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ANIMAL MODELS OF DYSTONIA: LESSONS FROM A MUTANT RAT

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Abstract

Dystonia is a motor sign characterized by involuntary muscle contractions which produce abnormal postures. Genetic factors contribute significantly to primary dystonia. In comparison, secondary dystonia can be caused by a wide variety of metabolic, structural, infectious, toxic and inflammatory insults to the nervous system. Although classically ascribed to dysfunction of the basal ganglia, studies of diverse animal models have pointed out that dystonia is a network disorder with important contributions from abnormal olivocerebellar signaling. In particular, work with the dystonic (dt) rat has engendered dramatic paradigm shifts in dystonia research. The dt rat manifests generalized dystonia caused by deficiency of the neuronally-restricted protein caytaxin. Electrophysiological and biochemical studies have shown that defects at the climbing fiber-Purkinje cell synapse in the dt rat lead to abnormal bursting firing patterns in the cerebellar nuclei, which increases linearly with postnatal age. In a general sense, the dt rat has shown the scientific and clinical communities that dystonia can arise from dysfunctional cerebellar cortex. Furthermore, work with the dt rat has provided evidence that dystonia (1) is a neurodevelopmental network disorder and (2) can be driven by abnormal cerebellar output. In large part, work with other animal models has expanded upon studies in the dt rat and shown that primary dystonia is a multi-nodal network disorder associated with defective sensorimotor integration. In addition, experiments in genetically-engineered models have been used to examine the underlying cellular pathologies that drive primary dystonia.

Keywords

Dystonia; Inferior olive; Purkinje cell; Caytaxin; TorsinA; Basal ganglia

Introduction

It is not possible to envision the field of movement disorders research without animal models. Normal and pathological motor behavior is the final produce of massive neural computations performed by tissues with precise but plastic three-dimensional structures with exacting connectivity patterns. Mammalian models are often essential for evaluating the efficacy of candidate drugs and devices that target specific receptors and/or structures in the

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brain. Accordingly, *in vitro* studies are often limited to biochemical and cell biology questions.

The choice of model system (e.g., patients, rodents, primates, *in silico*, test tube, or cultured neurons) is largely dictated by the particular hypothesis and overall experimental goals. The decision to utilize an animal model should be intimately incorporated with choice of species. Common invertebrate models include the roundworm (*Caenorhabditis elegans*) and fruit fly (*Drosophila melanogaster*). *C. elegans* only requires 3 days to develop to maturity, is translucent and very inexpensive, and can be frozen for long-term storage. *Drosophila* is highly amenable to sophisticated genetic manipulation with powerful tools such as the GAL4-UAS system. The mouse (*Mus musculus*) is the standard choice of mammalian species in most laboratories, particularly when genetic manipulations of the genome are planned. However, in recent years, transgenic techniques have also been applied to rats, ungulates and non-human primates. Primates are typically employed for pre-clinical testing of neuromodulatory devices.

Genetically dystonic rat

The genetically dystonic rat (SD-dt:JFL) is an autosomal recessive animal model of primary generalized dystonia. The dt rat is a spontaneous mutant discovered in the Sprague-Dawley (SD) strain. The dt rat develops a dystonic motor syndrome by Postnatal Day 12 (P12, Lorden et al. 1984). The mutation is fully penetrant and there is negligible variability to its expressivity. Mutations in the coding region of the gene (*TOR1A*) associated with DYT1 dystonia have been excluded in the dt rat (Ziefer et al. 2002). Dystonic rats exhibit both axial and appendicular dystonia that progresses in severity with increasing postnatal age. Neonatal dt rats can be reliably differentiated from normal littermates by P12. Prior to P10, normal and dt rats have an identical motor phenotype and display qualitatively similar motor activities in the open field (e.g., head elevation, grooming, crawling, and quadruped stability). Dystonia is reduced when the animals are at rest and disappears during sleep. Without surgical intervention, dt rats develop a progressive, severe, generalized dystonia that involves both the limbs and truncal musculature, and, invariably, leads to death prior to P40 despite gavage feedings and other supportive measures.

There are no gross differences between normal and dt rats in terms of brain morphology. Microscopic analysis of cresyl violet, hematoxylin and eosin, Luxol fast blue, periodic acid-Schiff and silver-stained central and peripheral nervous tissues from dt rats has shown no differences from normals (Lorden et al. 1984, LeDoux et al. 1995). Because they are a central component of the basal ganglia, the morphology of striatal neurons was examined with Golgi impregnation in both dt and normal rats; no abnormalities were detected in the mutants (McKeon et al. 1984).

Anatomical studies in the dt rat have focused on olivocerebellar pathways. Dystonic rats and normal littermates do not exhibit differences in Purkinje cell number, volume of the cerebellar nuclei, soma size of cerebellar nuclear neurons, molecular layer thickness, or granular cell layer thickness, although, in vermian and paravermian tissues at P20, Purkinje cells are 5–11% smaller in dt rats than in normal littermates (Lorden et al. 1985, 1992). This effect is not generalized since there are no differences in the size of pyramidal neurons in the hippocampus. Furthermore, Purkinje cell dendritic arborizations are qualitatively similar between normal and dt rats. The inferior olivary projection to cerebellar cortex was studied with both anterogradely and retrogradely transported horseradish peroxidase. The connectivity pattern was consistent with studies in normal rats, and cerebellar cortical terminations were normal at the level of light microscopy (Stratton 1991).

The second messenger, cyclic guanosine monophosphate (cGMP), together with cGMP-dependent protein kinases types I and II are expressed at high levels in cerebellar Purkinje cells (de Vente et al. 2001). Basal levels of cGMP are decreased in dt rat cerebellar cortex (Lorden et al. 1985). In addition, the increase in cerebellar cGMP seen in normal rats after the systemic administration of harmaline is much smaller in dt rats. These findings suggest that a defect in neurotransmission, probably postsynaptic, is present in dt rat cerebellar cortex.

Profound changes in GABAergic markers were then isolated to the cerebellar nuclei and Purkinje cells in the dt rat. In the dt rat cerebellar nuclei, glutamic acid decarboxylase (GAD) activity was found to increase with increasing postnatal age (Oltmans et al. 1986, Beales et al. 1990). Opposite changes were noted in cerebellar nuclear GABA_A receptors. In contrast, there were no changes in GABA levels or binding of the benzodiazepine ligand, muscimol (Beales et al. 1990, Lutes et al. 1992). The distribution, size and density of GAD-immunoreactive puncta in the cerebellar nuclei was examined in normal and dt rats; the only abnormality noted was a relative decrease in puncta density at P25 in dt rats (Lutes et al. 1992). With quantitative *in situ* hybridization, GAD₆₇ mRNA was increased in Purkinje cells and decreased in the cerebellar nuclei of dt rats (Naudon et al. 1998). In aggregate, these findings are most compatible with increased activity of Purkinje cell GABAergic synapses within the dt rat cerebellar nuclei. The changes in cerebellar nuclear GABA_A receptors and GAD₆₇ transcript are probably compensatory responses.

To test the hypothesis that cerebellar dysfunction is critical to the expression of the dt rat motor phenotype, groups of dt rats and normal littermates underwent cerebellectomy (CBX) at P15 (LeDoux et al. 1993). The entopeduncular nuclei were lesioned with kainic acid in a separate group of dt rats. Age-matched unoperated dt rats served as controls. CBX included the dorsal portion of the lateral vestibular nucleus, a structure that receives direct projections from cerebellar Purkinje cells. After CBX, normal and dt rats could not be differentiated by behavioral observation or simple tests of motor function. Although non-operated rats performed better than CBX rats on tests of righting and tended to perform better on a climbing task, the differences were not striking. This is due, in part, to the milder effects of CBX on both young animals and rodents when compared to adults and higher species, respectively. CBX rats were, however, unable to perform more complex motor tasks such as narrow beam walking that are not difficult for non-operated rats. The group of dt rats with bilateral lesions of the entopeduncular nucleus showed no significant improvement in motor function. After CBX, dt rats survive into adulthood without additional treatment. The lifespan of post-CBX dt rats is equivalent to that of normal littermates. Furthermore, post-CBX dt rats are able to mate and rear their offspring.

Electrophysiological studies in awake dt rats have been essential for the rational explanation of olivocerebellar network abnormalities in the mutants. In comparison with normal rats, cerebellar nuclear cells from awake dt rats as young as P12 show bursting firing patterns (Fig. 1). Bursting activity increases with increasing postnatal age in dt rats (LeDoux et al. 1998). Many cerebellar nuclear cells from older dt rats exhibit rhythmic bursting activity that shows little modification in response to either sensory stimuli or motor activity. The bursting firing patterns displayed by cerebellar nuclear neurons from dt rats can be attributed to a low-threshold inactivating calcium-dependent conductance that generates rebound excitation following transient membrane hyperpolarization (Llinás and Mühlethaler 1988).

With single-unit awake recordings from cerebellar cortex, there was a trend for Purkinje cell simple-spike frequency to be higher in dt than in age-matched normal rats. In distinction, Purkinje cell complex-spike frequency was markedly lower in normal than in dt rats (Fig. 2).

As demonstrated in Fig. 2, Purkinje cells from dt rats, particularly cells from the vermis or older animals, exhibited rhythmic bursting simple-spike firing patterns.

Inferior olivary firing rates were lower in dt rats than in normal littermates (Stratton and Lorden 1991). In contrast, there were no significant differences in inferior olivary post-harmaline firing rates between normal and dt rats (Stratton and Lorden 1991). After the systemic administration of harmaline, complex-spike frequency increased in both normal and dt rats although it remained lower in the mutants. As seen in Fig. 3, harmaline-stimulated complex spike activity was also more rhythmic and produced greater suppression of simple spikes in normal than in dt rats (LeDoux and Lorden 2002). These electrophysiological findings along with earlier biochemical data indicate that dt rats harbor a defect in their climbing fiber input to Purkinje cells.

In order to identify the mutant gene responsible for the dt rat motor syndrome, a female spontaneously hypertensive rat (SHR) was bred with a post-CBX male dt rat. Microsatellite markers were spaced approximately 5 cM apart along the 20 rat autosomes. The SHR dam and post-CBX male dt rat (F0 generation) were bred twice to yield a total of 23 phenotypically-normal heterozygotic F1 rats. Heterozygote F1 rats (SD-dt/SHR) were crossbred to produce 875 F2 offspring. The genotypes of phenotypically normal F2 rats (heterozygote versus homozygote) were determined by breeding with post-CBX dt rats. After the first round of genotyping 69 F2 rats, three markers (D7Rat36, D7Rat35 and D7Rat32) showed strong linkage disequilibrium with the dystonic phenotype. More refined mapping was performed with additional microsatellite markers in 875 F2 rats.

In brief, haplotype analyses, recombination rates, and, in part, the SHRSP $\times$ BN genetic map were used to order the microsatellite markers and determine that the mutant gene was likely located between D7Rat188 and D7Rat113 (6.795–10.973Mb) within Contig NW_047773. Syntenic relationships among rat, human and mouse genomes were then rigorously examined *in silico*. We established that chromosomal loci syntenic with the “dt” locus (Chr 7q11) were Chr 12q13.13/ 19p13.3 and Chr 10D3/10C1 in human and mouse, respectively. Candidate genes within the dt locus were screened for mutations using semi-quantitative reverse transcriptase (RT)-PCR. Primers were designed to amplify the entire coding regions of genes within the dt locus. Forty of the 105 genes within the “dt” locus were screened prior to detecting low expression levels of *Atcay* in dt rats.

Atcay cDNA, 5'-UTR, exon, and splice site sequences showed no differences between dt rats and normal littermates. Since *Atcay* coding sequences were normal, Southern blotting with a 5'-UTR cRNA probe was performed in search of an insertional mutation within 5'-*Atcay*. Restriction enzymes *PvuII* and *MspI* produced normal sized restriction fragments. In contrast, *TaqI* and *PstI* generated larger fragments with dt rat genomic DNA. Restriction site mapping identified an insertion in Intron 1.

PCR primers were designed to broadly flank the putative insertion site in Intron 1 of dt rat *Atcay* (*Atcay*^{dt}). The 429-bp insertion was identified as the 3'-long terminal repeat (3'-LTR) of rat intracisternal A-particle (IAP) elements. The 3'-LTR insertion target site in the dt rat contains a 6-bp duplication (AACATA) such that a total of 435 bp are added to *Atcay* Intron 1 in dt rats (*Atcay*^{dt}). The transcriptional orientations of *Atcay* and the 3'-LTR insertion are in the same direction. The 3'-LTR insertion contains transcriptional elements (Sp1, CAAT box, and TATA box), a 15-bp triplet repeat, and a polyadenylation signal (AGTAAA).

The 3'-LTR insertion in Intron 1 dramatically reduces the production of *Atcay* transcript. With Northern blots, three *Atcay* transcripts (1.8, 2.5 and 4.2 kb) were present in both cerebellar and cerebral cortical tissues from normal rats. In contrast, these bands were not clearly detectable in dt rats. As determined with relative quantitative RT-PCR (QRT-PCR),

mean cerebellar and cerebral cortical *Atcay* transcript levels in P15 dt rats were only 4.1% and 1.2% of normal, respectively. In comparison, mean cerebellar and cerebral cortical *Atcay* transcript levels in P15 heterozygotes were 53.1% and 60.0% of normal, respectively. Western blotting with polyclonal antibodies generated in mouse and rabbit were used to examine the consequences of the insertional mutation on translation of caytaxin. Marked reductions in cerebellar caytaxin levels were seen with both antibodies. In harmony with the results of QRT-PCR, an intermediate reduction of cerebellar caytaxin levels was seen in heterozygote littermates. The SD rat *Atcay* gene (AY611623) contains 13 exons with an open reading frame of 1116 bp that encodes a 372-amino acid protein with 97% and 91% identity to mouse and human caytaxin amino acid-sequences, respectively.

Three *Atcay* transcripts were identified in brain but none were present in heart, spleen, lung, liver, muscle, kidney or testis. QRT-PCR was performed with RNA extracted from cerebral cortex, striatum, thalamus, hippocampus and cerebellum. *Atcay* transcript levels peaked at P7 in hippocampus, increased linearly from P1 to P36 in cerebellum, and showed minimal developmental regulation in cerebral cortex. With *in situ* hybridization, *Atcay* transcript was localized to dorsal root ganglia, peripheral ganglia, and seemingly all neuronal populations in brain. In cerebellum, *Atcay* transcript was present in the molecular, Purkinje and granular layers; transcript density in the molecular layer peaked at P14.

A listing of caytaxin model systems is provided in Table 1. Cayman ataxia is limited to inhabitants of Grand Cayman Island (Bomar et al. 2003). To date, *ATCAY* mutations have not been identified in other populations with ataxia or dystonia. Unfortunately, detailed descriptions of the “Cayman ataxia” phenotype have not been published in refereed journals, and, as such, the presence of dystonia cannot be excluded. Clinical manifestations of “Cayman ataxia” may include psychomotor retardation, ataxic gait, “scoliosis,” nystagmus. Cerebellar atrophy was detected on computed tomography imaging. Cayman ataxia is autosomal recessive and apparently non-progressive.

Although not well studied, murine models of caytaxin deficiency show evidence of dystonia in addition to ataxia. Jittery homozygotes exhibit a zigzag gait when attempting to run and have great difficulty righting after placement on their backs (Kapfhamer et al. 1996). By P14, jittery homozygotes crouch on their heels in a squatting position and cannot run without falling. Forelimb dystonic spasms occur by P16. The jittery allele of *Atcay* has a B1 element inserted into exon 4, which, if transcribed and translated, would produce a truncated protein of only 62 amino acids (Gilbert et al. 2004). Hesitant homozygotes can be discerned at P14 by an unusual hesitant walking motion. After the third postnatal week, the hindlimbs of hesitant homozygotes are maintained in a stiff extended position causing the posterior trunk to rise abnormally during ambulation. The posturing of hesitant mice is consistent with dystonia and similar to waddles mice (Jiao et al. 2005, Jinnah et al. 2005). Homozygote sidewinder mice are phenotypically similar to jittery mice (Bomar et al. 2003).

Caytaxin contains a hydrophobic binding pocket within a CRAL-TRIO domain, a motif common to certain proteins that bind lipophilic molecules like α -tocopherol, retinal, phosphatidylcholine, and the phosphatidylinositols (Bomar et al. 2003). Although caytaxin is highly conserved among mammals, the entirety of its sequence shows weaker homology with invertebrate proteins. The CRAL-TRIO domain can also be found in plants and fungi. A CRAL-TRIO domain is the major component of the well-characterized SEC14 protein found in yeast. Some patients with mutations in the gene that encodes α -tocopherol transfer protein, another protein containing a CRAL-TRIO domain, exhibit dystonia as a major component of their phenotype. Structural analysis suggests that the ligand-binding pocket of caytaxin is more polar than that of α -tocopherol transfer protein. By extrapolation, it is

plausible that caytaxin binds a lipophilic ligand essential to calcium signaling in cerebellar Purkinje cells.

The binding cavity of caytaxin is suited for an amphipathic ligand like a phosphatidylinositol (Bomar et al. 2003, Panagabko et al. 2003, Xiao and LeDoux 2005). Compatible with this hypothesis, genes encoding the phosphatidylinositol 4-phosphate adaptor protein 1, phospholipid scramblase 3, and inositol polyphosphate phosphatase-like 1 were upregulated in dt rat cerebellar cortex (Xiao et al. 2007). Conversely, genes encoding inositol polyphosphate 1-phosphatase (INPP1), inositol polyphosphate-5-phosphatase A and frequenin were downregulated in the dt rat. INPP1 removes the 1 position phosphate from inositol 1,3,4-triphosphate and inositol 1,4-diphosphate, thereby producing inositol 3,4-diphosphate and inositol 4-phosphate, respectively (Inhorn and Majerus 1988).

In addition to phosphatidylinositol signaling pathways, data derived from oligonucleotide microarrays and QRT-PCR identified calcium homeostasis and extracellular matrix interactions as domains of cellular dysfunction in dt rats (Xiao et al. 2007). In dt rats, genes encoding the corticotropin releasing hormone receptor 1 (CRH-R1, *Crhr1*) and calcium-transporting plasma membrane ATPase 4 (PMCA4, *Atp2b4*) showed the greatest up-regulation with QRT-PCR. Immunocytochemical experiments demonstrated that CRH-R1 and PMCA4 were upregulated in cerebellar cortex of mutant rats. CRH-R1 and PMCA4 were upregulated in distinct cellular compartments within the molecular layer of cerebellar cortex. CRH-R1 was upregulated in Purkinje cells whereas PMCA4 was localized to parallel fibers. Along with previous electrophysiological and pharmacological studies, our data indicate that caytaxin plays a critical role in the molecular response of Purkinje cells to climbing fiber input. Caytaxin may also contribute to maturational events in cerebellar cortex.

Caytaxin is also known as BNIP-H where BNIP is the acronym for Bcl-2 nineteen kilodalton interacting protein and H applies to BNIP-2 homology. “Bcl” derives from B-cell lymphoma. BNIP-2 was originally identified as a partner for the anti-apoptotic protein Bcl-2. BNIP-2 contains a protein-protein interaction domain known as the BNIP-2 and Cdc42GAP homology (BCH) domain (Low et al. 2000). Expression of BNIP-2 is associated with Cdc42-dependent morphological changes such as cell elongation and membrane protrusions (Zhou et al. 2005).

Caytaxin may be developmentally regulated by the ubiquitin E3 ligase CHIP (C-terminus of Hsc70-interacting protein) which has been shown to polyubiquitinate caytaxin *in vitro* (Grelle et al. 2006). Another *in vitro* study suggests that caytaxin may interact with kidney-type glutaminase (KGA) which converts glutamine to glutamate (Buschdorf et al. 2006). Overexpression of caytaxin in PC12 cells relocalized KGA from the mitochondria to neurite terminals. More recently, Buschdorf and colleagues (2008) have shown that caytaxin also interacts with Pin1, a peptidyl-prolyl cis/trans isomerase. *In vitro*, nerve growth factor (NGF) appears to stimulate the interaction of caytaxin with Pin1. Moreover, Pin1 was shown to disrupt caytaxin-KGA complex formation.

Caytaxin may be at the molecular interface between dystonia and ataxia. Caytaxin deficiency is associated with variable degrees of ataxia and dystonia in jittery, hesitant and sidewinder mice. Although caytaxin and associated signaling pathways appear essential for the developmental and/or physiology of cerebellar cortex, the roles of individual network elements (e.g., granule and Purkinje cells) in the pathobiology of dystonia and ataxia have not been delineated. Using RNA derived from cerebellar cortex *in toto*, we have shown that caytaxin deficiency disrupts phosphatidylinositol signaling pathways, calcium homeostasis and extracellular matrix interactions (Xiao et al 2007). However, the relative contributions

of pre- and post-synaptic elements, and, similarly, primary and secondary effects on changes in gene expression could not be established given the widespread neuronal distribution of caytaxin during development. In aggregate, currently available data suggest that (1) caytaxin is required for the normal development of Purkinje cells and climbing fiber-Purkinje cell synapses, (2) CHIP may regulate caytaxin levels in adult brain, and (3) the temporal specificity of caytaxin expression in Purkinje and granule cells may determine motoric expressivity (dystonia vs. ataxia).

Other genetic models

Engineered

Vertebrate—Numerous knock-out (KO), knock-in (KI) and transgenic models of DYT1 and other types of dystonia have been generated in mice (Table 2). In addition, Dang et al. (2006) produced a torsinA knock-down model. Many of these models were reviewed by Zhao et al. (2008). Although comprehensive, the list provided in Table 2 does not list all published genetic models of dystonia. Importantly, none of these models manifest dystonia. On the other hand, one or more morphological, neurochemical, behavioral, cellular and neurophysiological abnormalities have been reliably characterized in KO, KI and transgenic models of DYT1 dystonia. The KI models have high etiological validity and some would argue, face validity, given that penetrance of the classic Δ GAG mutation is less than 40%.

Sharma and colleagues (2005) developed three transgenic models to study DYT1 dystonia: human mutant torsinA 1 (hMT1), human mutant torsinA 2 (hMT2) and human wild-type torsinA (hWT). The hMT2 and hWT mice exhibit weak expression of their transgenes (Sharma et al. 2005, Zhao et al. 2008). In contrast, hMT1 mice express transgene 3.90–4.95 $\times$ higher than human brain (Zhao et al. 2008). The hMT1 torsinA transgenic mouse developed by Sharma and colleagues (2005) shows impaired amphetamine-induced dopamine release (Balcioglu et al. 2007), increased striatal dopamine turnover (Zhao et al. 2008), impaired beam walking (Zhao et al. 2008), increased hind-base widths (Zhao et al. 2008), impaired bidirectional synaptic plasticity (Martella et al. 2009) and reduced activity of the dopamine transporter (Hewett et al. 2010). No abnormalities of the nuclear envelope have been detected in the hMT1 mouse model (Zhao et al. 2008).

Martella and colleagues (2009) reported that long-term depression (LTD) could not be elicited in medium spiny neurons from hMT1 mice. Moreover, long-term potentiation (LTP) in medium spiny neurons was enhanced in hMT1 mice in comparison to wild-type littermates. LTD was rescued in hMT1 medium spiny neurons either by lowering endogenous levels of acetylcholine or exposure to a muscarinic antagonist. In related work, a dopamine agonist, quinpirole, caused membrane depolarization and increase firing rates in striatal cholinergic interneurons from hMT1 mice (Pisani et al. 2006). In aggregate, these electrophysiological and neurochemical findings point out an imbalance between dopaminergic and cholinergic signaling in DYT1 dystonia.

Homozygous, but not heretozygous, KO and KI models exhibit structural abnormalities of the nuclear envelope (Goodchild et al. 2005). Morphological studies in other DYT1 models have shown evidence of torsinA- and ubiquitin-positive cytoplasmic inclusion bodies (Shashidharan et al. 2004, Dang et al. 2005, Grundmann et al. 2007) and bleb formation at the nuclear envelope (Grundmann et al. 2007).

Recent models have been engineered to explore the role of specific brain regions and pathways in the pathobiology of DYT1 dystonia (Yokoi et al. 2008, Page et al. 2010). Yokoi and colleagues (2008) generated a cerebral cortex-specific DYT1 conditional KO mice by mating *Tor1a*-loxP mice with *Emx1*-cre KI mice. The conditional KO mice showed normal

development of barrel fields in somatosensory cortex, hyperactivity and increased slips on a beam-walking task. Given the potential role of dopaminergic neurotransmission in DYT1 dystonia, Page and co-workers (2010) expressed human wild-type torsinA and Δ E-torsinA in mice under control of the tyrosine hydroxylase (TH) promoter. Expression of mutant and wild-type torsinA was limited to dopaminergic neurons of the midbrain (Page et al. 2010). There was no detectable change in striatal dopamine turnover. However, defective dopamine release was detected with microdialysis and fast cycle voltammetry. Similar to KI and other transgenic models, the TH- Δ E-torsinA mice exhibited increased slips on a beam-walking task.

Models of paroxysmal non-kinesigenic dyskinesia (DYT8) and myoclonus-dystonia syndrome (MDS/DYT11) have been generated and show many features of the corresponding human disorders. However the DYT11 model developed by Yokoi and colleagues (2006) does not manifest dystonia. A mouse model of SCA27 exhibits paroxysmal dystonia. Unfortunately, additional study of the DYT8 and DYT11 models has been lacking. As such, the underlying neurophysiological underpinnings of the corresponding movement disorders remain largely unknown.

Invertebrate—The fruit fly and roundworm provide viable tools for studying the *in vivo* cellular biology of movement and neurodegenerative disorders. Both, for example, harbor well-defined collections of dopaminergic neurons. Studies in invertebrates have contributed to our understanding of torsinA biology. For instance, Caldwell and colleagues (2003) provided evidence that torsinA plays a chaperone role by showing that overexpression of both human and *C. elegans* torsin proteins reduces polyglutamine-dependent protein aggregation. Expression of Δ E torsinA in flies caused temperature-dependent locomotor deficits, enlarged boutons at neuromuscular junctions and possible defects in TGF- β signaling (Koh et al. 2004). Of note, overexpression of Smad2, a downstream effector of the TGF- β signaling pathway, suppressed motoric and ultrastructural defects in the Δ E-torsinA flies.

Chen and colleagues (2010) used *C. elegans* to test the hypothesis that selective defects in torsinA function would result in increased vulnerability to endoplasmic reticulum (ER) stress. Their analyses showed that torsinA normally serves a protective function to maintain a homeostatic threshold against ER stress. Loss of torsinA or mutations that limit its translocation to the ER or block ATPase activity were shown to impair the ER stress response.

Invertebrate model systems, particularly *C. elegans*, also facilitate high-throughput testing of candidate drugs targeting specific biological processes. Just recently, the first screen for candidate small molecule therapeutics for dystonia was completed in *C. elegans* (Cao et al. 2010). Two classes of antibiotics, quinolones and aminopenicillins, were found to enhance activity of wild-type torsinA. In downstream corroborative analyses, a motoric defect in Δ E torsinA knock-in mice was rescued by systemic administration of ampicillin.

Spontaneous mutants

Rodents, mainly spontaneously mutant mice with movement disorders, have revealed molecules and cellular pathways involved in motor control. Examples of deficient or defective proteins in mice with motor dysfunction (ataxia and/or dystonia) include the type VIII alpha subunit of voltage-gated sodium channel in *Med^J* mice (Burgess et al. 1995), delta2 glutamate receptor in *lurcher* mice (Zuo et al. 1997), potassium inwardly-rectifying channel subfamily J member 6 in *weaver* mice (Patil et al. 1995), ATP/GTP binding protein 1 in *Purkinje cell degeneration* mice (Fernandez-Gonzalez et al. 2002), and P/Q type calcium channel α -1A subunit in *tottering* mice (Fletcher et al. 1996). Several rodents with

spontaneous mutations manifest dystonia as a core feature of their motor phenotypes (Table 3). Genetically engineered mice may, sometimes unexpectedly, also exhibit dystonia. For instance, an FGF14 knock-out mouse (Table 2) was found to manifest paroxysmal dystonia along with interictal ataxia (Wang et al. 2002).

All of the mutations listed in Table 3 are autosomal recessive although the causal mutations have not been identified in the *dt^{SZ}* hamster or *hph-1* mouse. Review of Table 3 reveals that virtually all genes associated with dystonia in spontaneous mutants are involved in Purkinje cell calcium signaling. For example, deficiency of carbonic anhydrase type VIII (CAR8) produces a distinctive lifelong gait disorder in waddles mice (Jiao et al. 2005) and quadrupedal gait in humans (Türkmen et al. 2009). Although CAR8 lacks catalytic activity, it does reduce the affinity of IP3R1 for IP3. CAR8 is concentrated in cerebellar Purkinje cells (Jiao et al. 2005). Consequently, CAR8 deficiency is predicted to cause motor dysfunction by altering calcium homeostasis and disrupting the normal physiology of Purkinje cells. Similar consequences could also be attributed to deficiency of IP3R1 and PMCA2. As is the case for caytaxin, CAR8 and other Purkinje cell proteins associated with dystonia may contribute to the normal ultrastructural maturation of cerebellar cortex (Hirasawa et al. 2007).

Pharmacological and neural lesion models

Models generated with wild-type animals have proven useful for identifying neural structures from which dystonia may arise. Table 4 lists many of the most informative models that have been described in the refereed literature. Generalized and segmental dystonia can be generated via manipulations of the cerebellum, red nucleus and basal ganglia (Table 4). In contrast, hand-forearm dystonia has been associated with dedifferentiation of somatosensory cortex, and abnormal head-neck postures can be produced by lesions or stimulation of the midbrain, particularly the interstitial nucleus of Cajal. Although there is no consensus regarding specific neuroanatomical sites of origin, animal models of the focal dystonias (blepharospasm, cervical dystonia, and hand-forearm dystonia) suggest that maladaptive sensorimotor integration may be a pathophysiological event common to all of the dystonias.

Studies of blepharospasm by Evinger and colleagues have been particularly informative (Schicatano et al. 1997, Peshori et al. 2001). In brief, a two-factor model may explain the origin of blepharospasm. The first factor is subclinical reduction in striatal dopamine, which typically occurs with normal aging. Reduced striatal dopamine may be associated with increases in trigeminal blink excitability and duration. The second factor is an external ophthalmic insult such as dry eye. This model is compatible with age-of-onset and ocular co-morbidities in humans with blepharospasm (Hallett et al. 2008).

Conclusions

Largely precipitated by work in animal models, a role for the cerebellum in the pathophysiology of human dystonia has received increasing attention in recent years (Jinnah and Hess 2006, Perlmuter and Thach 2007, Argyelan et al. 2009) and is supported by data from a variety of clinical fronts: lesion localization in secondary cervical dystonia (LeDoux and Brady 2003), functional imaging studies in DYT1 dystonia (Eidelberg et al. 1998), the syndrome of dystonia with cerebellar atrophy (DYTCA) (Le Ber et al. 2006), and dystonia as a presenting or prominent feature of several hereditary ataxias. LeDoux and Brady (2003) showed that structural lesions associated with secondary cervical dystonia were commonly localized to the cerebellum and its afferents. The cerebellum also contributes to primary dystonias. Using [18F]-fluorodeoxyglucose and positron emission tomography, similar patterns of hypermetabolism were detected in both manifesting and nonmanifesting carriers

of DYT1 mutations (Eidelberg et al. 1998). In movement-free conditions, both manifesting and non-manifesting carriers showed increased metabolic activity in the lentiform nuclei, cerebellum, and supplementary motor areas. In movement-related conditions, manifesting carriers showed increased metabolic activity in the midbrain, cerebellum, and thalamus. Le Ber and colleagues (2006) coined the acronym DYTCA for familial syndromes with the combination of dystonia and mild slowly-progressive ataxia. Their 12 patients from 8 families displayed appendicular dystonia, often with spasmodic dysphonia. Some subjects also had cervical or facial dystonia. Similar series of patients had been previously reported by Fletcher et al. (1988) and Kuoppamäki et al. (2003). These reports suggest that both ataxia AND dystonia may arise from defective olivocerebellar pathways in humans. Moreover, some of these cases could be allelic with Cayman ataxia, with phenotypic differences being due to genetic background and type of mutation (e.g., missense vs. splice site).

In coming years it is quite likely that genetic/molecular etiologies will be identified for an ever increasing percentage of primary dystonia cases. In this context, it is important to recognize that the current clinical definition of dystonia may preclude unification of molecular etiologies. Similarly, there may be no universal “upstream” signature circuit abnormality common to all the dystonias. Simply put, dystonia may be a relatively common downstream manifestation of a variety of hereditary and acquired insults to the nervous system. Despite these caveats, some common ground is likely and animal models should contribute to identification of genetic causes of human disorders, understanding disease pathogenesis and pinpointing neural network abnormalities to guide neurosurgical intervention. Moreover, animal models may be used to develop preventative treatments in individuals with pathological allelic variants and small molecule pharmacotherapeutics in subjects with manifest disease. It should be emphasized that rigorous study of select hereditary dystonias may provide insight into the pathobiology of the more common sporadic dystonias. For case in point, work with the dt rat has contributed to our understanding of motor systems by showing that abnormal signaling in cerebellar cortex can lead to abnormal cerebellar output with bursting firing patterns in cerebellar output. As a result, neuromodulation of cerebellar cortex and/or the cerebellar nuclei may be rediscovered as targets for functional neurosurgery in patients with dystonia. In summation, integration of data from animal models and functional imaging in humans suggests that maladaptive sensorimotor learning secondary to developmental defects in cerebellar cortex as a consequence of impaired calcium homeostasis in Purkinje cells contributes to the pathogenesis of adult-onset primary dystonia. It is also possible that the manifestation of dystonia requires interactions among pathological olivocerebellar and striatal circuits (Neychev et al. 2008).

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Fig. 1. Single-unit extracellular recordings from cerebellar nuclei in dt rats at P12, P17, and P24. (A) Representative portion of the spike trains. (B) Interspike interval histograms with parameters from a double-gamma distribution. (C) Autocorrelations. (D) Ratemeter histograms.

Fig. 2. Spontaneous Purkinje cell spike trains from normal (A) and dt (B) rats. Complex spikes are marked with arrows.

Fig. 3. Harmaline-stimulated Purkinje cell spike trains from normal (A) and dt (B) rats. Complex spikes are marked with arrows.

Table 1

Caytaxin model systems

Model	Mutation Location	Type of Mutation	Transcripts	Phenotype
Cayman ataxia (human)	Exon9/Intron 9	C→G missense G→T splice site	Unknown	gait ataxia, hypotonia, mental retardation, nystagmus, intention tremor, dysarthria
Dystonic rat	Intron 1	IAP element insertion	all markedly reduced	severe generalized dystonia, die by 4–5 weeks
Jittery mouse	Exon 4	B1 element insertion, Alu-related mutagenesis	aberrant largest transcript, smaller transcript markedly reduced	severe truncal and limb ataxia, dystonic forelimb spasms, die by 3–4 weeks
Hesitant mouse	Intron 1	IAP element insertion	aberrant larger transcripts with small amounts of normal transcript	mild ataxia, dystonia
Sidewinder mouse	Exon 5	2-bp deletion	all markedly reduced	severe truncal and limb ataxia, dystonic forelimb spasms, die by 3–4 weeks

Table 2

Other genetic models

Human Disorder(s)	Human Gene	Human Protein	Model	Important Results	Reference (s)
Engineered-vertebrate					
DYT1	<i>TOR1A</i>	torsinA	<i>Tor1a</i> -KO mouse	vesicle formation within the nuclear envelope	Goodchild et al. 2005
DYT1	<i>TOR1A</i>	torsinA	transgenic mouse: human ΔE torsinA CMV promoter	increased striatal dopamine turnover, deficits on a raised beam task,	Sharma et al. 2005, Zhao et al. 2008
DYT1	<i>TOR1A</i>	torsinA	transgenic mouse: human ΔE torsinA NSE promoter	40% of mice display an abnormal motor phenotype with circling, self-clasping and hyperactivity; ubiquitin + perinuclear aggregates in pons; reduced striatal DOPAC/dopamine ratios	Shashidharan et al. 2005
DYT1	<i>TOR1A</i>	torsinA	<i>Tor1a</i> -Ki mouse	impairments on beam walking task, protein aggregates in the pontine nuclei	Dang et al. 2005
DYT1	<i>TOR1A</i>	torsinA	<i>Tor1a</i> -KI mouse	homozygotes have vesicles in the nuclear envelope	Goodchild et al. 2005
DYT1	<i>TOR1A</i>	torsinA	<i>Tor1a</i> -knockdown mouse	hyperactivity & increased slips on beam-walking task; reduced striatal DOPAC	Dang et al. 2006
DYT1	<i>TOR1A</i>	torsinA	transgenic mouse: human ΔE torsinA PrP promoter	hyperactivity & defects on rotarod testing; inclusion formation in brainstem nuclei, nuclear envelope bleb formation; increased brainstem DOPAC	Grundmann et al. 2007
DYT1	<i>TOR1A</i>	torsinA	cerebral cortex <i>Tor1a</i> -KO via cross with <i>Emx</i> -cre mice	hyperactivity & increased slips on beam-walking task	Yokoi et al. 2008
DYT1	<i>TOR1A</i>	torsinA	transgenic mouse: human ΔE torsinA TH promoter	reduced cocaine-induced striatal dopamine, impaired beam walking	Page et al. 2010
DYT8 (PNKD)	<i>MR-1</i>	myofibrillogenesis regulator	MR-1 transgenic: BAC clones modified to contain A7V and A9V mutations	dystonic attacks precipitated by caffeine and ethanol	Nakayama et al. 2005
DYT11 (MDS)	SGCE	e-sarcoglycan	<i>Sgce</i> -KO mouse	myoclonus, motor deficits, hyperactivity, anxiety, & depression	Yokoi et al. 2006
SCA27	<i>FGF14</i>	fibroblast growth factor-14	<i>Fgf14</i> -KO mouse	ataxia, paroxysmal dystonia	Shakkottai et al. 2009
Engineered-invertebrate					
DYT1	<i>TOR1A</i>	torsinA	<i>Drosophila</i> -transgenic overexpression Δ GAG human torsinA	enlarged boutons neuromuscular junction, disruption of TGF- β signaling	Koh et al. 2004
DYT1	<i>TOR1A</i>	torsinA	<i>Drosophila</i> -RNAi downregulation of torp4A	progressive retinal degeneration	Muraro & Moffat 2006
DYT1	<i>TOR1A</i>	torsinA	<i>C. elegans</i> -transgenic overexpression of human torsinA	reduction in polyglutamine repeat-induced protein aggregation	Caldwell et al. 2003

Human Disorder(s)	Human Gene	Human Protein	Model	Important Results	Reference (s)
DYT1	TOR1A	torsinA	<i>C. elegans-ges-1</i> intestinal promoter transgenic expression of WT and mutant human torsinA	torsinA mutations (Δ E torsinA, reduced ATPase activity, other) increase sensitivity to endoplasmic reticulum stress	Chen et al. 2010

* Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; KI, knock-in; KO, knock-out; MDS, myoclonus-dystonia syndrome; MR-1, myofibrillogenesis regulator; PNKD, paroxysmal nonkinesigenic dyskinesia; PrP, prion protein; TH, tyrosine hydroxylase; WT, wild-type

Spontaneous mutants

Table 3

Model	Human Disorder(s)	Gene	Protein	Function of Encoded Protein	Reference(s)
Dystonia musculorum mouse	epidermolysis bullosa simplex (epithelial isoform)	<i>BPAG1</i>	neural isoform bullous pemphigoid antigen	hemi-desmosomal protein	Brown et al. 1995
<i>dt^{sc}</i> hamster		<i>unknown</i>	unknown	unknown	Richter et al. 1991
Tottering mouse	SCA6, FHM, EA-2	<i>CANCA1A</i>	$\alpha 1A$ calcium channel	calcium channel	Fureman et al. 2002
Stargazer mouse	chronic neuropathic pain	<i>CACNG2</i>	$\gamma 2$ calcium channel subunit	calcium channel	Leitch et al. 2009
Opisthotonus mouse	SCA15/SCA16	<i>ITPR1</i>	inositol triphosphate receptor 1 (IP3R1)	intracellular calcium homeostasis	Novak et al. 2010
<i>lph-1</i> mouse	DYT5 (dopa-responsive dystonia)	<i>unknown</i>	GTP cyclohydrolase type 1	rate-limiting enzyme for synthesis of BH4	Hyland et al. 2003
Ducky mouse		<i>CACNA2D2</i>	$\alpha 2\delta$ calcium channel subunit	calcium channel	Donato et al. 2006
Lethargic mouse	epilepsy	<i>CACNB4</i>	$\beta 4$ calcium channel subunit	calcium channel	Devanagondi et al. 2007
Waddles mouse	quadrapedal gait	<i>CAR8</i>	carbonic anhydrase-related protein type VIII	intracellular calcium homeostasis via IP3R1 binding	Jiao et al. 2005, Turkmen et al. 2009
Wriggle mouse Sagami		<i>PMCA2</i>	plasma membrane Ca^{2+} -ATPase	calcium transport	Takahashi et al. 1999
Myshkin (Myk) mouse	DYT12 (rapid-onset dystonia Parkinsonism)	<i>ATP1A3</i>	Na^{+} - K^{+} -ATPase $\alpha 3$ isoform	Na^{+} - K^{+} -ATPase	Clapcote et al. 2009

* Abbreviations: BH4, tetrahydrobiopterin; EA-2, episodic ataxia type 2; FHM, familial hemiplegic migraine; SCA6, spinocerebellar ataxia type 6;

Table 4

Pharmacological and neural lesion models

Disorder modeled	Species	Lesion/treatment	Key features	Reference
Focal dystonia				
blepharospasm	rat	partial unilateral 6-OHDA lesion of the SNpc & partial unilateral denervation of the orbicularis oculi muscle	blepharospasm	Schicatano et al. 1997
unilateral hemifacial spasm/blepharospasm	cat	unilateral injection of serotonin into the facial motor nucleus	unilateral blepharospasm/hemifacial spasm	LeDoux et al. 1998
cervical dystonia	monkey	electrolytic lesions-medial midbrain tegmentum	contraversive torticollis	Foltz et al. 1959
cervical dystonia	monkey	lesions of mesencephalic tegmentum	ipsiversive torticollis	Battista et al. 1976
cervical dystonia	monkey	microstimulation interstitial nucleus of Cajal	ipsiversive head rotation	Klier et al. 2002
cervical dystonia	monkey (marmoset)	6-OHDA lesion ascending nigrostriatal pathway	ipsiversive torticollis that resolved with apomorphine	Sambrook et al. 1979
writer's cramp	monkey	intensive sensorimotor training	hand-forearm dystonia	Byl et al. 1996
Generalized dystonia	mouse	injection of kainic acid into cerebellar cortex	severe truncal and appendicular dystonia	Pizoli et al. 2002
	mouse	systemic administration of L-type calcium channel agonists	severe truncal and appendicular dystonia	Jinnah et al. 2000
	rat	injection of sigma receptor ligands into the red nucleus	cervical and truncal dystonia	Walker et al. 1988
	rabbit	fetal hypoxia-ischemia	mixed dystonia-spasticity	Derrick et al. 2004
	mouse/rat	systemic 3-nitropropionic acid	mild dystonia, bradykinesia	Guyot et al. 1997