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Research Paper

Altered postnatal maturation of striatal GABAergic interneurons in a phenotypic animal model of dystonia

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ABSTRACT

GABAergic disinhibition has been suggested to play a critical role in the pathophysiology of several basal ganglia disorders, including dystonia, a common movement disorder. Previous studies have shown a deficit of striatal GABAergic interneurons (IN) in the *dt*^{sz} mutant hamster, one of the few phenotypic animal models of dystonia. However, mechanisms underlying this deficit are largely unknown. In the present study, we investigated the migration and maturation of striatal IN during postnatal development (18 days of age) and at age of highest severity of dystonia (33 days of age) in this hamster model. In line with previous findings, the density of GAD67-positive IN and the level of parvalbumin mRNA, a marker for fast spiking GABAergic IN, were lower in the *dt*^{sz} mutant than in control hamsters. However, an unaltered density of Nkx2.1 labeled cells and Nkx2.1 mRNA level suggested that the migration of GABAergic IN into the striatum was not retarded. Therefore, different factors that indicate maturation of GABAergic IN were determined. While mRNA of the KCC2 cation/chloride transporters and the cytosolic carboanhydrase VII, used as markers for the so called GABA switch, as well as BDNF were unaltered, we found a reduced number of IN expressing the alpha1 subunit of the $GABA_A$-receptor (37.5%) in *dt*^{sz} hamsters at an age of 33 days, but not after spontaneous remission of dystonia at an age of 90 days. Since IN shift expression from alpha2 to alpha1 subunits during postnatal maturation, this result together with a decreased parvalbumin mRNA expression suggest a delayed maturation of striatal GABAergic IN in this animal model, which might underlie abnormal neuronal activity and striatal plasticity.

1. Introduction

GABAergic interneurons (IN) control the striatal microcircuitry, and thereby the activity of medium spiny neurons (MSN; striatal output neurons) and the basal ganglia-thalamocortical network (Ramanathan et al., 2002). Subtypes of GABAergic IN include, in particular, fast-spiking IN (FSIs), positive for calcium-binding protein parvalbumin (PV) (Hu et al., 2014), IN that express calcium-binding protein calretinin and IN that contain nitric oxide synthase (NOS) (Ramanathan et al., 2002). There is evidence from studies in human patients and animal models that reduced inhibition by striatal PV IN could be involved in basal ganglia disorders, including dystonia (Richter and Richter, 2014), Tourette syndrome (Pappas et al., 2014), Parkinson's disease (Gittis and Kreitzer, 2012) and Huntington's disease (Reiner et al., 2013). Since IN express unique receptors such as specific $GABA_A$ receptor ($GABA_AR$) subunits (Waldvogel et al., 1999) insights into their pathophysiological role can lead to the discovery of new therapeutic targets for these incurable disorders.

Dystonia is characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive movements and postures (Albanese et al., 2013). The basal ganglia play an important role in the pathophysiology of dystonia. Abnormal neuronal activity in the striatum, the input structure of the basal ganglia, can lead to disturbed inhibition of thalamocortical projections and to changes in striatal and cortical plasticity (Breakefield et al., 2008; Gittis and Kreitzer, 2012; Kohling et al., 2004). Increasing evidence from animal models and human patients suggest that this maladaptive plasticity can be related

Abbreviations: BDNF, brain-derived neurotrophic factor; CAVII, cytosolic carboanhydrase isotyp 7; $GABA_AR$, $GABA_A$ receptor; IN, interneurons; PV, parvalbumin; KCC2, potassium chloride transporter 2.

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to disturbed GABAergic inhibition (Garibotto et al., 2011; Gittis et al., 2011; Reiner et al., 2013; Weise et al., 2011). The dt^{sz} mutant hamster, a phenotypic unique rodent model of paroxysmal dystonia, replicates these alterations. In this model, age-dependent reductions of PV-positive IN (Gernert et al., 2000; Hamann et al., 2007), calretinin-positive IN (Hamann et al., 2005) and NOS-positive IN (Sander et al., 2006) were found by quantification of cells labeled for the respective phenotypic markers. This deficit results in reduced inhibition of striatal projection neurons and in consequence leads to a decreased basal ganglia output, verified by several electrophysiological recordings (Avchalumov et al., 2014; Bennay et al., 2001; Gernert et al., 2002; Gernert et al., 2000; Kohling et al., 2004). Identification of the cause of reduced IN in this hamster model could reveal novel disease mechanisms and therapeutic targets.

Reduction of GABAergic striatal IN in the dt^{sz} mutant hamster occurs at the maximum expression of dystonia, at 33 days of age, which is only 12 days after weaning. In correlation with the remission of dystonic symptoms, the density of GABAergic IN normalizes at about 60–70 days of age (Hamann et al., 2007). This early and age dependent deficit points towards a developmental impairment which could either be a delayed migration of the IN into the striatum and/or a retarded maturation.

During embryonic development cells of the medial ganglionic eminence, expressing the homeodomain protein Nkx2.1, migrate into the forming striatum where they differentiate into the cholinergic, calretinin-positive, or PV-positive IN. These IN maintain expression of Nkx2.1 into adulthood, which is therefore an early and stable marker of striatal IN (Hamasaki et al., 2003; Marin et al., 2000). Important factors for the postnatal differentiation of GABAergic IN are the brain-derived neurotrophic factor (BDNF) (Eto et al., 2010; Lessmann and Brigadski, 2009), the GABA-switch related proteins potassium chloride transporter 2 (gene: Slc12a5, protein: KCC2) and cytosolic carboanhydrase isotyp 7 (CAVII) (Yeo et al., 2009), as well as the expression of specific $GABA_AR$ subunits (Fritschy, 2015; Waldvogel et al., 1999). BDNF co-regulates the expression of the transporter KCC2 (Yeo et al., 2009), which increases on postnatal day 6 leading to a shift from excitatory to inhibitory GABA-signalling of $GABA_AR$ (Rivera et al., 2005). $GABA_AR$ are composed of five different subunits including two α-, two β- and one γ-subunit for each receptor. Further classification separates subunits into α1–6 und β1–3 (Mohler, 2011). Importantly, mature GABAergic IN typically express α1β2γ2 $GABA_AR$, whereby MSN predominantly express α2β3γ2 $GABA_AR$ (Fritschy, 2015; Pirker et al., 2000; Waldvogel et al., 1999). Interestingly, premature GABAergic IN express α2β2γ2 $GABA_AR$ and switch to α1 subunit containing receptors during postnatal development (Cuzon Carlson and Yeh, 2011).

In order to further determine the developmental alterations in striatal IN, we investigated the expression of Nkx2.1, PV-mRNA, BDNF, the GABA-switch and $GABA_AR$ subunit alpha1 ($GABA_AR$-α_1) in the dt^{sz} hamster in comparison to controls. Our results provide evidence that maturation of GABAergic IN and not migration of GABAergic precursor IN into the striatum is delayed in the dystonic hamster model.

2. Material and methods

2.1. Animals

All experiments were performed with groups of female and male dt^{sz} mutant Syrian golden hamsters (*Mesocricetus auratus auratus*) (inbred line) at an age of 18, 33 or 90 days and on age- and sex-matched control hamsters. N numbers for experiments were between 4 and 17 as indicated in the Results section. The dt^{sz} mutant hamster shows a dystonic phenotype in response to stress with a maximum severity at 33 days of age and remission after 60–70 days of age (Hamann et al., 2007; Richter and Loscher, 1998). Both hamster lines were tested at 21 days of age for expression of dystonic phenotype in response to stress as described previously (Richter and Loscher, 1998). As expected, all dt^{sz} hamsters developed dystonic symptoms after stress induction, while none of the control hamsters showed a dystonic phenotype. The animals were bred and kept in institutional facilities on a 14 h light/10 h dark cycle. Hamsters were single-housed after weaning in makrolon cages (Type III) at 23 °C ± 2 °C and relative humidity of about 60%. Food (Altromin standard diet) and water were available ad libitum. All experiments and animal care were conducted under the aspects of reduction, refinement and replacement and following the German Animal Welfare Act (TVV44/12, TVV41/13) as well as the European guidelines (Directive 2010/63/EU). All analyses were performed blindly with respect to the genotype of the animals. The number and age of hamsters used in each experiment are given in the text and figures.

2.2. Tissue preparation for molecular biology

Groups of hamsters were euthanized at 33 days or 18 days of age by an overdose of pentobarbital (100 mg/kg bodyweight). The animals were briefly perfused transcardially with 0.1 M phosphate buffered saline (PBS) at pH 7.4 supplemented with 20 units of heparin/ml. Thereafter the brain was removed from the skull and dissected into the two hemispheres on an ice-cold glass plate. The right hemisphere was embedded and snap frozen in −35 °C cold 2-methyl-butane and stored at −80 °C (used for quantitative real-time PCR). The left hemisphere was dissected directly on the ice cold glass plate into motoric cortex, striatum and remaining tissue. This tissue was placed in pre-weighed cryotubes and weighed on a microbalance, flash frozen in liquid nitrogen and stored at −80 °C (used for the BDNF-ELISA).

2.3. Quantitative real-time PCR

Striatum and motor cortex were dissected from 100 μm slices of frozen embedded brain tissue inside a cryostat (Hyrax C 50, Zeiss, Germany) at −18 °C. Total RNA was isolated by using the RNeasy Plus Mini Kit (Qiagen, CA, USA) following the manufacture instructions. First-strand cDNA synthesis was performed using the qScript cDNA super Mix (Quanta BioSciences, MD, USA) according to the manufacturers protocol. For real time qPCR the KAPA Probe Fast Universal qPCR Master Mix (peqlab/VWR, Germany) was used with a total volume of 5 μl and amplification was performed on a PikoReal 96 Real-Time PCR system (ThermoScientific, Waltham, USA) according to manufacturers protocols. The following TaqMan Gene Expression Assays were used: BDNF (Mm01334044), Hprt (mM015455399), Gapdh (HS99999915_21), PV (Mm00443100), Nkx2.1 (Mm00447558), CAVII (Mm00551727) and Slc12a5 (Mm00803929). Data was analyzed using the PikoReal Software 2.1 (ThermoScientific). To determine the relative mRNA expression, GeNorm was used and candidate reference genes *Gapdh* and *Hprt* were chosen according to average expression stability as described previously (Richter et al., 2014; Vandesompele et al., 2002). Gapdh was also previously found to be stably expressed in striatal samples of this hamster model (Avchalumov et al., 2014).

2.4. BDNF ELISA

A sandwich-ELISA-system was used to quantify BDNF level in striatum (Quantikine ELISA Human BDNF Immunoassay, R&D systems, MN, USA) as described previously (Petzold et al., 2015). The samples were homogenized in lysis buffer (137 mM NaCl, 20 mM Tris HCl; 1% NP40; 2500 μl 10% glycerol, 1 mM PMSF, 10 mM aprotinin, 1 mM leupeptin). The solution was flash frozen in liquid nitrogen and thawed three times for breaking the cell membranes. The ELISA-System was performed according to the manufactures protocols. The protein concentration was determined by using the Pierce BCA Protein Assay Kit (ThermoScientific, Rockford, IL, USA) in accordance with the manufactures protocols. The results of the BDNF ELISA were normalized to protein or wet weight of the brain.