

Review

Cellular and molecular mechanisms involved in the selective vulnerability of striatal projection neurons in Huntington's disease

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Summary. Neurodegenerative disorders affecting the central nervous system, such as Alzheimer's disease, Parkinson's disease, Huntington's chorea (HD) and amyotrophic lateral sclerosis are characterized by the loss of selected neuronal populations. Another striking feature shared by these diseases is the deposition of proteinaceous inclusion bodies in the brain, which may be intracytoplasmatic or intranuclear, or even extracellular. However, the density and prevalence of aggregates are not always directly related to neurodegeneration. Although some of these diseases are the result of mutations in known proteins, with HD a clear example, the expression and location of the affected protein do not explain the selective neurodegeneration. Therefore, other intrinsic mechanisms, characteristic of each neuronal population, might be involved in the neurodegenerative process. In this review we focus on several proposed mechanisms such as excitotoxicity, mitochondrial dysfunction and altered expression of trophic factors, which could account for the pathogenesis of HD.

Key words: Aggregates, Huntingtin, Excitotoxicity, Mitochondria, Trophic factors

Neuropathology of HD

HD is a progressive neurological disorder characterized by dysfunction and death of neurons in specific brain regions. Neurodegeneration occurs most prominently in the striatum, although loss of neurons in the cerebral cortex has also been reported (DeLa Monte et al., 1988; Mann et al., 1993). As a result, HD patients suffer motor, cognitive and emotional disturbances,

which lead to progressive dementia and death 15-20 years after the onset of clinical symptoms (Martin and Gusella, 1986; Bates et al., 2002).

Neuronal degeneration within affected brain regions is also cell-specific. Thus, in the striatum, the GABAergic medium-sized spiny projection neurons are preferentially lost (Ferrante et al., 1991), whereas interneurons are relatively spared (Ferrante et al., 1985, 1987; Vonsattel et al., 1985). Furthermore, different subpopulations of projection neurons die in a progressive manner. Striatal neurons that send their axons to the external segment of the globus pallidus and express enkephalin are affected earlier and more severely than striatal neurons projecting to the substantia nigra pars reticulata and the internal segment of the globus pallidus, that are enriched in substance P and dynorphin (Reiner et al., 1988; Albin et al., 1992; Ritchfield et al., 1995; Mitchell et al., 1999; Glass et al., 2000). Although cortical neuronal loss is less severe, there is about a 30% reduction in neocortical regions such as the associative frontal, temporal and parietal regions, and primary somatosensory cortices (Heinsen et al., 1994). The degeneration of cortical neurons also follows a specific pattern: only large projection neurons, especially in layers V and VI and to a lesser extent in layer III, are affected (Cudkowicz and Kowall, 1990; Hedreen et al., 1991; Sotrel et al., 1991; Wagster et al., 1994; MacDonald and Halliday, 2002).

Huntingtin and its mutation

Although the clinical features of HD and its autosomal-dominant pattern of inheritance were first described in 1872 (Huntington, 1872), the gene whose mutation causes HD was not identified until 1993 (HDCRG, 1993; Bates, 2005). *HD* gene mutation was found to be an expansion of a trinucleotide CAG repeat, which results in long stretches of polyglutamine (polyQ) in the N-terminal portion of the encoded protein of about 350 Kb, which is called huntingtin (htt; HDCRG, 1993).

The number of CAG repeats in the unaffected population varies from 6 to 35. People with 35-39 repeats might or might not develop HD, whereas those with repeats of 40 and above will always show the disease (Myers et al., 1988). There is an inverse relationship between the age of onset and CAG repeat size, with longer repeats causing earlier onset and more severe pathology (Duyao et al., 1993; HD CRG, 1993).

The identification of *HD* gene mutation was a breakthrough in HD research. This discovery led to the development of several genetic models that have allowed the study of early disease events and the potential of several therapeutic agents (Hickey and Chesselet, 2003; Beal and Ferrante, 2004; Levine et al., 2004). However, direct correlation between *HD* gene mutation and neuronal dysfunction and death has not yet been established. Thus, although the degeneration is specific to some neuronal subpopulations, the protein is ubiquitously expressed throughout the central nervous system, in peripheral tissues and during embryonic development (Strong et al., 1993; Bhide et al., 1996).

To understand the pathological activity of mutant htt, the function of wild-type htt must first be known. The physiological roles of htt are still not fully understood, although several functions have been suggested. Htt's location in many subcellular compartments has hampered the definition of its function. It is present in cell bodies, dendrites and nerve terminals, in association with a number of organelles such as the Golgi apparatus, endoplasmic reticulum, synaptic vesicles, microtubules and mitochondria (DiFiglia et al., 1995; Sharp et al., 1995; Trotter et al., 1995; Gutekunst et al., 1998). Several studies have indicated that htt may be a scaffold protein involved in orchestrating sets of proteins for intracellular transport and signaling processes. In fact, htt has been implicated in vesicle transport and cytoskeletal anchoring (Gutekunst et al., 1998) as well as in clathrin-mediated endocytosis, neuronal transport processes and postsynaptic signaling (reviewed in Harjes and Wanker, 2003; Landles and Bates, 2004; Li and Li, 2004). Furthermore, htt is localized in the nucleus, where it interacts with proteins involved in gene transcription (Harjes and Wanker, 2003; Landles and Bates, 2004; Li and Li, 2004). Another essential role of htt is its contribution to cell survival. Mice lacking htt die at embryonic day 7.5 (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995) and the conditional deletion of htt in the forebrain leads to neurodegeneration (Dragatsis et al., 2000). The function of htt as an anti-apoptotic protein has also been shown in striatal cells subjected to serum deprivation or to 3-nitropropionic acid (3-NP) toxicity (Rigamonti et al., 2001). Therefore, it has been suggested that a loss of htt function may contribute to the neuropathology of HD (Cattaneo et al., 2005). However, there is also evidence that a gain in function is associated with the expanded polyQ domain that activates the neurodegenerative process. In fact, individuals whose htt expression is reduced by 50% as the result of a deletion in the HD

gene do not develop *HD* (Ambrose et al., 1994) and homozygous patients for HD gene have a clinical symptom onset indistinguishable from HD heterozygotes (Myers et al., 1989). Therefore, whether neural degeneration in *HD* results from the loss or a gain of function in the mutated form of htt remains controversial.

Aggregation of mutant htt

One of the pathological hallmarks of *HD* is the presence of inclusion bodies in neurons (Fig. 1). The exact mechanism by which mutant htt forms insoluble aggregates is unclear, but there is close correlation between the length of the CAG repeat and the density of aggregates (Vonsattel et al., 1985; Myers et al., 1988; Becher et al., 1998). This led to the suggestion that a protein with an expanded glutamine tract has an altered structure that facilitates self-association (Ross and Poirier, 2004, 2005). The fact that antibodies to ubiquitin (a signal of degradation by the proteasome) label htt aggregates (Ross and Poirier, 2004) suggests that the accumulation of mutant htt could also be related to impairment of the ubiquitin-proteasome system (Hernandez et al., 2004; Ross and Pickart, 2004; Venkatraman et al., 2004). However, the presence of ubiquitin and proteasome components in aggregates could also represent cellular defenses for degrading the soluble pool of mutant htt, as the inhibition of ubiquitin-mediated proteolysis suppresses aggregates but accelerates polyQ htt-induced cell death (Saudou et al., 1998).

Although htt is mainly localized in the cytoplasm, aggregates have also been detected in the nucleus. These intra-nuclear inclusions can only be detected by antibodies recognizing epitopes very close to the polyQ stretch, suggesting that they represent short truncated derivatives of htt protein (DiFiglia et al., 1997). In fact, it has been shown that aggregation, for any given polyQ, is greater for N-terminal truncated protein than for full-length protein (Cooper et al., 1998; Hackam et al., 1998; Martindale et al., 1998). In addition, short htt fragments containing a pathological polyQ expansion, when expressed in transgenic animals or cultured cells, are more toxic than full-length htt containing the identical number of glutamines (Davies et al., 1997; Hackam et al., 1998; Lunke and Mandel, 1998; Reddy et al., 1998; Schilling et al., 1999; Hodgson et al., 1999). Several lines of evidence indicate that htt cleavage and nuclear localization are necessary to induce degeneration (Saudou et al., 1998; Kim et al., 1999; Peters et al., 1999; Wellington et al., 2000). Accordingly, htt can be cleaved by different proteases (Wellington et al., 2000; Kim et al., 2001; Lunke et al., 2002; Gafni et al., 2004), but their respective contribution *in vivo* is still unclear.

The expression of htt and the localization of intranuclear inclusions in HD brains do not correlate exactly with the pattern of selective degeneration. Htt expression in the striatum, the most affected area in HD,

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is less abundant than in other brain regions (Landwehrmeyer et al., 1995). Furthermore, double-labeling of individual striatal neurons showed low-to-moderate htt immunoreactivity in GABAergic medium-sized striatal projection neurons (Ferrante et al., 1997; Gorfinkel-An et al., 1997; Fusco et al., 1999), whereas striatal interneurons, which are spared in HD, showed high htt immunoreactivity (Sapp et al., 1997; Fusco et al., 1999). In contrast, htt content is high in all cortical pyramidal neurons, especially those projecting to the striatum (Ferrante et al., 1997; Fusco et al., 1999). In HD brains, neurons with intranuclear inclusions do not correspond exactly with those that degenerate (Kuemmerle et al., 1999). Although intranuclear inclusions are present in the striatum, they are much more frequent in the cerebral cortex (Gutkunst et al., 1995; Sieradzan et al., 1999). Furthermore, within the striatum they are predominantly observed in spared large interneurons (Kuemmerle et al., 1999). This differential distribution cannot be attributed to a greater neuronal loss in the striatum, because these inclusions are equally rare in low-grade cases (Gutkunst et al., 1999). It has been suggested that this distinct accumulation of mutant htt aggregates between cortical and striatal neurons might be related to higher htt expression levels in the cortex or to different mechanisms for the recognition and elimination of misfolded proteins in these two brain regions (Sieradzan and Mann, 2001).

Unlike intranuclear inclusions, the presence of neuropil aggregates in the cerebral cortex and striatum, in any pathological grade, correlates with HD

neuropathology (Gutkunst et al., 1999). Analysis of post-mortem brains of HD patients at early stages of the disease showed the presence of dystrophic neurites prior to cell death (DiFiglia et al., 1997; Sapp et al., 1999). The presence of neuritic aggregates suggested that they could play an important role in the pathological process by dysregulating synaptic function and inducing neurite degeneration (Li et al., 2000).

Many mechanisms regulate the critical concentration of the aggregate precursor in a given cell, such as subcellular compartmentalization, differential processing of the full-length protein and expression levels (Bates, 2003). In fact, the continued expression of mutant htt is required for the development of a progressive HD phenotype (Lunkes and Mandel, 1998; Yamamoto et al., 2000). This has been shown by use of a conditional transgenic mouse model expressing exon 1 of the human *HD* (with 94Q) gene under the control of a tetracycline-dependent promoter which allows programmable switching off of the transgene expression (Yamamoto et al., 2000). Blockade of htt expression in symptomatic mice, with neuronal atrophy and dysfunction but without neuronal loss, leads to a disappearance of inclusions and an amelioration of the behavioral phenotype, demonstrating that a continuous influx of the mutant protein is required to maintain inclusions and symptoms (Yamamoto et al., 2000; Martin-Aparicio et al., 2001; Diaz-Hernandez et al., 2004). Similarly, htt gene silencing improved behavioral and neuropathological abnormalities in a mouse model of HD at a stage where there was no neuronal loss (Harper et al., 2005).

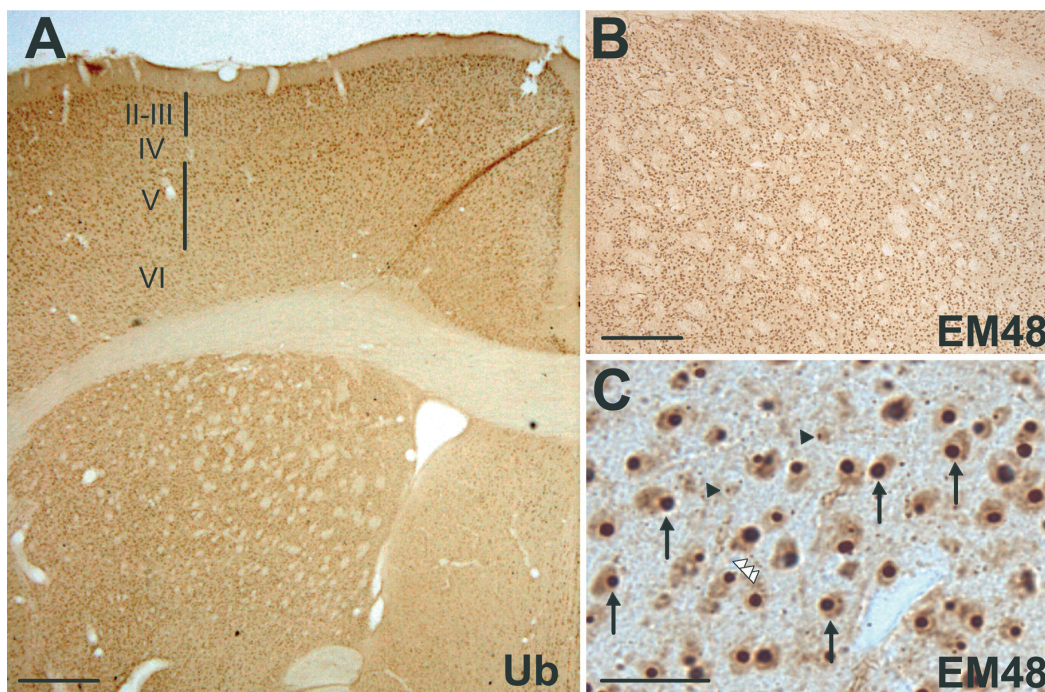


Fig. 1. Cellular aggregates of mutant htt are a characteristic hallmark of HD. **A.** Low magnification shows larger intranuclear ubiquitin-positive aggregates in the cerebral cortex than in the striatum of the transgenic mouse model R6/1. Aggregates are mainly located in layers II-III and V of the cerebral cortex. **B, C.** Aggregates can also be detected using a specific antibody against mutant htt (EM48). Intranuclear aggregates are detected at low magnification in the striatum (**B**). At higher magnification, small aggregates can also be detected in the neuropil (**C**). Arrows indicate large intranuclear aggregates, white arrowheads indicate small aggregates located in neuronal processes, black arrowheads indicate glial aggregates. Scale bars: A, 500 μ m; B, 250 μ m; and C, 50 μ m.

Interestingly, a very recent report shows that in the conditional mouse model of HD, switching off the transgene expression in advanced stages of disease, once neuronal loss has taken place in the brain, recovers motor dysfunction, reverses the vast majority of inclusions and attenuates additional neuronal loss (Diaz-Hernandez et al., 2005).

Although HD brains are characterized by the presence of aggregates, the exact role of these aggregates in HD pathogenesis is not clear. Aggregate formation is thought to be toxic and to cause neurodegeneration (Davies et al., 1997; DiFiglia et al., 1997; Ordway et al., 1997; Becher et al., 1998), to be uncoupled with toxicity (Kim et al., 1997; Leavitt et al., 1999) or even to be neuroprotective (Saudou et al., 1998; Arrasate et al., 2004; Ravikumar et al., 2004). In addition, the lack of correlation between aggregate localization and neuronal vulnerability in HD suggests that selective neurodegeneration might result not only from the specific distribution of toxic htt products but also from intrinsic neuronal properties. Therefore, each neuron subtype has a unique cellular context for affecting htt toxicity. Indeed, other mechanisms have been related to the development of neurodegenerative processes in HD, such as excitotoxicity, mitochondrial dysfunction and trophic factors.

Excitotoxicity in HD

The striatum receives large glutamatergic input from corticostriatal afferents, which makes it a structure at risk of glutamate-mediated excitotoxic injury. Corticostriatal projections extend from cortical neurons in layers III, V and deep layer VI to any striatal region (Gerfen, 1992; Flaherty and Graybiel, 1994). Glutamate exerts its action *via* ionotropic glutamate receptors, which are ligand-gated cationic channels (N-methyl-D-aspartate: NMDA; α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate: AMPA; and kainate: KA), or metabotropic glutamate receptors coupled to second-messenger systems *via* G proteins (Hollmann and Heinemann, 1994). Excitotoxicity, which results from over-stimulation of ionotropic glutamate receptors, has been related to the neuronal death observed in several neurodegenerative disorders (Choi, 1988). The excitotoxic hypothesis was first considered in the context of HD when it was observed that administration of glutamate receptor agonists in the striatum reproduces some of the biochemical features of HD (Coyle and Schwarcz, 1976; Schwarcz and Kohler, 1983; Beal et al., 1986). Among the glutamate receptor agonists tested, intrastriatal injection of quinolinic acid (QUIN; an NMDA receptor agonist) most closely mimics the neuropathology of HD in rats (Beal et al., 1986, 1989) and in non-human primates (Ferrante et al., 1993).

In human HD brains, striatal NMDA receptors significantly decrease even in pre-symptomatic stages of the disease (London et al., 1981; Dure et al., 1991), suggesting that neurons that express these receptors

strongly may be the most vulnerable (Young et al., 1988; Albin and Gilman, 1990). However, NMDA receptors are abundantly expressed on both striatal projection neurons and interneurons (Standaert et al., 1994; Landwehrmeyer et al., 1995). Moreover, other brain regions that are not affected in HD, such as the hippocampus, cerebellum and brainstem, contain similar or higher levels of glutamate receptors than the striatum, suggesting that other mechanisms could account for the regional specificity of HD pathology. In fact, it has been suggested that the selective loss of striatal neurons could be related to differences in the subunit composition of the NMDA receptors. These receptors are composed of combinations of NR1 and NR2A-D subunits (Hollmann and Heinemann, 1994). NR1 subunit is required for NMDA receptors to be functional, but NR2 subunit confers other important properties such as an increase in channel permeability and deactivation time, and changes in the sensitivity to Mg^{2+} or glycine (Schoepfer et al., 1994). Striatal projection neurons and interneurons display important differences in NMDA receptor subunit composition. Almost all populations of striatal neurons express high levels of NR1 subunit (Chen and Reiner, 1996; Chen et al., 1996; Ghasemzadeh et al., 1996), but striatal projection neurons predominantly express NR2B subunit together with small amounts of NR2A, whereas interneurons predominantly express NR2D with low levels of NR2A and NR2C (Standaert et al., 1994; Landwehrmeyer et al., 1995; Chen et al., 1999). Interestingly, NMDA receptors containing NR2C or NR2D subunits have lower affinity for QUIN than those containing NR2A and NR2B (Buller et al., 1994), showing that the lack of NR2A/B in striatal interneurons may thus confer resistance to NMDA-mediated excitotoxicity. In support of this hypothesis, it has been demonstrated that projection neurons and interneurons show different responses to glutamate receptor activation (Calabresi et al., 2000; Cepeda et al., 2001). Moreover, mutant htt selectively increases the current flowing through NMDA receptors comprising NR1/NR2B subunits (Chen et al., 1999; Zeron et al., 2001, 2002). Therefore, it has been suggested that the relative expression levels of NR2B compared with other NR2 subunits could account for the regional variation in neuronal degeneration severity in HD. Consistent with this, brain regions not affected in HD such as the cerebellum and brainstem express low levels of NR2B subunit (Hollmann and Heinemann, 1994; Monyer et al., 1994; Portera-Cailliau et al., 1996; Goebel and Poesch, 1999; Thompson et al., 2000).

All these results raised the question of whether mutant htt expression modulates the activity of NMDA receptors. It has been reported that htt is associated with post-synaptic density protein 95 (PSD-95), which is involved in the post-synaptic clustering of NMDA receptors (Sun et al., 2001). Mutant htt may alter the structure of the postsynaptic apparatus, since the presence of polyQ expansion significantly reduces its binding to PSD-95 (Sun et al., 2001). Furthermore,

mutant htt increases tyrosine phosphorylation of NMDA receptors via PSD-95, contributing to their sensitization (Song et al., 2003). Therefore, the interaction between mutant htt and associated cytoskeletal proteins, which are known to modulate NMDA receptor channel function and/or subcellular localization (Scannevin and Haganir, 2000), may increase NMDA receptor-mediated excitotoxicity. However, results obtained in various transgenic animal models of HD are controversial. Transgenic mice expressing exon 1 of the human HD gene with 115 (R6/1) or 155 (R6/2) CAG repeats show resistance to QUIN-induced striatal excitotoxicity (Hansson et al., 1999), whereas mice expressing full-length htt with 72 CAG repeats (YAC72) show increased sensitivity (Zeron et al., 2002). These differences have been attributed to the number of CAG repeats or the length of the mutant htt (Zeron et al., 2002). Furthermore, R6/1 and R6/2 animals develop resistance to QUIN gradually, which correlates with striatal cells expressing mutant htt being better able to handle cytoplasmic calcium overload after QUIN injection (Hansson et al., 2001a). These results suggested that exon 1 of mutant htt induces a sub-lethal grade of excitotoxicity that may cause an adaptation of striatal neurons to excitotoxic insult, resulting in an up-regulation of defense mechanisms (Hansson et al., 2001a). In fact, enhanced Akt signaling was an early response to NMDA receptor activation in mutant htt knock-in cells (Gines et al., 2003a). Furthermore, a reduction in the phosphorylation of NR1 subunit and PSD-95-like protein levels was observed in a transgenic mouse model of HD, which could result in the protection of striatal neurons from excitotoxicity (Jarabek et al., 2004).

Mitochondrial dysfunction in HD

In addition to excitotoxicity, mitochondrial dysfunction has been implicated in the physiopathology of HD (Beal, 2000; Rego and Oliviera, 2003). Hence, defects in energy metabolism affecting the brain and peripheral tissues have been reported in HD (Jenkins et al., 1993; Antonini et al., 1996; Lodi et al., 2000). Magnetic resonance spectroscopy studies showed a defect in oxidative energy metabolism in HD, suggesting an involvement in striatal degeneration (Jenkins et al., 1993, 1998; Browne et al., 1997). Additionally, biochemical studies disclosed that mitochondrial defect parallels severity and neuronal loss in HD brain. Complex II/III deficiencies, with a smaller decrease in complex-IV activity, were observed in the striatum but not in other brain areas such as cerebellum or cortex (Gu et al., 1996; Browne et al., 1997; Tabrizi et al., 1999). Furthermore, mitochondria from HD patient lymphoblasts display stress-induced mitochondrial depolarization that correlates with the CAG-repeat number (Sawa et al., 1999) and increased susceptibility to both calcium and mitochondrial inhibitors (Panov et al., 2002). In support of the hypothesis that

mitochondrial dysfunction plays a role in HD pathogenesis, systemic administration of 3-NP (an irreversible inhibitor of mitochondrial complex II) in rats and non-human primates produces striatal degeneration similar to that observed in HD (Beal et al., 1993a; Brouillet et al., 1995). Another complex II inhibitor, malonate, has also been used successfully to reproduce HD in animal models (Beal et al., 1993b; Greene et al., 1993).

Studies of various transgenic animal models have also suggested the involvement of energy impairment in HD pathogenesis. Magnetic resonance spectroscopy disclosed a decrease in N-acetylaspartate in R6/1 and R6/2 transgenic mice that could reflect impaired mitochondrial energy production (Jenkins et al., 2000; Van Dellen et al., 2000). In fact, increased sensitivity to 3-NP was found in cultured mutant knock-in striatal cells (Gines et al., 2003b; Ruan et al., 2004) as well as in striatal primary cultures obtained from YAC72 and YAC128 mutant mice (Shehadeh et al., 2006). However, contradictory results were obtained in R6/1 and R6/2 mice after treatment with mitochondrial complex II inhibitors. Larger (Bogdanov et al., 1998) or smaller (Hickey and Morton, 2000) striatal lesions than littermate controls were observed in R6/2 mice after systemic injection of 3-NP. Furthermore, R6/1 and R6/2 mice displayed resistance to malonate treatment that was dependent on CAG repeat length and age of onset (Hansson et al., 2001b).

How mutant htt compromises the function of complex II has not yet been established, although studies *in vitro* show that polyQ constructs directly promote mitochondrial dysfunction during graded calcium load (Panov et al., 2003). Furthermore, it has been shown that mutant htt is associated with the outer mitochondria membrane and decreases the calcium threshold required to induce mitochondrial transition pore opening (Choo et al., 2004). It has also been hypothesized that mutant htt affects the transcriptional regulation of specific genes that encode for mitochondrial metabolic proteins that decrease in HD mouse models (Luthi-Carter et al., 2000, 2002; Chan et al., 2002).

A link between chronic mitochondrial anomalies and excitotoxicity has also been suggested, since NMDA receptor antagonists can block the effect of mitochondrial inhibition (Henshaw et al., 1994; Greene and Greenamyre, 1995). Furthermore, inhibitors of succinate dehydrogenase strengthen, in the long term, NMDA-mediated excitation in striatal projection neurons but not in cholinergic interneurons (Calabresi et al., 2001). In support of these results, it has recently been shown that treatment with 3-NP induces higher depolarization of projection neurons in presymptomatic R6/2 HD transgenic mice than in wild-type mice, whereas the effect of 3-NP on cholinergic interneurons was similar in both genotypes (Saulle et al., 2004). These results suggest that the diverse membrane changes induced by inhibition of mitochondrial complex II may contribute to cell-type-specific neuronal death in HD.

Although the striatum is the brain region most vulnerable to 3-NP toxicity, cell loss in several areas of the cerebral cortex anatomically connected to the striatum also occurs after onset of striatal degeneration (Mittoux et al., 2000, 2002). Interestingly, metabolic compromise induces the death of cultured striatal and cortical neurons to the same extent, though through different molecular mechanisms (Galas et al., 2004). In addition, striatal and cortical cells expressing mutant htt display differential vulnerability to several other stimuli such as excitotoxicity or oxidative stress (Snider et al., 2003). Finally, striatal mitochondria are more vulnerable to Ca^{2+} -induced transition permeability than cortical mitochondria (Brustovetsky et al., 2003), which could account for the selective degeneration of striatal neurons in HD.

Trophic factors in HD

Neurotrophic factors are involved in the regulation of many neuronal aspects such as survival, maintenance and differentiation (Murphy et al., 1997; Huang and Reichardt, 2001; Airaksinen and Saarma, 2002; Chao, 2003). These proteins exert their selective actions on neuronal populations through the activation of specific receptors (Stahl and Yancopoulos, 1994; Airaksinen et al., 1999; Chao, 2003; Huang and Reichardt, 2003). Trophic factors have been suggested as good candidates for treatment of several neurodegenerative diseases, because they exert neuroprotective action on affected neuronal populations (Alexi et al., 2000; Aebischer and Ridet, 2001; Thoenen and Sendtner, 2002). Regarding HD, most of the studies on the regulation of trophic factors and protective effects have been performed in the excitotoxic or the 3-NP models. Among the trophic factors described up to now, neurotrophins, glial cell line-derived neurotrophic factor (GDNF) family and ciliary neurotrophic factor (CNTF) have been studied the most. Neurotrophins and their receptors, as well as GDNF-family members and receptors, are differentially regulated by intrastriatal injection of glutamate receptor agonists in the adult rat striatum (Canals et al., 1998, 1999; Marco et al., 2002a) or at different postnatal stages (Checa et al., 2000, 2001). This endogenous regulation following excitotoxicity suggests a possible contribution of trophic factors to the maintenance of striatal neuronal survival. Indeed, we and others have shown the neuroprotective effects of neurotrophins and GDNF family members against excitotoxicity (reviewed in Alberch et al., 2002, 2004).

The neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5, prevent the atrophy and death of striatal projection neurons induced by intrastriatal QUIN (Martinez-Serrano and Bjorklund, 1996; Alexi et al., 1997; Araujo and Hilt, 1997; Bemelmans et al., 1999; Perez-Navarro et al., 1999a, 2000a; Kells et al., 2004) or KA (Gratacos et al., 2001) injection. Furthermore, BDNF also protects striatal

projection neurons against neuronal injury and improves motor impairment induced by 3-NP toxicity (Ryu et al., 2004). Of all the neurotrophins tested, BDNF provides the strongest protection against striatal neuronal death (Perez-Navarro et al., 2000a; Gratacos et al., 2001), whereas NT-3 is the most efficient in regulating the phenotypical changes (atrophy and reduction in expression levels of enkephalin and SP) induced by QUIN injection (Perez-Navarro et al., 1999a). GDNF family members also prevent striatal neuronal degeneration. GDNF and neurturin protect these neurons against QUIN (Perez-Navarro et al., 1996, 2000b; Kells et al., 2004) or KA (Gratacos et al., 2001) injection, whereas GDNF also prevents striatal degeneration induced by systemic administration of 3-NP (Araujo and Hilt, 1998). However, these trophic factors exert specific actions, since GDNF acts only on striatonigral neurons (Perez-Navarro et al., 1999b) while striatopallidal neurons are only neuroprotected by neurturin (Marco et al., 2000b). Several studies have described the neuroprotective action of CNTF on striatal neurons. This trophic factor prevents degeneration of striatal projection neurons and behavioral changes induced by intrastriatal QUIN injection (Emerich et al., 1996, 1997a, 1998; de Almeida et al., 2001; Regulier et al., 2002; Emerich, 2004) or 3-NP treatment in rats (Mittoux et al., 2002). Furthermore, it also exerts neuroprotective effects in primates against intrastriatal injection of QUIN (Emerich et al., 1997b) or treatment with 3-NP (Mittoux et al., 2000). These positive results obtained with CNTF led to a phase-I study to evaluate the safety of intracerebral administration of this protein in subjects with HD (Bachoud-Levi et al., 2000). For this study, polymer-encapsulated cells engineered to secrete human CNTF were used to deliver this trophic factor over a two-year period. Improvements in electrophysiological results that correlate with capsules releasing the largest amount of CNTF were shown. Although this phase-I study shows the safety and tolerability of this gene approach, the survival of engineered cells is variable and needs to be improved (Bloch et al., 2004). The effect of some of these trophic factors has also been examined in transgenic models of HD. The injection of lentiviral vectors expressing CNTF in the striatum of YAC72 mice decreases the behavioral deficit of these animals (Zala et al., 2004). Treatment with CNTF or BDNF prevents apoptotic striatal cell death induced by transfection of mutant htt (Saudou et al., 1998).

Recently, much attention has focused on the study of BDNF and its role in the neurodegeneration observed in HD, as this neurotrophin is transcriptionally regulated by htt: wild-type htt increases *BDNF* gene transcription in cortical neurons, while mutant htt decreases it (Zuccato et al., 2001, 2003). Accordingly, BDNF expression and protein levels are lower in cortical and striatal tissues from HD patients (Ferrer et al., 2000; Zuccato et al., 2001). Furthermore, different transgenic mouse models of HD such as YAC72 (Zuccato et al., 2001), N171-82Q (Duan et al., 2003), R6/2 (Zhang et al., 2003) and knock-

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in (Gines et al., 2003b) show decreased levels of BDNF in cortical and striatal areas. As BDNF promotes the survival of cultured striatal neurons (Ventimiglia et al., 1995; Gavalda et al., 2004) and has potent neuroprotective effects on striatal projection neurons (Perez-Navarro et al., 1999a, 2000a, 2005; Gratacos et al., 2001), it has been hypothesized that BDNF deficit contributes to the selective degeneration of striatal projection neurons that occurs in HD. Interestingly, wild-type htt also enhances vesicular transport of BDNF along microtubules, a function that is attenuated both in the disease context and by reducing the levels of wild-type htt (Gauthier et al., 2004). Furthermore, excitotoxic lesion in the striatum increases BDNF mRNA levels in the cortex, suggesting that this BDNF could be anterogradely transported to provide trophic support to damaged striatal neurons (Canals et al., 2001). Recently, it has been shown that striatal neurons require cortical BDNF to acquire normal dendrite morphology and to

survive long-term (Baquet et al., 2004). All these results point to cortical BDNF as an important factor in the regulation of the function and survival of striatal neurons. The functional effects of endogenous BDNF levels on the physiopathology of HD have been studied in a double mutant mouse (bDM) expressing mutant htt and low levels of BDNF. These animals show advanced onset of motor dysfunctions, with a more severe lack of motor coordination. This abnormal behavior correlates with a specific loss of striatal enkephalinergic projection neurons, which can be prevented by treatment with BDNF (Canals et al., 2004). Dopaminergic neuronal dysfunction, with a reduction in dopaminergic neurons labeled with a retrograde marker, striatal dopamine content and dopamine receptor expression, has also been observed in bDM (Pineda et al., 2005), which could contribute to the motor disturbances observed in HD.

The involvement of endogenous BDNF in the physiopathology of HD has also been shown in HD patients. There is a polymorphism in the *bdnf* gene leading to a valine (Val)-to-methionine (Met) substitution at position 66 in the prodomain (Val66Met) which alters intracellular trafficking and activity-dependent secretion of BDNF (Egan et al., 2003; Chen et al., 2004). Human heterozygous for this polymorphism have differential susceptibility to several neuropsychiatric disorders (Ventriglia et al., 2002; Neves-Pereira et al., 2002, 2005; Sen et al., 2003). In HD patients, BDNF Val66Met is associated with a later onset of motor abnormalities (Alberch et al., 2005), although the mechanism by which BDNF Val66Met interacts with mutant htt to modulate the onset of HD is still unknown.

Therapeutic approaches

Most of the drugs developed for treating HD are based on the involvement of excitotoxicity and mitochondrial dysfunction in neuronal degeneration. Several clinical trials have been conducted: using NMDA antagonists (remacemide: Kiebertz et al., 1996; ketamine: Murman et al., 1997), glutamate-release inhibitors (baclofen: Shoulson et al., 1989; lamotrigine: Kremer et al., 1999; riluzole: Huntington Study Group, 2003) or mitochondrial support agents (creatine: Tabrizi et al., 2005; coenzyme Q10: Huntington Study Group, 2001). None of these studies showed great benefits: only a trend toward improvement in chorea was observed after treatment with remacemide and a reduction in chorea was seen 8 weeks after riluzole administration. In another clinical trial, a combination of coenzyme Q10 and remacemide was examined, with no significant effects on functional decline in early HD (Huntington Study Group, 2001).

Effective therapies for neurodegenerative disorders are those that prevent or inhibit early pathological events before neuronal death occurs. Treatment with neurotrophic factors is a promising therapy because these proteins regulate many aspects of neuronal

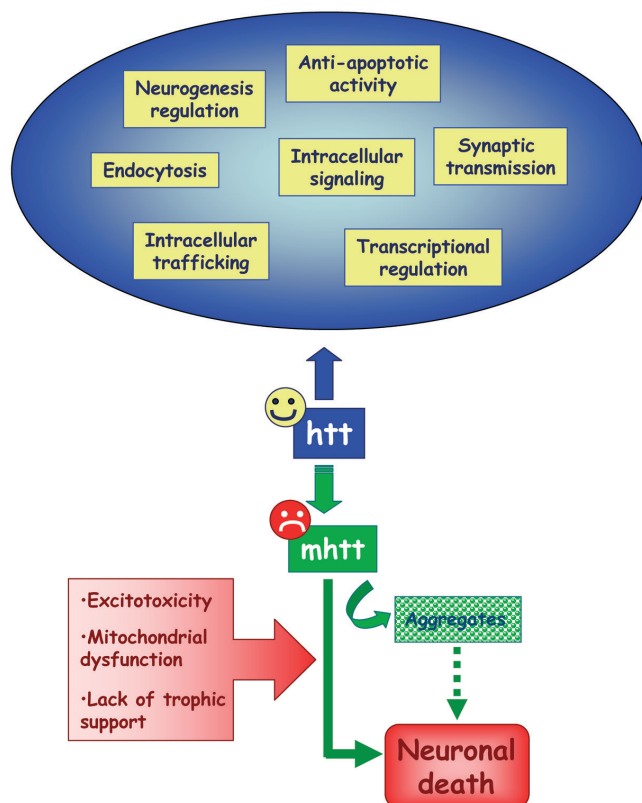


Fig. 2. Schematic diagram showing proposed functions for wild-type htt and mechanisms involved in neuronal cell death in the presence of mutant htt. Wild-type htt (htt) regulates neuronal development and controls several important neuronal functions in adulthood. When htt is mutated (mhtt) it forms aggregates in the cytoplasm and nucleus but the contribution of aggregates to neuronal cell death is not clear. Several mechanisms, intrinsic of each neuronal population, modulate neuronal degeneration in the presence of mhtt.

function. Furthermore, they prevent neuronal cell death in animal models of neurodegenerative diseases (Alberch et al., 2004). Among the trophic factors analyzed in the context of HD, treatment with BDNF is the most promising approach to stopping the disease or slowing its progression. However, BDNF needs the presence of its receptor TrkB to exert its protective effects. In this context, we recently observed that mutant htt also affects TrkB expression (Gines et al., 2006). GDNF family members could also be relevant to the treatment of HD, since GDNF and neurturin exert distinct protective actions on striatal projection neurons against excitotoxicity. GDNF prevents the death of striatonigral neurons, while neurturin is specific for the striatopallidal neurons, suggesting that the use of one of these two trophic factors may help to recover the equilibrium between both striatal projection pathways. Thus, a combined approach using distinct trophic factors, depending on the stage of the disease's progress, would lead to positive neuroprotective effects in HD.

Some neurotrophic factors have been tested in clinical trials, though without success (Aebischer and Ridet, 2001; Apfel, 2002; Thoenen and Sendtner, 2002). One problem might be related to inappropriate delivery techniques. To use trophic factors such as therapeutic molecules, consistent delivery systems have to be developed that will allow localized delivery that can be regulated. Different alternatives have been tested, such as mini-pumps releasing trophic factors directly into the central nervous system, viral vectors or cell delivery (Aebischer and Ridet, 2001). Another important unresolved issue is the timing of therapeutic intervention. Interestingly, a recent study, using a novel imaging approach, shows a progressive regional grey matter loss in pre-symptomatic HD mutation carriers over a period of two years before the onset of significant clinical decline (Kipps et al., 2005). Therefore, this imaging technique could become a powerful tool in neuroprotection trials.

Conclusion

Today there is evidence that HD is not a result of one single mechanism and that multiple pathological pathways participate in this disease (Fig. 2). Extensive evidence also indicates that the precise biological function and protein context in a given subset of neurons may determine their vulnerability to mutant htt. Therefore, the study of the interactions between htt and intrinsic neuronal properties may help us to understand the physiopathology of HD and to develop new therapeutic strategies.

Acknowledgements. Financial support was obtained from the Ministerio de Educación y Ciencia (SAF2005-01335; SAF2005-00147; SAF2005-02333); Redes Temáticas de Investigación Cooperativa (G03/167; G03/210, Ministerio de Sanidad y Consumo); Fondo de Investigaciones Sanitarias (Instituto de Salud Carlos III); and Fundació La Caixa.

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Accepted May 19, 2006