Neuroscience 202 (2012) 363–370

CELL TYPE-SPECIFIC LOCALIZATION OF OPTINEURIN IN THE STRIATAL NEURONS OF MICE: IMPLICATIONS FOR NEURONAL VULNERABILITY IN HUNTINGTON'S DISEASE

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Abstract—Striatal neuropathology of Huntington's disease (HD) involves primary and progressive degeneration of the medium-sized projection neurons, with relative sparing of the local circuit interneurons. The mechanism for such a patterned cell loss in the HD striatum continues to remain unclear. Optineurin (OPTN) is one of the proteins interacting with huntingtin and plays a protective role in several neurodegenerative disorders. To determine the cellular localization pattern of OPTN in the mouse striatum, we employed a highly sensitive immunohistochemistry with the tyramide signal amplification system. In this study, we show that OPTN appeared as a cytoplasmic protein within the subsets of the striatal neurons. Of particular interest was that OPTN was abundantly expressed in the interneurons, whereas low levels of OPTN were observed in the medium projection neurons. This cell type-specific distribution of OPTN in the striatum is strikingly complementary to the pattern of neuronal loss typically observed in the striatum of patients with HD. We suggest that OPTN abundance is an important cellular factor in considering the cell type-specific vulnerability of striatal neurons in HD.

Key words: Huntington's disease, optineurin, huntingtin, striatum, interneurons, neurodegeneration.

Huntington's disease [HD (MIM 143100)] is an autosomal dominant neurodegenerative disorder that causes late-onset motor, cognitive, and psychiatric disturbances (Albin and Tagle, 1995). HD is the result of disrupted regulation of transcriptional machinery due to the expansion of a CAG trinucleotide repeat in the *huntingtin* (*Htt*) gene, which translates into an abnormally long polyglutamine (polyQ) tract in the huntingtin (Htt) protein (The Huntington's Disease Collaborative Research Group, 1993). The neuropathology of HD is characterized by primary and progressive degeneration of the medium spiny neurons (MSNs) in the striatum, with relative sparing of striatal interneurons (Ferrante et al., 1985, 1987; Kowall et al., 1987; Reiner et al., 1988; Albin et al., 1990; Cicchetti et al., 2000). However, the precise mechanism by which such cell type-specific loss of striatal neurons occurs in HD is yet to be elucidated.

Optineurin (OPTN) is a ubiquitous protein with high expression levels in the central nervous system (Li et al., 1998; Rezaie et al., 2005). It is a negative regulator of the nuclear factor-κB (NF-κB) signaling (Zhu et al., 2007; Mrowka et al., 2008; Sudhakar et al., 2009; Nagabhushana et al., 2011) and a multifunctional protein involved in protein and vesicular trafficking (Sahlender et al., 2005; Park et al., 2010), glutamate receptor signaling (Anborgh et al., 2005), and transcription activation (Moreland et al., 2000). OPTN has been identified as a gene mutated in some cases of hereditary glaucoma that causes retinal degeneration leading to visual loss (Rezaie et al., 2002), and our recent study has shown that loss of OPTN can induce degenerative loss of motor neurons in familial amyotrophic lateral sclerosis (Maruyama et al., 2010). These findings suggest that OPTN could be a common factor involved in protection against cell death in neurodegeneration. Of particular interest is that OPTN is one of the Htt-interacting proteins (Hattula and Peränen, 2000), which exerts a protective effect on glutamate-induced neurotoxicity associated with mutant Htt (Anborgh et al., 2005). To seek an association between expression of OPTN and specific patterns of neuropathology in HD, we here examined the cellular localization patterns of OPTN in the mouse striatum.

EXPERIMENTAL PROCEDURES

All procedures involving the use of animals and analysis of brain anatomy were approved by the Institutional Care and Use Committees of the University of Tokushima.

Animals and tissue preparation

Male C57BL/6 mice (Nihon SLC Co., Shizuoka, Japan), 8–10 weeks of age, were used ($n=5$). Mice were intraperitoneally injected with a lethal dose of pentobarbital (Sigma, St Louis, MO, USA) and were transcardially perfused with 0.01 M phosphate-buffered saline (PBS) at pH 7.4, followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at pH 7.4. The brains were

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Abbreviations: ChAT, choline acetyltransferase; CR, calretinin; DARPP-32, dopamine and cAMP-regulated phosphoprotein of 32 kDa; HD, Huntington's disease; Htt, huntingtin; MSNs, medium spiny neurons; NPY, neuropeptide Y; OPTN, optineurin; PB, phosphate buffer; PBS, phosphate-buffered saline; PV, parvalbumin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TSA, tyramide signal amplification.

0306-4522/12 $36.00
doi:10.1016/j.neuroscience.2011.11.059

removed, post-fixed overnight in the same fixative at 4 °C, and stored in a 10%–30% sucrose gradient in 0.1 M PB at 4 °C for cryoprotection. Sections were cut on a cryostat at 10- or 15-μm thickness, and they were stored in PBS containing 0.05% NaN_3 until use.

Western blot assay

Brains obtained from deeply anesthetized C57BL/6 mice (Nihon SLC Co., Shizuoka, Japan; $n=2$), 9–10 weeks of age, were homogenized in 0.05 M Tris–HCl at pH 7.2 containing 0.025 M KCl, 0.005 M $MgCl_2$, and 0.32 M sucrose. The protein lysates (20 μg of protein) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and separated proteins were then transferred onto a polyvinylidene difluoride membrane. The membranes were incubated with rabbit polyclonal antibody against OPTN (1:1000; Cayman Chemical, Ann Arbor, MI, USA) (Table 1), and were then incubated with horseradish peroxidase-conjugated anti-rabbit IgG. The bound antibodies were detected by chemiluminescence staining (ECL plus kit, GE Healthcare, Buckingham, UK).

Single-label immunohistochemistry

Immunostaining was performed on free-floating sections by using the tyramide signal amplification (TSA) method (Sako et al., 2011; Morigaki et al., 2011). Rabbit polyclonal antibody against OPTN (1:10,000; Cayman Chemical) was used as a primary antibody (Table 1). The bound primary antibodies were detected by the Histofine Simple Stain Kit (Nichirei, Tokyo, Japan) and the TSA system with Cyanine 3 (Perkin Elmer, Shelton, CT, USA).

Double-label immunohistochemistry

For double immunostaining, the striatal sections were first stained for OPTN with the TSA-Cyanine 3 system according to the previously described protocols. To remove the bound antibodies, the stained sections were incubated in 0.1 M glycine-HCl at pH 2.2 at room temperature for 30 min. After incubation with PBS for 1 h, they were labeled with rabbit polyclonal antibodies against choline acetyltransferase (ChAT) (1:20,000; Millipore, St. Louis, MO, USA), parvalbumin (PV) (1:100,000; Abcam, Cambridge, UK), neuropeptide Y (NPY) (1:20,000; Affiniti, Nottingham, UK), calretinin (CR) (1:100,000; Chemicon, Temecula, CA, USA), or dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) (1:20,000; Cell Signaling, Denver, MA, USA) (Table 1). Goat polyclonal antibody against Htt (1:20,000; Santa Cruz Biotechnology, Santa Cruz, MA, USA) was also used (Table 1). The bound antibodies were detected by the Histofine Simple Stain Kit (Nichirei, Tokyo, Japan) and the TSA system with fluorescein (Perkin Elmer, Shelton, CT, USA).

Digital imaging and densitometric analyses

Digital microscopy images were captured using an Olympus BX51 microscope (Olympus, Tokyo, Japan), imported into Adobe Photoshop CS4, and processed digitally. Cell density analyses were made at a level of the striatum in the anterior-to-posterior coordinate (AP: +0.5 to +1.0 mm) (Hof et al., 2000) for each mouse ($n=5$). We counted the number of cells labeled for each marker within a 0.5×0.5-mm^2 field in the striatum, according to the methods that we previously reported (Sato et al., 2008; Sako et al., 2010). Among the striatal cells strongly labeled for OPTN, the percent population of those cells co-localized with ChAT, PV, NPY, or CR was also calculated. To estimate the cytoplasmic density of OPTN labeling in the striatal neurons labeled for ChAT, PV, NPY, CR, or DARPP-32, double-immunostaining of the striatal sections with these antibodies was simultaneously performed with the same protocols. High-power photomicrographs of labeled neurons were obtained using a 100× oil-immersion objective, and they were digitally changed to the non-colored images in a gray scale. We measured the optical density of OPTN labeling within the cytoplasm of striatal neurons ($n=20$) doubly labeled for ChAT, PV, NPY, CR, or DARPP-32 from each mouse ($n=5$). The mean cytoplasmic density of OPTN labeling was then calculated in each striatal neuron subclass.

Statistical analysis

All quantitative data were expressed as means±SEM values. The Student's *t*-test (two-tailed, paired) was used for two group comparisons. *P*-values less than 0.05 were considered statistically significant.

Table 1. Antibodies used for immunohistochemistry with tyramide signal amplification (TSA) system

Antibody to	Immunogen (C) carboxy terminal (N) amino terminal	Source	Dilution
Optineurin	Synthetic peptide Amino acids: 575–591 (C)	Cayman (Ann Arbor, MI, USA) Rabbit polyclonal antibody No. 0420152-1	1:10,000
Choline acetyltransferase	Human placental enzyme	Millipore (St. Louis, MO, USA) Rabbit polyclonal antibody No. AB143	1:20,000
Parvalbumin	Purified parvalbumin	Abcam (Cambridge, UK) Rabbit polyclonal antibody No. ab11427	1:100,000
Neuropeptide Y	Synthetic porcine NPY	Affiniti (Nottingham, UK) Rabbit polyclonal antibody No. NA 1233	1:20,000
Calretinin	Recombinant rat calretinin	Chemicon (Temecula, CA, USA) Rabbit polyclonal antibody No. AB149	1:100,000
DARPP-32	Synthetic peptide for the residues around Thr34 of human DARPP-32	Cell Signaling (Denver, MA, USA) Rabbit polyclonal antibody No. 19A3	1:20,000
Huntingtin	Synthetic peptide (N)	Santa Cruz (Santa Cruz, CA, USA) Goat polyclonal antibody No. SC-8767	1:20,000