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COMMENTARY

Autophagy, mitochondria and 3-nitropropionic acid joined in the same model

Rosa A González-Polo, José M Bravo-San Pedro, Rubén Gómez-Sánchez, Elisa Pizarro-Estrella, Mireia Niso-Santano and José M Fuentes

Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED). Departamento de Bioquímica y Biología Molecular y Genética, E. Enfermería y T.O., Universidad de Extremadura, Cáceres, Spain

Correspondence
José M Fuentes, Departamento de Bioquímica y Biología Molecular y Genética, E. Enfermería, Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Universidad de Extremadura, Avda Universidad, s/n, 10003 Cáceres, Spain. E-mail: jfuentes@unex.es

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Huntington's disease (HD) is a neurodegenerative disorder caused by a mutation in the gene encoding the huntingtin protein. Although the precise mechanism by which neuronal degeneration occurs is still unclear, several elements are important to its development: (1) altered gene expression and protein synthesis, (2) mitochondrial damage and (3) improper regulation of the autophagy programme. In this issue of *British Journal of Pharmacology*, Galindo and co-workers provide the first evidence for a role of the mitochondrial permeability transition pore (mPTP) in mitochondrial fragmentation and autophagy activation. In a model of cell death induced by 3-nitropropionic acid (3-NP) in human neural cells, the authors describe clear functions for mPTP and Bax, but not the mitochondrial fusion/fission machinery, mitochondrial fragmentation and autophagy (mitophagy). This commentary summarises the significance of this relationship and suggests several points for future development.

LINKED ARTICLE
This article is a commentary on Solesio *et al.*, pp. 63–75 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2012.01994.x

Abbreviations
3NP, 3-nitropropionic acid; HD, Huntington's disease; mTOR, mammalian target of rapamycin; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species

Commentary

Huntington's disease (HD) is an autosomal dominant disease with a relatively high prevalence (1/10 000). HD typically presents in adults and is characterized by personality changes, cognitive impairment and psychiatric and movement disorders. Among the movement disorders, the most common type is chorea, but dystonias, myoclonus and rigidity can also arise. HD invariably leads to early death because of the lack of effective treatments to cure the disease or delay its progression (Krainc, 2010).

HD is caused by a mutation in a gene encoding a protein called huntingtin, which consists of 3144 amino acids with a molecular weight of approximately 350 kDa. The mutation is an expansion of a triplet repeat (CAG) encoding a glutamine repeat sequence. Healthy individuals have between 7 and 34 repeats, whereas HD patients have many more repeats, and the number of repeats is inversely related to age at disease onset. A number of repeats greater than 40 causes the disease, and a number above 80 is associated with childhood- or adolescent-onset disease. Although this protein is ubiquitous and is expressed in all cell types, HD mainly affects sites within the brain, including the striatum, cortex, thalamus and subthalamic nucleus. Although the striatal neurons are the most severely affected, HD is not simply an alteration of the striatum, and in the advanced stages of the disease, damage in other brain regions is evident (Krainc, 2010).

From the pathological point of view, HD is characterized by the presence of cytoplasmic inclusions of huntingtin. Although the degradation mechanism of this protein has not yet been fully elucidated, two degradative pathways are clearly involved: the ubiquitin–proteasome system (UPS) and autophagy. However, the expanded glutamine sequences are not a good substrate for the proteasome, which may explain

why autophagy is particularly important in HD (Bence *et al.*, 2001). Autophagy is one of the most intriguing mechanisms in cell biology. Essentially, this process consists of the sequestering of portions of the cytoplasm (cytosol and/or organelles) in membranous structures called autophagosomes and their subsequent degradation by lysosomal enzymes (He and Klionsky, 2009). Initially viewed as a mechanism of cell death, autophagy has more recently been recognized as a process by which cells can adapt to changes and stress, including nutrient deprivation, hypoxia, DNA damage and altered mitochondrial or ER stress, among others (Levine and Kroemer, 2008). When cells die by autophagy, there is a massive vacuolization that constitutes a failed attempt to adapt; death occurs through a mechanism similar to that of apoptosis (Kroemer and Levine, 2008). Certain genetic or pharmacological interventions impair autophagy as a side effect of protecting cells against various stresses, especially in the CNS (Madeo *et al.*, 2009). Accordingly, autophagic dysfunction is emerging as an active topic in the study of neurodegenerative diseases in which misfolded proteins accumulate, including HD (Filonova *et al.*, 2000). In HD, the critical role of autophagy is demonstrated by the presence of aggregates of highly ubiquitinated huntingtin protein in the lysosomes of the affected neurons. Furthermore, the molecular mechanisms involved in the process of autophagy are altered in the neurons of HD patients. Thus, the huntingtin mutant is capable of altering the autophagic machinery by binding to beclin-1 (a protein that forms part of the class III PI3-kinase complex involved in activating macroautophagy), thus decreasing protein degradation and increasing the half-life of the very long huntingtin mutant (Shibata *et al.*, 2006). Moreover, the expression of beclin-1 is known to decrease with age (Shibata *et al.*, 2006), thereby reducing the cell's ability to induce autophagy during aging and promoting the accumulation of mutant huntingtin and the progression of the disease. Another finding that highlights the involvement of autophagy in HD is that the mammalian target of rapamycin (mTOR) is present in polyglutamine aggregates both in cell models and in animal or human brain tissue, resulting in a decrease in the activity of mTOR (Ravikumar *et al.*, 2004). In this context, treatment with rapamycin (which stimulates autophagy) reduces huntingtin accumulation and neurodegeneration in cellular models of HD. *In vivo*, rapamycin also reduces huntingtin aggregation and neurological deficits in mouse models of HD. These observations emphasize that the inadequate regulation of autophagy may be a factor in the origin and development of this disease.

Furthermore, mitochondrial dysfunction has been associated with the pathogenesis of HD, although the precise mechanism by which this part of the process develops has not yet been fully elucidated. However, anatomopathological studies and behavioural studies in animal models using mitochondrial toxins support this hypothesis. Of all of the substances used for this purpose, 3-nitropropionic acid (3NP) is the most commonly used, and it shares many mechanisms of neurotoxicity with mutant huntingtin. Thus, cellular and animal models (Kumar *et al.*, 2011) using 3NP are particularly useful for studying the possible synergistic effects of mitochondrial dysfunction in cellular pathways affected by the presence of mutant huntingtin in the context of neuronal degeneration (Brouillet *et al.*, 2005).

The work of Galindo' group (Solesio *et al.*, 2012) published in this issue, is of considerable interest, as it brings together several of the elements above that are relevant to the study of HD: mitochondrial damage, 3NP and autophagy. The main contribution of this paper is that it shows a direct correlation between formation of the mitochondrial permeability transition pore (mPTP) and autophagy induced by 3NP treatment. Interestingly, activation of autophagy preceded the apoptotic process and was mediated, at least partially, by reactive oxygen species (ROS) and mPTP formation. Although there are substantial data that show common elements between the regulation of apoptosis and that of autophagy and the involvement of certain proteins in both mechanisms, Solesio *et al.*, (2012) showed that Bax, a key element in the apoptotic process (Perez-Alvarez *et al.*, 2009), was not necessary for mPTP formation preceding the induction of autophagy following exposure to 3NP. This phenomenon has been observed previously in cellular models of other neurodegenerative diseases, such as Parkinson's disease (PD), in which the PD-related neurotoxin paraquat was able to induce an early autophagy that preceded apoptosis (Gonzalez-Polo *et al.*, 2007a,b). This evidence supports the theory that a neurotoxic stimulus (typically involving mitochondrial impairment and/or ROS production) initiates the development of the autophagy programme as a mechanism of neuroprotection leading to the removal of the relevant proteins or organelles (typically by oxidation or nitrosylation). If the stimulus persists or the autophagy is insufficient, it triggers apoptosis, leading to the death of the neuron. However, the work of Solesio *et al.*, (2012) concluded that 3NP-induced ROS did not appear to constitute a significant signalling link between 3NP and the mitochondrial fragmentation machinery, but that mPTP formation was the key event in 3NP toxicity. Its inhibition by cyclosporin A blocked other effects, including ROS production. Interestingly, the participation of the mitochondrial fission pathway was excluded because 3NP did not induce the translocation of the GTPase dynamin related protein-1 (Drp1) to the mitochondria. This result differs from previous reports, in which mitochondrial fission increases the response of cell models of HD to apoptotic stimuli (Costa *et al.*, 2010) or in the overexpression of mutant huntingtin (Song *et al.*, 2011). Solesio *et al.*, 2012) propose that 3NP-induced mitochondrial fragmentation can occur through an mPTP-dependent mechanism without the activation of the fission machinery. This proposal is supported by three crucial experimental results: (1) cyclosporin A was effective in blocking the mitochondrial alterations that were induced by 3NP; (2) 3NP did not recruit Drp1 to the mitochondria; and (3) the mitochondrial division inhibitor (Mdivi-1) failed to inhibit the mitochondrial fragmentation caused by 3NP.

In conclusion, the authors provide an interesting addition to our knowledge of changes in cell signalling relevant to the study of HD. However, many mechanisms remain to be elucidated, such as (a) verifying the presence of a complete and correct autophagic flux, (b) identifying the precise mechanism by which autophagy is induced upon exposure to 3NP, (c) understanding the effect of this activation in the presence of the mutant form of huntingtin, (d) determining whether the pharmacological activation of autophagy is able to prevent the accumulation of polyglutamine and mitigate the

toxic effects of 3NP and (e) determining whether there are common regulatory mechanisms in the sequential activation of apoptosis and autophagy. Such investigations will allow a better understanding of the process by which neuronal degeneration occurs in HD.

Conflict of interest

None of the authors declare any conflict of interest.

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