Neurobiology of Disease 42 (2011) 152–161

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Review

Animal models of dystonia: Lessons from a mutant rat

Mark S. LeDoux *

University of Tennessee Health Science Center, Department of Neurology, 855 Monroe Avenue, Link Building, Suite 415, Memphis, TN 38163, USA

ARTICLE INFO

Article history:
Received 2 September 2010
Revised 15 October 2010
Accepted 9 November 2010
Available online 21 November 2010

Keywords:
Dystonia
Inferior olive
Purkinje cell
Caytaxin
TorsinA
Basal ganglia

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. This article is part of a Special Issue entitled "Advances in dystonia".

Contents

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 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

* Fax: +1 901 448 7440.
E-mail address: mledoux@uthsc.edu
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2010.11.006

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 post-synaptic, 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, GAD_{67} 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 GAD_{67} 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 (Llinas and Muhlethaler, 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