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GLIAL ELEMENTS CONTRIBUTE TO STRESS-INDUCED torsinA EXPRESSION IN THE CNS AND PERIPHERAL NERVOUS SYSTEM

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Abstract—DYT1 dystonia is caused by a single GAG deletion in exon 5 of *TOR1A*, the gene encoding torsinA, a putative chaperone protein. In this study, central and peripheral nervous system perturbations (transient forebrain ischemia and sciatic nerve transection, respectively) were used to examine the systems biology of torsinA in rats. After forebrain ischemia, quantitative real-time reverse transcriptase-polymerase chain reaction identified increased torsinA transcript levels in hippocampus, cerebral cortex, thalamus, striatum, and cerebellum at 24 h and 7 days. Expression declined toward sham values by 14 days in striatum, thalamus and cortex, and by 21 days in cerebellum and hippocampus. TorsinA transcripts were localized to dentate granule cells and pyramidal neurons in control hippocampus and were moderately elevated in these cell populations at 24 h after ischemia, after which CA1 expression was reduced, consistent with the loss of this vulnerable neuronal population. Increased *in situ* hybridization signal in CA1 stratum radiatum, stratum lacunosum-moleculare, and stratum oriens at 7 days after ischemia was correlated with the detection of torsinA immunoreactivity in interneurons and reactive astrocytes at 7 and 14 days. Sciatic nerve transection increased torsinA transcript levels between 24 h and 7 days in both ipsilateral and contralateral dorsal root ganglia (DRG). However, increased torsinA immunoreactivity was localized to both ganglion cells and satellite cells in ipsilateral DRG but was restricted to satellite cells contralaterally. These results suggest that torsinA participates in the response of neural tissue to central and peripheral insults and its sustained up-regulation indicates that torsinA may contribute to remodeling of neuronal circuitry. The striking induction of torsinA in astrocytes and satellite cells points to the potential involvement of glial elements in the pathobiology of DYT1 dystonia. **© 2008 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: DYT1, dystonia, reactive astrocytes, hippocampus, satellite cells, dorsal root ganglia.

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Abbreviations: AAA+, *A*TPases *a*ssociated with a variety of cellular *a*ctivities; ANOVA, analysis of variance; C_T, threshold cycle; DC, direct current; DRG, dorsal root ganglia; eDG, external dentate gyrus; ER, endoplasmic reticulum; GFAP, glial fibrillary acidic protein; iDG, internal dentate gyrus; IR, immunoreactivity; PB, phosphate buffer; PBS, phosphate-buffered saline; PNS, peripheral nervous system; QRT-PCR, relative quantitative multiplex real-time reverse transcriptase polymerase chain reaction; ROI, region of interest; SLM, stratum lacunosum-moleculare; SO, stratum oriens; SR, stratum radiatum; 4-VO, four-vessel occlusion.

DYT1 dystonia obeys an autosomal dominant inheritance pattern with reduced penetrance, usually begins in childhood and frequently generalizes. The causal mutation is a GAG deletion in the *TOR1A* gene that removes a single glutamic acid residue near the carboxy terminus of the encoded protein torsinA (Ozelius et al., 1997, 1999). TorsinA belongs to the *A*TPases *a*ssociated with a variety of cellular *a*ctivities (AAA+) superfamily of proteins. TorsinA possesses an N-terminal signal sequence, a single AAA+ module that includes Walker A and Walker B nucleotide binding motifs, sensor 1 and sensor 2 regions, and two biochemically-confirmed glycosylation sites (Ozelius et al., 1997, 1999; Neuwald et al., 1999; Kamm et al., 2004; Callan et al., 2007). Members of the AAA+ family function as molecular chaperones for protein quality control (protein complex assembly, operation, disassembly, protein folding, unfolding, and degradation), membrane fusion and vesicular transport, and cytoskeletal regulation (Neuwald et al., 1999; Vale, 2000; Ogura and Wilkinson, 2001).

In cultured cells, the majority of torsinA is located in lumen of the endoplasmic reticulum (ER) and associates peripherally with the ER membrane, possibly via binding to an integral ER membrane protein (Hewett et al., 2003; Kuner et al., 2003; Liu et al., 2003; Callan et al., 2007). When overexpressed, torsinA is enriched at the nuclear envelope (Goodchild and Dauer, 2004; Naismith et al., 2004). In normal brain, torsinA is present in neuron perikarya and extends distally to the tips of dendrites and axons (Konakova et al., 2001; Konakova and Pulst, 2001; Augood et al., 2003; Kamm et al., 2004).

An assortment of studies has indicated that torsinA may function as a chaperone for unfolded or degraded proteins and may facilitate movement of polytopic proteins to the cell surface (Torres et al., 2004). TorsinA has been found localized to Lewy bodies in Parkinson's disease brain and inclusion bodies in trinucleotide repeat diseases (Shashidharan et al., 2000; Sharma et al., 2001; Walker et al., 2003). Overexpression of torsinA suppresses aggregation of α-synuclein in human H4 neuroglioma cells (McLean et al., 2002), and polyglutamine-induced protein aggregation in *C. elegans* (Caldwell et al., 2003). TorsinA facilitates clearance of another dystonia-related protein, ε-sarcoglycan, by the ubiquitin proteosome system and protects PC12 cells against a variety of cellular insults including serum deprivation and oxidative stress (Kuner et al., 2003; Shashidharan et al., 2004; Esapa et al., 2007). Similarly, torsinA protects dopaminergic neurons from oxidative stress in mice (Kuner et al., 2004) and *C. elegans* (Cao et al., 2005).

The chaperone functions of torsinA are critical during developmental processes which seemingly involve inter-

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action with cytoskeletal elements (Ferrari-Toninelli et al., 2004; Kamm et al., 2004; Hewett et al., 2006). The expression of torsinA is developmentally regulated with the highest levels of transcript and protein seen during the prenatal and early postnatal periods (Xiao et al., 2004). The expression of torsinA is particularly intense in cerebellar cortex and striatal cholinergic interneurons at postnatal day 14, a period of intense dendritogenesis and synaptogenesis in these regions (Xiao et al., 2004; Vasudevan et al., 2006). A torsinA homologue, OOC-5, present in *C. elegans*, plays an essential role in Par protein localization. Mutations of *ooc-5* result in polarity defects in *C. elegans* embryos (Basham and Rose, 2001). Attenuated torsinA expression promotes neurite outgrowth in SH-SY5Y human neuroblastoma cells (Ferrari-Toninelli et al., 2004). Conversely, overexpression of mutant torsinA interferes with neurite extension (Hewett et al., 2006). In aggregate, these studies suggest that torsinA may be part of the molecular machinery required for topologically-precise neuritogenesis and/or associated nuclear rotation.

Since penetration of the DYT1 *TOR1A* ΔGAG deletion is only 30–40% (Ozelius et al., 1997), environmental factors may contribute to the development of dystonia in individuals who harbor this mutation. Trauma to the CNS and peripheral nervous system (PNS), hyperthermia, cerebral ischemia and structural disease of the brain and spinal cord are known causes of secondary dystonia (Jankovic and Van der Linden, 1988; LeDoux and Brady, 2003). Similarly, these and other stressors (e.g. intense sensorimotor training) may trigger the onset of dystonia in genetically predisposed individuals (Treves and Korczyn, 1986). Despite these important clinical associations, understanding of torsinA responses to neural perturbations *in vivo* is limited. Our study evaluated the temporal and spatial expression of torsinA in response to central and peripheral injury in models of transient forebrain ischemia and sciatic nerve transection, respectively.

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and with approval of the Institutional Animal Care and Use Committee. All efforts were made to limit the numbers of animals used and minimize their suffering. Wistar rats (Hilltop Laboratory Animals, Inc., Scottdale, PA, USA) were used for transient forebrain ischemia since the effects of four-vessel occlusion (4-VO) have been well characterized in animals of this source and strain (Pulsinelli and Brierley, 1979; Ueda and Nowak, 2005). The sciatic nerve transections were performed in Sprague–Dawley rats (Harlan, Indianapolis, IN, USA).

Transient forebrain ischemia

Adult male Wistar rats weighing 250–300 g were subjected to transient 4-VO ischemia (Pulsinelli and Brierley, 1979) with direct current (DC) potential monitoring as previously described (Ueda and Nowak, 2005). In brief, surgical procedures were carried out under general inhalational anesthesia with 1–2% halothane in 70% N_2 and 30% O_2. The vertebral arteries were electrocauterized at the first cervical segment and Silastic occluding devices were placed around the common carotid arteries. On the following day, rats were re-anesthetized and placed in a stereotaxic frame. Epidural and rectal temperatures were monitored and maintained at 37 °C. Hippocampal DC potentials were recorded using glass microelectrodes. The carotid occluding devices were tightened to produce cerebral ischemia, as verified by 10–20 mV shifts in DC potential. All hemispheres included in these studies exhibited ischemic depolarizations of 7–9 min duration in hippocampus, previously shown to produce consistent loss of CA1 neurons (Ueda and Nowak, 2005). After release of occlusions, scalp incisions were closed, and rats were allowed to recover from anesthesia. Control rats underwent sham surgical procedures that included vertebral artery cauterization and electrode placement without carotid occlusion. Brain tissues for RNA extraction and *in situ* hybridization were obtained at five post-ischemic survival intervals (6 h, 24 h, 7 days, 14 days and 21 days), whereas tissues for immunocytochemistry were collected at three post-ischemic survival intervals (24 h, 7 days, and 14 days). Preparations from three ischemic and three sham control rats were obtained at each survival interval.

For RNA extraction and *in situ* hybridization, rats were anesthetized with 5% halothane prior to decapitation. Brains were rapidly removed from the cranial vault and sectioned in the midsagittal plane. For *in situ* hybridization, hemispheres were frozen in isopentane (−40 °C), sectioned parasagittally at 16 μm and stored at −80 °C in sealed slide boxes with desiccant capsules. For RNA extraction, cerebellum, striatum, thalamus, hippocampus and cerebral cortex were dissected from the hemisphere and homogenized in RNAwiz™ (Ambion, Austin, TX, USA) on ice within 2–3 min after decapitation.

For immunocytochemistry, rats were overdosed with pentobarbital (100 mg/kg, i.p.) prior to transcardiac perfusion with heparinized saline and then 4% paraformaldehyde/0.1 M phosphate buffer (PB). Brains were post-fixed for 2 h, blocked, and incubated in a cryoprotectant solution (30% sucrose/0.1 M PB, pH 7.4) for at least 48 h. Blocks were sectioned at 20 μm and collected on SuperFrost®-Plus glass slides (Fisher Scientific, Pittsburgh, PA, USA).

Sciatic nerve transection

Three-month-old male Sprague–Dawley rats (250–300 g) were subjected to left sciatic nerve transection under ketamine/xylazine (87/13 mg/kg, i.p.) anesthesia. The rat sciatic nerve was readily identified in the posterior thigh between the vastus lateralis and caput vertebralis muscles. In each rat, the left sciatic nerve was completely transected except for the medial epineurium which was left intact to allow for apposition of the cut ends to facilitate axonal regeneration. Wounds were closed and animals were allowed to recover from anesthesia in individual cages. Four age-, weight- and gender-matched non-surgical control rats were only subjected to anesthesia. Four surgical rats were employed at each of five post-transection intervals (24 h, 3 days, 7 days, 14 days and 28 days). Three rats in each group were utilized for RNA extraction and one was used for immunocytochemical examination of the dorsal root ganglia (DRG).

For RNA extraction, rats were overdosed with pentobarbital (100 mg/kg, i.p.) prior to transcardiac perfusion with saline and then RNA*later* (Ambion) as described in LeDoux et al. (2006). The vertebral column was sharply dissected from the remainder of the carcass and placed in a 50 ml conical tube containing RNA*later*. The vertebral column was kept moist with RNA*later* while the right and left lumbar (L3–L6) DRG were microsurgically isolated and collected into separate pools for subsequent RNA extraction.

For immunocytochemistry, rats were overdosed with pentobarbital (100 mg/kg, i.p.) prior to transcardiac perfusion with heparinized saline and then 4% paraformaldehyde/0.1 M PB. The vertebral column was dissected and kept moist with fixative while the right and left lumbar (L4–L5) DRG were isolated and collected into separate vials. DRG were post-fixed for an additional 2 h and then transferred to a cryoprotectant solution for at least 48 h.

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