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The neurodegenerative process in a neurotoxic rat model and in patients with Huntington's disease: Histopathological parallels and differences

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ABSTRACT

Although Huntington's disease (HD) occurs only in humans, the use of animal models is crucial for HD research. New genetic models may provide novel insights into HD pathogenesis, but their relevance to human HD is problematic, particularly owing to a lower number of typically degenerated and dying striatal neurons and consequent insignificant reactive gliosis. Hence, neurotoxin-induced animal models are widely used for histopathological studies. Unlike in humans, the neurodegenerative process (NDP) of the HD phenotype develops very fast after the application of quinolinic acid (QA). For that reason, we compared three groups of rats in more advanced stages (1–12 months) of the QA lesion with 3 representative HD cases of varying length and grade. The outcomes of our long-term histological study indicate that significant parallels may be drawn between HD autopsies and QA-lesioned rat brains (particularly between post-lesional months 3 and 9) in relation to (1) the progression of morphological changes related to the neuronal degeneration, primarily the rarefaction of neuropil affecting the density as well as the character of synapses, resulting in severe striatal atrophy and (2) the participation of oligodendrocytes in reparative gliosis. Conversely, the development and character of reactive astrogliosis is principally conditioned by the severity of striatal NDP in the context of neuron–glia relationship. Despite the above-described differences, morphological patterns in which the components of striatal parenchyma react to the progression of NDP are similar in both human and rat brains. Our study specifies the possibilities of interpreting the morphological findings gained from the QA-induced animal model of HD in relation to HD post-mortem specimens.

Introduction

Huntington's disease (HD) is a rare neurological disorder caused by a genetic mutation in the IT15 gene (The Huntington's Disease Collaborative Research Group, 1993). Progressive cell death in the basal ganglia and cortex, followed by a decline in cognitive, motor and psychiatric functions are characteristic of this disease. A grading system that defines the extent of striatal degeneration using five grades (grades 0–4) has been described by Vonsattel et al. (1985). HD is a devastating disease leading to death over a period of 10–20 years. In addition to autopsies, animal models of HD are widely used to study pathology of the disease from different points of view, taking into consideration possible treatment (e.g. Vonsattel, 2008). The most common are excitotoxic models in which cell death is induced by intracerebral instillation of a neurotoxic acid, formerly kainic acid, later ibotenic acid and finally quinolinic acid. Quinolinic (quolinic, quinolinate) acid (QA) is an excitatory amino acid (a NMDA-selective glutamate agonist and an endogenous metabolite of tryptophan), whose properties (a preferential degeneration of GABA-ergic neurons and a relative sparing of NDPH diaphorase- and cholinergic interneurons) and simple application account for its wide use in creating a practicable (though not ideal) animal model of HD (e.g. Beal et al., 1986; Björklund et al., 1986). On the other hand, the application of 3-nitropropionic acid, resulting in mitochondrial impairment, is only rarely used. Both mechanisms of neurodegeneration have also been confirmed in the brains of HD patients.

In 1993, the huntingtin mutation was discovered (Gusella et al., 1993) and recent models, which include transgenic, knock-in,

Abbreviations: CAG, cytosine–adenine–guanine; CNP-ase, 2′, 3′-cyclic nucleotide 3′-phosphodiesterase; CNS, central nervous system; CN, caudate nucleus; DAB, 3,3′-diaminobenzidine-tetrahydrochloride; GFAP, glial fibrillary acidic protein; GP, globus pallidus; H&E, hematoxylin and eosin; HD, Huntington's disease; IC, internal capsule (*capsula interna*); IT15, interesting transcript 15; LV/LVs, lateral ventricle/ventricles; MAP2, microtubule-associated protein 2; mhtt, mutant huntingtin; NDP, neurodegenerative process; NDPH, nicotinamide adenine dinucleotide phosphate; NF, bundles of myelinated nerve fibers; NG2, NG2 chondroitin-sulfate proteoglycan; NMDA, N-methyl-D-aspartic acid; OPCs, oligodendrocyte progenitor cells; PBS, phosphate buffered saline; Pu, putamen; QA, quinolinic (quolinic, quinolinate) acid.

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knock-out, and virally-inserted mutated polyQ tract models, have been generated. The R6/1 and R6/2 transgenic mice were the first and still widely used transgenic mice models (Mangiarini et al., 1996; Bates et al., 1997; Bates and Gonitel, 2006). However, owing to the large number of CAG repeats (114 and 150, respectively) both strains represent the HD models of rapid progression of the disease with only short lifespan of mice. A major drawback of all genetic models is a lower number of typically degenerated and dying striatal neurons. Also, the concomitant reactive gliosis is not typically developed in this type of striatal NDP (e.g. Kántor et al., 2006). On the other hand, the essential advantage is the presence of mutant huntingtin (mhtt), particularly in the form of intranuclear inclusions (for reviews see: Menalled and Chesselet, 2002; Wang and Qin, 2006; Ramaswamy et al., 2007) similar to those found in HD autopsies.

Although the animal models of HD have been reviewed in several studies (Wang and Qin, 2006; Ramaswamy et al., 2007; Vonsattel, 2008; Ferrante, 2009) a detailed long-term comparative histopathological study of QA-lesioned animal brains and HD post-mortem specimens is not available.

Astrocytes are the most numerous cells in the CNS. They provide structural, trophic and metabolic support to neurons, including the modulation of synaptic activity and synaptogenesis (Chen and Swanson, 2003). The diversity of the astroglial response to different types of brain injury is well documented (Fawcett and Asher, 1999; Sofroniew and Vinters, 2010), however, the morphological alterations of reactive astrocytes during the progression of NDP of HD phenotype in the context of neuron–glia relationship still remain obscure. Decades of pathological and physiological studies have focused on neuronal degeneration in this disorder, and it is becoming increasingly evident that astrocytes also play important roles in different neurological diseases (Maragakis and Rothstein, 2006). It has been proposed that reactive astrocytes play an important role in CNS disorders via loss of normal astrocyte functions or gain of abnormal effects (Sofroniew and Vinters, 2010).

In accordance with the above-described facts, the aim of our present study was to describe the morphological patterns in which the components of striatal parenchyma react to the progression of NDP. We present here our findings in the brains of rats surviving 1–12 months after the striatal QA lesion and three representative cases of HD autopsies of varying lengths and grades, and discuss the possibilities of parallels between them.

Materials and methods

Experimental design

Adult male Wistar rats (220–240 g body weight at the beginning of the experiment) were used. All procedures were performed in accordance with the directive of the EEC (86/609/EEC) and the use of animals in present experiments was reviewed and approved by the Animal Ethical Committee of Charles University in Prague, Faculty of Medicine in Hradec Králové. Rats were divided into 3 (I–III) groups in relation to the survival time period chosen to be approximately comparable with 3 cases of HD. Rats in group I were sacrificed at 1 month, in group II at 3 and 6 months, in group III at 9 and 12 months after the intrastriatal injection of quinolinic acid. In each of 5 subgroups, five rats with unilateral QA lesion (in a right hemisphere), two sham-lesioned and two intact control rats were examined.

Quinolinic acid lesion and sham-lesion

Rats were anesthetized and prepared for surgery as previously described in detail (Mazurová et al., 2006). Quinolinic acid (Sigma–Aldrich, Czech Republic; concentration 120 nmol, i.e. 20 μg/2 μl aqua pro inj.) was stereotaxically injected into the striatum of the right hemisphere at the following coordinates: A 1.2; L 2.5; V 5.0 and 4.5 (A: anterior to bregma, L: lateral from midline, V: ventral from dura; all coordinates are in mm; tooth bar at 0 level) using the automatic Syringe Pump SP310 (World Precision Instruments, Berlin, Germany). In sham-lesioned animals, saline (2×1 μl) was injected into the brain instead of the QA.

The animals were transcardially perfused under deep anesthesia with 4% neutral buffered formaldehyde. Each brain was removed from the skull, transversely cut in 3 mm thick blocks at the same level (using the Brain Blocker, Better Hospital Equipment Corp., NY, USA), post-fixed by immersion for 3 days and embedded in paraffin.

Subjects' case history

The brains of three patients with approx. 2-, 8- and 20-year clinical manifestation of HD (sex/age: ♀/52, ♂/38, ♀/52) were studied. Two control brains of patients (sex/age: ♂/56, ♀/43) with no history of neurologic disorder were also taken for the study. The clinical features of HD (described in autopsy records) were characteristic for the given stages in all three patients. (However, detailed neurological records or results of genetic testing were not available because old archival material was used). Surprisingly, the total brain weight was markedly reduced in all HD cases independent of the sex and duration of the illness (duration/sex/weight: 2 years/♀/1160 g, 8 years/♂/1150 g, 20 years/♀/1120 g). The weight of the control male brain was 1400 g and 1510 g of the female brain. The cause of death was not related to CNS injury in any of the cases studied. The severity of striatal histopathological changes was graded according to Vonsattel et al. (1985).

Paraffin blocks of brain tissue from autopsies were taken from the neostriatum (the caudate nucleus and putamen) at the level of the globus pallidus and at the level of the nc. accumbens. The blocks were donated by Fingerland's Department of Pathology, Faculty Hospital in Hradec Králové.

Histology and immunohistochemistry

Histological processing was the same for both the experimental material and autopsies. Serial coronal sections through both hemispheres (in a series of 15 sections; 6 μm thick) were prepared by conventional histological processing. The first section in each series was stained with H&E, the next sections were stained using Bodian's impregnation of neurofibrils and Luxol Fast Blue for myelin detection in a selected series. For immunohistochemical labelling, the following monoclonal antibodies were used in selected series: anti-GFAP (Sigma–Aldrich, Prague, Czech Republic; dilution 1:400), anti-CNP-ase (Sigma–Aldrich, Prague; dilution 1:500), anti-synaptophysin (Dako, Czech Republic; dilution 1:20), anti-MAP2 (Sigma–Aldrich, Prague; dilution 1:500) and anti-ß-III-tubulin (Exbio, Prague, Czech Republic; dilution 1:20).

In brief, deparaffinized and rehydrated sections were incubated in an aqueous solution of H_2O_2 (dilution 5:1) for 20 min to reduce endogenous peroxidase activity. Pretreatment in a microwave 3×5 min at 800 W in a sodium citrate buffer (pH 6.0) and washing in 0.01 M PBS was carried out. For CNP-ase detection, digestion using 0.1% pepsin in 0.1 N HCl (for 30 min at 37 °C) was necessary. Incubation with primary monoclonal antibodies (*see above*) was performed overnight at 4 °C. Sections were then washed and incubated with biotinylated anti-mouse secondary antibody (Jackson ImmunoResearch Lab., West Grove, PA, USA; dilution 1:500) for 45 min at room temperature, and subsequently with a streptavidin conjugate of peroxidase (Dako, Brno, Czech Republic; dilution 1:300) for 45 min. Visualization of bound antibody was performed using 3,3′-diaminobenzidine-tetrahydrochloride (DAB,