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Huntington's disease and mitochondrial alterations: emphasis on experimental models

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Abstract Huntington's disease (HD) is an inheritable neurological disorder coursing with degeneration of basal ganglia and producing chorea and dementia. One common factor accounting for neurodegeneration in this disorder is mitochondrial deterioration at both morphologic and functional levels. The development of experimental models in animals or cell preparations to resemble pathologic and pathogenic conditions of this disorder has served for more than four decades to describe part of the mechanistic alterations that could be occurring in mitochondria of HD patients, and the subsequent design of therapeutic alternatives where mitochondrial alterations are the primary target. In this miniriview we describe some of the most relevant studies at the experimental level, giving support to the hypothesis that mitochondria play a central role in HD pathogenesis.

Keywords Huntington's disease · Mitochondrial alterations · Energy depletion · Neurodegeneration · Experimental models

Mitochondria and neuronal damage: basic concepts

Mitochondrial structure and function are regulated by biogenesis, fission, fusion, transport and degradation. Mitochondria are responsible for major biochemical functions needed for cellular homeostasis, and represent the main source of ATP. An adequate balance in these processes (mitochondrial dynamics) is essential for neuronal signaling, plasticity and neurotransmitter release. In addition, mitochondria are considered as mediators of cell survival and death since their proteins are factors regulating apoptosis. Indeed, mutations or abnormal expression of these factors are linked to neurodegenerative disorders (Büeler 2010).

In neuronal cells, maintenance of the respiratory chain complexes ensures the preservation of resting membrane potential (Martin et al. 1994). The appropriate maintenance of resting potential clearly depends on the activity of membrane ATPases, which in turn preserve the electronegative gradient by modulating ion flux in cells. Mitochondria are recognized as essential for neuronal function because these cells posses a limited glycolytic capacity, turning them highly dependable on mitochondrial oxidative phosphorylation (OXPHOS) to fulfill the high energy requirements. Therefore, neuronal viability can be affected either by alterations in the capacity of neurons to maintain basal levels of energy, or simply through a sudden necessity to quickly respond to major energetic requirements (Lees 1993). OXPHOS is the major source of free radicals, including hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and superoxide anion ($O_2^{\cdot -}$), all of them being byproducts of the cell respiratory cycle (Lemasters et al. 1999). Reactive oxygen species (ROS) generated by mitochondria have shown to target different molecules, including diverse mitochondrial components (lipids, proteins, DNA, etc.). In fact, the low reparative capacity of mDNA makes itself a preferential target for further oxidative damage. Hence, mitochondrial dysfunction is a key event during the pathogenic cascade leading to necrotic or apoptotic cell death (Lemasters et al. 1999; Kroemer and Reed 2000). Upon oxidative stress and excessive cytoplasmic Ca^{2+} upload conditions, mitochondria suffer a considerable loss

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of impermeability at the internal membrane, thereby leading to a full collapse of mitochondrial membrane potential ($\Delta\psi m$), in a process currently known as permeability transition (PT). When PT is accompanied by mitochondrial swelling and cytochrome c release into the cytoplasm, the whole event activates certain caspases accounting for cell death (Murphy et al. 1999; Kroemer and Reed 2000). Under normal conditions, antioxidant defense systems are capable of reducing the deleterious actions of ROS; however, an accelerated production of ROS induced by altered mitochondria can decrease or even block these systems, leading to loss of ATP caused by transmembrane ATPases' disruption, and further necrotic cell death by osmotic collapse (Dykens 1997, 1999). Moreover, a severe loss of mitochondrial function initiates apoptosis in response to a wide variety of stressors. For instance, excitotoxicity comprises excessive stimulation of glutamate receptors, including N-methyl-D-aspartate (NMDA) and other voltage-dependent and metabotropic receptors. Glutamate-mediated enhanced levels of intracellular calcium during excitotoxic episodes modify mitochondrial integrity and accelerate: ROS formation, leading to cell death (Dykens et al. 1987; Dykens 1994). Altogether, this evidence has served to suggest mitochondria as a key modulator for neuronal viability or death during excitotoxicity (Simpkins et al. 2010).

Huntington's disease and mitochondrial alterations

Since a mechanistic point-of-view, one of the most fascinating neurodegenerative disorders associated with mitochondrial alterations is Huntington's disease (HD), an autosomic dominant disease likely caused by a genetic mutation in position IT15. HD occurs when the gene for protein huntingtin (Htt), localized in region 4p16.3 at the short arm of chromosome 4, displays an expansion of cytosine-adenine-guanine (CAG) trinucleotide in exon 1, leading to the formation of mutant Htt (mHtt) which exhibits large repetitions of polyglutamine (Gusella et al. 1983; The Huntington's Disease Collaborative Research Group 1993). In adults, HD is characterized by severe psychiatric disturbances, including irritability, aggressiveness and depression, and these signs precede involuntary motor alterations. Progression of these motor alterations—also known as choreiform movements—can be observed in three phases: 1) Slight involuntary movements are first accompanied by tremor; 2) Progressively, during the second phase (or hyperkinetic phase), patients lose coordination of the body due to the presence of abrupt involuntary movements (chorea), involving malfunction of muscles from head, thorax and limbs, thus limiting the capacities of patients to perform daily-tasks. A progressive decline in cognitive functions, accompanied by a massive loss of body weight, are characteristic of this phase; 3) In final phase, approximately 20 years after its onset, choreiform movements are replaced by rigidity and bradikinesia (Harper 1992).

The striatum is the most affected brain region in HD. Damage to this region is characterized by selective degeneration of medium-size spiny neurons, although in advanced stages the brain cortex is also affected, mostly presenting a notorious atrophy in pyramidal neurons from layers III, V and VI at motor and associative cortexes (Cudkowicz and Kowall 1990; DiFiglia 1997; MacDonald and Halliday 2002). The abundant formation of inclusions, as well as a notorious loss of striatal GABAergic and cortical glutamatergic pyramidal neurons, are also evident.

A considerable amount of evidence suggests that mitochondrial dysfunction is directly or indirectly involved in HD (Reddy et al. 2009). By mean of the Positron Emission Tomography (PET), hypermetabolism has been observed in the caudate, putamen and cortex from symptomatic HD patients, as well as in non-symptomatic patients carrying the HD gene (Kuhl et al. 1982). Accordingly, glucose metabolism and O_2 formation are significantly decreased in basal ganglia and cerebral cortex from symptomatic patients (Kuwert et al. 1990; Beal 1992). The decreased mitochondrial metabolism is likely due to an alteration in the activity of mitochondrial complexes I and IV (Brenuan et al. 1985; Borlongan et al. 1997), a concept that is supported by ultrastructural studies in which abnormal mitochondria were observed in both early- and late-onset HD patients (Struys-Ponsar et al. 1994). In addition, defects in mitochondrial enzymes, such as succinate dehydrogenase (SDH, complex II) and aconitase, have been described in *post mortem* brains from HD patients (Tabrizi et al. 1999). Furthermore, Chen et al. (2007) described mitochondrial abnormalities and oxidative damage in peripheral blood from HD patients. Lim et al. (2008) also reported that mHtt expression induces mitochondrial calcium homeostasis disruption, and similar results were obtained from mitochondria isolated from cultured cells expressing mHtt (Milakovic et al. 2006), thus suggesting that mitochondrial dysfunction plays a central role in HD pathogenesis. Supporting evidence on this topic includes studies where lactate levels have been found increased in cerebrospinal fluid from HD patients (Jenkins et al. 1998), while altered enzyme activity involved in ATP production has been described in *post mortem* HD brain tissues: a reduction in the activity of mitochondrial complexes II, III and IV during the progression of HD was evidenced (Browne et al. 1997). More recently, a decreased metabolic ratio of brain glucose was observed in the striata of HD patients in early phases, suggesting that alterations in glycolytic metabolism is part of the initial degenerative—and probably causative -

signals in this disorder (Powers et al. 2007). Noteworthy, all these alterations are restricted to basal ganglia, a brain area with a higher metabolic activity. Another relevant study to this topic revealed a modest, but still significant reduction in the activity of glyceraldehide-3-phosphate dehydrogenase (GAPDH) in *post mortem* caudate tissue from HD patients (Kish et al. 1998), an observation that has been reinforced by experiments showing reduced GAPDH activity in fibroblasts from HD patients (Cooper et al. 1998). These findings are relevant since GAPDH is a cytoplasmic protein that has been shown to interact with mutant Htt (Burke et al. 1996), although to date it is yet unknown whether this interaction is readily contributing to the pathogenesis of HD. In addition, other alterations have been reported on mitochondrial enzymes from the Krebs cycle, including pyruvate dehydrogenase and aconitase (Butterworth et al. 1985; Tabrizi et al. 1999). Altogether, these findings indicate that major alterations in energy metabolism are present in HD patients even before initial manifestations and signs begin, which might be contributing to facilitation of excitotoxic neuronal damage.

Of consideration, some of the metabolic defects described in *post mortem* HD brains are also present in transgenic mice models of this disorder; for instance, a deficient activity of mitochondrial complex II and aconitase have been described in R6/2 transgenic mice (Browne and Beal 2004). Shortly thereafter, mitochondrial dysfunction in HD was correlated with an altered function of PGC-1α - a known transcriptional co-activator associated with the expression of mitochondrial genes—since presymptomatic patients showed a reduction in the levels of mRNA for this co-activator (Cui et al. 2004). Moreover, experiments in the mitochondria isolated from myoblasts both from presymptomatic and symptomatic HD subjects indicate that mitochondrial deficit described for this disorder participates in membrane depolarization induced by mHtt, release of cytochrome c and increase of caspases 3, 8 and 9 activities (Ciammola et al. 2006). Decreased mitochondrial ATP generation in lymphoblasts from HD patients has shown to be concomitant with a reduction in mitochondrial membrane potential and further alterations in calcium upload (Sawa et al. 1999; Panov et al. 2002, 2005; Seong et al. 2005; Squitieri et al. 2006). These lymphoblasts also showed to be more vulnerable to mitochondrial toxins, such as 3-nitropropionic acid (3-NP) and sodium cyanide, in a manner which is dependent on the length of polyQ tract of mHtt (Sawa et al. 1999; Seong et al. 2005).

On the other hand, mitochondrial DNA damage and suppression has been reported both in brain tissue of HD patients and animal models (Banoei et al. 2007; Acevedo-Torres et al. 2009). These alterations might be linked with an abnormal transcriptional regulation of nuclear-encoded mitochondrial genes. In parallel, mHtt can interact with different transcription factors, including TATA binding proteins (Huang et al. 1998; Pérez et al. 1998), Sp1 (Shimohata et al. 2002) and the nuclear scaffold protein NAKAP (Sayer et al. 2005), thereby suggesting that mHtt interferes with genes expression, as well as activity and transcriptional regulation in HD neurons. This concept has been supported by recent studies carried out both in brain tissue from HD subjects and knock-in mice over-expressing mHtt, as well as in striatal neuronal cell cultures obtained from knock-in mice expressing a 111 tract of polyglutamine in which the interaction between mHtt, mitochondrial bioenergetics and PGC1α (a potent suppressor of Reactive Oxygen Species) was investigated. These studies showed a decreased expression in mRNA of PGC-1α in the three models, suggesting that mHtt interferes with the formation of complex CREB/TAF4, which in turn regulates transcription and codification of the gene for PGC-1α (Cui et al. 2004; St-Pierre et al. 2006; Weydt et al. 2006); as a consequence, mitochondria might be suffering severe degenerative changes, thus compromising cell stability. In this regard, Wang et al. (2009) recently suggested that HD can be reflecting abnormal patterns in mitochondrial fission/fusion balance.

Altogether, these studies demonstrate that mitochondrial damage is strongly involved in HD pathogenesis; albeit the issue on whether these alterations are contributing to triggering the disease or merely reflect epiphenomena from other major mechanisms occurring in HD brains, remains to be elucidated in future investigations (Fig. 1).

Experimental models of HD and mitochondrial damage

mHtt and HD models exhibiting mitochondrial damage

The Htt gene is widely expressed in the Nervous System and several other organs (Strong et al. 1993). Therefore, it is not surprising at all that CAG length expansions vary among different tissues, and even between different structures in the Nervous System: expansions are shorter in cerebellum than in frontal cortex and striatum, always prevailing neuronal over glial expression (Strong et al. 1993; Furtado and Mazurek 1996). In normal brains, Htt is mostly localized in the cytoplasmic domain, whereas its subcellular distribution is completely different in brains of HD patients: brain cortex and striatum often present dense perinuclear cumuli of the protein. Important properties of polyglutamine expansions are their capacity to recruit and interact with other proteins, as well as its effect on gene transcription, sometimes simulating the action of certain transcription factors through its glutamine-enriched domains. The presence of small portions of mHtt in different subcellular structures—including the nucleus, plas-

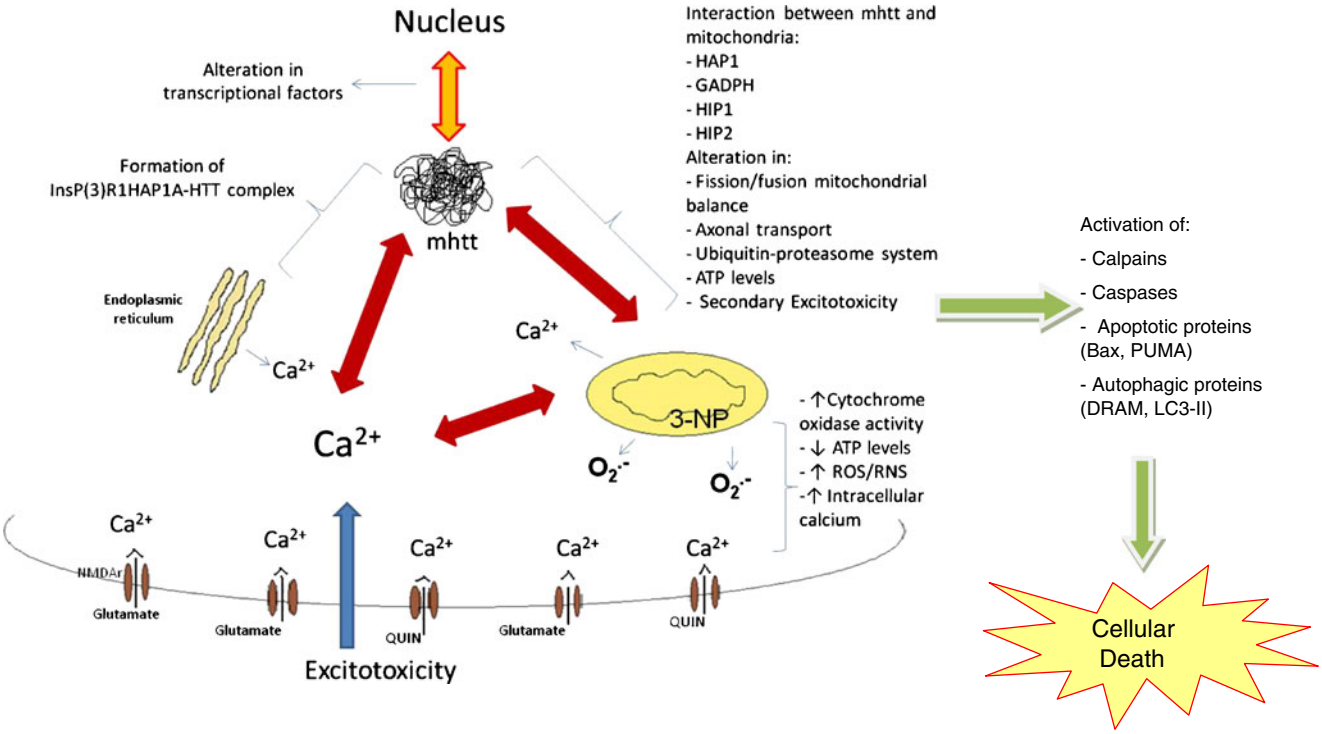

Fig. 1 Schematic representation of the many interactions that mitochondria are sustaining at different cellular levels to fall in dysfunctional status, thus contributing to pathological conditions in Huntington's disease via energy defects. Mitochondria are affected by (and at the same time can be influencing) aberrant conditions like excitotoxicity, dysfunctional mutant huntingtin, oxidative/nitrergic stress, defective signals from the nucleus, altered calcium homeostasis, endoplasmic reticulum stress, etc. Derived from these alterations, mitochondria trigger signals affecting cell functioning, ultimately leading to activation of apoptotic, necrotic and/or autophagic pathways, and further cell death

ma membrane, mitochondria, liposomes and endoplasmic reticulum—has been reported in some studies, suggesting that these depositions could alter functions of the structure in turn (Kegel et al. 2002, 2005; Panov et al. 2002; Choo et al. 2004; Truant et al. 2006; Atwal et al. 2007; Strehlow et al. 2007; Orr et al. 2008).

Different studies have demonstrated that mHtt is associated with mitochondrial alterations and dysfunction of microtubules regulating mitochondrial axonal transport to nerve terminals, directly affecting neuronal transmission and producing synaptic damage and selective neuronal loss (Trushina et al. 2004; Chang et al. 2006; Orr et al. 2008). Among those proteins interacting with mHtt are GADPH, Htt-associated protein 1 (HAP1), and the protapototic proteins HIP1 and HPI2 (the later known to be conjugated with ubiquitin) (Li et al. 1995). mHtt also activates transcription factors such as CA150, a peptide containing a repetition of a glutamine-alanine tract that has been found augmented in the brains of HD patients (Holbert et al. 2001).

Recent findings of Tang et al. (2004) revealed that inositol(1,4,5)-triphosphate receptor (InsP(3)R1), an intracellular channel releasing calcium from the endoplasmic reticulum, is relevant as it can form a triple complex with HAP1 and mHtt (InsP(3)R1-HAP1A-Htt) in the brain. Noteworthy, mHtt, but not wild-type Htt, can activate InsP(3)R1, thereby facilitating calcium release to the cytoplasm with direct stimulation of cellular function in a HAP1-dependent manner. In turn, an altered intracellular calcium homeostasis could be affecting mitochondrial function.

Both wild-type and mutant Htt are catalytically processed by the proteasome, but the velocity of degradation is inversely proportional to the length of polyglutamines expansion (Jana et al. 2001). In this regard, the inhibition of ubiquitine/proteasome system activity seems to duplicate the amount of ubiquitinated aggregates, indicating that intracellular inclusions are generated when the capacity of this system to degrade mHtt is compromised (Waelter et al. 2001). Htt aggregation could be occurring by a process involving overloading of abnormal protein, thus exceeding the degradative capacity of the proteasome (Waelter et al. 2001). Recent in vitro evidence demonstrates that Htt glial expression induces the formation of astrocytic inclusions, thereby blocking synaptic glutamate reuptake and creating a scenario for excitotoxicity (Shin et al. 2005).

mHtt aggregates are associated with mitochondria. In fact, mHtt directly affects mitochondrial membrane potential toward alterations in calcium homeostasis (Petrasch-

Parwez et al. 2007), also disrupting mitochondrial axonal transport (Chang et al. 2006; Orr et al. 2008). More recently, mHtt was reported to cause mitochondrial fragmentation through an inhibition of mitochondrial fusion, accompanied by reduced ATP levels (Wang et al. 2009). Noteworthy, GTPase protein Mitofusin 2 (Mfn2) overexpression, and its dominant negative mutant dynamin-like protein 1 (DLP1), not only prevented mitochondrial fragmentation, but recovered ATP levels (Wang et al. 2009), suggesting that mitochondrial fission induced by mHtt leads to mitochondrial dysfunction. Furthermore, knock-down for DLP1 is known to be rescued from motility defects associated with mHtt overexpression in a HD model in *C. elegans* (Wang et al. 2009), suggesting that mitochondrial fission induced by mHtt is also mediating aberrant motility associated with neuronal dysfunction.

On the other hand, similar to the case of *post mortem* HD tissues, degenerating mitochondria have been found in brain, liver and muscle from different HD mutant models (R6/2, N171-82Q and $Hdh^{Q111/Q111}$ mice), and this effect matches with the onset of HD-like signs (Panov et al. 2002; Yu et al. 2003; Choo et al. 2004). In the same transgenic models, decreased levels of N-acetyl-aspartate (NAA) have been observed. This effect could be evoked by a mHtt-induced alteration in energy metabolism since creatinine, an pro-energetic molecule, attenuated the fall in NAA levels *in vivo* (Andreassen et al. 2001).

In 2000, Tabrizi and coworkers reported a 30% reduction in complex IV activity from the electron chain transport, accompanied by decreased aconitase in mitochondria isolated from the striatum and cortex from R6/2 mice. A considerable number of modifications associated with oxidative metabolism have also been described in the brains of these R6/2 mice, including increased levels of carboxylation in aconitase, voltage-dependent 1 anion-channel and creatinine kinase. Additional studies in liver mitochondria from knock-in $Hdh^{Q111/Q111}$ symptomatic mice, and in brain mitochondria from presymptomatic full-length YAC72 mice, suggest that mHtt expression results in a reduction of mitochondrial capacity to sequester calcium (Panov et al. 2002; Choo et al. 2004), which seems to be associated with an increased susceptibility to activation during mitochondrial PT. Panov et al. (2002) demonstrated that alterations in the capacity of brain mitochondria from YAC72 mice to sequestrate calcium were evident at 3–4 months of age, while behavioral and pathological changes emerged several months later, suggesting that the defects in mitochondrial calcium buffering properties can be part of a direct effect of mHttt in mitochondria. This conclusion was reached through additional experiments showing that incubation of mitochondria from wild-type mice or human lymphoblast with constructed proteins containing polyglutaine expansions also reduced mitochondrial membrane potential in a manner which is dependent on the expansion itself (Choo et al. 2004; Panov et al. 2003).

Binding of p53 (tumor suppressor gene) to mHtt in neuronal cell cultures has been shown to enhance the localization of mHtt in the nucleus, augmenting its transcriptional activity (Bae et al. 2005). p53 levels are also reported as increased in brains from symptomatic HD patients, transgenic mice for HD and $STHdh^{Q111/Q111}$ clone cells. Consequently, p53 deficiency reduces the expression of mHtt in Hdh^{Q140} transgenic mice, whereas its inactivation prevents mitochondrial defects in cells expressing mHtt, and ameliorates behavioral and biochemical (fall in complex IV activity) events observed in transgenic mice for HD (Bae et al. 2005; Ryan et al. 2006).

Phenotypic model of HD produced by quinolinic acid

Quinolinic acid (2,3-pyridin dicarboxylic acid or QUIN) is an endogenous metabolite of L-tryptophan which is synthesized as a byproduct of the kynurenine pathway (Vender 1975). QUIN is known to act as a N-methyl-D-aspartate receptor (NMDAr) agonist. This toxic metabolite has been largely considered as a neurotoxin capable of resembling different features of HD in rodents and non-human primates, and more recently, has been found augmented in the brain of HD at early stages, supporting the concept that it might be involved in pathogenesis of this disorder.

Since the 80's, QUIN injection into the rat hippocampus was known to produce decreased levels of ATP (Vezzani et al. 1987), but it was not until the 90's when Bordelon et al. (1997) formally evaluated alterations in energy metabolism as part of the toxic cascade evoked by QUIN by assessing the involvement of respiratory chain alterations in ATP depletion. Their findings showed that QUIN is able to induce a reduction in oxygen consumption about 6 h after its infusion. Therefore, mitochondrial dysfunction and decreased ATP and NAD levels are involved in the toxic cascade evoked by QUIN through an excessive cytoplasmic calcium influx, supporting the concept that mitochondrial alterations represent a key event for cell death in excitotoxic models. Indeed, the early effects of QUIN in the striatum comprise a massive influx of calcium ions that, in turn, would be responsible for subsequent abnormalities in energy metabolism, further leading to neurodegeneration and cell death.

Fernandes et al. (2008) conducted experiments demonstrating that the striatal infusion of QUIN to laboratory animals produces early alterations in sarco/endoplasmic reticulum-$Ca(2^{+})$-ATPase (SERCA) activity, which implies affected mechanisms of Ca^{2+} homeostasis and signaling. Accordingly, since QUIN-induced excitotoxicity comprises a fall in bioenergetics by disruption of calcium homeostasis

and ROS formation, and this is clearly dependent on the fall in mitochondrial membrane potential. Korde et al. (2005) showed that an uncoupling mitochondrial agent, 2,4-dinitrophenol, exerted protection against QUIN toxicity. Furthermore, some Krebs' cycle intermediaries, such as malate, citrate and oxalacetate, have been shown to protect brain lipid membranes from lipid peroxidation induced by QUIN (Puntel et al. 2005), supporting a role of energy metabolism as a mediator of toxicity in excitotoxic/pro-oxidant models.

Interestingly, the stimulation of cellular metabolism in this model constitutes a potential neuroprotective condition (Maus et al. 1999; Sheline et al. 2000). For instance, pyruvate can be employed by cells as an energy substrate and metabolic precursor, producing protective effects against QUIN-induced toxicity (Ryu et al. 2003). Moreover, there is experimental evidence suggesting that QUIN-induced lesions can be the consequence of oxidative stress, and that pyruvate, but not lactate, can exert protection by blocking this effect. These results were accompanied by further evidence suggesting that microglia and astrocytes actively contribute to neurodegeneration, augmenting ROS formation in the presence of QUIN. In turn, Maksimovic et al. (2001) showed that cytochrome c oxidase, a component of the respiratory chain, is increased in striatum and hippocampus from animals treated with QUIN (150 nM). This reinforces the concept that QUIN can produce a disrupted energy metabolism as a primary target.

A recent study indicated that oxidative phosphorylation and transference of cellular energy are compromised by high concentrations of QUIN in the striatum of young rats, and these effects were suggested to be due to NMDAr overstimulation (Ribeiro et al. 2006). In addition, QUIN is known to readily exert an in vitro effect on energy metabolism in brain tissue from young rats, as the toxin augmented glucose reuptake by 55%, while decreasing the activity of complex II of the respiratory transport chain by 35% (Schuck et al. 2007). Inhibition of complex II was prevented by pre-incubation of cortical homogenates with superoxide dismutase plus catalase, clearly indicating that this effect is mediated by ROS generated in mitochondria. Our group has evaluated the effects of different antioxidants, either in vitro or *in vivo*, in the toxic model induced by QUIN. Among these agents are iron porphyrinate as a peroxynitrite decomposition catalyst, S-allylcysteine as a typical free radical scavenger, and L-carnitine as energy precursor and antioxidant (Pérez-De la Cruz et al. 2005, 2008, 2009; Silva-Adaya et al. 2008; Elinos-Calderon et al. 2009, 2010). Findings collected throughout these studies support the basic concept that antioxidants can rescue nerve tissue from both oxidative damage and mitochondrial dysfunction, and that prevention of these events is reflected in the preservation of major functional aspects of the brain, including neurochemical and behavioral markers. Therefore, preserving mitochondrial function is a key target for therapeutic approaches at the experimental level in this model.

Modeling HD by 3-nitropropionic acid

3-nitropropionic acid (3-NP) is a micotoxin capable of irreversibly inhibiting succinate dehydrogenase (SDH or complex II), an enzyme typically located in the inner mitochondrial membrane. SDH is responsible for oxidation of succinate to fumarate. Its blockade typically induces a reduction of ATP synthesis followed by neuronal cell death (revised in Túnez et al. 2010); thereby causing neurotoxicity in animals and humans that resembles HD characteristics (Ludolph et al. 1991). Indeed, the HD model produced by 3-NP is widely accepted this far as a representation of an altered energy metabolism occurring in this disorder (Túnez et al. 2010). The brain lesions caused by systemic administration of 3-NP are highly specific of the striatum, further involving the hippocampus, thalamus and cortex (Beal et al. 1993; Borlongan et al. 1997; Brouillet et al. 1999). 3-NP also produces depleted levels of glutathione and increases ROS formation and nitrotyrosine as a byproduct of nitrergic stress. Interestingly, several antioxidants, such as coenzyme Q10, N-acetylcysteine, melatonin, dehydroepiandrosterone, and others, have been shown to protect nerve tissue from 3-NP-induced damage, suggesting that oxidative/nitrergic stress typically generated by mitochondrial dysfunction are major contributors to degeneration (La-Fontaine et al. 2000; Nam et al. 2005; Túnez et al. 2004, 2005). In addition, the mitochondrial respiratory phase 3 linked to NAD^+, and the activity of complex I, were directly compromised by 3-NP in rat brain cortex (Pandey et al. 2008). Kumar and Kumar (2009) reported that 3-NP administration for 14 consecutive days produced HD-like symptoms that comprised body-weight changes, locomotor activity, deficient rotarod performance, oxidative damage and dysfunction of complexes I, II and IV in the rat striatum, cortex and hippocampus. PC12 cells treated with 3-NP also presented high levels of H_2O_2, simultaneous to depleted ATP levels, and these effects can be prevented by overexpression of Bcl2 (Mandavilli et al. 2005). SDH inhibition by 3-NP also causes excessive mitochondrial fission linked to neuronal cell death (Liot et al. 2009). Accordingly, treatment of cultured striatal cells with 3-NP results in decreased mitochondrial function, membrane potential and ATP levels (Gines et al. 2003; Nasr et al. 2003; Milakovic and Johnson 2005; Seong et al. 2005).

Mirandola et al. (2010) demonstrated that mitochondria isolated from the striatum are more vulnerable to 3-NP than those from cortex, cerebellum, liver and heart, due to an apparent higher susceptibility to PT. This effect was potentiated in this region by slight variations in calcium

levels after glutamatergic receptor activation. These observations were further supported by studies showing that moderate activation of NMDAr by QUIN, combined with mitochondrial inactivation by 3-NP, can synergically augment striatal degeneration primarily involving intracellular Ca^{2+} deregulation, with an apparent lack of sensitization of NMDAr (Jacquard et al. 2007). Taking these experiments as a frame, we collected evidence that mitochondrial dysfunction and oxidative damage can be synergically evoked when the toxic condition 3-NP + QUIN is tested (Pérez-De la Cruz et al. 2008). Of note, several antioxidants can successfully ameliorate the cell damage induced by 3-NP, suggesting that mitochondrial damage evoked by this toxin is dependent on ROS formation.

Using time-lapse fluorescence imaging, Liot et al. (2009) demonstrated that 3-NP produces two different signals in mitochondria: one signal is a direct effect on mitochondrial bioenergetics, causing a dramatic and rapid loss of ATP together with an increase of ROS with no further alterations in morphology or neuronal viability; the second signal occurs some hours later and involves the activation of NMDAr, leading to a second peak of ROS formation, as well as enhanced mitochondrial fission and cell death. Moreover, exposure of cultured cells to 3-NP and cyanide activate different transduction pathways, including phospholipase A and araquidonic acid metabolism by cyclooxygenase (Gunasekar et al. 1998), and these components pocess the ability to directly activate or inactivate NMDAr at a glycine site. Alternatively, ROS/RNS generated by mitochondrial inhibition can affect antioxidant enzymes such as catalase or superoxide dismutase (Prabhakaran et al. 2002; Mandavilli et al. 2005), thereby influencing NMDAr activity.

On the other hand, 3-NP also induces elevated levels of proapoptotic proteins, including Bax and PUMA, as well as some autophagic proteins, including DRAM and LC3-II. It is assumed that this effect, in parallel with mitochondrial disruption, is due to the upregulation that 3-NP exerts on p53, since an inhibitor of this protein decreased the mentioned markers (Zhang et al. 2009).

Finally, it has been described that human HD cybrids are more susceptible than control cybrids to cell degeneration dependent on aberrant mitochondrial function induced by 3-NP and a classical apoptotic inducer, staurosporine (Ferreira et al. 2010). HD cybrids treated with 3-NP caused the release of cytochrome c from mitochondria, the activation of caspase 3 and the subsequent release of mitochondrial AIF, and all these effects are likely to be mediated by mitochondrial translocation of Bax. In contrast, control cybrids were more vulnerable to necrotic cell death when treated with 3-NP, suggesting differential mechanisms among the experimental conditions tested.

Concluding remarks

We briefly explored the importance of mitochondrial alterations in HD, with particular emphasis in experimental models. Derived from the collected and revised studies, different major factors are hypothesized to interact with mitochondria to induce cell death: mHtt aberrant functioning, excitotoxicity, oxidative stress, etc. Yet, it remains unclear which of these factors are pivotal to trigger cell death in HD via mitochondrial dysfunction. For instance, oxidative stress gives sense to this hypothesis and deserves intense exploration since recent evidence support its active participation in HD (Duran et al. 2010). In contrast, that mHtt is known to be present in HD subjects in practically all organs, it remains unclear how aggregates appear and why the first clinical manifestations of HD occur in adulthood, thus leaving the mHtt with more questions than answers. In addition, the interaction of excitotoxicity with mitochondrial alterations and energy depletion remains as an intriguing issue deserving further investigation. In the meantime, it is clear enough that mitochondrial alterations obey to different stressors in HD and evoke conditions involved in cell death. In light of this evidence, therapeutic strategies at the clinical level should be oriented to target these alterations in order to recover mitochondrial function and structure.

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