

Integrative Hypothesis for Huntington's Disease: A Brief Review of Experimental Evidence

V. PÉREZ-DE LA CRUZ, A. SANTAMARÍA

Laboratory of Excitatory Amino Acids, National Institute of Neurology and Neurosurgery Manuel Velasco Suárez, Mexico City, Mexico

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Summary

Huntington's disease (HD) is a demential, neurodegenerative inheritable disease affecting middle-aged patients. HD is characterized by uncontrolled choreiform movements, psychiatric symptoms and cognitive decline. Histopathological changes in HD brains reveal a considerable damage to basal ganglia, particularly affecting middle-sized spiny neurons from the caudate-putamen region. Neurochemical changes are specifically oriented to deplete GABAergic and cholinergic systems, while molecular alterations include an increased expression of CAG trinucleotide at exon 1 from the huntingtin (*htt*) gene, as well as aggregation of mutant *htt*. Although several hypotheses regarding the mechanisms by which neurotoxicity is triggered in HD brains have been suggested on the basis of experimental evidence, so far it remains not clear which of them are predominant or whether they are complementary. Recent experimental evidence through transgenic mice models reveal an interesting interaction between expanded CAG triplets, mutant *htt*, and the increase in toxic metabolites from the kynurenine pathway. Further evidence supports the assumption that different toxic mechanisms (i.e. excitotoxicity, energy metabolism impairment, inflammatory events, oxidative stress, etc.) are confluent and depend on each other. In this review we will briefly summarize some of those findings and propose a final integrative hypothesis for HD.

Key words

Huntington's disease • Huntingtin aggregation • Excitotoxicity • Energy metabolism deficit • Oxidative stress • Inflammation

Huntington's disease

Huntington's disease (HD) is a neurodegenerative disorder produced by CAG trinucleotide expansion at the huntingtin (*htt*) gene. As a neurodegenerative disorder, HD is characterized by

dementia and degeneration of basal ganglia (caudate-putamen) and brain cortex, thus producing abnormal choreiform movements. HD affects humans at middle age (around 35-50 years of age) and presents an autosomic dominant inheritance pattern. Mutation is located at the short arm of chromosome 4, being *htt* its mutated protein

(H.D.C.R.G. 1993). Wild-type htt presents up to 35 glutamine residues, while its mutant form shows 38 or more residues. Therefore, it is accepted as a diagnostic parameter that HD patients present more than 35 CAG repeats at exon 1 of htt gene, although a juvenile variant of the disease (with an early onset) may exhibit up to 250 CAG repeats (H.D.C.R.G. 1993, Duyao *et al.* 1993). Until now, the precise role of htt in HD is unknown, although the mutant protein might be responsible for alterations in some components of the cytoskeleton, thus leading to affected axonal transport and cell death.

Biochemical alterations found in the caudate of patients with HD are a consequence of selective cell death in neurons from basal ganglia. These changes include decreased levels of γ -aminobutyric acid (GABA) and its synthesis enzyme glutamate decarboxylase (GAD), acetylcholine (ACh) and its synthesis enzyme choline-acetyl transferase (CAT), and some peptides specifically localized in middle-sized spiny neurons (Shoulson 1984). In addition, important alterations in the number of N-methyl-D-aspartate receptors (NMDA) have been described, suggesting that some components of the glutamatergic transmission may be involved as causative factors in HD (Ellerby 2002).

In HD, three major stages can be distinguished according to the severity of the disease: early, middle and late. At a behavioral level, HD is characterized by irritability, obsessions and compulsions, hallucinations, impaired memory, altered family dynamics, decreased executive functions and dementia, all of them inexorably progressing along the different stages. The cognitive deficit is accompanied by motor alterations which include both involuntary movements and alterations in voluntary movements. Motor restlessness, dystonia and chorea are characteristic involuntary movements from HD patients, while voluntary movements can be affected by bradykinesia, lack of coordination and delayed initiation of movements, as well as impairment in their modulation (The Huntington's Disease Society of America, 2006). While neurochemical changes might specifically be responsible for some of these motor alterations, it is still unclear whether the complex and progressive pattern of motor and cognitive deficits is the result of multiple compromised neurotransmitter systems or specific neuronal populations affected. In this regard, it is assumed that the glutamatergic component in HD might be related to early excitation stages, while these changes can also be dependent on the depletion of GABA. Affected dopaminergic and cholinergic systems have also

been involved in HD and constitute a source of continuous investigation.

Altogether, the features described above support the idea that those hypotheses helping to explain the alterations in HD need to involve the generation of excitotoxic events, the alteration in energy metabolism and mitochondrial dysfunction, the resulting oxidative stress, and the htt aggregation, all leading to neurotoxicity. Behavioral correlates are also relevant to evidence of similar patterns in HD and its models, especially those resembling locomotor abnormalities. Thanks to the simultaneous occurrence of some of these mechanisms in different paradigms mimicking HD, it is now possible to consider all these hypotheses as complimentary. Indeed, the purpose of using different animal models of HD is to try to elucidate those toxic mechanisms leading to neuronal damage and their possible interactions. Such models follow different routes but certainly, none of them exclude each other. They can be classified into three major categories: 1) excitotoxic models, 2) impaired energy metabolism models, and 3) transgenic models. Therefore, given the information that has been collected through several years of research on both these paradigms and the human disorder, we may now suggest the following non-excluding major hypothesis for neurotoxicity observed in HD: a) excitotoxicity through NMDA receptor overactivation accompanying oxidative stress and inflammatory events, b) energy metabolism impairment leading to metabolic damage, oxidative damage and secondary excitotoxicity, and c) deletion of huntingtin protein, leading to its aggregation, compromise of structural components of nerve cells and further metabolic damage.

The aim of this Minireview is to provide the reader with evidence that these models share common mechanisms to formulate a single integrative hypothesis of Huntington's disease.

Excitotoxic models: quinolinic acid and HD

In general terms, excitotoxicity involves a drastic increase in intracellular Ca^{2+} concentrations in response to an overexposure of neurons to the effects of excitatory amino acids (EAA), such as glutamate and its analogues. In particular, NMDA receptors play a relevant role in the neurotoxicity induced by EAA since it is a widely distributed channel complex permeable to Ca^{2+} . When the receptor-channel complex is stimulated by endogenous or exogenous agonists, a sudden and drastic

influx of this ion into the neuronal cell may trigger metabolic lethal pathways involving proteolytic enzymes (proteases and endonucleases), thus increasing the formation of reactive oxygen (ROS) and nitrogen (RNS) species (Rami *et al.* 1997). Increased Ca^{2+} influx is also accompanied by enhanced neurotransmitter release that usually propagates cell damage through a positive feedback, thus leading cells to apoptotic or necrotic death. These mechanisms are currently involved in both acute (trauma) and chronic (Alzheimer's disease, Parkinson's disease, HD, etc.) degenerative disorders (Siesjo 1981, Choi 1988, Saito *et al.* 1993).

Different compounds have been employed to produce excitotoxic models of HD, among them are NMDA, kainic acid, ibotenic acid and quinolinic acid (QUIN). The effects of some of these toxins have been investigated in the rat brain with the aim to compare the extent of neuronal damage that they produce (Foster *et al.* 1983, Schwarcz *et al.* 1983, Beal *et al.* 1986). After looking at the morphologic alterations and biochemical profiles in the striatum of the lesioned animals, some changes resembling alterations in HD brains, such as decreased levels of GABA, ACh and substance P, as well as selective axon-sparing lesions, were observed. However, QUIN exerted more selective and specific effects, which are also similar to those of HD, as it produced no major changes in somatostatin or neuropeptide Y.

Concerning behavioral findings, Shear *et al.* (1998) compared intra-striatal injections of QUIN vs. 3-nitropropionic acid (3-NP, a mitochondrial toxin) in rats. They found that both toxins caused an increase in locomotor activity two weeks after the lesion, but only QUIN-induced hyperactivity lasted up to four weeks, although it was demonstrated that both toxins are equally capable to affect learning. In addition, a study in non-human primates revealed that QUIN-treated animals showed nocturnal hyperactivity, abnormal posture, and impairment in the successful responses in an object-retrieval task test (Roitberg *et al.* 2002). Altogether, these and other behavioral studies suggest that QUIN also mimics early stages of HD at behavioral levels. Furthermore, on the basis of these observations and its own endogenous nature, QUIN has been suggested as an agent potentially involved in the pathogenesis of HD. This consideration has been reinforced by recent findings demonstrating that the levels of this metabolite, together with those of another kynurenine metabolite responsible of ROS formation, 3-hydroxykynurenine (3-HK), are

increased in the striatum and cortex of HD brains (Guidetti *et al.* 2004). Such evidence has served to establish the formal concept of the "kynurineric hypothesis" which will be briefly mentioned in this work.

The kynurenine pathway is a metabolic route typically located in glial cells and reduces tryptophan into several metabolites with different redox functions. QUIN is an endogenous metabolite at the kynurenine pathway acting as a selective agonist at the NMDA receptor (Stone 1993), and produces membrane depolarization and Ca^{2+} influx into neuronal cells, resulting in damage when tested at toxic concentrations. Different metabolic alterations produced by QUIN include the reduction in the consumption of mitochondrial O_2 , decreased levels of ATP and NAD^+ , and important changes in the concentrations of glutamate and aspartate (Bordelon *et al.* 1997). QUIN is also responsible for some other toxic effects in the brain, such as increased levels of intracellular Ca^{2+} , decreased GABA levels, lipid peroxidation and oxidative stress, and further apoptotic and necrotic cell death (Foster *et al.* 1983, During *et al.* 1989, Santamaría and Ríos 1993). Moreover, the toxin reproduces the pattern of selective GABAergic and cholinergic cell loss found in HD, mainly affecting middle-size striatal spiny neurons (Bordelon *et al.* 1997, El-Defrawy *et al.* 1986), thus bringing phenomenological validity to the model of HD produced by QUIN.

Regarding its pro-oxidant potency, QUIN has been associated with the stimulation of tumor processes through a possible formation of hydroxyl radicals ($\bullet\text{OH}$) (Harman 1993). More recently, the toxin was demonstrated to directly induce the formation of $\bullet\text{OH}$ radicals under *in vivo* conditions in rat corpus striatum in a mechanism which seems to be independent of NMDA receptors (Santamaría *et al.* 2001a). Other recent reports emphasize the involvement of nitric oxide ($\text{NO}\bullet$) and superoxide anion ($\text{O}_2^{\bullet-}$) in the neuronal damage induced by QUIN, thus demonstrating its potential ability to produce the highly toxic RNS peroxynitrite (ONOO^-), in the course of days (Noack *et al.* 1998, Ryu *et al.* 2004) or even hours (Pérez-De La Cruz *et al.* 2005). The origin of ONOO^- in this model has been tracked toward the capability of QUIN to induce both the expression and the activity of inducible NO synthase (iNOS) (Ryu *et al.* 2004), thereby increasing the levels of $\text{NO}\bullet$ while decreasing the activity of the enzyme responsible of detoxifying $\text{O}_2^{\bullet-}$, superoxide dismutase (SOD). Such an altered cellular environment is propitious for the further formation of more ROS and RNS. Indeed, the roles of

NO and NOS in the pattern of QUIN toxicity have been largely discussed. While the actions of NOS have been described to be apparently bidirectional (Schmidt *et al.* 1995), the use of some NOS inhibitors, such as the general inhibitors L-NARG (Santamaría *et al.* 1999) and L-NAME (Pérez-Severiano *et al.* 1998), or the neuronal NOS inhibitor L-MIN (Bazzett *et al.* 1997), result in some protective actions against QUIN-induced neurotoxicity and oxidative damage, the last suggesting that nNOS might play, besides iNOS, an important role during excitotoxic insults. In contrast, some authors have recently proposed a hypothesis that some of these inhibitors prevent the QUIN-induced decrease in glutamate binding, alternative toxic mechanisms (i.e. oxidative damage, NO-htt interactions, etc.) might be an integral part of the pattern of toxicity elicited by QUIN (Lisý and Šťastný 2002). It remains to elucidate whether these NO- and NOS-mediated patterns take part in HD. Nevertheless, all these evidences have served for several groups to pay attention to the ability of QUIN to exert its neurotoxic actions through mechanisms independent of NMDA receptors, because QUIN is not a potent NMDA receptor agonist. Therefore, oxidative stress seems to be an attractive alternative to partially explain the neurotoxic events evoked by QUIN. Some mechanisms involved in the oxidative capacity of QUIN are: 1) the formation of QUIN-Fe²⁺ chemical complexes which in turn are mediating the generation of ROS and RNS (Goda *et al.* 1996, Štípek *et al.* 1997, Murakami *et al.* 1998, Iwahashi *et al.* 1999), 2) the alteration of some endogenous antioxidant systems (Rodríguez-Martínez *et al.* 2000), and 3) the direct generation of ROS and RNS in brain tissue (Santamaría *et al.* 2001a). Furthermore, these observations have been supported by the successful use of antioxidants with different modes of action against QUIN-induced neurotoxicity (Behan *et al.* 1999, Rossato *et al.* 2002, Santamaría *et al.* 2003, Pérez-Severiano *et al.* 2004, Pérez-de la Cruz *et al.* 2005), thus emphasizing the active role that ROS and RNS are playing in the pattern of toxicity produced by this molecule.

Due to these findings, there is a question whether this animal model mimics only some features of the pattern of neuronal damage observed in the human disorder mediated by excitotoxic events, or if it also represents some other toxic aspects besides those related to NMDA receptor activation. This topic is particularly relevant not only for HD, but also for some other human alterations. Due to the endogenous nature of QUIN, this toxic metabolite is involved in inflammatory disorders of

the nervous system, i.e. AIDS-dementia complex and hepatic encephalopathy (Heyes *et al.* 1992, Kerr *et al.* 1997), in which its levels are increased by monocytic cells during the inflammatory events. In this regard, the regulation that the kynurenine pathway exert on monocyte-derived macrophages formation has been investigated. It was found that QUIN and other tryptophan metabolites may modulate these responses in a NMDA receptor-dependent manner (Chiarugi *et al.* 2001a). On the basis of these observations, NMDA receptor antagonists and poly(ADP-ribose) polymerase inhibitors are thought to be promising candidates as therapeutic agents in HD. Another mechanism accounting for inflammation-mediated QUIN-induced toxicity is related to the expression of cytokines (interleukins, tumor necrosis factors, etc.) in astrocytes (Pemberton *et al.* 1997, Croitoru-Lamoury *et al.* 2003). Bacterial, viral, parasitic and fungal infections affecting the CNS, as well as meningitis, septicemia and autoimmune diseases are characterized by elevated levels of QUIN (Heyes *et al.* 1992), suggesting that kynurenine pathway may be involved in major toxic events in these disorders. These findings related to QUIN and inflammatory responses are quite relevant for HD, which is a disease with inflammatory components. They will provide insights to common toxic mechanisms in neurological diseases and related experimental models.

Energy metabolism deficit models: 3-nitropropionic acid and HD

The CNS is particularly vulnerable to variations in energetic resources due to the elevated metabolism of neuronal cells and their constant demand on substrates. Therefore, any alteration in energy metabolism represents a potential risk for loss of neuronal viability (Lees 1993). Changes in the availability of energy substrates (such as oxygen or glucose deprivation) produce major imbalances in ion concentrations, thus affecting membrane potential and further depolarization (Martin *et al.* 1994) since membrane ATPases are not capable of sustaining the resting potential. Ca²⁺ then becomes essential for these events, as a drastic increase in its cytosolic concentrations through the opening of voltage-dependent membrane channels and voltage-dependent NMDA receptor-channel complexes triggers excitotoxic events already described. In the last years several disorders associated with alterations in energy metabolism have been described. Such is the case of HD,

in which decreased levels of glucose and oxygen in basal ganglia and brain cortex have been reported (Beal 1992). Specific changes in respiratory metabolism are likely to occur in the activity of complexes I and IV (Brennan *et al.* 1985, Borlongan *et al.* 1997), and to a smaller extent, also in the activity of complex II. In this regard, one of the most recent and encouraging line of research refers to the promising use of creatine against alterations in HD and related experimental models. Creatine, a guanidino compound produced endogenously and acquired exogenously through the diet, is a critical component in maintaining ATP levels and ameliorating the severity of many of the pathogenic mechanisms associated with HD (Ryu *et al.* 2005). The positive effects obtained so far with this compound in HD patients and related models demonstrate the active role that energy metabolism failure plays in the triggering of toxic mechanisms leading to degenerative events.

In order to reproduce the metabolic alterations observed in HD at experimental levels, different animal models have been designed. Among them we can mention those of malonate and 3-nitropropionic acid (3-NP). We will now focus our attention on 3-NP, a fungal toxin that causes neurotoxicity in animals and humans (Ludolph *et al.* 1991). Brain lesions produced by a systemic administration of 3-NP are more or less specific of the striatum, although hippocampus, thalamus and brain cortex are also affected (Borlongan *et al.* 1997). For this reason, 3-NP has been widely used as a suitable model for disruption of energy metabolism, and more specifically as a model of HD (Beal *et al.* 1993, Borlongan *et al.* 1997, Brouillet *et al.* 1999). The primary mechanism of action of this toxin involves the inhibition of complex II (succinate dehydrogenase (SDH)) at the mitochondrial electron transport chain. SDH is an important enzyme located at the inner domain of the mitochondrial membrane and is responsible for oxidation of succinate to fumarate. When the enzyme is irreversibly blocked by 3-NP, decreased levels of ATP and neuronal cell death processes may be observed (Coles *et al.* 1979, Tunez *et al.* 2004).

With regard to behavioral findings, several studies have defined three major stages produced by 3-NP when administered to animals: 1) somnolence, 2) uncoordinated march with stereotypical padding and rolling movements, 3) lateral and ventral recumbence (Borlongan *et al.* 1995a, Koutouzis *et al.* 1994a). Further studies in mice showed that the systemic administration of 3-NP resulted in basal ganglia lesions with an initial

decrease in motor activity followed by occasional episodes of hyperactivity and abnormal movements (i.e. tremor, head bobbing, circling, tail rigidity and elevation) (Ludolph *et al.* 1991). On the other hand, repeated administration of 3-NP (each 4 days for a total of 28 days) evoked an early hyperkinetic pattern during the first two weeks followed by a pronounced hypokinetic pattern during the last two weeks, and such alterations were also dependent on the age (Borlongan *et al.* 1995a, 1997, Koutouzis *et al.* 1994b). When taken together, all these findings are suggesting that 3-NP is more likely mimicking either juvenile onset or late stages of HD (Borlongan *et al.* 1995b, Shear *et al.* 1998).

Far from being excluding, these observations are convergent and integrative when compared with other hypotheses and the human disease (Fig. 1). In this regard, the assumption that the impairment of energy metabolism may result in excitotoxic events was demonstrated by Novelli *et al.* (1988). These authors showed that some inhibitors of oxidative phosphorylation or Na⁺,K⁺-ATPase induce glutamate to become neurotoxic at concentrations that normally do not produce toxic effects. The mechanism directly involved in such an effect is related to the reduction in ATP levels, which is crucial for maintaining the membrane resting potential in neuronal cells (Beal 1994). Further studies, where microdialysis as an experimental tool has been employed, revealed only moderate increases in extracellular glutamate concentrations in the brains of 3-NP-treated rats, in contrast with increased levels of lactate as an evidence of an altered energy metabolism (Beal 1994, Wullner *et al.* 1994). These results are consistent with the concept that 3-NP causes excitotoxicity by making neurons vulnerable to endogenous basal levels of glutamate. In addition, it is known that 3-NP also changes the Ca²⁺ homeostasis under *in vitro* conditions, thus involving excitotoxicity. In turn, neuronal cell death induced by excitotoxicity has been related to the formation of ROS and RNS (Lafon-Cazal *et al.* 1993, Alexi *et al.* 1998), supporting a key role of oxidative stress in the pattern of toxicity elicited by 3-NP (La Fontaine *et al.* 2000). Systemic administration of 3-NP to animals also decreases the levels of glutathione while it enhances the formation of free radicals and oxidized proteins in the striatum (Schulz *et al.* 1996, Binienda *et al.* 1998). Furthermore, the use of well-known endogenous and exogenous antioxidants, such as coenzyme Q₁₀, N-acetylcysteine, melatonin, S-allylcysteine and dehydroepiandrosterone resulted in protective effects against the neuronal damage induced by

the toxin (La Fontaine *et al.* 2000, Tunez *et al.* 2004, 2005, Nam *et al.* 2005, Herrera-Mundo *et al.* 2006, Pérez-de la Cruz *et al.* 2006). Although the precise mechanisms by which antioxidants may protect against 3-NP toxicity still remain unclear, these effects might be related either to scavenging of free radicals leaked from the altered chain transport, or to preventive actions on the

blockade of succinate dehydrogenase. Nevertheless, all these evidences are pointing to the co-existence of common toxic patterns among models of different origin, since both QUIN and 3-NP seem to share and produce features of HD leading to neurodegeneration through comparable mechanisms, despite their obvious differences.

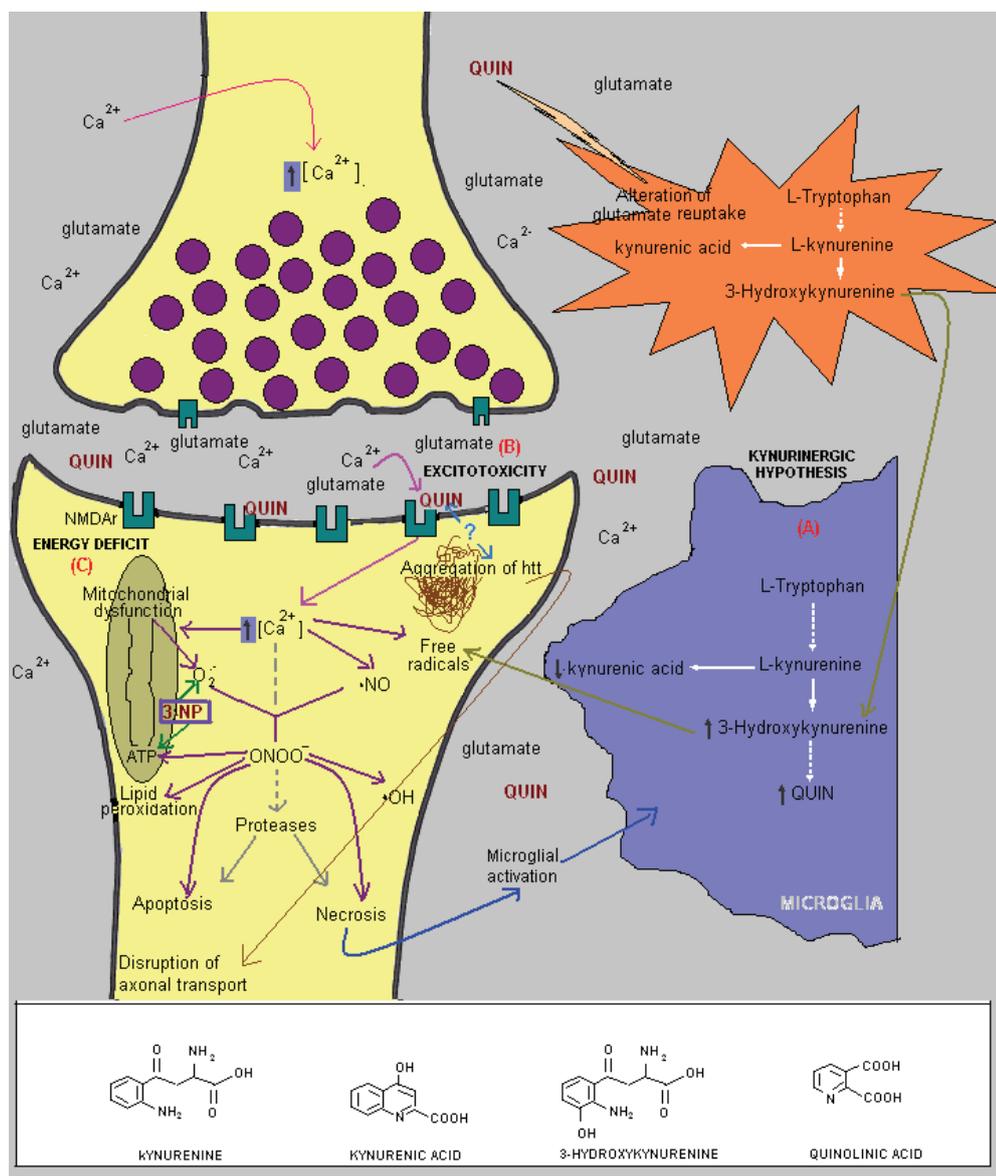


Fig. 1. Different experimental hypo-theses to explain neurotoxicity and cell death in Huntington's disease (HD). The kynurineric hypothesis (A) is based on the imbalance between the neurotoxic metabolites quinoate (QUIN) and 3-hydroxykynurenine (3-HK) with respect to the levels of the neuro-protective, modulatory and antioxidant metabolite kynurenic acid (KYNA), all of them coming from the kynurenine pathway. Such imbalance is produced during the early stages of the development of HD. Increased amounts of QUIN and 3-HK will act at an extracellular level, and then QUIN will produce excitotoxic events mediated by overactivation of NMDA receptors (B), thus causing increased intracellular Ca^{2+} concentrations, which in turn will lead to major alterations in synaptic and mitochondrial functions, generation of ROS and RNS, activation of proteases, phospholipases and endonucleases, etc. 3-HK will also act in favor of an enriched pro-oxidant cellular environment, thereby producing oxidative stress. Altogether, these factors may account for neuronal cell death (either necrotic or apoptotic), and the consequent activation of microglia with corresponding increase in

the formation of toxic metabolites. On the other hand, energy metabolism impairment (C) (primary mechanism by which 3-nitropropionic acid (3-NP), and probably huntingtin (htt) aggregation cause their toxic effects) will produce alterations in the membrane resting potential and a secondary excitotoxic glutamatergic pattern, thus contributing to the general scheme of neuronal damage. Until now, the origin and the precise role of htt aggregation on neuronal damage in HD are unclear, but it might be related to initial disruption in the axonal transport, and probably to excitotoxic damage. The contribution of astrocytes is also relevant due to the fact that, during stressing and microglial activation conditions, these cells generate more 3-HK. In addition, the glutamate re-uptake by astrocytes is affected by QUIN, and so there will be increasing extracellular concentrations of glutamate and more risk of oxidative damage. In summary, the kynurineric hypothesis is tightly related to excitotoxicity, energy deficit and htt aggregation since the last compromises the basic physiology of neurons, thereby releasing chemical messengers to the extracellular space, stimulating microglia and leading to increased levels of 3-HK/QUIN with energy failure and further neuronal death.

Transgenic models

The recent development of transgenic models brings new alternatives for the study of possible toxic mechanisms occurring in HD. From the different transgenic models available, R6/1 and R6/2 mice are among the most explored as yet. Both strains express exon 1 of *htt* gene from humans presenting around 115 and 150 CAG triplet repeats, respectively (Mangiarini *et al.* 1996). In general terms, R6 mice express only exon 1 (from a total of 67 exons of the full gene), which includes the tract of polyglutamine (Mangiarini *et al.* 1996, Smith *et al.* 2005), encoding for only 3 % of the N-terminal region of the protein. Interestingly, these animals develop some symptoms resembling specific alterations in HD, including motor dysfunction, loss of body weight and muscle mass, and marked neuropathological changes such as the formation of aggregates in the brain cortex and neostriatum (Petersen *et al.* 2002, Smith *et al.* 2005). Important neurochemical alterations have been observed in these animals from a functional point of view: by means of microdialysis studies, it has been shown that R6/1 mice exhibit changes in amino acid neurotransmitter release in the striatum (Nicnocaill *et al.* 2001). However, in contrast with human HD, levels of dopamine and serotonin are often reduced in R6/1 animals (Reynolds *et al.* 1999, Ariano *et al.* 2002, Yohrling *et al.* 2002), while the content of synaptic proteins (Morton *et al.* 2001, Morton and Edwardson 2001, Lievens *et al.* 2002, Modregger *et al.* 2002) and the glial transport system – which normally removes released glutamate from the extracellular space – (Lievens *et al.* 2001) appear both to be affected. In addition, striatal cells from R6/1 mice seem to suffer from oxidative stress, as evidenced by the increase in markers of oxidative damage to DNA. Such oxidative damage may probably be mediated in R6/1 animals by specific alterations in the activity of superoxide dismutase and the increase in the activity of NOS, while in R6/2 mice it may be related to the reduction in mitochondrial function (Tabrizi *et al.* 2000, Bogdanov *et al.* 2001, Santamaría *et al.* 2001b, Pérez-Severiano *et al.* 2002). Although both cases support the hypothesis of oxidative damage to the striatum in HD due to the formation of ROS and RNS, R6/1 mice are thought to be more related to oxidative damage. Since the striatal cells from R6/1 animals are suffering from oxidative stress, this strain should be considered as contrasting with other models of mutant mice (i.e. R6/2 mice or other models not tested yet for these alterations), in which a given mutation is not specifically associated with

oxidative stress, even if they may present neurotransmitter changes. This consideration should support the integrative hypothesis for HD, as long as more information can be obtained from other mutant models in the near future.

Behavioral changes in these mice have also served also as possible correlates for mimicking HD. R6/1 mice display locomotor abnormalities since 38 weeks of age, and they include weaving motion and a reduction in stride length (Clifford *et al.* 2002). Motor coordination deficit in a stationary beam tasks is evident in these animals between 8-9 weeks of age, and becomes more pronounced at 14 weeks of age (van Dellen *et al.* 2000). Recent evidence suggests that R6/1 mice show a biphasic motor pattern directly related to age (initial hyperactivity followed by hypoactivity, comparable with that of 3-NP). Bolivar *et al.* (2004) hypothesized that this was due to the fact that the initial impairment of motor circuitry results in early behavioral changes (hyperactivity), further leading to a decreased neural transmission, which is responsible for hypoactivity. In contrast, R6/2 mice develop a severe neurologic disorder with emerging important symptoms (tremor, poor coat condition and hindlimb claspings) since 8 weeks of age (Mangiarini *et al.* 1996). Behavioral analyses of these mice also reveal age-related dystonic movements, impairment in motor performance, grip strength and body weight, which worsen with time until their death (Carter *et al.* 1999, File *et al.* 1998, Lione *et al.* 1999, Stack *et al.* 2005). However, despite the fact that obvious symptoms appear at 8 weeks of age, there is evidence suggesting that behavioral, cellular, and molecular alterations occur between the third and the fourth weeks of age. Thus, behavioral changes at these earlier phase include deficit in spatial learning, recall of contextual fear-conditioned memory and aberrant locomotor activity. More recently, Hickey *et al.* (2005) described early motor deficits (from 28 to 40 days) in climbing, running activity on running wheels and open field activity. The early reduction in activity is similar to that observed in HD patients since they significantly reduce their activity despite the choreiform pattern (van Vugt *et al.* 2001).

On the other hand, Guidetti *et al.* (2006) have directly implicated an active role of the kynurenine pathway in the R6/2 transgenic mice model, as well as in the yeast artificial chromosome 128 mice (YAC128, which expresses the full-length mutant human *htt* gene and carries 128 CAG repeats) and the Hdh^{Q92} y Hdh^{Q111} (expressing the mutant *htt* with 92 and 111 residues of glutamine, respectively). In all these models, the levels of

two toxic metabolites of the pathway, 3-HK and QUIN, were increased in the brain cortex and the striatum, and some of these changes were paralleled with neurobiological signs of HD. Of particular interest were those findings showing that each model increased such metabolites at different stages, R6/2 mice is the model producing the earliest changes (since 4 weeks of age), while the others started after several months (Guidetti *et al.* 2006). This is particularly relevant as R6/2 might be suggested as a model more closely related to an early onset of HD (juvenile HD). Thus, an early increase in QUIN and 3-HK in this model may correlate with a prompt onset in HD, supporting the hypothesis that kynurenine pathway may be involved in HD, as its toxic metabolites might mediate the cortical and striatal degeneration seen in the disease through a mechanism triggered by the aberrant genome, a question of primary interest deserving further detailed investigation.

As a final remark, compensatory mechanisms have been suggested to be present in transgenic R6 animals against different toxins. Hansson *et al.* (1999) tested the resistance to the toxic actions of QUIN in R6/1, where QUIN produced massive damage to wild type, but no to transgenic mice, suggesting that these animals develop an over-regulatory response on anti-apoptotic defenses. Other studies revealed compensatory mechanisms in the brain of R6/2 mice, which are protecting the brain from the toxic actions of 3-NP, QUIN and kainic acid (Hickey and Morton 2000, Morton and Leavens 2000). In other words, at some stages of the phenotypic expression of the pathology in mice, their brains are still “fighting back” against general toxicity, and thus, the same strategies are offering protection against excitotoxicity. Despite these findings, it has not yet been investigated what is precisely happening in HD brains regarding these compensatory events. Based on the actual status of knowledge, we emphasize that the simultaneous occurrence of common toxic mechanisms in human HD during the progression of the disease cannot be excluded.

Kynurineric hypothesis

One of the most interesting and promising hypotheses suggested for HD is based on significant alterations in tryptophan metabolism, and is tightly related with QUIN and other kynurenine neuroactive metabolites, such 3-HK and kynurenic acid (KYNA). In particular, 3-HK and QUIN are produced in the spinal cord of experimental allergic encephalopathy and

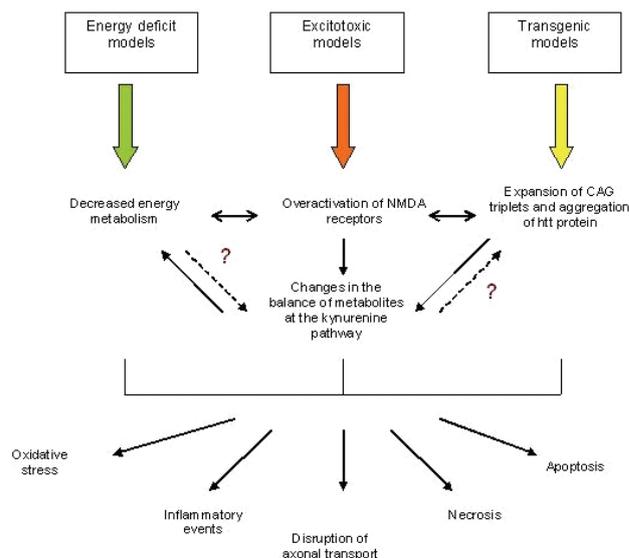


Fig. 2. Suggested interactions between the different hypotheses proposed to explain neurotoxicity and cell death in Huntington's disease.

multiple sclerosis (Chiarugi *et al.* 2001b). This points to their active role in neurodegeneration also in these two models and other diseases, despite the fact that they have been suggested to exert toxic effects in the CNS by different mechanisms – apoptosis for 3-HK and necrosis for QUIN (Chiarugi *et al.* 2001c). Several studies have documented abnormalities in different components of the kynurenine pathway at the neostriatum of HD patients (Beal *et al.* 1992, Connick *et al.* 1989, Pearson and Reynolds 1992, Schwarcz *et al.* 1988). However, these reports are based on samples from HD patients at terminal stages, biochemical measurements are compromised by the effect of neuronal cell loss, thereby producing obvious limitations for supporting the kynurineric hypothesis. Recently, Guidetti *et al.* (2000) observed significant increases in 3-HK levels both in HD patients at stage I and in transgenic mice for full-length mutant htt. In both cases, these changes were related to alterations in the balance of 3-HK and KYNA, pointing to an abnormal metabolism of the kynurenine pathway. It remains to be demonstrated, whether these alterations are playing a key role in the neurotoxic events produced during the early stages of HD.

Conclusions and perspectives

According to the information we have reviewed, the mechanisms by which neuronal degeneration and cell death are being generated in HD may include excitotoxicity, energy deficit, oxidative stress,

inflammatory processes, and protein aggregation. A sequence of the occurrence of these events is described in Figure 1. Far from being excluding, all these factors are somehow closely related (Fig. 2). With the recent development of new experimental models for simulating the alterations in HD, as well as new clinical evidence, fresh findings emphasize an active role of the kynurenine pathway in human disease (Guidetti *et al.* 2006). Dysbalance among toxic and neuroprotective metabolites at this pathway and its recent link with transgenic mice models – where energy disorders and excitotoxicity are commonly occurring – constitute an important approach to find clues for further investigation and elucidation of the key events triggering neurodegenerative processes in HD. Despite these findings, the precise manner, in which

these hypotheses are interacting, is still subject of intense investigation. In this regard, here we have provided a brief evaluation of the most relevant experimental models and their phenomenological nature, however, their constructive and predictive validity for HD will be enhanced by further experimental evidence.

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Corresponding author

V. Pérez-De La Cruz, A. Santamaría, Laboratorio de Aminoácidos Excitadores, Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez, Insurgentes Sur 3877, México D.F. 14269, México. E-mails: absada@yahoo.com, veped@yahoo.com.mx