335-44 (2003)
49. Lambert AJ & M.D. Brand: Superoxide production by NADH:ubiquinone oxidoreductase (complex I) depends on the pH gradient across the mitochondrial inner membrane. *Biochem J* 382, 511-7 (2004)
50. Beal MF: Mitochondria take center stage in aging and neurodegeneration. *Ann Neurol* 58, 495-505 (2005)
51. Vinogradov AD & V.G. Grivennikova: Generation of superoxide-radical by the NADH:ubiquinone oxidoreductase of heart mitochondria. *Biochemistry* 70, 120-7 (2005)
52. Nicholls DG: Mitochondrial function and dysfunction in the cell: its relevance to aging and aging-related disease. *Int J Biochem Cell Biol* 34, 1372-81 (2002)
53. Raha S & B.H. Robinson: Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem Sci* 25, 502-8 (2000)
54. Boveris A, E. Cadenas & A. O. Stoppani: Role of ubiquinone in the mitochondrial generation of hydrogen peroxide. *Biochem. J* 156, 435-444 (1976)

Mitochondria and neurodegenerative diseases

55. Cadenas E, A. Boveris, C. I. Ragan & A. O. Stoppani: Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. *Arch. Biochem. Biophys.* 180, 248-257 (1977)
56. Zhang L, L. Yu & C.A. Yu: Generation of superoxide anion by succinate-cytochrome c reductase from bovine heart mitochondria. *J Biol Chem* 273, 33972-6 (1998)
55. Li N, K. Ragheb, G. Lawler, J. Sturgis, B. Rajwa, J.A. Melendez & J.P. Robinson: Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J Biol Chem* 278, 8516-25 (2003)
56. Dawson TM & V.L. Dawson: Molecular pathways of neurodegeneration in Parkinson's disease. *Science* 302, 819-22 (2003)
57. Dawson TM & V.L. Dawson: Neuroprotective and neurorestorative strategies for Parkinson's disease. *Nat Neurosci* 5, 1058-61 (2002)
58. Sherer TB, R. Betarbet R & J.T. Greenamyre: Environment, mitochondria, and Parkinson's disease. *Neuroscientist* 8,192-7 (2002)
59. Sherer TB, P.A. Trimmer, K. Borland, J.K. Parks, J.P. Jr Bennett & J.B. Tuttle: Chronic reduction in complex I function alters calcium signalling in SH-SY5Y neuroblastoma cells. *Brain Res* 891, 94-105 (2001)
60. Dauer W & S. Przedborski: Parkinson's disease: mechanisms and models. *Neuron* 39, 889-909 (2003)
61. Giasson BI, Lee VM. Are ubiquitination pathways central to Parkinson's disease? *Cell.* 114, 1-8 (2003)
62. Fornai F, O.M. Schluter, P. Lenzi, M. Gesi, R. Ruffoli, M. Ferrucci, G. Lazzeri, C.L. Busceti, F. Pontarelli, G. Battaglia, A. Pellegrini, F. Nicoletti, S. Ruggieri, A. Paparelli & T.C. Sudhof: Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proc Natl Acad Sci U S A* 102, 3413-8 (2005)
63. Collins MA & E.J. Neafsey: Potential neurotoxic "agents provocateurs" in Parkinson's disease. *Neurotoxicol Teratol* 24, 571-7 (2002)
64. Nagatsu, T: Isoquinolines neurotoxins in the brain and Parkinson's disease. *Neurosci Res* 29, 99-111 (1997)
65. McNaught KS, P.A. Carrupt, C. Altomare, S. Cellamare, A. Carotti, B. Testa, P. Jenner & C.D. Marsden: Isoquinoline derivatives as endogenous neurotoxins in the aetiology of Parkinson's disease. *Biochem Pharmacol* 56, 921-33 (1998)
66. Maruyama W, G. Sobue, K. Matsubara, Y. Hashizume, P. Dostert & M. Naoi: A dopaminergic neurotoxin, 1(R), 2(N)-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, N-methyl(R)salsolinol, and its oxidation product, 1,2(N)-dimethyl-6,7-dihydroxyisoquinolinium ion, accumulate in the nigro-striatal system of the human brain. *Neurosci Lett* 223, 61-4 (1997)
67. Naoi M, S. Matsuura, T. Takahashi & T. Nagatsu: A N-methyltransferase in human brain catalyses N-methylation of 1,2,3,4-tetrahydroisoquinoline into N-methyl-1,2,3,4-tetrahydroisoquinoline, a precursor of a dopaminergic neurotoxin, N-methylisoquinolinium ion. *Biochem Biophys Res Commun* 161, 1213-9 (1989)
68. Suzuki T, S. Fujita, S. Narimatsu, Y. Masubuchi, M. Tachibana, S. Ohta & M. Hirobe: Cytochrome P450 isozymes catalyzing 4-hydroxylation of parkinsonism-related compound 1,2,3,4-tetrahydroisoquinoline in rat liver microsomes. *FASEB J* 6, 771-6 (1992)
69. Morikawa N, M. Naoi, W. Maruyama, S. Ohta, Y. Kotake, H. Kawai, T. Niwa, P. Dostert & Y. Mizuno: Effects of various tetrahydroisoquinoline derivatives on mitochondrial respiration and the electron transfer complexes. *J Neural Transm* 105, 677-88 (1998)
70. Antkiewicz-Michaluk L, I. Romanska, I. Papla, J. Michaluk, M. Bakalarz, J. Vetulani, A. Krygowska-Wajs & A. Szczudlik: Neurochemical changes induced by acute and chronic administration of 1,2,3,4-tetrahydroisoquinoline and salsolinol in dopaminergic structures of rat brain. *Neuroscience* 96, 59-64 8 (2000)
71. McNaught KS, U. Thull, P.A. Carrupt, C. Altomare, S. Cellamare, A. Carotti, B. Testa, P. Jenner & C.D. Marsden: Effects of isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on mitochondrial respiration. *Biochem Pharmacol* 51, 1503-11 (1996)
72. Seaton TA, J.M. Cooper & A.H. Schapira: Free radical scavengers protect dopaminergic cell lines from apoptosis induced by complex I inhibitors. *Brain Res* 777, 110-8 (1997)
73. Niwa T, N. Takeda, N. Kaneda, Y. Hashizume & T. Nagatsu: Presence of tetrahydroisoquinoline and 2-methyl-tetrahydroquinoline in parkinsonian and normal human brains. *Biochem Biophys Res Commun* 144, 1084-9 (1987)
74. Saitoh T, Y. Mizuno, T. Nagatsu & M. Yoshida: Effect of long-term administration of 1,2,3,4-tetrahydroisoquinoline (TIQ) on striatal dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) content in mice. *Neurosci Lett* 92, 321-4 (1988)
75. Kohno M, S. Ohta & M. Hirobe: Tetrahydroisoquinoline and 1-methyl-tetrahydroisoquinoline as novel endogenous amines in rat brain. *Biochem Biophys Res Commun* 40, 448-54 (1986)

Mitochondria and neurodegenerative diseases

76. Ayala A, J. Parrado, J. Cano & A. Machado: Reduction of 1-methyl 1,2,3,4-tetrahydroisoquinoline level in substantia nigra of the aged rat. *Brain Res* 638, 334-6 (1994)
77. Absi E, A. Ayala, A. Machado & J. Parrado: Protective effect of melatonin against the 1-methyl-4-phenylpyridinium-induced inhibition of complex I of the mitochondrial respiratory chain. *J Pineal Res.* 29, 40-7 (2000)
78. Parrado J, E. Absi, A. Ayala, A. Castano, J. Cano & A. Machado: The endogenous amine 1-methyl-1,2,3,4-tetrahydroisoquinoline prevents the inhibition of complex I of the respiratory chain produced by MPP(+). *J Neurochem* 75, 65-71 (2000)
79. Ramsay RR, M.J. Krueger, S.K. Youngster & T.P. Singer: Evidence that the inhibition sites of the neurotoxic amine 1-methyl-4-phenylpyridinium (MPP+) and of the respiratory chain inhibitor piericidin A are the same. *Biochem J* 273, 481-4 (1991)
80. Absi E, J. Parrado J, A. Ayala & A. Machado: Decrease of 1-methyl-1,2,3,4-tetrahydroisoquinoline synthesizing enzyme activity in the brain areas of aged rat. *Brain Res* 955, 161-3 (2002)
81. Talpade DJ, J.G. Greene, D.S. Jr Higgins & J.T. Greenamyre: *In vivo* labelling of mitochondrial complex I (NADH:ubiquinone oxidoreductase) in rat brain using [(3)H]dihydrorotenone. *J Neurochem* 75, 2611-2621 (2000)
82. Heikkila RE, W.J. Nicklas, I. Vyas & R.C. Duvoisin: Dopaminergic toxicity of rotenone and the 1-methyl-4-phenylpyridinium ion after their stereotaxic administration to rats: implication for the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. *Neurosci Lett* 62, 389-94 (1985)
83. Marking L: Oral toxicity of rotenone to mammals. *U. S. Fishand Wildlife Serv. Investigations in Fish Control* 94, 1-5 (1988)
84. Thiffault C, J.W. Langston & D.A. Di Monte: Increased striatal dopamine turnover following acute administration of rotenone to mice. *Brain Res* 885, 283-288 (2000)
85. Ferrante RJ, J.B. Schulz, N.W. Kowall & M.F. Beal: Systemic administration of rotenone produces selective damage in the striatum and globus pallidus, but not in the substantia nigra. *Brain Res* 753, 157-162 (1997)
86. Betarbet R, T.B. Sherer, G. MacKenzie, M. Garcia-Osuna, A.V. Panov & J.T. Greenamyre: Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3, 1301-1306 (2000)
87. Sherer TB, J.H. Kim, R. Betarbet & J.T. Greenamyre: Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp Neurol* 179, 9-16 (2003)
88. Alam M & W.J. Schmidt: L-DOPA reverses the hypokinetic behaviour and rigidity in rotenone-treated rats. *Behav Brain Res* 153, 439-46 (2004)
89. Coulom H & S. Birman: Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila melanogaster*. *J Neurosci* 24, 10993-10998 (2004)
90. Alam M, A. Mayerhofer & W.J. Schmidt: The neurobehavioral changes induced by bilateral rotenone lesion in medial forebrain bundle of rats are reversed by L-DOPA. *Behav Brain Res* 151, 117-124 (2004)
91. Sherer TB, R. Betarbet, A.K. Stout, S. Lund, M. Baptista, A.V. Panov, M.R. Cookson & J.T. Greenamyre: An *in vitro* model of Parkinson's disease: linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. *J Neurosci* 22, 7006-7015 (2002)
92. Sherer TB, R. Betarbet, C.M. Testa, B.B. Seo, J.R. Richardson, J.H. Kim, G.W. Miller, T. Yagi, A. Matsuno-Yagi & J.T. Greenamyre: Mechanism of toxicity in rotenone models of Parkinson's disease. *J Neurosci* 23, 10756-10764 (2003)
93. Sousa SC, E.N. Maciel, A.E. Vercesi & R.F. Castilho: Ca²⁺-induced oxidative stress in brain mitochondria treated with the respiratory chain inhibitor rotenone. *FEBS Lett* 543, 179-183 (2003)
94. Sipos I, L. Tretter & V. Adam-Vizi: Quantitative relationship between inhibition of respiratory complexes and formation of reactive oxygen species in isolated nerve terminals. *J Neurochem* 84, 112-118 (2003)
95. Bashkatova V, M. Alam, A. Vanin & W.J. Schmidt: Chronic administration of rotenone increases levels of nitric oxide and lipid peroxidation products in rat brain. *Exp Neurol* 186, 235-241 (2004)
96. He Y, S.Z. Imam, Z. Dong, J. Jankovic, S.F. Ali, S.H. Appel & W. Le W: Role of nitric oxide in rotenone-induced nigro-striatal injury. *J Neurochem* 86, 1338-1345 (2003)
97. Ryu EJ, H.P. Harding, J.M. Angelastro, O.V. Vitolo, D. Ron & L.A. Greene: Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. *J Neurosci* 22, 10690-10698 (2002)
98. Gao HM, B. Liu & J.S. Hong: Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. *J Neurosci* 23, 6181-6187 (2003)
99. Sherer TB, R. Betarbet, J.H. Kim & J.T. Greenamyre: Selective microglial activation in the rat rotenone model of Parkinson's disease. *Neurosci Lett* 341, 87-90 (2003)

Mitochondria and neurodegenerative diseases

100. Gao HM, J.S. Hong, W. Zhang & B. Liu: Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. *J Neurosci* 22, 782-790 (2002)
101. Qin L, M.L. Block, Y. Liu, R.J. Bienstock, Z. Pei, W. Zhang, X. Wu, B. Wilson, T. Burka & J.S. Hong: Microglial NADPH oxidase is a novel target for femtomolar neuroprotection against oxidative stress. *FASEB J* 19, 550-557 (2005)
102. Kielian TL & F. Blecha: CD14 and other recognition molecules for lipopolysaccharide: a review. *Immunopharmacology* 29, 187-205 (1995)
103. Holst O, A.J. Ulmer, H. Brade, H.D. Flad & E.T. Rietschel: Biochemistry and cell biology of bacterial endotoxins. *FEMS Immunol Med Microbiol* 16, 83-104 (1996)
104. Castano A, A.J. Herrera, J. Cano & A. Machado: Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *J Neurochem* 70, 1584-1592 (1998)
105. Herrera AJ, A. Castano, J.L. Venero, J. Cano & A. Machado: The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system. *Neurobiol Dis* 7, 429-447 (2000)
106. Liu B, L. Du & J.S. Hong: Naloxone protects rat dopaminergic neurons against inflammatory damage through inhibition of microglia activation and superoxide generation. *J Pharmacol Exp Ther* 293, 607-617 (2000)
107. Gayle DA, Z. Ling, C. Tong, T. Landers, J.W. Lipton & P.M. Carvey: Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor- α , interleukin-1 β , and nitric oxide. *Brain Res Dev Brain Res* 133, 27-35 (2002)
108. Jeohn GH, C.L. Cooper, B. Wilson, R.C. Chang, K.J. Jang, H.C. Kim, B. Liu & J.S. Hong: p38 MAP kinase is involved in lipopolysaccharide-induced dopaminergic neuronal cell death in rat mesencephalic neuron-glia cultures. *Ann NY Acad Sci* 62, 332-346 (2002)
109. Gao HM, J.S. Hong, W. Zhang & B. Liu: Synergistic dopaminergic neurotoxicity of the pesticide rotenone and inflammogen lipopolysaccharide: relevance to the aetiology of Parkinson's disease. *J Neurosci* 23, 1228-1236 (2003)
110. Hoglinger GU, J. Feger, A. Prigent, P.P. Michel, K. Parain, P. Champy, M. Ruberg, W.H. Oertel & E.C. Hirsch: Chronic systemic complex I inhibition induces a hypokinetic multisystem degeneration in rats. *J Neurochem* 84, 491-502 (2003)
111. Fornai F, P. Lenzi, M. Gesi, M. Ferrucci, G. Lazzeri, C.L. Busceti, R. Ruffoli, P. Soldani, S. Ruggieri, M.G. Alessandri & A. Paparelli: Fine structure and biochemical mechanisms underlying nigrostriatal inclusions and cell death after proteasome inhibition. *J Neurosci* 23, 8955-8966 (2003)
112. Hoglinger GU, G. Carrard, P.P. Michel, F. Medja, A. Lombs, M. Ruberg, B. Friguet & E.C. Hirsch: Dysfunction of mitochondrial complex I and the proteasome: interactions between two biochemical deficits in a cellular model of Parkinson's disease. *J Neurochem* 86, 1297-1307 (2003)
113. Shamoto-Nagai M, W. Maruyama, Y. Kato, K. Isobe, M. Tanaka, M. Naoi & T. Osawa: An inhibitor of mitochondrial complex I, rotenone, inactivates proteasome by oxidative modification and induces aggregation of oxidized proteins in SH-SY5Y cells. *J Neurosci Res* 74, 589-597 (2003)
114. Betarbet R, T.B. Sherer & J.T. Greenamyre: Ubiquitin-proteasome system and Parkinson's diseases. *Exp Neurol* 191, Suppl 1:S17-27 (2005)
115. Ahmadi FA, D.A. Linseman, T.N. Grammatopoulos, S.M. Jones, R.J. Bouchard, C.R. Freed, K.A. Heidenreich & W.M. Zawada: The pesticide rotenone induces caspase-3-mediated apoptosis in ventral mesencephalic dopaminergic neurons. *J Neurochem* 87, 914-921 (2003)
116. Kitamura Y, M. Inden, A. Miyamura, J. Kakimura, T. Taniguchi & S. Shimohama: Possible involvement of both mitochondria- and endoplasmic reticulum-dependent caspase pathways in rotenone-induced apoptosis in human neuroblastoma SH-SY5Y cells. *Neurosci Lett* 333, 25-28 (2002)
117. Wang HG, N. Pathan, I.M. Ethell, S. Krajewski, Y. Yamaguchi, F. Shibasaki, F. McKeon, T. Bobo, T.F. Franke & J.C. Reed: Ca²⁺-induced apoptosis through calcineurin dephosphorylation of BAD. *Science* 284, 339-43 (1999)
118. Molina-Jimenez MF, M.I. Sanchez-Reus & J. Benedi: Effect of fraxetin and myricetin on rotenone-induced cytotoxicity in SH-SY5Y cells: comparison with N-acetylcysteine. *Eur J Pharmacol* 472, 81-87 (2003)
119. Newhouse K, S.L. Hsuan, S.H. Chang, B. Cai, Y. Wang & Z. Xia: Rotenone-induced apoptosis is mediated by p38 and JNK MAP kinases in human dopaminergic SH-SY5Y cells. *Toxicol Sci* 79, 137-146 (2004)
120. Wang X, Z.H. Qin, Y. Leng, Y. Wang, X. Jin, T.N. Chase & M.C. Bennett: Prostaglandin A1 inhibits rotenone-induced apoptosis in SH-SY5Y cells. *J Neurochem* 83, 1094-1102 (2002)
121. Watabe M & T. Nakaki: Rotenone induces apoptosis via activation of bad in human dopaminergic SH-SY5Y cells. *J Pharmacol Exp Ther* 311, 948-953 (2004)
122. Tai KK & D.D. Truong: Activation of adenosine triphosphate-sensitive potassium channels confers

Mitochondria and neurodegenerative diseases

- protection against rotenone-induced cell death: therapeutic implications for Parkinson's disease. *J Neurosci Res* 69, 559-566 (2002)
123. Tai KK, Z.A. McCrossan & G.W. Abbott: Activation of mitochondrial ATP-sensitive potassium channels increases cell viability against rotenone-induced cell death. *J Neurochem* 84, 1193-1200 (2003)
124. Lapointe N, M. St-Hilaire, M.G. Martinoli, J. Blanchet, P. Gould, C. Rouillard & F. Cicchetti: Rotenone induces non-specific central nervous system and systemic toxicity. *FASEB J* 18, 717-719 (2004)
125. Fleming SM, C. Zhu, P.O. Fernagut, A. Mehta, C.D. DiCarlo, R.L. Seaman & M.F. Chesselet: Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. *Exp Neurol* 187, 418-29 (2004)
126. Kweon GR, J.D. Marks, R. Krencik, E.H. Leung, P.T. Schumacker, K. Hyland & U.J. Kang: Distinct mechanisms of neurodegeneration induced by chronic complex I inhibition in dopaminergic and non-dopaminergic cells. *J Biol Chem* 279, 51783-51792 (2004)
127. Zhu C, P. Vourc'h, P.O. Fernagut, S.M. Fleming, S. Lacan, C.D. DiCarlo, R.L. Seaman & M.F. Chesselet: Variable effects of chronic subcutaneous administration of rotenone on striatal histology. *J Comp Neurol* 478, 418-26 (2004)
128. Sakka N, H. Sawada H, Y. Izumi Y, T. Kume T, H. Katsuki, S. Kaneko, S. Shimohama & A. Akaike: Dopamine is involved in selectivity of dopaminergic neuronal death by rotenone. *Neuroreport* 14, 2425-2458 (2003)
129. Ren Y, W. Liu, H. Jiang, Q. Jiang & J. Feng: Selective vulnerability of dopaminergic neurons to microtubule depolymerization. *J Biol Chem* 280, 34105-34112 (2005)
130. Maragos WF, J. Zhu, M.D. Chesnut & L.P. Dvoskin: Mitochondrial toxin inhibition of [(3)H]dopamine uptake into rat striatal synaptosomes. *Biochem Pharmacol* 63, 1499-505(2002)
131. Marey-Semper I, M. Gelman & M. Levi-Strauss: The high sensitivity to rotenone of striatal dopamine uptake suggests the existence of a constitutive metabolic deficiency in dopaminergic neurons from the substantia nigra. *Eur J Neurosci* 5, 1029-34 (1993)
132. Bougria M, J. Vitorica, J. Cano & A. Machado: Implication of dopamine transporter system on 1-methyl-4-phenylpyridinium and rotenone effect in striatal synaptosomes. *Eur J Pharmacol* 291, 407-15 (1995)
133. Santiago M, L. Granero L, A. Machado & J. Cano: Complex I inhibitor effect on the nigral and striatal release of dopamine in the presence and absence of nomifensine. *Eur J Pharmacol* 280, 251-6 (1995)
134. Degli Esposti M. Inhibitors of NADH ubiquinone reductase: an overview. *Biochim Biophys Acta* 1364, 222-35 (1998)
135. Lümnen P. Complex I inhibitors as insecticides and acaricides. *Biochim Biophys Acta* 1364, 287-96 (1998)
136. Butters N, D. Sax, K. Montgomery & S. Tarlow: Comparison of the neuropsychological deficits associated with early and advanced Huntington's disease. *Arch Neurol* 35, 585-589 (1978)
137. Pillon B, B. Dubois, A. Ploska & Y. Agid: Severity and specificity of cognitive impairment in Alzheimer's, Huntington's, and Parkinson's diseases and progressive supranuclear palsy. *Neurology* 41, 634-643 (1991)
138. Randolph C. Implicit, explicit, and semantic memory functions in Alzheimer's disease and Huntington's disease. *J Clin Exp Neuropsychol* 13, 479-494 (1991)
139. Diamond R, R.F. White, R. Myers, C. Mastromauro, W.J. Koroshetz, N. Butters, D.M. Rothstein, M.B. Moss & J. Varterling: Evidence of presymptomatic cognitive decline in Huntington's disease. *J Clin Exp Neuropsychol* 14, 961-975 (1992)
140. Martin JB & J. Gusella: Huntington's disease: pathogenesis and management. *N Engl J Med* 315, 1267-1276 (1986)
141. Kremer B, B. Weber B & M.R. Hayden: New insights into the clinical features, pathogenesis and molecular genetics of Huntington's disease. *Brain Pathol* 2, 321-335 (1992)
142. The Huntington's Disease Collaborative Research Group: A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* 72, 971-83 (1993)
143. Jenkins BG, W.J. Koroshetz, M.F. Beal & B.R. Rosen: Evidence for impairment of energy metabolism *in vivo* in Huntington's disease using localized proton NMR spectroscopy. *Neurology*. 43, 2689-2695 (1993)
144. Kuhl DE, M.E. Phelps, C.H. Markham, E.J. Metter, W.H. Riege & J. Winter: Cerebral metabolism and atrophy in Huntington's disease determined by 18FDG and computed tomographic scan. *Ann Neurol* 12, 425-434 (1982)
145. Young AB, J.B. Penney, S. Starosta-Rubenstein, D.S. Markel, S. Berent, B. Giordani, R. Ehrenkauffer, D. Jewett & R. Hichwa: PET scan investigations of Huntington's disease: cerebral metabolic correlates of neurologic features and functional decline. *Ann Neurol* 20, 296-303 (1986)
146. Mazziotta JC, M.E. Phelps, J.J. Pahl, S.C. Huang, L.R. Baxter, W.H. Riege, J.M. Hoffmann, D.E. Kuhl, A.B.

Mitochondria and neurodegenerative diseases

- Lanto, J.A. Wapenski & C.H. Markham: Reduced cerebral glucose metabolism in asymptomatic subjects at risk for Huntington's disease. *N Engl J Med* 316, 357–362 (1987)
147. Berent S, B. Giordani, S. Lehtinen, D. Markel, J.B. Penney, H.A. Buchtel, S. Starosta-Rubinstein, R. Hichwa & A.B. Young: Positron emission tomographic scan investigations of Huntington's disease: cerebral metabolic correlates of cognitive function. *Ann Neurol* 23, 541–546 (1988)
148. Kuwert T, H.W. Lange, K.J. Langen, H. Herzog, A. Aulich & L.E. Feinendegen: Cortical and subcortical glucose consumption measured by PET in patients with Huntington's disease. *Brain* 113, 1405–1423 (1990)
149. Martin WRW, C. Clark, W. Ammann, A.J. Stoessl, W. Shtyel & M.R. Hayden: Cortical glucose metabolism in Huntington's disease. *Neurology* 42, 223–229 (1992)
150. Brennan WAJ, E.D. Bird & J.R. Aprille: Regional mitochondrial respiratory activity in Huntington's disease brain. *J Neurochem* 44, 1948–1950 (1985)
151. Mann VM, J.M. Cooper, F. Javoy-Agid, Y. Agid, P. Jenner & A.H.V. Schapira: Mitochondrial function and parental sex effect in Huntington's disease. *Lancet* 336, 749 (1990)
152. Parker Jr WD, S.J. Boyson, A.S. Luder & J.K. Parks: Evidence for a defect in NADH: ubiquinone oxidoreductase (complex I) in Huntington's disease. *Neurology* 40, 1231–1234 (1990)
153. Browne SE, A.C. Bowling, U. MacGarvey, M.J. Baik, S.C. Berger, M.M. Muqit, E.D. Bird & M.F. Beal: Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Ann Neurol* 41, 646–653 (1997)
154. Ludolph AC, F. He, P.S. Spencer, J. Hammerstad & M. Sabri: 3-Nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin. *Can J Neurol Sci* 18, 492-8 (1991).
155. Liu X, X. Luo X & W. Hu: Studies on the epidemiology and etiology of moldy sugarcane poisoning in China *Biomed Environ Sci* 5, 161-77 (1992)
156. He F, S. Zhang, F. Qian & C. Zhang: Delayed dystonia with striatal CT lucencies induced by a mycotoxin (3-nitropropionic acid). *Neurology* 45, 2178-83 (1995)
157. Alston TA, L. Mela & H.J. Bright: 3-Nitropropionate, the toxic substance of *Indigofera*, is a suicide inactivator of succinate dehydrogenase. *Proc Natl Acad Sci USA* 74, 3767-71. (1977)
158. Coles CJ, D.E. Edmondson & T.P. Singer: Inactivation of succinate dehydrogenase by 3-nitropropionate. *J Biol Chem* 254, 5161-7 (1979)
159. Beal MF, E. Brouillet, B.G. Jenkins, R.J. Ferrante, N.W. Kowall, J.M. Miller, E. Storey, R. Srivastava, B.R. Rosen & B.T. Hyman: Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. *J Neurosci* 13, 4181-92 (1993)
160. Brouillet E, B.G. Jenkins, B.T. Hyman, R.J. Ferrante, N.W. Kowall, R. Srivastava, D.S. Roy, B.R. Rosen & M.F. Beal: Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. *J Neurochem* 60, 356-9 (1993)
161. Wullner U, A.B. Young, J.B. Penney & M.F. Beal: 3-Nitropropionic acid toxicity in the striatum. *J Neurochem* 63, 1772-81 (1994)
162. Borlongan CV, T.K. Koutouzis, T.S. Randall, T.B. Freeman, D.W. Cahill & P.R. Sanberg: Systemic 3-nitropropionic acid: behavioral deficits and striatal damage in adult rats. *Brain Res Bull* 36, 549-56 (1995)
163. Brouillet E, P. Hantraye, R.J. Ferrante, R. Dolan, A. Leroy-Willig, N.W. Kowall & M.F. Beal: Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proc Natl Acad Sci U S A* 92, 7105-9 (1995)
164. Palfi S, R.J. Ferrante, E. Brouillet, M.F. Beal, R. Dolan, M.C. Guyot, M. Peschanski & P. Hantraye: Chronic 3-nitropropionic acid treatment in baboons replicates the cognitive and motor deficits of Huntington's disease. *J Neurosci* 16, 3019-25 (1996)
165. Beal MF, E. Brouillet, B.G. Jenkins, R. Henshaw, B. Rosen & B.T. Hyman: Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate. *J Neurochem* 61, 1147–1150 (1993)
166. Ferrante RJ, N.W. Kowall, M.F. Beal, E.P.Jr Richardson, E.D. Bird & J.B. Martin: Selective sparing of a class of striatal neurons in Huntington's disease. *Science* 230, 561-3 (1985)
167. Graveland GA, R.S. Williams & M. DiFiglia: Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science* 227, 770-3 (1985)
168. Ferrante RJ, N.W. Kowall & E.P.Jr Richardson: Proliferative and degenerative changes in striatal spiny neurons in Huntington's disease: a combined study using the section-Golgi method and calbindin D28k immunocytochemistry. *J Neurosci* 11, 3877-87 (1991)
169. Guyot MC, P. Hantraye, R. Dolan, S. Palfi, M. Maziere & E. Brouillet: Quantifiable bradykinesia, gait abnormalities and Huntington's disease-like striatal lesions in rats chronically treated with 3-nitropropionic acid. *Neuroscience* 79, 45-56 (1997)

Mitochondria and neurodegenerative diseases

170. Albin RL & J.T. Greenamyre: Alternative excitotoxic hypotheses. *Neurology* 42, 733–738 (1992)
171. Zeevalk GD & W.J. Nicklas: Evidence that the loss of the voltage-dependent Mg²⁺ block at the N-methyl-D-aspartate receptor underlies receptor activation during inhibition of neuronal metabolism. *J Neurochem* 59, 1211–1220 (1992)
172. Lipton SA & P.A. Rosenberg: Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* 330, 613–22 (1994)
173. Greene JG, S.S. Sheu, R.A. Gross & J.T. Greenamyre: 3-Nitropropionic acid exacerbates N-methyl-D-aspartate toxicity in striatal culture by multiple mechanisms. *Neuroscience* 84, 503–510 (1998)
174. Schulz JB, J. Lindenau, J. Seyfried & J. Dichgans: Glutathione, oxidative stress and neurodegeneration. *Eur J Biochem* 267, 4904–11 (2000)
175. Gould DH, M.P. Wilson & D.W. Hamar: Brain enzyme and clinical alterations induced in rats and mice by nitroaliphatic toxicants. *Toxicol Lett* 27, 83–9 (1985)
176. Brouillet E, M.C. Guyot, V. Mittoux, S. Altairac, F. Conde, S. Palfi & P. Hantraye: Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat. *J Neurochem* 70, 794–805 (1998)
177. Pang Z & J.W. Geddes: Mechanisms of cell death induced by the mitochondrial toxin 3-nitropropionic acid: acute excitotoxic necrosis and delayed apoptosis. *J Neurosci* 17, 3064–73 (1997)
178. Erecinska M & D. Nelson: Effects of 3-nitropropionic acid on synaptosomal energy and transmitter metabolism: relevance to neurodegenerative brain diseases. *J Neurochem* 63, 1033–41 (1994)
179. Matthews RT, L. Yang, B.G. Jenkins, R.J. Ferrante, B.R. Rosen, R. Kaddurah-Daouk & M.F. Beal: Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J Neurosci* 18, 156–63 (1998)
180. Riepe M, N. Hori, A.C. Ludolph, D.O. Carpenter, P.S. Spencer & C.N. Allen: Inhibition of energy metabolism by 3-nitropropionic acid activates ATP-sensitive potassium channels. *Brain Res* 586, 61–6 (1992)
181. Blandini F, R.H. Porter & J.T. Greenamyre: Glutamate and Parkinson's disease. *Mol Neurobiol* 12, 73–94 (1996)
182. Dawson R, M.F. Beal, S.C. Bondy, D.A. Di Monte & G.E. Isom: Excitotoxins, aging, and environmental neurotoxins: implications for understanding human neurodegenerative diseases. *Toxicol Appl Pharmacol* 134, 1–17 (1995)
183. Riepe M, A. Ludolph, M. Seelig, P.S. Spencer & A.C. Ludolph: Increase of ATP levels by glutamate antagonists is unrelated to neuroprotection. *Neuroreport* 5, 2130–2 (1994)
184. Greene JG, R.H. Porter, R.V. Eller & J.T. Greenamyre: Inhibition of succinate dehydrogenase by malonic acid produces an "excitotoxic" lesion in rat striatum. *J Neurochem* 61, 1151–1154 (1993)
185. Greene JC & J.T. Greenamyre: Characterization of the excitotoxic potential of the reversible succinate dehydrogenase inhibitor malonate. *J Neurochem* 64, 430–436 (1995)
186. Beal MF: Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Ann Neurol* 31, 119–30 (1992)
187. Beal MF: Aging, energy, and oxidative stress in neurodegenerative diseases. *Ann Neurol* 38, 357–366 (1995)
188. Reynolds D. S, R.J. Carter & A.J. Morton: Dopamine modulates the susceptibility of striatal neurons to 3-nitropropionic acid in the rat model of Huntington's disease. *J Neurosci* 18, 10116–10127 (1998)
189. Fu YT, F.S. He, S.L. Zhang & J.S. Zhang: Lipid peroxidation in rats intoxicated with 3-nitropropionic acid. *Toxicol* 33, 327–331 (1995)
190. Schulz JB, R.T. Matthews, B.G. Jenkins, R.J. Ferrante, D. Siwek, D.R. Henshaw, P.B. Cipolloni, P. Mecocci, N.W. Kowall, B.R. Rosen BR. *et al.* Blockade of neuronal nitric oxide synthase protects against excitotoxicity *in vivo*. *J Neurosci* 15, 8419–8429 (1995)
191. La Fontaine MA, J.W. Geddes, A. Banks & D.A. Butterfield: 3-nitropropionic acid induced *in vivo* protein oxidation in striatal and cortical synaptosomes: insights into Huntington's disease. *Brain Res* 858, 356–62 (2000)
192. Schulz JB, D.R. Henshaw, U. MacGarvey & M.F. Beal: Involvement of oxidative stress in 3-nitropropionic acid neurotoxicity. *Neurochem Int* 29, 167–71 (1996)
193. Beal MF, R.J. Ferrante, R. Henshaw, R.T. Matthews, P.H. Chan, N.W. Kowall, C.J. Epstein & J.B. Schulz: 3-Nitropropionic acid neurotoxicity is attenuated in copper/zinc superoxide dismutase transgenic mice. *J Neurochem* 65, 919–22 (1995)
194. Bogdanov MB, R.J. Ferrante, G. Mueller, L.E. Ramos, J.C. Martinou & M.F. Beal: Oxidative stress is attenuated in mice overexpressing BCL-2. *Neurosci Lett* 262, 33–6 (1999)
195. Calkins MJ, R.J. Jake, D.A. Johnson, K. Chan, Y.W. Kan & J.A. Johnson: Protection from mitochondrial complex II inhibition *in vitro* and *in vivo* by Nrf2-mediated transcription. *Proc Nat Acad Sci USA* 102, 244–249 (2005)

Mitochondria and neurodegenerative diseases

196. Gould DH & D.L. Gustine: Basal ganglia degeneration, myelin alterations, and enzyme inhibition induced in mice by the plant toxin 3-nitropropanoic acid. *Neuropathol Appl Neurobiol* 8, 377–393 (1982)
197. Hassel B & U. Sonnewald: Selective inhibition of the tricarboxylic acid cycle of GABAergic neurons with 3-nitropropionic acid *in vivo*. *J Neurochem* 65, 1184–1191 (1995)
198. Greene JG & J.T. Greenamyre: Manipulation of membrane potential modulates malonate-induced striatal excitotoxicity *in vivo*. *J Neurochem* 66, 637–643 (1996)
199. Hamilton BF & D.H. Gould: Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: a type of hypoxic (energy deficient) brain damage. *Acta Neuropathol (Berl)* 72, 286–97 (1987)
200. Behrens M, J. Koh, L.M. Canzoniero, S.L. Sensi, C.A. Csernansky & D.W. Choi: 3-Nitropropionic acid induces apoptosis in cultured striatal and cortical neurons. *Neuroreport* 6, 545–548 (1995)
201. Hamilton BF & D.H. Gould: Correlation of morphologic brain lesions with physiologic alterations and blood-brain barrier impairment in 3-nitropropionic acid toxicity in rats. *Acta Neuropathol (Berl)* 74, 67–74 (1987)
202. Maragos WF & F.S. Silverstein: The mitochondrial inhibitor malonate enhances NMDA toxicity in the neonatal rat striatum. *Brain Res Dev Brain Res* 88, 117–21 (1995)
203. Calabresi P, P. Gubellini, B. Picconi, D. Centonze, A. Pisani, P. Bonsi, P. Greengard, R.A. Hipskind, E. Borrelli & G. Bernardi: Inhibition of mitochondrial complex II induces a long-term potentiation of NMDA-mediated synaptic excitation in the striatum requiring endogenous dopamine. *J Neurosci* 21, 5110–20 (2001)
204. Saulle E, P. Gubellini, B. Picconi, D. Centonze, D. Tropepi, A. Pisani, M. Morari, M. Marti, L. Rossi, M. Papa, G. Bernardi & P. Calabresi: Neuronal vulnerability following inhibition of mitochondrial complex II: a possible ionic mechanism for Huntington's disease. *Mol Cell Neurosci* 5, 9–20 (2004)
205. Beal MF, N.W. Kowall, D.W. Ellison, M.F. Mazurek, K.J. Swartz & J.B. Martin: Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* 321, 168–171 (1986)
206. Figueredo-Cardenas G, C.L. Harris, K.D. Anderson & A. Reiner: Relative resistance of striatal neurons containing calbindin or parvalbumin to quinolinic acid-mediated excitotoxicity compared to other striatal neuron types. *Exp Neurol* 149, 356–72 (1998)
207. Pezzi S, N. Checa & J. Alberch: The vulnerability of striatal projection neurons and interneurons to excitotoxicity is differentially regulated by dopamine during development. *Int J Dev Neurosci* 23, 343–9 (2005)
208. Uitti RJ, A.H. Rajput, E.M. Ashenurst & B. Rozdilsky: Cyanide-induced parkinsonism: a clinicopathologic report. *Neurology* 35, 921–5 (1985)
209. Klawans HL, R.W. Stein, C.M. Tanner & C.G. Goetz: A pure parkinsonian syndrome following acute carbon monoxide intoxication. *Arch Neurol* 39, 302–4 (1982)
210. Nishino H, Y. Shimano, M. Kumazaki & T. Sakurai: Chronically administered 3-nitropropionic acid induces striatal lesions attributed to dysfunction of the blood-brain barrier. *Neurosci Lett* 186, 161–164 (1995)
211. Nishino H, Y. Shimano, M. Kumazaki, T. Sakurai, H. Hida, I. Fujimoto & A. Fukuda: Hypothalamic neurons are resistant to the intoxication with 3-nitropropionic acid that induces lesions in the striatum and hippocampus via the damage in the blood-brain barrier. *Neurobiology* 3, 257–267 (1995)
212. Nishino H, M. Kumazaki, A. Fukuda, I. Fujimoto, Y. Shimano, H. Hida, T. Sakurai, S.B. Deshpande, H. Shimizu, S. Morikawa & T. Inubushi: Acute 3-nitropropionic acid intoxication induces striatal astrocytic cell death and dysfunction of the blood-brain barrier: involvement of dopamine toxicity. *Neurosci Res* 27 343–355 (1997)
213. Fukuda A, Deshpande SB, Shimano Y, Nishino H. Astrocytes are more vulnerable than neurons to cellular Ca²⁺ overload induced by a mitochondrial toxin, 3-nitropropionic acid. *Neuroscience*. 87, 497–507 (1998)
214. Kim GW, Y. Gasche, S. Grzeschik, J.C. Copin, C.M. Maier & P.H. Chan: Neurodegeneration in striatum induced by the mitochondrial toxin 3-nitropropionic acid: role of matrix metalloproteinase-9 in early blood-brain barrier disruption? *J Neurosci* 23, 8733–42 (2003)
215. Shimizu K, K. Ohtaki, K. Matsubara, K. Aoyama, T. Uezono, O. Saito, M. Suno, K. Ogawa, N. Hayase, K. Kimura & H. Shiono: Carrier-mediated processes in blood-brain barrier penetration and neural uptake of paraquat. *Brain Res* 906, 135–42 (2001)
216. Sokoloff L, M. Reivich, C. Kennedy, M.H. Des Rosiers, C.S. Patlak, K.D. Pettigrew, O. Sakurada & M. Shinohara: The [¹⁴C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28, 897–916 (1997)
217. Kuroiwa T, M. Terakado, T. Yamaguchi, S. Endo, M. Ueki, R. Okeda: The pyramidal cell layer of sector CA 1 shows the lowest hippocampal succinate dehydrogenase activity in normal and postischemic gerbils. *Neurosci Lett* 206, 117–120 (1996)

Mitochondria and neurodegenerative diseases

218. Pang Z, G.H. Umberger & J.W. Geddes: Neuronal loss and cytoskeletal disruption following intrahippocampal administration of the metabolic inhibitor malonate: lack of protection by MK-801. *J Neurochem* 66, 474–484 (1996)
219. Cohen G: Monoamine oxidase, hydrogen peroxide, and Parkinson's disease. *Adv Neurol* 45, 119–125 (1986)
220. McLaughlin BA, D. Nelson, M. Erecinska & M.F. Chesselet: Toxicity of dopamine to striatal neurons *in vitro* and potentiation of cell death by a mitochondrial inhibitor. *J Neurochem* 70 2406–2415 (1998)
221. Maragos WF, R.J. Jakel, Z. Pang & J.W. Geddes: 6-Hydroxydopamine injections into the nigrostriatal pathway attenuate striatal malonate and 3-nitropropionic acid lesions. *Exp Neurol* 154 637–644 (1998).
222. Xia XG, N. Schmidt, P. Teismann, B. Ferger & J.B. Schulz: Dopamine mediates striatal malonate toxicity via dopamine transporter-dependent generation of reactive oxygen species and D2 but not D1 receptor activation. *J Neurochem* 79, 63-70 (2001)
223. Ferger B, O. Eberhardt, P. Teismann, C. de Groote & J.B. Schulz: Malonate-induced generation of reactive oxygen species in rat striatum depends on dopamine release but not on NMDA receptor activation. *J Neurochem* 73 1329–1332 (1999)
224. Moy LY, G.D. Zeevalk & P.K. Sonsalla: Role for dopamine in malonate-induced damage *in vivo* in striatum and *in vitro* in mesencephalic cultures. *J Neurochem* 74, 1656–1665 (2000)
225. Alexi T, P.E. Hughes, B. Knusel & A.J. Tobin: Metabolic compromise with 3-nitropropionic acid produced striatal apoptosis in Sprague–Dawley rats but not in BALB/c ByJ mice. *Exp Neurol* 153, 74–93 (1998)
226. Kim GW, J.C. Copin, M. Kawase, S.F. Chen, S. Sato, G.T. Gobbel & P.H. Chan: Excitotoxicity is required for induction of oxidative stress and apoptosis in mouse striatum by the mitochondrial toxin, 3-nitropropionic acid. *J Cereb Blood Flow Metab* 20, 119–129 (2000)
227. Ouary S, N. Bizat, S. Alterac, H. Menetrat, V. Mittoux, F. Conde, P. Hantraye & E. Brouillet: Major strain differences in response to chronic systemic administration of the mitochondrial toxin 3-nitropropionate in rats: Implications for neuroprotection studies. *Neuroscience* 97, 521–530 (2000)
228. Portera-Caillau C, J.C. Hedreen, D.L. Price & V.E. Koliatsos: Evidence for apoptotic cell death in Huntington disease and excitotoxic animal models. *J Neurosci* 15, 3775–3787 (1995)
229. Butterworth NJ, L. Willians, J.Y. Bullock, D.R. Love, R.L. Faull & M. Dragunow: Trinucleotide (CAG) repeat length is positively correlated with the degree of DNA fragmentation in Huntington's disease striatum. *Neuroscience* 87, 49–53 (1998)
230. Rosenstock TR, A.C. Carvalho, A. Jurkiewicz, R. Frussa-Filho & S.S. Smali: Mitochondrial calcium, oxidative stress and apoptosis in a neurodegenerative disease model induced by 3-nitropropionic acid. *J Neurochem* 88, 1220–1228 (2004)
231. Fernandez-Gomez FJ, M.F. Galindo, M. Gomez-Lazaro, V.J. Yuste, J.X. Comella, N. Aguirre & J. Jordan: Malonate induces cell death via mitochondrial potential collapse and delayed swelling through an ROS-dependent pathway. *Br J Pharmacol* 144, 528-37 (2005)
232. Bizat N, J.N. Hermel, F. Boyer, C. Jacquard, C. Creminon, S. Ouary, C. Escartin, P. Hantraye, S. Krajewski & E. Brouillet: Calpain is a Major Cell Death Effector in Selective Striatal Degeneration Induced *In vivo* by 3-Nitropropionate: Implications for Huntington's Disease. *J Neurosci* 23, 5020 –5030 (2003)
233. Yang L, S. Sugama, R.P. Mischak, M. Kiaei, N. Bizat, E. Brouillet, T.H. Joh & M.F. Beal: A novel systemically active caspase inhibitor attenuates the toxicities of MPTP, malonate, and 3NP *in vivo*. *Neurobiol Dis* 17, 250-9 (2004)
234. Ankarcona M, J.M. Dypbukt, S. Orrenius & P. Nicotera: Calcineurin and mitochondrial function in glutamate-induced neuronal cell death. *FEBS Lett* 394, 321-4 (1996)
235. Agostinho P & C.R. Oliveira: Involvement of calcineurin in the neurotoxic effects induced by amyloid-beta and prion peptides. *Eur J Neurosci* 17, 1189-96 (2003)
236. Liu J, J.D. Jr Farmer, W.S. Lane, J. Friedman, I. Weissman & S.L. Schreiber: Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 66, 807-15 (1991)
237. Griffith JP, J.L. Kim, E.E. Kim, M.D. Sintchak, J.A. Thomson, M.J. Fitzgibbon, M.A. Fleming, P.R. Caron, K. Hsiao & M.A. Navia: X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12-FK506 complex. *Cell* 82, 507-22 (1995)
238. Almeida S, A. Dominguez, L. Rodrigues, C.R. Oliveira & A.C. Rego: FK506 prevents mitochondrial-dependent apoptotic cell death induced by 3-nitropropionic acid in rat primary cortical cultures *Neurobiol Dis* 17, 435–444 (2004)
239. Antonawich FJ, S.M. Fiore-Marasa & C.P. Parker CP: Modulation of apoptotic regulatory proteins and early activation of cytochrome C following systemic 3-nitropropionic acid administration. *Brain Res Bull* 57, 647-9 (2002)
240. Garcia M, P. Vanhoutte, C. Pages, M.J. Besson, E. Brouillet & J. Caboche: The mitochondrial toxin 3-nitropropionic acid induces striatal neurodegeneration via a

Mitochondria and neurodegenerative diseases

c-Jun N-terminal kinase/c-Jun module. *J Neurosci* 22, 2174–2184 (2002)

241. Yu Z, D. Zhou D, G. Cheng & M.P. Mattson: Neuroprotective role for the p50 subunit of NF-kappaB in an experimental model of Huntington's disease. *J Mol Neurosci*. 15, 31-44 (2000)

242. Narasimhan P, R. Sklar, M. Murrell, R.A. Swanson & F.R. Sharp: Methylmalonyl-CoA mutase induction by cerebral ischemia and neurotoxicity of the mitochondrial toxin methylmalonic acid. *J Neurosci* 16, 7336-46 (1996)

243. Royes LF, M.R. Figuera, A.F. Furian, M.S. Oliveira, L.G. da Silva, C.R. Malfatti, P.H. Schneider, A.L. Braga, M. Wajner & C.F. Mello: Creatine protects against the convulsive behavior and lactate production elicited by the intrastriatal injection of methylmalonate. *Neuroscience* 118, 1079-90 (2003)

244. Strauss K. A. & D.H. Morton: Type I glutaric aciduria, part 2: a model of acute striatal necrosis. *Am J Med Genet C Semin Med Genet* 121, 53–70 (2003)

245. Phelka AD, M.J. Beck & M.A. Philbert: 1,3-Dinitrobenzene inhibits mitochondrial complex II in rat and mouse brainstem and cortical astrocytes. *Neurotoxicology* 24, 403-15 (2003)

246. Tjalkens RB, A.D. Phelka & M.A. Philbert: Regional variation in the activation threshold for 1,3-DNB-induced mitochondrial permeability transition in brainstem and cortical astrocytes. *Neurotoxicology* 24, 391-401 (2003)

247. Goralski KB & K.W. Renton: Brain inflammation enhances 1-methyl-4-phenylpyridinium-evoked neurotoxicity in rats. *Toxicol Appl Pharmacol* 196, 381-9 (2004)

248. Bennett MC, G.W. Mlady, M. Fleshner & G.M. Rose: Synergy between chronic corticosterone and sodium azide treatments in producing a spatial learning deficit and inhibiting cytochrome oxidase activity. *Proc Natl Acad Sci USA* 93, 1330-4 (1996)

Key Words: Parkinson's Disease, Huntington's disease, Rotenone, 3-NPA, isoquinolines, Review

Send correspondence to: Professor Alberto Machado, Departamento de Bioquímica, Bromatología, Toxicología y Medicina Legal, Facultad de Farmacia, c/ Profesor García González 2. 41012-Sevilla, Spain. Tel. and Fax: 34-9546752, E-mail: machado@us.es

<http://www.bioscience.org/current/vol12.htm>